Interruption of Transmission of *Onchocerca volvulus* in the Oaxaca Focus, Mexico

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Abstract. All endemic communities of the Oaxaca focus of onchocerciasis in southern Mexico have been treated annually or semi-annually with ivermectin since 1994. In-depth epidemiologic assessments were performed in communities during 2007 and 2008. None of the 52,632 *Simulium ochraceum* s.l. collected in four sentinel communities was found to contain parasite DNA when tested by polymerase chain reaction-enzyme-linked immunosorbent assay (PCR-ELISA), resulting in an upper bound of the infection rate in the vectors of 0.07/2,000. The prevalence of microfilariae (mf) in the cornea and/or anterior chamber of the eye was also zero (0 of 1,039 residents examined; 95%-UL = 0.35%). Similarly, all 1,164 individuals examined by skin biopsy were mf negative (95%-UL = 0.31%), and sera collected from 3,569 children from 25 communities did not harbor Ov16 IgG4-antibodies (95%-UL = 0.09%). These meet the criteria for absence of morbidity and parasite transmission in the Oaxaca focus. As a result mass treatments with ivermectin were halted in 2009.

INTRODUCTION

Human onchocerciasis is caused by the filarial parasitic nematode *Onchocerca volvulus*, which in Latin America is transmitted by new world black flies (*Simulium* spp.) in six countries (Brazil, Colombia, Ecuador, Venezuela, Guatemala, and Mexico), where 525,543 individuals are at risk.¹ In Mexico, onchocerciasis occurs in three distinct foci (Southern Chiapas, Northern Chiapas, and Oaxaca) where *S. ochraceum* s.l. is the main vector. The Oaxaca focus contains 98 affected communities, none of which are hyperendemic (11 of the communities are mesoendemic, and 87 hypoendemic). The population at risk in Oaxaca (44,919 individuals) comprises about 10% of the total at risk population in the Americas (525,543 individuals). The predominant inhabitants of the focus are indigenous people of the Zapoteco, Chinanteco, and Cuicateco ethnic groups. The most important economic activity in the communities of the Oaxaca focus is coffee cultivation.

Introduction of the parasite into the Oaxaca focus probably resulted from human movements from Oaxaca to and from the endemic areas of Chiapas or Guatemala during religious pilgrimages to Esquipulas.² Historically, the first cases of onchocerciasis were discovered in 1924 in the community San Miguel Tiltepec in the Oaxaca focus.³ Since then, there have been continuous efforts by residents of communities, operational workers, health authorities, and researchers to control the disease. In 1927, the first parasitological studies based on the analysis of nodules were performed in Oaxaca. In 1931, an onchocerciasis control program was launched based on mass identification and removal of onchocercomas, which are subcutaneous masses containing the adult worms. In 1947, Dr. Luis Mazzotti discovered the utility of diethylcarbamazine (DEC) for the diagnosis and treatment of onchocerciasis, and DEC treatments of patients was added to the nodulectomy program from 1948 through to the 1980s. Since the 1990s, onchocerciasis control in Mexico has relied on the mass distribution of

Mectizan (ivermectin) to the at-risk communities. Annual mass ivermectin distribution treating to all eligible residents from the at-risk communities began in 1994, and in 1997 the strategy was modified to provide mass treatments every 6 months.

The goal of the Onchocerciasis Elimination Program for the Americas (OEPA) is to eliminate new ocular morbidity caused by infection with *O. volvulus* and interrupt transmission of the parasite by the year 2012. Ultimately, the goal of the program is to eliminate the parasite transmission in all affected countries of the region, which requires a 3-year period of surveillance after treatment interventions have stopped to verify no recrudescence of transmission occurs. The World Health Organization (WHO)⁴ and OEPA⁵ have established a series of epidemiologic and entomologic criteria to be achieved to declare onchocerciasis eliminated. World Health Organization/OEPA criteria include: 1) the elimination of new ocular morbidity (defined as a prevalence of <1% of *O. volvulus* microfilariae [mf] in the cornea and/or anterior chamber of the eye), and 2) transmission criteria related to human epidemiological and vector entomological indices. A reduction of new infections to an incidence rate of less than one new case per 1,000 individuals (<0.1%)⁴ has been practically defined as lack of specific antibodies to *O. volvulus* in children. The sample size required to calculate a one-sided 95% confidence interval (CI) for a point prevalence that excludes 0.1% is 3,000 children. WHO/OEPA entomological criterion for interruption of transmission is to show the absence, or near absence, of infective-stage larvae of *O. volvulus* in the vector population (i.e., a rate of less than one infective fly per 1,000 parous flies). Practically, because polymerase chain reaction (PCR) using *O. volvulus*-specific DNA probes are generally applied to examine pools of flies, parity cannot be determined, so the threshold used is less than one infective fly per 2,000 flies tested (assuming 50% of these are parous flies).^{4,5} A minimum sample size of 10,000 flies is required to be examined by PCR collected per each monitored community to reach this standard.

