Rapid Suppression of *Onchocerca volvulus* Transmission in Two Communities of the Southern Chiapas Focus, Mexico, Achieved by Quarterly Treatments with Mectizan

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Abstract. The impact of quarterly Mectizan (ivermectin) treatments on transmission, microfilaremia, and ocular lesions was evaluated in two formerly hyperendemic communities (Las Golondrinas and Las Nubes II) located in the main endemic focus for onchocerciasis in Southern Chiapas, Mexico. The data suggest that *Onchocerca volvulus* transmission has been suppressed after elimination of microfilaremia in these two communities. Increasing the frequency of Mectizan treatment to four times per year appears to have resulted in the rapid suppression of transmission in communities with residual transmission.

INTRODUCTION

Onchocerciasis, caused by infection with the filarial parasite *Onchocerca volvulus*, remains a major public health threat worldwide. It afflicts an estimated 30–40 million infected people, of which approximately 779,000 have been blinded by the infection.1 In Mexico, the major endemic focus of onchocerciasis is located in Southern Chiapas (Soconusco), an area that is contiguous with the Huehuetenango focus in Guatemala. For the Americas overall, the endemic areas in Mexico and Guatemala are home to 71% of the regional infected population.

The overall goal of the Onchocerciasis Elimination Program in the Americas (OEP) is to first eliminate onchocerciasis as a public health problem, and then to completely eliminate the reservoir of infection in the six endemic countries of Latin America. To assist in this process, the World Health Organization (WHO) developed a series of guidelines to certify that an area is free of onchocerciasis.2 Two different measures of transmission suppression were recommended by WHO. In areas where pre-treatment data were available, WHO defined suppression of infectivity as a 99% reduction in transmission from pre-treatment rates. Where pre-treatment data are not available, transmission suppression was defined by the WHO guidelines as an “absence or near absence of L3 infection in the vector population and the absence of infection in humans.” The WHO did not specify quantitative metrics to the term “near absence.”

The onchocerciasis program in Mexico began treatment with Mectizan in 1989, initially treating only symptomatic individuals. In 1995, this strategy was modified to provide twice-annual treatment to every eligible resident in the at-risk communities. In 2003, the strategy was further modified in the majority of the formerly hyperendemic communities in the Southern Chiapas focus. In these communities, treatment was provided four times per year (in 50 communities), whereas a semi-annual treatment schedule was maintained in the formerly meso and hypoendemic communities of this focus. This change in strategy was due in part to the fact that data collected in 2001 suggested that transmission was still persisting in this focus.4,5 This entomologic data was supported by parasitologic findings that also indicated transmission was continuing in this focus. The data suggested that despite having achieved a remarkable reduction in the mobility, new cases continued to appear in these communities, mainly in children of < 5 years of age.

The Mectizan program has achieved high rates of coverage in endemic communities in Chiapas over the past four years (2003–2006). Mectizan coverage of the eligible population has remained above 85% each year from 2003 to 2006, with a mean coverage of 90.1% (range = 86.3–94%). The data presented here represent the results obtained from an entomologic follow-up study conducted in 2004 and 2006 in Las Nubes II and Las Golondrinas, two formerly hyperendemic communities that began the four annual treatment regimen in 2003 and 2004, respectively. This data has been compared with a pre-treatment study conducted in 1991 in Las Golondrinas. The prevalence of dermal microfilariae (mf) and ocular pathology were also assessed in these two communities.

MATERIALS AND METHODS

Study area. There are 559 communities endemic for onchocerciasis in the Southern Chiapas focus.6 A total of 39 (7%) of these communities are formerly hyperendemic (historically high transmission and an *O. volvulus* prevalence skin biopsy of > 60%), 209 communities (37%) are formerly mesoendemic (historically moderate transmission and an *O. volvulus* prevalence skin biopsy of > 20% and < 60%), and 311 (56%) are formerly hypoendemic for onchocerciasis (his-
torically low transmission and rate of positive biopsy \( \leq 20\% \); WHO, 1995).\(^2\) The two sentinel communities included in this study (both formerly hyperendemic) were Las Golondrinas (15°26'06"N, 92°39'17"W, elevation = 920 meters), and Las Nubes II (15°18'09"N, 92°28'25"W, elevation = 1080 meters). This study was conducted during the period lasting from 2003 to 2006 in Las Nubes II and during a period lasting from 2004 to 2006 in Las Golondrinas.

