CURRENT STATUS OF ONCHOCERCIASIS IN COLOMBIA

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Abstract. To assess the current epidemiologic status of onchocerciasis in Colombia two surveys were undertaken in 1995 in a suspected new focus on the border between Colombia and Ecuador and in the known focus located on the Micay River. No new focus was found along the Colombia-Ecuador border. In the known focus, communities along the upper Micay River and its tributaries were surveyed; 655 adults underwent physical examinations and skin biopsies. Infected individuals were found almost exclusively in the community of Naicona, where prevalence of infection was 40% (36 of 91). Polymerase chain reaction detection of onchocercal DNA in skin snips correlated with the skin-snip biopsy results. The prevalence of punctate keratitis, the only ocular manifestation found, was 33%. A rapid entomologic assessment demonstrated Simulium exiguum infected with Onchocerca volvulus. This is the first finding in Colombia of naturally infected black flies and confirms S. exiguum as a vector species. These data will be used for implementing a control program using periodic ivermectin distribution.

The first confirmed case of onchocerciasis in Colombia was detected in 1965 in a patient presenting loss of visual acuity. Microfilariae were observed both in the anterior chamber of the eye and in a skin biopsy. This index case led to the description of the only known onchocerciasis focus in Colombia, located near the town of Lopez on the Micay River on the Pacific Coast. Previous epidemiologic surveys conducted in 1965, 1977, and 1989 suggested that onchocerciasis transmission in Colombia was decreasing without any interventions. Over this 24-year period prevalence measured by skin biopsy positivity decreased from 15% to 4.1%. These passive studies repeatedly showed that the infected individuals inhabited areas near the headwaters of the Micay River. An active epidemiologic study was carried out in the nine communities located along the upper Micay River to define the actual geographic location and prevalence of onchocerciasis infection in this area.

Studies carried out in the province of Esmeraldas in northwestern Ecuador in 1988 showed that migrant workers who had arrived recently from the adjacent department of Narino in southwestern Colombia were infected with Onchocerca volvulus. This finding suggested that onchocerciasis could exist along the Colombia-Ecuador border.

To assess the current epidemiologic status of onchocerciasis in the Lopez de Micay focus and to verify the existence of other foci on the Pacific Coast, a modified rapid epidemiologic assessment using the standard procedure of skin-snip biopsies was used. Additionally, the usefulness of the polymerase chain reaction (PCR) for the diagnosis of O. volvulus infections was assessed. Entomologic studies were also carried out.

MATERIALS AND METHODS

Study areas. During 1995, two areas were assessed epidemiologically. As shown in Figure 1, area A is located in southwestern Colombia near the border with Ecuador and includes all settlements located between the Mira and Matate Rivers (1'42' N, 77'59' W), with an altitude between 100 and 240 meters above sea level. The population in this area is approximately 50% Amerindians and 50% Afro-Colombians. Area B comprised the localities in the previously described focus, plus those along the upper tributaries of the Micay River (2°56' N, 77°12' W), with an altitude 30–120 meters. Nearly all inhabitants here are Afro-Colombians. According to the system of Holdridge, both areas can be classified as tropical rain forest with an average temperature of 26–30°C and very high average rainfall (4,000–8,000 mm). Masses of humid air from the Pacific Ocean are pushed inland and condense upon reaching the mountain range, producing high precipitation and humidity in both areas. The localities are not readily accessible and can only be reached by small motor boat.

Population studied. The surveys were carried out using an updated census in each area: Following recommendations by the Task Force Group on Epidemiologic Characterization of Onchocerciasis (Richards FO and others, unpublished data), at least 10% of the inhabitants (male and female) 15 years of age or older who had lived in the area more than 10 years were included in the study. The population that was surveyed included all the villages along the upper Mira river in area A and all the villages on the upper Micay river in area B. In each of the communities visited, every individual was given a physical examination, including a search for dermatologic changes, palpation for subcutaneous nodules, and ocular examination for punctate keratitis and other corneal alterations. Data such as age, occupation, and current and past places of residence were recorded. Two skin biopsies were taken (see below). The PCR analysis was performed only on the samples taken in the Lopez de Micay focus.

A rapid ophthalmologic assessment (ROA) was carried out on parasitologic- and PCR-positive patients following guidelines established by the Onchocerciasis Elimination Program for the Americas (OEPA). First, visual acuity was evaluated with Snellen’s chart and then the anterior segment was examined using a slit-lamp (Bausch and Lomb, Rochester, NY) with standard 10× and 16× magnification. After pupillary reflexes were examined, a drop of 1% tropicamide
solution (Mydriacyl®; Alcon-Couvreur, Puurs, Belgium) was put in each eye every 3 min on three occasions. Patients were asked to sit with their head between their knees for 10 min to concentrate microfilariae in the anterior chamber. Twenty minutes later, patients were examined with a binocular indirect ophthalmoscope (Keeler Instruments, Inc., Broomal, PA) with a 20 diopter lens (Ocular Instruments, Inc., Bellevue, WA). When necessary, ocular pressure was measured with a Schiotz tonometer. Fundoscopic examination was performed with both indirect and direct ophthalmoscopes (Welch Allyn, Skaneateles, NY).