The data presented here report the in-depth epidemiological follow-up study conducted throughout 2008 necessary to declare onchocerciasis ocular morbidity eliminated and transmission interrupted in the Oaxaca focus, following the criteria for elimination established by WHO/OEPA. As a result of these data, health authorities decided to halt treatments

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with ivermectin in 2009, and subsequently initiate the post-treatment surveillance activities in this focus.

MATERIALS AND METHODS

Study area. In 1995, local health authorities selected four sentinel communities of the Oaxaca focus (Figure 1). They were La Esperanza (17°37'40"N, 96°22'10"W, elevation 1,600 m above sea level), Santiago Lalopa (17°25'4"N, 96°14'54"W, elevation 1,200 m), Santiago Teotlaxco (17°26'45"N, 96°19'14"W, elevation 1,225 m), and Santa María La Chichina (17°26'23"N, 96°17'80"W, elevation 1,360 m). In addition to the study of the sentinel communities, a large-scale serological study was performed in children resident in 21 extra-sentinel communities of highest historical endemicity.

Annual mass ivermectin distribution, offered to every eligible resident in the 30 communities in the Oaxaca focus, was initiated in 1994; from 1997 to 2008 mass treatments were provided twice a year. A total of 26 treatment rounds have been provided in Oaxaca over the last 13 years. As shown in Figure 2, ivermectin coverage of the eligible population has remained at a level of greater than 85% every year from 2001 throughout 2008.

The consistently high coverage on the eligible population in the Oaxaca focus is the result of several favorable factors. First, the onchocerciasis program is well established, having been initiated in the 1930s, and throughout the intervening period has consistently maintained a staff of workers exclusively dedicated to onchocerciasis control. Staff members are organized into brigades, and they visit their assigned communities every 3 months. Thus, the brigades become very familiar with their assigned communities, maintaining a detailed census of the residents. Second, ivermectin distribution is regularly performed at a central location in the community. If someone in the community does not attend the distribution event, a home visit is then conducted by the brigades to attempt to convince the person to undergo treatment. Finally, ivermectin distribution has always been accompanied with health education activities. These are directed toward preserving the interest and participation in the community by emphasizing the benefits associated with ivermectin treatment to the individual, for the individual, and to the community.

Entomologic study. Black flies (52,632 *S. ochraceum*) were collected by using standardized procedures⁶⁻⁸ during the peak *O. volvulus* transmission season lasting from December 2007 to

FIGURE 2. Coverage rate (percent) with ivermectin of the eligible population of the Oaxaca focus, Mexico, 1996–2008. The line at 85% indicates the coverage needed in a sustained fashion to interrupt transmission.

March 2008. In 2007, mass ivermectin distribution was conducted just before the peak transmission began. The collections were carried out during the first 50 minutes of each hour, beginning at 11:00 AM and ending at 4:50 PM.⁹ Collectors received ivermectin 1 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, although this probably overestimated the biting rate, because a proportion of the landing flies in a natural setting do not successfully obtain a blood meal. 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.

