

THE EFFECTS OF LONG-TERM COMMUNITY LEVEL TREATMENT WITH IVERMECTIN (MECTIZAN®) ON ADULT *ONCHOCERCA VOLVULUS* IN LATIN AMERICA

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Abstract. The objective of this study was to examine nodules from Mexico, Guatemala, and Ecuador collected over a one-year period (2001) to determine the effects of semi-annual ivermectin treatments on *Onchocerca volvulus* macrofilarial populations. Nodules were sectioned, stained with hematoxylin and eosin, and histologic findings were compared between countries and with historical data prior to the introduction of ivermectin into the region. Nodules from Ecuador had 10 times more dead or moribund worms than the historical control (66.6% versus 6.5%); nodules from patients from Mexico and Guatemala did not differ from the control. More than 80% of the female worms in each country were uninseminated and producing unfertilized oocytes. Nodules containing males differed in each country from the historical control ($P < 0.0001$), with presence of males ranging from 19.7% in Mexico to 13.6% in Ecuador versus 73% in the control. Nodules with females producing active microfilariae ranged from 7.8% (Mexico) to 2.7% (Ecuador) versus 60% in the historical control ($P < 0.0001$). Nodules from Ecuador and Mexico were significantly smaller in size than those from Guatemala or historical controls ($P < 0.0005$). These results depict a deteriorating condition of adult *O. volvulus* populations in Mexico, Guatemala and Ecuador, indicating that semi-annual ivermectin treatment of ≥ 6 years has had a profound effect on survival and reproduction of this species.

INTRODUCTION

Ivermectin (Mectizan®; Merck, Rahway, NJ) is an efficacious microfilaricidal drug that has been used extensively in Africa and Latin America for treatment of human onchocerciasis.¹ In the six Latin American countries where this disease is endemic, a treatment regimen of two times per year was recommended by the Onchocerciasis Elimination Program for the Americas (OEPA)² because previous controlled studies at the individual and community levels demonstrated that this approach blocked transmission of the parasite both in places where black fly vectors possessed a cibarial armature (*Simulium ochraceum*)³ and when they lacked this structure (*S. exiguum*).⁴ Mass treatment with ivermectin is therefore given every six months, aiming to reach at least 85% of the estimated 450,000 treatment-eligible persons in the region, to eliminate morbidity and interrupt transmission where possible.

An earlier study by Duke and others⁵ in Guatemala also showed that recurrent treatments at six month intervals for two years significantly increased the proportion of dead and moribund adult female worms, as well as reduced the production of microfilariae by surviving females. The number of adult male worms was reduced but not significantly. Because semi-annual treatments have been given in several OEPA countries since 1995, the aim of this study was to evaluate the long-term effects of ivermectin exposure on adult worm viability and fecundity. We report and contrast the effects of treatments in communities in Mexico, Guatemala, and Ecuador, and compare adult worm status with other long-term control efforts in Africa that emphasized vector control.

MATERIALS AND METHODS

Collection, fixation, and processing of nodules. Nodules were removed from persons seeking surgical therapy from Ministry of Health sponsored programs in Guatemala, Mexico and Ecuador during 2001. Data on age, sex, and number of ivermectin treatments were obtained when possible from these individuals or from program records. Semi-annual treatments have been underway in each country since 1995, with $\geq 80\%$ of eligible persons gradually receiving Mectizan® in each country as of 2001.⁶ Within the three countries, persons receiving surgery reported drug treatments ranging from as few as zero in Mexico and Guatemala to as many as 17 in Ecuador, indicating the variation encountered in a regional disease control program.

Surgery was performed using standard techniques⁷ and fixation, preservation, and sectioning of nodules followed the methods described by Boussinesq and others.⁸ Each excised nodule was placed immediately in approximately 20 times its volume in an ethanol (70%)/glycerol (10%)/water (20%) fixative in an individual tube. The fixative was changed after 24 hours and nodules were stored for further processing at Western Michigan University. This investigation was reviewed and approved by the Western Michigan University Institutional Review Board for Research Involving Human Subjects. Prior to processing, fixed nodules were cleaned of extraneous tissues, weighed, and embedded in paraffin wax. Sections were cut at 6 μm ; nodules exceeding 1 cm were cut at three levels (first quarter, middle, and last quarter) to insure optimal detection of worms. Sections were stained with hematoxylin and eosin and examined independently by two observers (CDM and BOD) with considerable knowledge of nodule histology.