All protocols were reviewed and approved by institutional ethical committees in accordance with the requirements of the Colombian Ministry of Health. Informed consent was obtained in all cases.

**Skin snip biopsies.** A biopsy was obtained from the right scapular region and the right iliac crest of each individual using a sclerocorneal punch chemically sterilized in 20% glutaraldehyde (Glutarex®; 3 M, Santa Fe de Bogota, Colombia). Each skin snip was put in a well of a 96-well plate with 60 µl of 0.9% saline solution. The 96-well plates were covered with Parafilm® (American Can Co., Neenah, WI) and incubated at room temperature (30°C) for 12 hr. Plates were read using an inverted microscope and microfilariae were counted in each positive biopsy.

**Analysis by PCR.** Once the plates were read, each skin snip was transferred to an Eppendorf® (Brinkman Instruments, Westbury, NY) vial containing 50 µl of 100 mM disodium EDTA for PCR analysis in the laboratory. The individual biopsy samples were studied using a modification of the gridred screening technique developed by T. Unnasch (University of Alabama, Birmingham, AL, unpublished data). Briefly, skin snips were treated with standard proteinase K (10 mg/ml)/10% sodium dodecyl sulfate with carrier salmon sperm DNA and incubated at 56°C for 1 hr, boiled in the presence of 1 M dithiotreitol for 30 min, and freeze-thawed three times. Twenty-five microliters of each sample was then distributed on a matrix with an equal number of columns and rows. For each row, all samples in it were pooled; similarly, all samples in each column were pooled. Each pool was then mixed with sodium iodide and glass beads, incubated at 40°C for 15 min, centrifuged, and the supernatant was discarded. The pellet was washed three times in ethanol and the DNA was eluted using distilled water. Pool screening PCR was then carried out by mixing 2 µl of a 1:10 dilution of the DNA, PCR water (Sigma, St. Louis, MO), and 10× PCR buffer, dNTP solution, primers (OVS-up: 5’BIOT-GATTYTTCCGRGCAAXARCGC-3’ and OVS-down: 5’GCXRTRTAAATXTGXAAATTC-3’; degenerate oligonucleotide primers for *O. volvulus* R = A or G; Y = C or T; X = A, G, C, or T), and Taq polymerase. After 40 cycles (one cycle = 1 min at 94°C, 2 min at 37°C, and 30 sec at 72°C), the double-stranded PCR products were added to microtiter plate wells containing adsorbed streptavidin. The detection of the PCR products by ELISA followed the methodology described by Nutman and others. The products were denatured with NaOH to remove the unbiotinylated strand, probed with a fluorescein-labeled oligonucleotide (5’-FL-CCCTAATCTCAAAAAACGGG-FL-3’) and allowed to hybridize under high-stringency conditions. Next, an anti-fluorescein antibody conjugated to alkaline phosphatase was used and the reaction was developed by adding a substrate (p-nitrophenyl phosphate). Samples were considered positive when a yellow color developed. Every ELISA was run with a positive control (pOVSI34, a plasmid containing 12 copies of the OV-150 repeat) and a negative control (water). Analyses of the PCR products consisted in detection of positivity in rows and columns. Samples shared by a positive row and a positive column were considered positive.

**Entomologic studies.** Preliminary entomologic studies were carried out to establish the anthropophilic simulid species present in the two areas. Simulid larvae and pupae were
collected in rivers and streams from submerged branches of the overhanging vegetation, submerged tree trunks, rocks, and organic debris. Pupae were isolated in a breeding chamber to obtain adults in the field laboratory. Adults were collected on the exposed limbs of human volunteers along the riverbanks. Captured adult flies were classified according to species.

A rapid entomologic assessment (RENTA) was carried out on August 14–17, 1996 in the community of Naiciona. Female blackflies were collected with an aspirator on two O. volvulus microfilariae positive human baits on four consecutive days during daylight hours between 6:00 AM and 6:00 PM. Engorged females were placed in plastic containers, anesthesized with chloroform, identified, and dissected in saline solution using a stereoscopic microscope. Ovaries were examined to determine parity; the head, thorax, and abdomen were separated in parous females for detection of first stage (L1), L2, and L3 larvae.

**Data analysis.** All collected data were registered in individual records for every subject examined. A database was constructed based on these records, using the Epi-Info program (USD, Inc., Stone Mountain, GA) version 5.01. All analyses were carried out using this program.