Flies were combined into pools containing a maximum of 50 individuals per pool and the heads and bodies separated as previously described.⁶ 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 enzyme-linked immunosorbent assay (ELISA) have been published elsewhere.^{6,10} The DNA extractions were carried out in sets of 20 samples each, with each set containing 18 fly pools and two sham extractions that served as contamination controls for the DNA extraction process. All 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 two 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 fly 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 this proportion expressed as the number of positive flies per 2,000 flies examined. Head pools were not analyzed from the four sentinel villages in

FIGURE 1. Map of the two southern Mexico states (marked in dark grey) showing the three endemic foci for onchocerciasis: The gray area indicates the Oaxaca focus, and the black areas indicate the Northern and Southern Chiapas foci.

which no evidence for infection in the vector bodies was found, as infection in bodies has previously been shown to be a more sensitive indicator of parasite vector contact than infection in heads.^{6,10} Thus, if no bodies were found to be positive, it was assumed that no parasite vector contact was detected, and that the prevalence of infectious flies (i.e., flies with infective stages in the head capsule) would be zero. All of the body pools collected from that community was screened, and PoolScreen version 2.0 was used to estimate the prevalence of infected flies in the community and the associated 95% CIs.¹¹

Seasonal transmission potentials (STP) for each sentinel village were calculated as the product of the seasonal biting rate, the proportion of flies carrying L3 larvae in the study season (from December 2007 through March 2008), and the average number of L3 larvae in each infective fly. As previously discussed, we assumed that after multiple rounds of Mectizan treatment, the number of infective larvae present in each infective fly would be close to one.¹⁰

The seasonal biting rate was calculated as the product of the geometric mean¹² of the number of flies collected per person per day and the total number of days in the transmission season, which included the months of December through March. The daily biting rate and the seasonal biting rate were estimated as previously described.¹³ 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 the Oaxaca focus, the level of transmission estimated during the peak of greatest transmission in 2008 was very low (because of the effect of 13 years of treatment with Mectizan). Therefore, the value of transmission potential outside of the peak transmission period is probably zero or near zero. The STP (transmission occurring during the peak transmission season of December through March) likely represents a fairly accurate estimate of ATP.

Serologic study. The prevalence of IgG4 antibodies to Ov16,^{14,15} a recombinant antigen of *O. volvulus*, was determined from two populations of children in the Oaxaca focus: those residents in the four sentinel villages, and school children selected from the overall population sample in the focus. All 242 children 10 years of age and under living in the four sentinel communities were tested. In 21 “extra-sentinel” communities (including all previously mesoendemic communities in the focus) 3,327 school children were also screened. In our study, we included 36 schools that were randomly selected out of 91 schools present in the focus. On average, there were 105 children per school. To recruit participants, explanatory meetings were held with all the members of the school community—parents, teachers, and administrators who provided the lists of all the students enrolled. Considering that not all of the school children participated, the final number of children included was 3,327.

Blood was collected by finger prick from each individual enrolled in the study and dried in the field, transported to the laboratory at 4°C, and kept refrigerated in sealed bags containing silica gel at -20°C until use, within a month of collection. Two 6-mm punches of blood saturated filter paper were placed in a phosphate-buffered saline-Tween (PBS-T) 0.05% and bovine serum albumin (BSA) 5% buffer and eluted overnight at 4°C. The elution was then run in duplicate in a standard ELISA,³ to detect IgG4 antibodies against the OV-16 recombinant antigen. A standard curve was used on each plate to identify positive samples and permit comparisons between plates and over days. The cut-off value was determined after analyzing OV-16 negative and OV-16 positive samples (from 10 par-

asitologically confirmed *O. volvulus* positive individuals). The cutoff was chosen as 40 arbitrary units by identifying the value that optimized both sensitivity and specificity. Any positive results were repeated before being reported as positive.

Ophthalmologic study. Ocular examinations were carried out by an ophthalmologist experienced in onchocerciasis ocular evaluations for OEPA. The examinations were done using a Topcon Optical SL-3D slit lamp (Kogaku Kikai KK, Tokyo, Japan). Exams focused on finding *O. volvulus* microfilariae in the cornea (MFC) and/or the anterior chamber of the eye (MFAC). Before the exam the patients kept a “head down position” (forehead in the lap) for 5 minutes to allow MFC and/or MFAC to settle in a visible position. A population of 1,039 residents, representing about 80% of the total population in the four sentinel communities, was examined.

Parasitologic study. A total of 1,164 individuals, representing 89% of the total population in the sentinel communities, participated in the survey. Two simultaneous skin biopsies were taken from each patient using a 1.5–2.0-mm corneoscleral biopsy punch, one from the left supra-scapular region and the right supra-iliac region. Skin biopsies were incubated overnight in buffered saline, and emerging mf was counted using an inverted microscope.