Nodules were cut through their long axis and the cut surface was imaged for digital analysis on a Nikon SMZ-U stereoscope (Nikon, Tokyo, Japan) using a Javelin Chromachip color video camera (Javelin Electronics, Tokyo, Japan) with MetaMorph software (Universal Imaging Corporation, West Chester, PA).

Data collection and analysis. The classification system developed by Duke and others⁹ was used to describe the condition of the adult worms, i.e., presence/number of adult worms in the nodule, viability, fecundity/reproductive status of females, spermatogenesis, and presence of microfilariae in the capsule of the nodule. The latter was considered an important parameter because it indicates the presence of an active infection in which the skin can be populated with microfilariae. Results were compared between countries and to published, historical data from Guatemala from 1988 before ivermectin had been introduced into the region.¹⁰ A chi-square test was used to analyze differences in these parameters; 95% confidence intervals were calculated and statistical significance was set at $P \leq 0.05$. Image data of nodules were subjected to both *t*-tests and one-way analysis of variance for comparisons of area and perimeter. These results were compared with size data of nodules from Ecuador prior to when ivermectin had been introduced into that country (Mackenzie CD, unpublished data). Statistical significance was set at $P \leq 0.05$.

RESULTS

A total of 295 patients sought surgical treatment, resulting in the removal and evaluation of 342 nodules (Table 1). Nodular material was therefore accumulated from communities within the three countries based on clinical presentation with no bias toward size, location on the body, or degree of previous drug exposure.

The status of worm populations varied significantly, with important differences noted between each country and the historical control. A significant number of patients from Ecuador had nodules containing dead or moribund worms ($n = 32$; 66.6%), whereas nodules from patients from Mexico and Guatemala did not differ from controls in this regard (Figure 1). When the proportion of viable adult females was compared, the population from Ecuador also differed from those in Mexico and Guatemala and the historical control, i.e., there was a highly significant increase in the number of moribund/

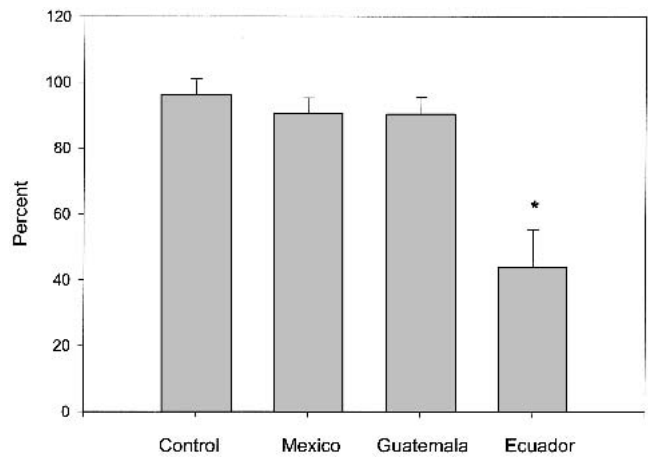


FIGURE 1. Percentage of live female *Onchocerca volvulus* by country in the study population versus the historical control before the introduction of ivermectin. Error bars represent the upper 95% confidence intervals and the asterisk indicates a significant difference from the control, Mexico, and Guatemala at the $P < 0.05$ level.

dead female worms per nodule observed in Ecuador ($P < 0.0001$), but not in Mexico and Guatemala ($P = 0.225$ and $P = 0.123$). However, when the reproductive status of the surviving worms was analyzed for each country and compared with the historical control, there were far fewer fecund worms (those that were inseminated and producing all embryonic stages up to microfilariae) than uninseminated worms producing unfertilized, degenerating oocytes (Figure 2). The impact of this phenomenon on reproduction was apparent when comparisons were made between females producing microfilariae that had migrated to the nodular capsule (Figure 3). Capsular microfilariae were observed in 7.8% of nodules from Mexico, 6.8% of nodules from Guatemala, and 2.7% of nodules from Ecuador versus 60% in the historical control ($P < 0.0001$).