**RESULTS**

**Epidemiologic and entomologic findings.** In study Area A, all villages (n = 9) were surveyed. Skin snip biopsies were taken from 407 individuals 15 years of age or older representing 75% of the population within this age group. All skin snip biopsies were negative. Although in a preliminary visit carried out in July 1995 *Simulium (Psilopelma) quadrivittatum* and *S. (Notoplexia) exiguum* female blackflies were collected on human baits, no adults were found during the visit in August. Only larvae and pupae of blackflies were collected in the rivers and streams near the different localities visited. The simuliid species identified in this area were *S. (Notoplexia) gonzalezi, S. (Psilopelma) quadrivittatum, S. (Hemicnetha) mexicanum, S. (Ectemnaspis) lutzianumlewisii,* and *S. (Ectemnaspis) bipunctatum* and *S. (Notoplexia) exiguum,* although very few specimens of the latter were collected.

Study Area B is the only described focus of onchocerciasis in Colombia. Skin snip biopsies were obtained from 19% of all the individuals 15 years of age or older (655 of 3,434) in all the communities on the upper Micay River and three of its tributaries. Microfilariae were observed in 39 individuals (6%). However, all positive samples came from three communities. Prevalence was highest in the locality of Naiciona, where 36 persons (40% of the biopsied individuals) were positive (Table 1). Of the three other infected subjects, two lived in Playa Grande, the next community downstream from Naiciona on the Chuare River, and had a history of frequent travel to Naiciona to engage in gold panning. The other positive individual was an 83-year-old man who presently lives in Cacahual but lived in Naiciona until the age of 75. Fifty-five percent of the positive patients were young adults (15–30 years of age) whose principal occupation was gold panning (58%). Prevalence was higher in males (56%), but this difference was not statistically significant.

Preliminary studies showed that *S. exiguum* was the predominant human biting species in this area, although *S. (Hemicnetha) mexicanum, S. (Ectemnaspis) lutzianumlewisii,* and *S. (Ectemnaspis) bipunctatum* were also found. Biting density in Naiciona was 6–13 times higher when compared with those observed in the other localities. Three hundred fifty female black flies were collected during the RENTA; 93% of the collected flies (324 of 350) were *S. exiguum;* the rest were *Psaroniocompsa* near *incrustatum* recorded for the first time in Naiciona. Three daytime activity peaks were found: between 8:00 AM and 9:00 AM, 11:00 AM and noon, and 3:00 PM and 5:00 PM. Nulliparous females were found more frequently in the morning hours whereas multiparous females were more frequent in the afternoon hours. *Psaroniocompsa* near *incrustatum* was more frequently found during the afternoon hours, particularly after 2:00 PM. Both species showed preference for biting on the lower limbs. Two hundred twenty-seven females were dissected in the field while the rest were conserved in isopropanol for future studies; one hundred thirty-three (59%) were parous and 94 (41%) were nulliparous. *Onchocerca volvulus* larvae were found in only three of 133 *S. exiguum* specimens; the natural parasite infection rate was 2.3%. Two blackflies had one L3 each while the third infected female had six L1.

**Parasitologic findings.** Iliac crest skin snips had greater positivity than scapular skin snips (86 versus 58%). Microfilarial (mf) densities, although not statistically significant, were also higher in iliac crest biopsies (average 10.5 mf /biopsy versus 3.6 mf /scapular region biopsy). Correlation between both biopsy sites was good (Kappa index = 0.6). The mean and community microfilarial loads, calculated according to OEPA guidelines, were 2.66 and 0.74 mf, respectively. Microfilarial loads were low (more than 50 % of the positive patients had 10 or less mf per biopsy).

**Clinical findings.** Clinical manifestations were few. Sixty-one percent of the skin snip–positive patients were asymptomatic. Palpable subcutaneous nodules were found in 17% of the examined individuals but no other dermal alterations were observed.

Forty-six of 51 eligible patients underwent ROA. The only ophthalmologic finding was punctate keratitis. This alteration was observed in 15 patients (33%) (12 males and three females). In seven patients bilateral involvement was present. In all cases, less than 20 microfilarial lesions were observed in the cornea. Only one patient presented a live microfilaria in the corneal stroma. The odds ratio (OR) of presenting punctate keratitis for microfilariae positive individuals was 9.1 (95% confidence interval [CI] = 3.3–24.6).

**Assessment of the PCR as a diagnostic method.** Samples from 594 individuals were processed for PCR analysis. All parasitologically positive skin snips were also positive.

### Table 1

<table>
<thead>
<tr>
<th>Locality</th>
<th>No. examined</th>
<th>No. positive</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naiciona</td>
<td>91</td>
<td>36</td>
<td>40</td>
</tr>
<tr>
<td>Playa Grande</td>
<td>70</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Cacahual</td>
<td>26</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>
by PCR. Additionally, samples from 14 individuals with negative skin snips were found positive by PCR. All these individuals were either inhabitants of Naiciona or had lived there. One person had a palpable nodule that was excised and *O. volvulus* were found. According to Cohen's scale, the concordance between parasitologic examination and PCR (0.83; 95% CI = 0.75–0.92) can be defined as almost perfect.