Statistical analysis. PoolScreen version 2.0 was used to calculate a prevalence of infection in the fly 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 previously) to calculate an estimated STP. *Simulium ochraceum* s.l. were collected during the peak transmission period of December 2007 through March 2008 from four sentinel communities in the Oaxaca focus endemic for *O. volvulus*. The proportion of individuals positive to infection with mf in skin snips, and in the cornea and/or anterior chamber of the eye, was calculated as the number of positive individuals divided by the total number examined and expressed as a percentage. The associated 95% exact CIs of the proportion of individuals harboring Ov16 antibodies were determined using the method of Miettinen (1970), as described in Armitage and Berry.¹⁶ The same method was used to estimate the 95% exact CIs surrounding the point prevalence of MFC, MFAC, and skin mf.

RESULTS

Entomologic study. A total of 52,632 flies were examined by PCR in 1,412 pools (La Esperanza: *N* = 343, Santa Maria La Chichina: *N* = 356, Santiago Teotlaxco: *N* = 367, and Santiago Lalopa: *N* = 346). The number of vectors collected was sufficient to comply with the WHO/OEPA guideline

TABLE 1
Prevalence of infective flies (expressed as rate per 2,000 flies examined) and seasonal transmission potential (third-stage larvae per person per season) estimated during 2008 in four sentinel communities in the focus of Oaxaca, Mexico*

Community	Seasonal biting rate	Prevalence of infective flies/2,000	Seasonal transmission potential
La Esperanza	48,427	0 (0.27)	0 (6.5)
Santa Maria la Chichina	31,836	0 (0.29)	0 (4.6)
Santiago Teotlaxco	32,686	0 (0.31)	0 (5.1)
Santiago Lalopa	72,794	0 (0.27)	0 (9.8)
Total	120,772	0 (0.07)	0 (4.2)

*Value represents point estimate and value in parentheses represents 95% upper limit confidence interval surrounding point estimate.

TABLE 2

Prevalence of IgG4 antibodies to Ov16 in four sentinel communities and 21* extra-sentinel communities in the focus of Oaxaca, Mexico

Santiago Lalopa	Santiago Teotlaxco	La Esperanza	Santa María la Chichina	Extra sentinel communities*	Total
0/92 (3.92%)	0/86 (4.1%)	0/20 (15.8%)	0/44 (8.0%)	0/3,327 (1.5%)	0/3,569 (0.09)

* San Juan Yae, San Bartolome Yatoni, Telea de Castro, San Juan Toavela, San Pedro Teutila, San Juan Zapotitla, San Pedro Sochipam, San Juan Zautla, San Felipe de León, Villa Alta, Arroyo de Banco, Santiago Progreso, Tanetze de Zaragoza, San Isidro Reforma, Santa María Zoogochi, Santo Domingo Roayaga, Lachiroag, Ayoziotepec, San Juan Yatzone, San Juan Yalahui, and San Mateo Yelta.

Value represents point prevalence and value in parentheses represents 95% upper limit confidence interval surrounding point estimate.

of having at least 10,000 flies tested from each community. The results are summarized in Table 1. All body pools were negative for *O. volvulus* DNA, which suggested no parasite-vector contact was occurring. For this reason, head pools from these communities were not examined. Upper limit confidence limits of prevalence of infective flies in all areas were well below the threshold of 1/2,000 (maximum 0.31/2,000). Upper confidence interval limits of the STPs ranged from 4.6 to 9.8 L3 per person per season (overall, the maximum value of the STP in Oaxaca was 4.2 L3 per person per season).

Serologic study. The results of the serological studies are shown in Table 2. No child among a total of 3,569 examined in the four sentinel communities and the 21 extra sentinel communities was positive for Ov16 IgG4 antibodies (95%-UL = 0.09%).

Ophthalmologic study. No MFC or MFAC were found among the 1,039 residents (95%-UL = 0.35%) in the four sentinel communities.

Parasitologic study. None of the 1,164 residents examined by skin biopsy were found to have mf (95%-UL = 0.31%) in the four sentinel communities (Table 3).