**Black fly collection and PCR.** Black flies were collected in 2004 and 2006 during the peak \( O. volvulus \) transmission season, which lasts from January to May, following standardized procedures.\(^4,7,8\) Mass Mectizan distribution was performed just before peak transmission began. The collection of insects was conducted simultaneously in two sites for each community; one in a nearby coffee plantation and the other within the community, and performed in teams of two, representing a bait and collector.\(^9\) Collections were carried out during the first 50 minutes of each hour, beginning at 11:00 a.m. and ending at 16:50 p.m. Collectors received Mectizan (ivermectin) 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). Black flies were collected before they began feeding. This landing rate was taken as an estimate of the biting rate. It is likely that the landing rate overestimated the biting rate because a proportion of the landing flies might not actually successfully bite. Thus, the transmission potential calculations provided below may be overestimated by a factor proportional to the number of flies that land but do not bite. Field-collected black flies were preserved in isopropanol at room temperature and returned to the laboratory. The *Simulium ochraceum* s.l. flies were separated by morphologic examination, and the few flies that were found to have taken a fresh blood meal were discarded. *Simulation ochraceum* s.l. females were divided into aliquots of 50 specimens each for further processing for DNA. The flies were placed in liquid nitrogen and subjected to vigorous agitation to separate the heads and bodies. The heads were purified from the bodies by passage through a 25-mesh sieve and processed separately.

The pools were then tested for the presence of *O. volvulus* parasites using a polymerase chain reaction (PCR) assay specific for *O. volvulus*. Details of the protocols for genomic DNA purification, primer sequences, PCR conditions, detection of PCR products by enzyme-linked immunosorbent assay (ELISA), and internal controls for the PCR assay have been presented previously.\(^4,5,10\)

Previous studies have shown that the prevalence of flies carrying immature stages of the parasite life cycle (infected flies) is uniformly greater than the prevalence of flies carrying infectious L3 (or infective flies).\(^4,5,11\) To maximize the efficiency of the screening process, body pools were first screened until a confirmed body positive pool was obtained. A confirmed body pool from the flies collected from a given community was taken as evidence for potential ongoing transmission, and screening of the body pools from that location was discontinued. All of the head pools collected from that community were screened, and PoolScreen version 2.0.16\(^12\) was used to estimate the prevalence of infectious flies in the community and the associated 95% confidence intervals (CIs). These proportions were expressed as the number of infective flies per 2,000 flies examined to maintain consistency with the current cut-off for suppression of transmission adopted by OEPa. If all of the body pools from a given community were screened and none were found to be positive, it was concluded that there was no evidence for ongoing transmission in the community. The prevalence of infected (and infective) flies was therefore taken to be zero, and the upper bound of the 95% CI for the prevalence of infected (and infective) flies was calculated using the PoolScreen algorithm as described above. Head pools collected from villages where no evidence for infection in the vector population was found were not analyzed further.\(^4,5\)

**Skin biopsies and ophthalmologic examination.** Parasitologic studies were carried out in May 2004 and 2006, after 13 and 15 years of Mectizan treatment (corresponding to 29 and 37 treatment rounds with ivermectin, respectively). Two skin biopsies were taken from each individual, one from the left scapula and one from the right buttock. Skin snips were taken using a 1.5–2.0 mm stolz corneoscleral biopsy punch, and mf were recorded.\(^7\) Ocular examinations were carried out by an ophthalmologist using a Topcon Optical SL-3D slit lamp (Kogaku Nikai KK, Tokyo, Japan), and included testing of visual acuity, and examination of the anterior segment of the eye for ocular opacities, oncho–specific punctuate keratic lesions, and prevalence of mf in the anterior chamber of the eye.