The scarcity of adult male worms (Figure 4) was particularly noticeable, with the numbers of nodules containing males differing significantly in each country from that seen in the historical controls ($P < 0.0001$). The presence of males in nodules ranged from 19.7% in Mexico to 13.6% in Ecuador versus 73% recorded in 1988 before ivermectin was introduced into the region (Table 1). The number of males per

TABLE 1
Evaluation of recurrent ivermectin treatment on adult *Onchocerca volvulus*

Parameter	Control	Mexico	Guatemala	Ecuador
No. of patients with nodules	46	135	112	48
No. of nodules examined	48	152	124	66
No. of patients with dead or moribund worms	3 (6.5)*	15 (11.1)*	13 (11.6)*	32 (66.6)*
Total no. of male worms	59	32	23	12
No. of dead male worms	0	0	0	1
No. of nodules with male worms	35 (73)*	30 (19.7)*	22 (17.7)*	9 (13.6)*
No. of male worms per nodule	1.22	0.21	0.18	0.18
Total no. of female worms	78	168	145	82
No. of dead/moribund female worms	3	16	14	46
No. of live female worms	75	152	131	36
No. of live female worms per nodule	1.56	1.00	1.06	0.54
No. of females producing microfilariae	45 (60)*	12 (7.8)*	9 (6.8)*	1 (2.7)*

* Pretreatment data from Guatemala reported by Duke and others.¹⁰ Values in parentheses are percentages.

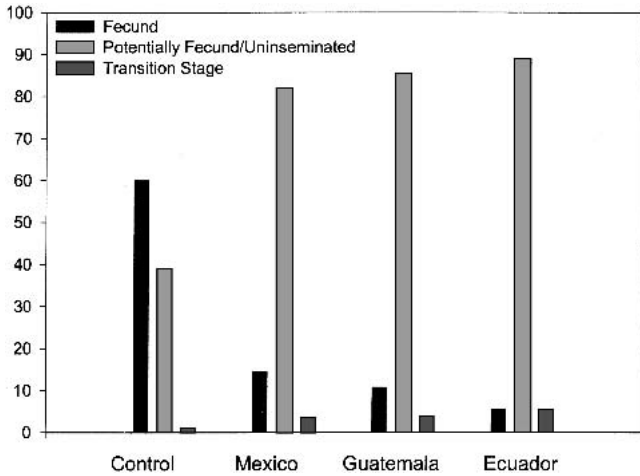


FIGURE 2. Reproductive status of surviving *Onchocerca volvulus* adult females by country (%). Fecund worms are inseminated and producing all embryonic stages up to microfilariae. Potentially fecund worms are uninseminated and producing oocytes that are not fertilized and are degenerating. Transition stage worms are changing from fecund to non-fecund or vice-versa.

nodule in each country was also significantly different from the historical control: 1.22 males per nodule versus 0.21, 0.18, and 0.18 for Mexico, Guatemala, and Ecuador, respectively.

Nodules from Ecuador and Mexico were significantly smaller in area and perimeter ($P < 0.05$) than those from Guatemala (Figure 5) and from historical controls ($P < 0.005$) collected in Ecuador prior to the use of ivermectin (Table 2). Nodules from Ecuador taken from treated persons also differed significantly in area and perimeter from those collected in Mexico ($P < 0.005$).

DISCUSSION

The results given here depict a deteriorating condition of adult *O. volvulus* populations as of 2001, indicating that recurrent ivermectin treatment has had a profound effect on