DISCUSSION

The epidemiologic parameters of transmission and morbidity presented in this study strongly suggest that transmission of *O. volvulus* is permanently interrupted and that neither ocular nor skin disease is now attributable to the infection in the Oaxaca focus. Regular semi-annual treatment rounds with high coverage were very important to reach the OEPA's goal of interruption of transmission and the likely elimination of onchocerciasis in this area. Likewise, the regional program based on ivermectin has already made significant progress for elimination in other nearby foci including the focus of Northern Chiapas in Mexico, the foci of Santa Rosa, Huehuetenango, and Escuintla-Guatemala in Guatemala, and that of López de Micay in Colombia, where transmission has also been judged to have been interrupted.^{5,17-21}

The entomological criteria for asserting interruption of transmission includes absence or near absence of infective-stage larvae of *O. volvulus* in the vector population, which has been operationally defined as < 1 infective fly/2,000. Martin-

Tellaecche and others²² showed that consecutive treatment with ivermectin resulted in a dramatic decrease of the prevalence of skin mf and nodules in Oaxaca. Despite this promising finding in the human population, during the first large-scale entomologic study of transmission in this area conducted in 2001 after 7 years of mass administration of ivermectin, three of four sentinel communities still had evidence for ongoing transmission.¹⁰ During the second large-scale entomologic study carried out in this area, conducted in 2004 and after 10 years of the ivermectin program, parasite DNA was detected in one single pool of 50 vector heads out of 170 such pools (8,500 flies) examined in one of the sentinel communities. No evidence for transmission was found in the other communities in a total of 13,650 flies' examined.²³

In addition to the 1/2,000 infective fly threshold, OEPA recommends the use of the ATP, (which in the present situation is equivalent to seasonal transmission potential) to assess the status of onchocerciasis transmission, because both of these measurements take into account the biting rate and the prevalence of infective flies. Annual transmission potential encompasses not only the vector competence and vector capacity, but also frequency of feeding on humans, longevity, biting density, how often it feeds, the size of the mf reservoir (prevalence, density, etc.). Unfortunately, the threshold ATP under which transmission does not occur is controversial. The estimated threshold ATP has ranged from 5 to 54 L3/person/year using mathematical modeling²⁴ and from 7.6 to 18 L3/person/year using field observations.^{4,25} All 95%-UL of potential STPs in the four sentinel villages were within this range (4.6–9.8, Table 1) during 2008, which suggests that if conditions remain unchanged, the parasite population is likely to be on the path to elimination. It should be noted that these are overestimated STPs because our calculations used landing rate as a proxy for biting rate, whereas only a proportion of the flies that land would actually be successful in obtaining a blood meal.

During the first serologic studies in the sentinel communities, carried out in 2001, IgG4 antibodies to Ov16 were detected by using an unmarketed immunochromatographic card test (ICT, AMRAD, Sydney, Australia²⁶) in a population of 210 participating persons. All 192 children (16 years of age and under) were negative, but 7/17 adults (41%) (17 years of age and over) harbored antibodies (Onchocerciasis Program in Oaxaca,

TABLE 3

Population (no. examined), prevalence of microfilaria in the cornea (MFC), and/or microfilariae (mf) in the anterior chamber of the eye (MFAC), and skin mf (by skin biopsy) in four sentinel communities in the focus of Oaxaca, Mexico*

Community	Population (no. examined)	Prevalence (%) of MFC and/or MFAC	Population (no. examined)	Prevalence of skin mf
La Esperanza	157 (112)	0 (3.23)	157 (121)	0 (2.9)
Santa María la Chichina	323 (225)	0 (1.6)	323 (238)	0 (1.5)
Santiago Teotlaxco	378 (307)	0 (1.19)	378 (359)	0 (0.97)
Santiago Lalopa	454 (395)	0 (0.92)	454 (446)	0 (0.8)
Total	1312 (1039)	0 (0.35)	1312 (1164)	0 (0.31)

Value represents point prevalence and value in parentheses represents 95% upper limit confidence interval surrounding point estimate. MFC = microfilariae in cornea; MFAC = microfilaria in anterior chamber.

unpublished data). In addition to ICT testing, 1,133 individuals of both sexes and ages participated in a seroconversion study using a tricoctail of recombinant antigens (OvMBP16, OvMBP11, and OvMBP10).²⁷ A cohort of 117 children 10 years of age and under, which were negative to antibodies in 2001, were re-tested in 2004 and none had seroconverted, which resulted in an estimated past exposure incidence of 0%.²³

