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, onchocercerciasis occurs in three distinct foci

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 tri,/F3 8.04 Tf1 0 0 /m 12 Tf3(Tf1 2r557.14/F3t g Tf1 0 0 1 350.59 515.e.14/F3t g T5)D

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 procedures[6-8] during the peak *O. volvulus* transmission season lasting from December 2007 to 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.

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 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 mean[12] 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

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, [5] 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 parasitologically 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 of having at least 10,000 flies tested from each community. The results are summarized in Table 1

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

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