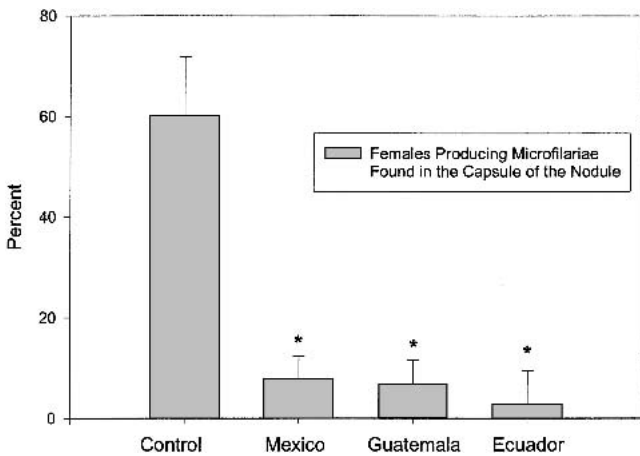


FIGURE 3. Reproductive status of adult *Onchocerca volvulus* following recurrent ivermectin treatment (female worms producing microfilariae by country). Error bars represent the upper 95% confidence intervals and asterisks indicate significant differences from the control at the $P < 0.05$ level.

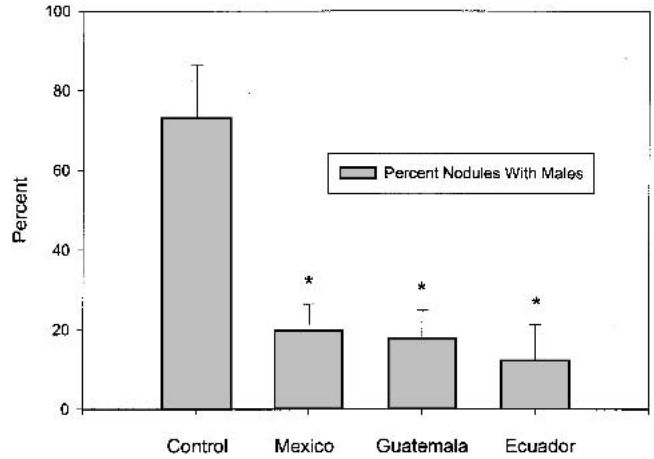


FIGURE 4. Reproductive status of adult *Onchocerca volvulus* following recurrent ivermectin treatment (percentage of nodules with live males by country). Error bars represent the upper 95% confidence intervals and the asterisks indicate significant difference from the control at the $P < 0.05$ level.

survival and reproduction of this species in Mexico, Guatemala, and Ecuador. We observed that fewer than 8% of the female worms in each country were producing microfilariae, a highly significant difference from the 60% seen in the pre-ivermectin historical controls. This change is probably due to a variety of inter-related factors associated with long-term exposure to ivermectin given at six-month intervals. Earlier controlled, clinical studies indicated that treatments at this

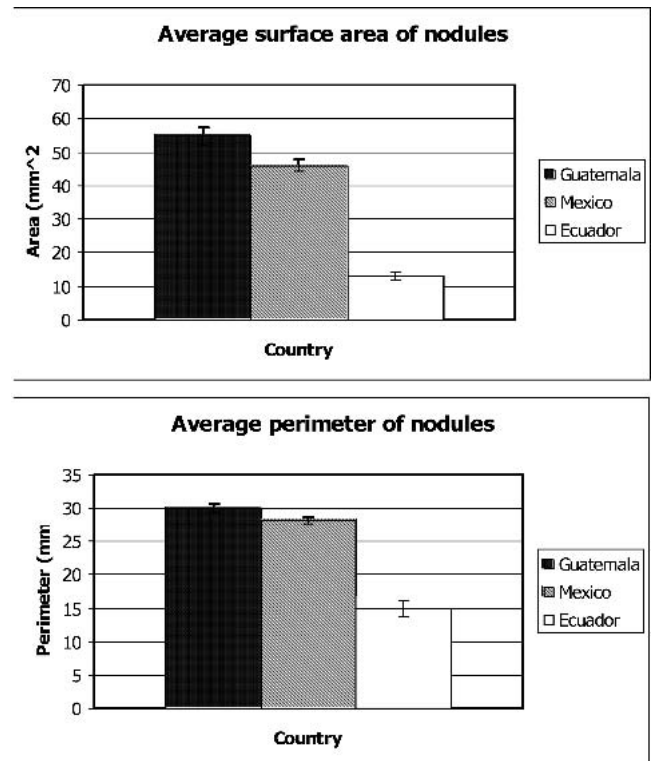


FIGURE 5. Digital analysis of nodule size (area in mm² and perimeter in mm) of material from Mexico, Guatemala, and Ecuador. Error bars show the standard error.