Subsequent serologic studies carried out in 2004 and 2008 also showed that 267 children 10 years of age or under did not harbor Ov16 antibodies (Onchocerciasis Program in Oaxaca, unpublished data and this work). This testing represented a serosurvey that encompassed all children from the sentinel communities. In 2008, we conducted a larger-scale serologic study to comply with the WHO requirements and reach the required statistical power to exclude 0.1% prevalence. We excluded children of preschool age (5 years of age and less) who are more difficult to access and unlikely to be infected or exposed in low transmission settings, the current situation in the Oaxaca focus. Because the durability of the Ov16 antibody is unknown, at the other end of the age spectrum adults greater than 20 years of age are likely to have antibody from previous exposure or infection. School-aged children seemed best to test, because the rate of new and detectable seroconversions is likely to rise most rapidly from 5 to 20 years of age.⁵

It is useful to consider previous impact study results from the Oaxaca focus. The number of individuals with nodules in the entire Oaxaca focus was *circa* 400 individuals in 1981, however, the last three individuals with nodules were seen in 1999 (Figure 3). The prevalence of MFAC and/or MFC in the four sentinel communities of the Oaxaca focus dropped from 1.7% to 4.1% in 1995–2000 to 0% in 2004–2008 (Figure 4). The Mectizan program also had a significant impact on the prevalence of skin mf (Figure 5). These results are similar to those observed in other endemic areas,^{5,8,28} showing the strong effect of Mectizan on skin and eye mf, thus preventing later severe ocular and dermic pathologies.

Although migration of infected flies from neighboring communities, such as San José Yaeé, Santa Ma. Yaviche, Santiago Yagayo, Santa Cruz Yagavila, San Juan Yagila, and Santa Ma. Zoogochi, could have occurred, this mechanism of re-introduction of infection is unlikely. The only communities located within the flight range of the vector (around 3.5 km)²⁹ have also been receiving ivermectin treatment at the same coverage levels as those of the sentinel communities, and consequently, the level of infection in migrating flies from these communities is likely to be similar to that in the local pop-

FIGURE 3. Number of individuals with nodules in the Oaxaca focus, Mexico, 1981–2007.

FIGURE 4. Overall and examined population for microfilariiae (mf) in the cornea and/or anterior chamber of the eye in participants from the four sentinel communities of the Oaxaca focus, Mexico, 1995–2008.

ulation. Similarly, the threat of infection from the focus of Southern and Northern Chiapas, the most proximal focus to Oaxaca is extremely small, given that these foci are separated by 540 km and 380 km (Figure 1), a distance that is over 100 times that of the flight range of the vector black fly.

It is extremely difficult to predict with certainty when treatments may be safely stopped, because transmission may rebound if the pressure on the parasite population is removed. As a result, the process for certification of onchocerciasis consists of four phases.^{4,13} Phase I includes ivermectin treatment until transmission is suppressed. In phase II, suppression is maintained through treatment of the mean reproductive lifespan of the adult female. In phase III, which the data suggest represent the current situation in Oaxaca, the adult parasite population should have died by senescence and removal of ivermectin treatment will not result in a resumption of transmission. To help guide the certification of elimination of the *O. volvulus* infection (phase IV), studies on parasite transmission in the post-treatment era in the Oaxaca focus will be needed. These are currently underway.

In conclusion, based on the entomologic and epidemiologic assessments presented in this study, the WHO/OEPA criteria indicating elimination of *O. volvulus* transmission have been met in the Oaxaca focus. In light of these results, local and federal Mexican health authorities agreed that ivermectin should be suspended in the Oaxaca focus in 2009, thus commencing an intensive epidemiological surveillance program to document that transmission will not re-develop.

FIGURE 5. Overall and examined population for microfilariiae (mf) in skin snips in participants from the four sentinel communities of the Oaxaca focus, Mexico, 1993–2008.

Received September 11, 2009. Accepted for publication November 22, 2009.

