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ENTOMOLOGICAL AND SEROLOGICAL ASSESSMENT OF ONCHOCERCA VOLVULUS TRANSMISSION IN THE NORTHERN CHIAPAS FOCUS (MEXICO)

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ABSTRACT – Entomological and serological surveys were performed during 2001 and 2005 in two communities (Altagracia and El Ámbar) of the Northern Chiapas (Mexico) focus to assess the parasite transmission and incidence of exposure to onchocerciasis. In Altagracia in 2001, infective flies were detected, leading to an estimated transmission potential of 1.4 L3 per person per year. In 2005, no infective flies were detected in Altagracia, leading to an estimated transmission potential of 2.4 L3 per person per year. In 2005, no infective flies were detected in Altagracia, leading to an estimated transmission potential of 2.4 L3 per person per year. No evidence for transmission was found during either year in El Ámbar. Within a sentinel cohort in these communities, 12% (15/126) were serologically positive for antibodies recognizing a cocktail of *O. volvulus* recombinant antigens in El Ámbar, while 7% (5/68) were serologically positive in Altagracia. The incidence in children five years old and younger (n = 27) within this sentinel cohort was 13% (2/15) in El Ámbar, and 8% (1/12) in Altagracia. Although no evidence for parasite transmission was seen in El Ámbar in the surveys conducted in 2001 and 2005, a few individuals in the sentinel cohort seroconverted during this period.

KEY WORDS: Ivermectin, blackflies, serology, ELISA, recombinant antigens, pool screening, PCR

INTRODUCTION

Onchocerca volvulus is endemic to three foci in Mexico: Southern Chiapas, Northern Chiapas, and Oaxaca. It was suggested that the onchocerciasis in the Northern Chiapas focus is a result of the annual seasonal in-migration of coffee workers from the Southern Chiapas focus. It was further hypothesized that onchocerciasis infections in the Northern Chiapas focus are solely a result of this seasonal in-migration, and that no independent transmission of the parasite occurs in this

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focus (DAVIES, 1968; VÁSQUEZ CASTELLANOS, 1991). However, recent entomological studies suggested that independent transmission of *O. volvulus* might be occurring in the Northern Chiapas focus (RODRÍGUEZ-PÉREZ ET AL., 2004, 2006).

The overall goal of the Onchocerciasis Elimination Program in the Americas (OEPA) is to eliminate onchocerciasis in the six countries of Latin America where it is endemic. To assist in this process, the World Health Organization (WHO) has developed a series of guidelines to certify that an area is free of onchocerciasis. Those guidelines focused on entomology are based on demonstrating transmission suppression in *Simulium* blackflies for a period of 12 years. In the foci where no pre-control entomological data exist (such as Northern Chiapas focus), transmission suppression is defined in the WHO guidelines as an 'absence or near absence' of L3 infection in the vector population. The WHO did not define "near absence" in terms of quantitative metrics. In practical terms, control efforts that result in transmission levels below that necessary to maintain the basic reproductive rate (R_o) at a value of 1.0 will eventually result in extinction of the parasite population. Therefore, from an operational point of view, maintaining transmission levels below those necessary to maintain the parasite population can be considered to meet the guideline of "near absence" of transmission.

Here we present the results of entomological studies of transmission of *O. volvulus* in Northern Chiapas. These studies are complemented by a longitudinal serological study of *O. volvulus* exposure in a sentinel human cohort in the same area. The data suggest that while some transmission may still exist in this area, the level of transmission is likely to be insufficient to maintain the parasite population.

MATERIALS AND METHODS

Study area

The present study was conducted during 2001 and 2005. Pre-selected sentinel communities were not in place in the Northern Chiapas focus because autochthonous transmission was thought not to occur in this focus. Thus, two communities (Altagracia [17° 01' 51"N, 92° 46' 02"W, elevation 1,300 m], and El Ámbar [17° 01' 29"N, 17° 01' 29"W, elevation 1,610 m]) were selected as advised the coordinator and brigade commander of the local onchocerciasis elimination program. The population affected in the Northern Chiapas focus is predominantly indigenous, consisting mainly of Chamula, Tzotzil, and Zoque Indians. The most important economic activity in these communities is coffee cultivation.