TABLE 2

Comparison of nodules (area and perimeter) from each country and with an historical control from Ecuador prior to the introduction of ivermectin

	Mean	SD	SE
Guatemala (n = 198)			
Area	54.91	35.83	2.55
Perimeter	30.1	8.71	0.62
Mexico (n = 225)			
Area*	45.96	26.98	1.8
Perimeter*	28.14	8.22	0.55
Ecuador (n = 101)			
Area*†	13.06	10.56	1.05
Perimeter*†	14.95	6.14	0.61
Historical control (n = 21)			
Area	55.11	36	2.67
Perimeter	31.13	9.12	0.76

* Significantly different from Guatemala and historical control.

† Significantly different from Mexico.

interval significantly affect the vigor and health of adult female worms after as few as four successive treatments.⁵ Six-month use of ivermectin at the community level for 3–4 years in Guatemala and Ecuador also impacted transmission of *O. volvulus* vertebrate infective stage larvae (L_3 s) so greatly that few, if any, L_3 s were available to produce new infections.^{3,11} Unlike vector control, wide-spread ivermectin treatment has an immediate dampening effect on transmission of new infections.^{3,12} Consequently, with long term six-month treatments, the input of L_3 s that might successfully develop to adult males and continue the insemination process in pre-existing infections or develop to young females that are highly fecund is severely restricted. For example, in Mexico, Guatemala, and Ecuador, only 14.5%, 10.7%, and 5.5%, respectively, of all living female worms examined were inseminated and producing microfilariae.

Male *O. volvulus* adults are also sensitive to long-term ivermectin exposure. Kläger and others¹³ reported that patients receiving 10 six-month doses of ivermectin over a six-year period had significantly fewer male worms per nodule than did those from patients receiving annual treatment over the same time frame (1.42 versus 1.01 live males per nodule). The ratio of live males to females was also reduced to approximately 1:1 per nodule. In that study, patients received five doses of ivermectin at six-month intervals and, after a rest period of 18 months, a second round of 5 six-month treatments. However, overall coverage rate in the community was low, approximately 30%, and as a result of ongoing transmission, patients were subjected to new infections during the six-year study.¹⁴

The potential inability to detect males accurately in thin sections, however, must also be considered. The bodies of dead males disintegrate and are difficult to find using either hematoxylin and eosin-stained sections or the collagenase method.^{8,15} In this study, only one dead male was seen. Thus, it is possible that the numbers of males may have been underestimated due to the use of a histologic method of examination (as opposed to collagenase digestion of nodules). However, several factors suggest otherwise. In describing the macrofilarial population of *O. volvulus* in hyperendemic zones in Cameroon, Boussinesq and others⁸ routinely detected male worms in sections stained with hematoxylin and eosin and noted that the numbers of male worms per nodule

and the male:female ratios were well within the ranges reported previously by other investigators in three African countries (Mali, Liberia, Burkino Faso) who had used the collagenase technique. A second study evaluating dosage levels and timing of ivermectin treatment gave essentially the same results.¹⁶ Furthermore, in evaluating the reproductive status of surviving female worms, a process which lends itself better to histologic methodology, more than 80% in each country were uninseminated, indicating that the counterpart male populations were considerably diminished and unable to maintain gene flow. This follows the trend reported earlier by Duke and others⁵ in which insemination rates were decreased by $\geq 25\%$ following 4 six-month treatments. Therefore, the comparable results using two different methods of evaluation support the paucity of male worms.

The numbers of male worms per nodule in the three OEPA countries in 2001 were less than those observed after extensive vector control in several countries in Africa (Figure 6). For example, all three OEPA countries had fewer males per nodule than those observed in Togo 12–13 years after transmission had been interrupted¹⁷ and in Burkina Faso 9–10 years after interruption of transmission.¹⁵ Because 2–3 years of vector control are required before transmission is reduced to ≤ 100 L_3 s per year,¹⁸ the effects of semi-annual treatments over 6–7 years at high coverage rates are generally equivalent to 10–13 years of vector control, and suggest that ivermectin may be macrofilaricidal for male worms as opposed to when a vector control approach is taken and male worms undergo normal senescence more slowly.