Acknowledgments: We are grateful to all people from the sentinel and extra-sentinel communities in the focus of Oaxaca who enthusiastically participated throughout this study. We thank the personnel of the Ministry of Health (the Mexican Onchocerciasis Elimination Program): Alfredo Rodríguez-Atanacio and Víctor Salazar-Bueyes for their valuable assistance during this study. We deeply appreciate the people that have participated with laboratory (PCR): Cristian Lizarazo-Ortega and Guadalupe C. Rodríguez-Castillejos of CIBIOGEM-IPN in Reynosa, Mexico, and field work: Rabi Hernández, Vicente Aragón-Canseco, and Gerardo-Jiménez. We also are indebted to Pedro Ruiz, Armando Hernández, Epifanio M. Zúñiga, and Daniel Coronel of the brigades of the Onchocerciasis Program in Oaxaca, who enthusiastically collaborated with the personnel of the Ministry of Health. We are much obliged to OEPA's ophthalmologist: Héctor Margeli-Pérez and Orlando Oliva-De León, whose enthusiastic participation made this study feasible. Last but not least, Nancy Cruz and Nidia Rizzo (Center for Health Studies of the University of Valley in Guatemala) made this study possible because of their participation in the serology testing. The authors thank all forgotten people that have indeed contributed to the completion of this study.

Financial support: This study was supported by CONACYT-México (grant 874080), and the Onchocerciasis Elimination Program for the Americas (The Carter Center), the Bill and Melinda Gates Foundation, Lions Clubs International Foundation, and Merck & Co for supporting local workers, lab supplies, and technicians. Merck & Co, through the Mectizan Donation Program, has donated all the ivermectin through the years. Mario A. Rodríguez-Pérez holds a scholarship from Comisión de Operación y Fomento de Actividades Académicas/ Instituto Politécnico Nacional. The Direction of Vector Borne Diseases of CENAVECE, Ministry of Health Mexico and the Health Services of the State of Oaxaca also supported this study as part of their tenure.