Mass drug administration

The onchocerciasis elimination program in Mexico began treatment with Mectizan in 1989, initially providing treatment only to nodule and/or Mazzotti reaction-positive individuals in residing hyperendemic communities. From 1991 to 1994, bi-annual treatment with Mectizan was extended to residents of mesoendemic communities and to 25% of the eligible residents of hypoendemic villages. From 1995 to 1997, the coverage in hypoendemic communities increased to 40% of eligible residents. From 1997 to the present, the strategy has been to provide mass bi-annual treatments to every qualified resident in all the at-risk communities (meso-, hypo-, and hyperendemic villages). Because all communities in the Northern Chiapas focus were classified as hypoen-

demic, mass ivermectin distribution in the Northern Chiapas focus was initiated in 1997. Ivermectin coverage of the eligible population in 2001, 2003, 2004, and 2005, was 81.5%, 88.5%, 86.5%, 87%, and 92.5, respectively. In the Northern Chiapas focus, there are 13 hypoendemic communities with a total population of 7,010, of which 6,465 (92%) subjects are eligible to receive ivermectin treatment.

Blackfly collection and PCR

Blackflies were collected following standardized procedures (RODRÍGUEZ- PÉREZ ET AL., 2004; WALSH ET AL., 1978; RODRÍGUEZ- PÉREZ ET AL., 1995) during the peak O. volvulus transmission season lasting from February to April 2001, and from February to May 2005. Collections were carried out during the first 50 minutes of each hour, beginning at 07:00 hours and ending at 16:50 hours. Collectors received Mectizan[™] one 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). Blackflies 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 (GRILLET ET AL., 2001). 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 blackflies were preserved in isopropanol at room temperature and returned to the laboratory. There, the S. ochraceum s. l. flies were separated by morphological examination, and the few flies that were found to have taken a fresh blood meal were discarded. Simulium ochraceum s. l. females were divided into aliquots of 50 specimens each for further processing for DNA.

These pools were then tested for the presence of *O. volvulus* parasites utilizing a PCR assay specific for *O. volvulus*. Details of protocols for genomic DNA purification, primer sequences, PCR conditions, detection of PCR products by ELISA, and the entomological data interpretation have been presented previously (RODRÍGUEZ-PÉREZ ET AL., 2004, 2006).

Blood samples and ELISA

Details of human blood sample collection, use of specific recombinant antigens, the ELISA protocol, and data interpretation were carried out as previously described (RODRÍGUEZ-PÉREZ ET AL., 1999).

Collections of sera from 30 individuals living in non-endemic communities close to the study area and with no history of *O. volvulus* infection were run as negative controls. The diagnostic cutoff value was considered to be the mean value of the readings from these negative controls plus seven standard deviations [about 99.9999999974%, or at least 98% according to the Chebyshev rules (WEIMER, 1993) if the values are within a normally distributed population].

Data analysis

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 (KATHOLI ET AL., 1995). In all cases, estimates for which the 95% CIs did not overlap were considered to be significantly different. The rates of infective flies (prevalence of infection in head pools) were used to calculate estimates of seasonal transmission potential. The seasonal transmis-

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sion 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 April during 2001, and from February through May during 2005), and the average number of L3 larvae in each infective fly. The seasonal biting rate was calculated as the product of the geometric mean of the number of flies collected per person/day and the total number of days in the months of February through April (= 89 days) during 2001, and from February through May (=120 days) during 2005 where the geometric mean =

 $\ln \left[\sum_{i=1}^{n} (Xi+1) \right]_{-1}$ with X being the number of collected flies per capture unit (50 min) and *n* the total

number of capture units. The geometric mean was then adjusted to bites per hour (multiplying by 0.83) and the product multiplied by the number of light hours (10 hours) (WHO, 2001 a, b). The associated 95% confidence intervals (CIs) were determined using the CIs formula for the mean of a population in an excel spreadsheet: $\frac{\sigma}{\sqrt{\pi}}$ where $1.96 = 1 - \alpha 0.05$; σ = standard deviation.

Because *S. ochraceum* s. l. females were not collected throughout the year, it was not possible to calculate precisely 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 MectizanTM). Therefore, the value of transmission potential outside of the peak transmission period is probably zero or near zero. It follows that, the seasonal transmission potential (transmission occurring during the peak transmission season of February through May) probably represents a fairly accurate estimate of ATP. We estimated that after multiple rounds of ivermectin treatment, the number of infective larvae present in each infective fly would be close to 1 (RODRÍGUEZ-PÉREZ ET AL., 2006).