**Skin snip PCR to detect parasite DNA.** The two skin snips from an individual or person were pooled and homogenized in a buffer containing 10 mm Tris HCl (pH 7.5), 1 mm EDTA. The homogenate was incubated at 55°C for 1 hour in 400 \(\mu\)L of proteinase K (400 \(\mu\)g/mL) and boiled for 30 minutes in the presence of 20 mm dithiothreitol to disrupt the parasite cuticle. Boiling was followed by a series of freeze-thaw steps to release the parasite DNA. A total of 3 volumes of NaI solution (6 M sodium iodide, 0.1 M sodium sulfite), 20 \(\mu\)L of glass slurry (Sephaglass), and 4 \(\mu\)L of carrier DNA (250 ng/mL) were then added to the homogenate. To purify the DNA, the glass slurry was pelleted out and washed in 1,200 \(\mu\)L of cold ethanol wash (10 mm Tris HCl [pH 7.5], 100 mm NaCl, 1 mm EDTA, 50% [v/v] ethanol). The washing step was repeated 3 times, and the pellet dried at room temperature. The pellet was suspended in 150 \(\mu\)L of TE (10 mm Tris HCl pH 7.5, 1 mm EDTA pH 8.0) at 55°C for 5 minutes. The glass slurry was pelleted out from this solution by centrifugation for 2 minutes, and the supernatant containing the purified DNA placed in microtiter plates. A total of 5 \(\mu\)L of these DNA solutions were used as template DNA in each PCR reaction. The PCR reaction, PCR conditions, and detection of PCR products by ELISA was essentially the same as that used for the detection of O-150 PCR reactions using black fly DNA as a template.\(^4,5\)

**Data analysis.** 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 Mexico, the level of transmission estimated during the peak of greatest transmission was very low (because of the effect of 12 years [32 rounds] of treatment with Mectizan). Therefore, the value of transmission potential outside of the peak transmission period is probably zero or near zero. The seasonal transmission potential (transmission occurring during the peak transmission season of January through May) thus likely represents a fairly accurate estimator of ATP. 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 1.\(^5\)
The prevalence of infection in the body pools and head pools and associated 95% CIs were determined using the algorithms available in the computer program PoolScreen version 2.0.16. The proportions of infective flies (prevalence of infection in head pools) were used to calculate estimates of seasonal transmission potential. The seasonal transmission potential was calculated as the product of the seasonal biting rate, the proportion of flies carrying L3 larvae in the study season (from January through May during 2006), and the average number of L3 larvae in each infective fly. The seasonal biting rate was calculated as the product of the geometric mean; \( \sqrt{\frac{n}{t}} \) the number of flies collected per person per day and the total number of days in the months of January through May.

The raw 50-minute fly count was first adjusted to a 60-minute time period by dividing each fly count by 0.83 (i.e., 5/6). A constant value of 1 was added to each adjusted value, and the geometric mean number of flies per person per hour was calculated. This mean hourly value was multiplied by 10 (the mean number of daylight hours during the transmission season at latitude in Chiapas)\(^{12,14,15} \) to obtain an estimate of the daily biting rate. To estimate the seasonal biting rate, the daily biting rate was multiplied by the number of days that correspond to the length of the transmission season from January to May.\(^{11} \) We verified that the log-transformation of the fly counts had rejected a null hypothesis of normality (Smirnov Kolmogorov test; SPSS Inc., Chicago, IL). The CIs were calculated on the log-transformed data. The antilog was calculated and the constant subtracted from the upper and the lower limit (95% CIs became asymmetric according to Kirkwood and Sterne).\(^{16} \) The geometric mean and the CIs (as they are in the same units as the means) were multiplied by 10 to obtain the CIs for the daily biting rates. To estimate the confidence interval of the ATP, the seasonal biting rate was multiplied by the CI of the proportion of infective flies.\(^{17,18} \)