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REFERENCES

- OEPA, 2008. *Onchocerciasis Elimination Program for the Americas*. Available at: <http://www.oepa.net/index.html>. Accessed November 15, 2009.
- Vásquez-Castellanos JL, 1991. Cafeticultura e historia social de la oncocercosis en el Soconusco, estado de Chiapas, México. *Salud Publica Mex* 33: 124–135.
- Pardo R, 1927. A Propósito de los ciegos de Tliltepec. *Gac Med Mex* 58: 195.
- World Health Organization, 2001. *Certification of Elimination of Human Onchocerciasis: Criteria and Procedures*. Geneva, Switzerland: World Health Organization.
- 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.
- Rodríguez-Pérez MA, Lilley BG, Domínguez-Vázquez A, Segura-Arenas R, Lizarazo-Ortega C, Mendoza-Herrera A, Reyes-Villanueva F, Unnasch TR, 2004. Polymerase chain reaction monitoring of transmission of *Onchocerca volvulus* in two endemic states in Mexico. *Am J Trop Med Hyg* 70: 38–45.
- Walsh JF, Davies JB, Le Berre LE, Garms R, 1978. Standardization of criteria for assessing the effects of *Simulium* control in onchocerciasis control programmes. *Trans R Soc Trop Med Hyg* 72: 675–676.
- Rodríguez-Pérez MA, Rodríguez MH, Margeli-López HM, Rivas-Alcalá AR, 1995. Effect of semiannual treatments of ivermectin on the prevalence and intensity of *Onchocerca volvulus* skin infection, ocular lesions, and infectivity of *Simulium ochraceum* populations in southern Mexico. *Am J Trop Med Hyg* 52: 429–434.
- Cupp EW, Ochoa JO, Collins RC, Cupp MS, Gonzales-Peralta C, Castro J, Zea-Flores G, 1992. The effects of repetitive community-wide ivermectin treatment on transmission of *Onchocerca volvulus* in Guatemala. *Am J Trop Med Hyg* 47: 170–180.
- Rodríguez-Pérez MA, Katholi CR, Hassan HK, Unnasch TR, 2006. Large-scale entomologic assessment of *Onchocerca volvulus* transmission by poolscreen PCR in Mexico. *Am J Trop Med Hyg* 74: 1026–1033.
- Katholi CR, Toé L, Merriweather A, Unnasch TR, 1995. Determining the prevalence of *Onchocerca volvulus* infection in vector populations by PCR screening of pools of black flies. *J Infect Dis* 172: 1414–1417.
- Williams CB, 1937. The use of logarithms in the interpretation of certain entomological problems. *Ann Appl Biol* 24: 404–414.
- Rodríguez-Pérez MA, Lutzow-Steiner MA, Cabrera AS, Lizarazo-Ortega C, Domínguez-Vázquez A, Sauerbrey M, Richards F Jr, Unnasch TR, Hassan HK, Hernández-Hernández R, 2008. Rapid suppression of *Onchocerca volvulus* transmission in two communities of the Southern Chiapas focus, Mexico achieved by quarterly treatments with Mectizan. *Am J Trop Med Hyg* 79: 239–244.
- Lobos E, Weiss N, Karam M, Taylor HR, Ottesen EA, Nutman TB, 1991. An immunogenic *Onchocerca volvulus* antigen: a specific and early marker of infection. *Science* 251: 1603–1605.
- Lipner EM, Dembele N, Souleymane S, Alley WS, Prevots DR, Toe L, Boatín B, Weil GJ, Nutman TB, 2006. Field applicability of a rapid-format anti-Ov-16 antibody test for the assessment of onchocerciasis control measures in regions of endemicity. *J Infect Dis* 194: 216–221.
- Armitage P, Berry G, 1994. *Statistical Methods in Medical Research*. Third edition. Oxford: Blackwell Scientific Publications.
- Sauerbrey M, 2008. The Onchocerciasis Elimination Program for the Americas (OEPA). *Ann Trop Med Parasitol* 102(Suppl 1): 25–29.
- Gonzalez RJ, Cruz-Ortiz N, Rizzo N, Richards J, Zea-Flores G, Domínguez A, Sauerbrey M, Catú 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.
- 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: CDC-CAP.
2009. Onchocerciasis (river blindness). Report from the eighteenth InterAmerican Conference on Onchocerciasis, November, 2008. *Wkly Epidemiol Rec* 84: 385–396.
- Rodríguez-Pérez MA, Unnasch TR, Domínguez-Vázquez A, Morales-Castro AL, Richards F Jr, Peña-Flores GP, Orozco-Algarra ME, Prado-Velasco G, 2010. Lack of active *Onchocerca volvulus* transmission in the Northern Chiapas focus, Mexico. *Am J Trop Med Hyg* 83: 15–20.
- Martin-Tellaeche A, Ramirez-Hernandez J, Santos-Preciado JI, Mendez-Galvan J, 1998. Onchocerciasis: changes in transmission in Mexico. *Ann Trop Med Parasitol* 92: S117–S119.
- Rodríguez-Pérez MA, Lizarazo-Ortega C, Hassan HK, Domínguez-Vázquez A, Méndez-Galván J, Lugo-Moreno BP, Sauerbrey M, Richards F Jr, Unnasch TR, 2008. Evidence for suppression of *Onchocerca volvulus* transmission in the Oaxaca focus in Mexico. *Am J Trop Med Hyg* 78: 147–152.
- Wada Y, 1982. Theoretical approach to the epidemiology of onchocerciasis in Guatemala. *Jpn J Med Sci Biol* 35: 183–196.
- Porter CH, Collins RC, Brandling-Bennett AD, 1988. Vector density, parasite prevalence, and transmission of *Onchocerca volvulus* in Guatemala. *Am J Trop Med Hyg* 39: 567–574.

26. Weil GJ, Steel C, Liftis F, Li B-W, Mearns G, Lobos E, Nutman TB, 2000. A rapid-format antibody card test for diagnosis of onchocerciasis. *J Infect Dis* 182: 1796–1799.
27. Rodriguez-Perez MA, Danis-Lozano R, Rodriguez MH, Bradley JE, 1999. Comparison of serological and parasitological assessments of *Onchocerca volvulus* transmission after 7 years of mass ivermectin treatment in Mexico. *Trop Med Int Health* 4: 98–104.
28. Vieira JC, Cooper PJ, Lovato R, Mancero T, Rivera J, Proaño R, López AA, Guderian RH, Rumbela J, 2007. Impact of long-term treatment of onchocerciasis with ivermectin in Ecuador: potential for elimination of infection. *BMC Med* 5: 9.
29. Collins RC, Ochoa JO, Cupp EW, Gonzales-Peralta C, Porter CH, 1992. Microepidemiology of onchocerciasis in Guatemala: dispersal and survival of *Simulium ochraceum*. *Am J Trop Med Hyg* 47: 147–155.