Antibody prevalence was defined as the proportion of antibody-positive results among persons who had optical values equal to or greater than the cut-off value. The cut-off for classifying a sample as positive was set at the mean of 30 negative controls plus 7 standard deviations (BLOCH ET AL., 1988). Test specificity at this cut off was 100%, while sensitivity was 96% when compared to the skin snip test (BRADLEY ET AL., 1993) and 97% sensitivity when tested on a sero-positive reference collection (RODRÍGUEZ-PÉREZ ET AL., 2003).

A total of 578 individuals of both sexes including 85 children 5 years old and younger participated in the study. The proportion of sero-converting individuals over the course of the study was estimated by following a cohort of 194 of these 578 individuals (including 27 children \leq 5 years of age) who were sero-negative to the test in 2001.

RESULTS

The prevalence of infected flies (*i.e.* parasite DNA detected in pools of bodies) and that of infectious ones(*i.e.* parasite DNA detected in pools of heads) were assessed using a PCR assay as described in Materials and Methods. A total of 197 pools of flies (both head and body pools) from Altagracia were screened in 2001. In 2005, a total of 71 pools were screened. Following the OEPA-approved protocol for PCR screening of vector pools, the body pools in 2005 were first screened for evidence of fly-parasite contact, as prevalence of the infection in bodies is uniformly greater than the prevalence of parasite in head pools (RODRíGUEZ-PÉREZ ET AL., 2006). Because none of the body pools screened in 2005 were PCR-positive, it was concluded that no evidence for parasite-vector contact existed, so head pools from this time were not examined further. Following the Same procedure in El Ámbar, 101 pools were screened in 2001 and 17 in 2005. Results of the PCR screens were used to calculate the prevalence of infective flies in the vector populations

Seasonal biting rate = Geometric mean of the number of bites per person/day multiplied by the total number of days in the months of February through April (= 89 days) during 2001, and from February through May (= 120 days) during 2005. The number in boldface represents the point estimate for the value in question and the numbers in normal type represent the 95% transmission Seasonal potential 0 0 Prevalence of infective flies per 10,000** 0 0 2005 Prevalence of infected flies **Estimated from the fact that no evidence for infection was seen in the fly population – see text per 10,000 (0-5.4)0 0 (2,061-2,412) biting rate 2,232 Seasonal 394 transmission potential Seasonal 1.4 0 Prevalence of infective flies (0.01 - 3.9)per 10,000 1.5 0 2001 Prevalence of infected flies per 10,000 (1.5 - 10.4)5.6 0 CI surrounding the point estimate. Seasonal biting (8,686-9,484) 3,044 9,077 rate Altagracia El Ámbar

(0-22.2)

(338-452)

(0-3.8)

(0-3.8)

(2, 773 - 3, 334)

Table 1. Prevalences of infected and infective flies and calculated seasonal transmission potentials for Altagracia and El Ambar, Northern Chiapas: Table 2. Number of individuals becoming serologically positive (% of incidence of exposure to onchocerciasis) from 2001 throughout 2005 in El Ámbar and Altagracia of the Northern Chiapas focus.

	El Ámbar	Altagracia
Age Group	Elisa + /Elisa –	Elisa + /Elisa –
0 – 5	2/15 (13)	1/12 (8)
≥ 6	13/111(12)	4/56 (7)

with a 95% confidence interval. These data were then combined with estimates of the biting rate (calculated from the fly collection data as described in Materials and Methods) to calculate the estimated seasonal transmission potential. The results of these calculations are summarized in Table 1. In 2001, the prevalence of infective flies was 1.5 per 10,000 (95% CI; 0.01-3.9 per 10,000), leading to an estimated transmission potential of just 1.4 L3 per person per year. In 2005, no evidence for infection in the vector population was detected from this community, leading to an estimated transmission potential of zero. Similarly, no evidence for infection in the vector population was seen in El Ámbar in either 2001 or 2005, again leading to an estimated transmission potential of zero for this community for both years (Table 1).

During the four years covered by this study, 17 of 167 initially serologically negative individuals ≥ 6 years old converted to seropositivity. Similarly, 3/27 children 5 years old and younger seroconverted during this period (Table 2).