Repetitive mating is a key factor in the maintenance of *O. volvulus* populations and it has been estimated that for ongoing production of microfilariae, insemination must take place at roughly three-month intervals.^{19,20} To maintain sufficient gene flow, Plaisier and others²¹ suggested that the probability of mating should be equal to the ratio of male:female worms, with a value of 1.0 (100% insemination) occurring when there are more male than female worms. Current male:female ratio values per nodule for the populations

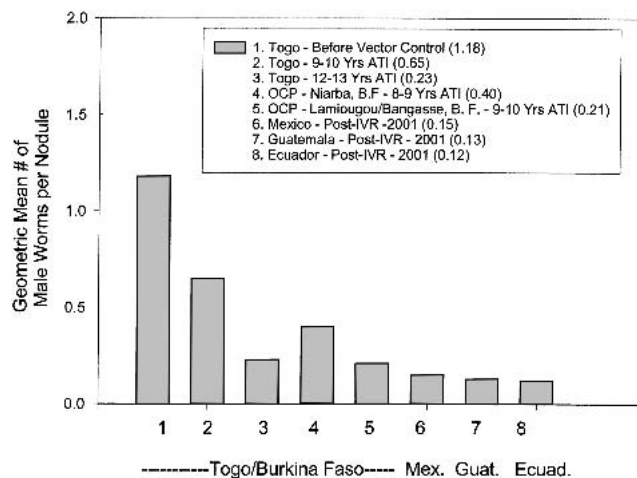


FIGURE 6. Comparison of living male worms per nodule following vector control in Africa versus ivermectin treatment every six months in Latin America through 2001. Yrs = years; ATI = after transmission interruption; OCP = Onchocerciasis Control Program in West Africa; B. F. = Burkina Faso; Post-IVR = after recurrent ivermectin treatments; Mex. = Mexico; Guat. = Guatemala; Ecuad. = Ecuador.

in each of the three OEPA countries evaluated fall well below the values seen in the historical control, with the level of non-inseminated females in each country in 2001 being greater than 80%. Because insemination is one of the fundamental mechanisms driving the basic reproductive rate, we believe that each population is in decline as a result of uncoupling this important biologic function by severely restricting new infections and thereby limiting mating opportunities. For example, the high degree of non-inseminated females reported here is similar to the pattern reported by Chavasse and others²² where insemination in *O. volvulus* was significantly reduced following multiple ivermectin treatments. Lok and others²³ also reported that the sexual competence of *Dirofilaria immitis* males was inhibited when worms received multiple treatments of milbemycin oxime, a macrocyclic lactone similar in structure to ivermectin.

The high rate of dead adult worms likely occurred because transmission of L₃s has been greatly reduced or interrupted in the communities where patients lived^{3,24,25} and the worm populations are undergoing senescence. In Ecuador, 65% of nodules had dead female worms, a figure that compares favorably with results in west Africa where approximately 75% of nodules contained dead female worms in areas where transmission had been blocked by vector control for 9–10 years.¹⁵ Likewise, approximately 70% of nodules had dead male worms, a figure somewhat lower than the 86% reported here for Ecuador.

The high percentage of dead female worms in Ecuador likely reflects the broad coverage and extensive use of ivermectin on a recurrent basis.¹¹ While precise treatment data were unavailable for many persons in the study, records available for 48 patients from Ecuador indicated a mean number of treatments per patient of 8.2. Duke and others⁵ reported that as few as four six-month treatments caused significant increases in the proportions of moribund and dead females and of live, uninseminated females when compared with corresponding controls. This observation was confirmed in Sierra Leone¹³ where treatments repeated at six-month intervals significantly decreased the proportion of living and gravid female worms and reduced overall reproduction by approximately 90%. The significantly smaller area and perimeter size of Ecuadorian nodules was also indicative of the large number of dead worms. This observation parallels those of Darge and others²⁶ who observed a mean reduction in nodule size of 27% when suramin was used as a macrofilaricide, with the proportion of dead female worms increasing from 17% at the end of therapy to 48% six months later and 61% at one year.

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