**DISCUSSION**

Although studies in Ecuador in 1988 suggested the possibility of the existence of a second onchocerciasis focus along the Colombian border, in the upper Mira River in the Department of Narino, the epidemiologic survey of all the localities along the upper Mira River demonstrated that no such focus exists. This is further confirmed by the negative results of a rapid epidemiologic assessment carried out in the Canton San Lorenzo in Ecuador, just across the border from where the present study was carried out.

All simulid species found in study Area A are also present in the Ecuadorian focus. *Simulium gonzalezi*, the species of which most abundant immature forms were found during August in the Mataje River, is also suspected to be a secondary vector of *O. volvulus* in Mexico and Guatemala. In both Ecuador as well as in Narino, Colombia, it can be found in breeding sites similar to those of *S. exiguum*. *Simulium quadrivittatum* is highly anthropophilic in Central America and Ecuador. In Ecuador, *S. quadrivittatum* is considered a secondary vector of *O. volvulus* in Ecuador. In the preliminary trip to Narino one month before the survey, this species was found to be biting humans. The very low number of *S. exiguum* pupae collected in the Mataje River in August 1995, compared with the high number of *S. gonzalezi* pupae, is probably due to the fact that their life cycles are not synchronous in this area. The lack of adult specimens can be attributed to seasonal variations in the number of flies or to the heavy rains that caused flooding of breeding sites in the weeks immediately preceding the study. According to inhabitants of this area, adult black flies are abundant in other months of the year.

Thirty years after its initial discovery, onchocerciasis transmission persists at low levels on the Colombian Pacific Coast, as demonstrated by the fact that more than 50% of the infected individuals were in the 15–30 years of age group. Transmission, however, does not occur on the Micay Crest and scapular region could be linked to the biting preferences of the vector.

The finding for the first time of naturally infected *S. exiguum* corroborates the experimental demonstration by Tidwell and others, of this species as a vector in this region. Previous studies had not recorded (*Psaroniocompsa*) near *incrustatum* as a human-biting species. The differences in the biting densities of *S. exiguum* found in this locality and the relatively isolated nature of the focus. In contrast with the findings of Tidwell and others, who found two daytime activity peaks between 8:00 AM and 10:00 AM and between 3:00 PM and 5:00 PM, an additional peak was found in this study between 11:00 AM and noon. Both species showed biting preference for the lower limbs. This finding agreed with those of Barreto and others and Tidwell and others, who demonstrated that *S. exiguum* had a biting preference for the lower limbs. It is necessary to complete the study doing monthly collections to establish the activity peaks throughout the year. The location of Naiciona at an altitude of 100 m on a shaded river bank with abundant vegetation makes it an ideal breeding place for simulids.

The skin snip biopsy is the standard method for diagnosing *O. volvulus* infection, particularly in areas where intensity of infection is high. Sensitivity of the test decreases markedly in areas where parasite loads are lower as is probably the case in the Micay focus. Theoretically, this problem could be overcome with the use of molecular-based tests. In the present study, the PCR increased the yield of positive samples by 37% (14 samples negative on parasitologic examination were positive by PCR). This figure is greater than that reported in other studies (25%) (Guderian R, unpublished data). The greater sensitivity of the PCR can be attributed to the fact that a variable percentage (15–25%) of the microfilariae present in the skin snip biopsy do not emerge from it and therefore cannot be detected by parasitologic examinations, as was the case with the skin snip–negative patient who had an onchocercal nodule. Therefore, the PCR could be useful in foci in which microfilarial densities are low, as is the case of the Colombian focus. However, the PCR does not preclude the necessity to perform an invasive method such as the skin biopsy.

Given the prevalence of infection in Naiciona, this focus can be considered as mesoendemic. Considering the small population size (336 inhabitants), the low microfilarial loads encountered, and its limited geographic location, control and eventually eradication in this, the only known focus in Colombia, seem feasible through implementation of a community-based ivermectin distribution program. However, migration studies will be essential to establish the movement patterns of the people in the area. To formulate indicators that will allow the program to measure the impact of control interventions, entomologic studies to determine the biting habits of the vector, annual transmission potential, and annual biting rate are required. To assure the long-term sustainability of the ivermectin distribution program, baseline
studies are needed to acquire the necessary knowledge base for undertaking effective health education in Naiconia. Cytogenetic studies of *S. exiguum* found in Colombia would be of interest since it has been suggested that it is different from the Cayapa cytotype found in Ecuador. Cytogenetic differences could account for possible differences in dispersion and vectorial capacity.

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