DISCUSSION

This is the first time that ELISA has been used in a follow-up study to assess the level of exposure to onchocerciasis in a sentinel cohort in the Northern Chiapas focus. During the four year period encompassed by this study (2001-2005), several sero-negative individuals sero-converted to O. volvulus positive as assayed by ELISA. A low annual incidence of exposure to onchocerciasis among all age categories, which included subjects born before and after implementation of the ivermectin program, indicates that few individuals were exposed to new infections. Some of the subjects \leq 10 years old had positive antibody assays. In a previous entomological study (RODRÍGUEZ-PÉREZ ET AL., 2004, 2006), PCR positive body and head S. ochraceum s. l. pools from a community in this focus were found. The serological data reported here provide further indirect evidence for the occurrence of independent transmission in this focus. As mentioned in the Introduction, it was hypothesized that the Northern Chiapas focus is a result of the annual seasonal in-migration of coffee workers from Southern Chiapas (VÁSQUEZ-CASTELLANOS, 1991; DAVIES, 1968). It was further suggested that onchocerciasis infections are solely a result of this seasonal in-migration and that no independent transmission of the parasite occurs in this focus (VÁSQUEZ-CASTELLANOS, 1991; DAVIES, 1968). If this were the case, the antibody positive individuals found in the area may indicate that they had migrated to areas with ongoing transmission. To date, little migration from the Northern to the Southern Chiapas focus for coffee harvesting has been observed. If antibodypositive subjects did not migrate to the areas at risk, it may be possible that exposure to the parasite occurred in the villages in which they were born. In support of this hypothesis, the incidence of exposure to onchocerciasis in children ≤ 5 years of age ranged from 8% to 13% within the 4-year period.

The serological data do not provide precise estimations of infection rates because it is possible that some individuals exposed to the parasite may develop specific antibodies but never get infected. Thus, detection of circulating antibodies to *O. volvulus* in an exposed population cannot be used to define the presence and level of infection, but it has the potential as an epidemiological tool to provide an estimate of exposure to infection. In addition, the serological method is a much less invasive technique than the skin biopsy method, since only finger prick blood samples are required; consequently, it was more widely accepted by subjects in the two communities of the Northern Chiapas focus (compliance rate = 70.3% in 2005 and 44.2 % in 2001).

As indicated to in the Introduction, in order for transmission to be significant from an epidemiological perspective, it must occur at a level that is sufficient to maintain the basic reproductive rate at a value that is equal to or greater than 1. Transmission below this level will be insufficient to maintain the population, eventually leading to extinction of the parasite. While the annual transmission potential necessary to maintain reproduction at a level sufficient to sustain the population is not precisely known, previous modeling studies using data from West Africa have suggested that the annual transmission necessary to maintain a reproductive rate of 1.0 may be between 12 and 29 (BASÁNEZ ET AL., 2007; DIETZ 1982). In Altagracia, the transmission potential was just 1.4 in 2001, while in 2005 it was zero. In El Ambar, the transmission potential was estimated to be zero for both 2001 and 2005. Even taking the product of the upper boundaries of the 95% confidence intervals for both the prevalence of infective flies and the annual biting rate the maximal possible transmission potential for Altagracia is estimated to be just 3.7 larvae per person per year in 2001, while in El Ambar the upper boundary for transmission potentials was 1.3 in 2001. All of these values are substantially below the minimal exposure estimate of 12 to 29 larvae per person per year necessary to maintain the basic reproductive rate at 1.0 or above. Thus, the serological and entomological data, when taken together, suggest that some exposure to O. volvulus is still occurring in the communities of Altagracia and El Ámbar. However, the level of transmission is not sufficient to maintain the parasite population, and in the absence of any perturbations, the O. volvulus population in Northern Chiapas is likely to be headed towards extinction. Additional serological studies need to be carried out before it can be confirmed that complete suppression of exposure to new parasite infections in the Northern Chiapas focus has been achieved. Such studies are currently under way.

In summary, the data presented confirmed the occurrence of parasite transmission in the Northern Chiapas focus. However, in the two communities studied, the level of transmission is likely to be below the level necessary to maintain the minimum reproductive rate of the parasite population. Additional entomological and serological surveys will need to be conducted before it can be said that onchocerciasis has been eliminated in the Northern Chiapas focus.

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