at risk in the entire focus of northern Chiapas (Figure 1). In the last 20–30 years, the social and economic structure of the indigenous population of the northern Chiapas focus has been changing: young people have been abandoning the endemic area and migrating to industrialized urban cities, particularly in northern Mexico and the United States. In contrast, the seasonal migration of workers from northern Chiapas to the coffee plantation areas of

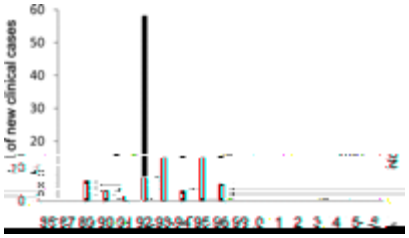

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FIGURE 2. Number of new clinical cases of

be so also. However, that approach did not allow the researchers to definitively state that the flies did not contain infectious-stage parasites.

Here, we report data obtained from an in-depth epidemiological follow-up study of onchocerciasis in northern Chiapas conducted throughout 2006. We also report data showing an absence of infective larvae in the flies collected in the entomological study conducted in 2005.

polymerase chain reaction (PCR) assay specific for *O. volvulus* . Details of protocols for genomic DNA purification, primer sequences, PCR conditions, and detection of PCR products by enzymelinked immunosorbent assay (ELISA) have been published elsewhere. [6, 7] PoolScreen version 2.0 was used to estimate the infective proportion in the vector population of the community and the associated 95% CIs. 13 This proportion was expressed as the number of positive flies per 2,000 flies examined. Seasonal transmission potentials (STPs) 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 February to May 2005), and the average number of L3 larvae in each infective fly. Before Mectizan distribution,

reported elsewhere. [10, 18] Blood was collected by finger prick from each individual enrolled in the study, 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. Blood collections and IgG 4-Ov16 ELISA assays to monitor exposure in the under 10 cohort were carried out as previously described. [10]

Ophthalmologic study. Ocular examinations were carried out by an ophthalmologist experienced in onchocerciasis ocular evaluations. The examinations were done using a Topcon Optical SL-3D slit lamp (Kogaku Kikai KK, Tokyo, Japan). Exams focused on finding *O. volvulus* mf 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 682 individuals 10 years of age and over, representing about 50% of the total population in the two sentinel communities, were examined during 2006.

Parasitologic study. A total of 986 individuals representing 71% of the total population in the two sentinel communities participated in the survey. Two simultaneous skin biopsies were taken from each patient using a 1.5- to 2.0-mm corneoscleral biopsy punch: one biopsy from the left supra-scapular region and one biopsy from the right supra-iliac region. Skin biopsies were incubated overnight in buffered saline, and emerging microfilariae were counted using an inverted microscope.

Statistical analysis. Poolscreen v2.0 was used to calculate a prevalence of infectivity 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 above) to calculate an estimated STP. *S. ochraceum* s.l. were collected during the peak transmission period of February through May 2005 from two sentinel communities in the northern Chiapas focus endemic for *O. volvulus.* The proportion of individuals positive to infection with mf in skin snips and mf 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 exact 95% CIs of the proportion of individuals harboring Ov16 antibodies were determined using the Miettinen method described by Armitage and Berry. [19] The same method was used to estimate the 95% exact CIs surrounding the point prevalence of MFC, MFAC, and skin mf.

RESULTS

Entomological study. A total of 4,400 host-seeking *S. ochraceum* s.l. females were collected during the peak transmission period of February through May 2005 from two sentinel communities in the northern Chiapas focus endemic for *O. volvulus* . A total of 88 head pools, each containing 50 heads (Altagracia: *N* = 71; El Ambar: *N* = 17) were examined by PCR. The results of this analysis are summarized in Table 1. All head pools were negative for *O. volvulus* DNA. Thus, the point estimate of the prevalence of infective flies was zero and had an upper bound on the 95% CI of 0.9/2,000. Similarly, the upper bound of the 95% CI for the STP was 1.2 L3/season.

Serological study. Of the 305 children 10 years and under (representing all the children in that age group in the sentinel communities) that were tested for IgG4 antibodies, 0 were positive. Therefore, the estimated prevalence of *O. volvulus* exposure in the sentinel communities was 0% (Table 2).

Ophthalmologic study. No MFC and/or MFAC were found among the 682 residents tested of 1,391 individuals in the two sentinel communities (Table 2 RnETlog

DISCUSSION

The absence of new clinically defined cases of onchocerciasis in the entire northern Chiapas focus during the last decade (Figure 2) provides indirect support to the hypothesis that parasite transmission is no longer ongoing in this focus. This finding suggests that endemic onchocerciasis no longer represents a serious health risk to the endemic community in northern Chiapas. However, it is still possible that new cases might be introduced into this region from migration from other areas of Mexico where transmission is still ongoing, such as southern Chiapas. This risk is likely to be less than past levels, because the population in the northern Chiapas focus no longer conducts seasonal migration to southern Chiapas at the levels seen historically. However, the risk of reinfection from southern Chiapas, although decreased from historic levels, still exists.

No evidence for infective parasites in the vector, or for new parasite exposure in the human population, was found in the two sentinel communities examined in this study, supporting the conclusion that there is currently no ongoing transmission of *O. volvulus* in the northern Chiapas focus. As a result, community- wide treatments with ivermectin were halted in northern Chiapas in 2008, and post-treatment surveillance activitiesin this focus were initiated. Thus, northern Chiapas joins the five other foci in Latin America where transmission seems to have been interrupted: Oaxaca in Mexico, Santa Rosa, Huehuetenango, and Escuintla-Guatemala in Guatemala, and López de Micay in Colombia. [10, 18, 20– 23]

The entomological criteria for asserting interruption of transmission includes the absence or near absence of infective-stage larvae of *O. volvulus* in the vector population, which has been operationally defined as < 1 infective fly per 2,000 examined. [9, 10] During the first entomologic study carried out 2l.0.0220.73 416.23n1 0 0 1A

Although migration of infected flies from neighboring communities could

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