The associated 95% exact CIs for pre-treatment and post-treatment prevalence of skin mf in the community as well as the prevalence of corneal opacities (punctuate keratitic lesions), and the prevalence of mf in the anterior chamber of the eye were determined using the method of Miettinen (1970) as described in Armitage and Berry.\(^{19} \)

RESULTS

The \( S. \) ochraceum s.l. flies were collected during the peak transmission period of January through May from two communities in the Southern Chiapas focus that were formerly hyperendemic for \( O. \) volvulus. The flies were grouped into pools of 50 specimens by month and community. The heads and bodies in each pool were separated and screened by PCR for the presence of \( O. \) volvulus larvae, using the screening strategy described in the Materials and Methods.

In 2004, 2 of 106 pools of \( S. \) ochraceum s.l. bodies from Las Golondrinas were PCR positive, whereas 1 of 125 body pools from Las Nubes II was positive. Thus, evidence for vector-parasite contact was found in both communities. A total of 192 head pools from Las Golondrinas and 125 head pools from Las Nubes II were then screened. One head pool was positive from Las Golondrinas, whereas two head pools from Las Nubes II were positive, leading to calculated prevalence of infective flies of 0.3/2,000 (95% CI = 0.0003–0.82/2,000) and 0.8/2,000 (95% CI = 0.05–1.8/2,000), respectively.

In 2006, after two years of quarterly Mectizan treatment rounds per year, all of the \( S. \) ochraceum s.l. body pools screened from Las Golondrinas (\( N = 221 \) pools, or 11,050 flies) were PCR negative, and just one body pool from Las Nubes II was positive (\( N = 83 \) pools, or 4,150 flies). A total of 221 pools of heads (11,050 individuals) were screened from Las Golondrinas and 91 pools (4,550 individuals) were screened from Las Nubes II. All of the head pools were PCR negative, suggesting an absence of flies carrying infective larvae (Table 1).

The results of the PCR assays were used to calculate a prevalence of infective flies in the vector populations, together with an associated 95% CI. The prevalence of infective flies was then combined with estimates of the biting rate to calculate an estimated seasonal transmission potential. In 2004, the point prevalence of infective flies was 0.31 and 0.8 for Las Golondrinas and Las Nubes II, leading to an estimated seasonal transmission potential of 14.5 and 10.6 L3s per person per year, respectively. In 2006, the point prevalence of infective flies in Las Golondrinas was 0, leading to an estimated seasonal transmission potential of 0 (95% upper limit \( [UL] = 9.8 \) L3 per person per year. Similar to the situation described for Las Golondrinas, no infective flies were found in Las Nubes II in 2006, leading to an estimated seasonal transmission potential of 0 (95% UL = 9.5) L3 per person per year.

The prevalence of infection (as estimated by the presence of skin mf by skin snips) in Las Golondrinas before treatment began was 78% (95% CI = 72.9–82.6%). In 2004, after 13 years of treatment, the prevalence of skin microfiladermia was reduced to 2.8% \( (P < 0.05; \) Wilcoxon rank sum test). In 2006, after 15 years of treatment and following two years of quarterly treatments, this prevalence was further reduced to 0.3%. Because the skin snip PCR (O-150) diagnostic test to

### TABLE 1
Prevalence of infected and infective flies (expressed as rate per 2,000 flies examined), and seasonal transmission potential (L3s per person per year) estimated in two communities of the Southern Chiapas endemic focus, Mexico

<table>
<thead>
<tr>
<th>Community/year</th>
<th>Seasonal biting rate</th>
<th>Prevalence of infected flies</th>
<th>Prevalence of infective flies</th>
<th>Seasonal transmission potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>LasGolondrinas2001*</td>
<td>20,830 (19,117–22,688)</td>
<td>14.5 (9.1–20.6)</td>
<td>0.47 (0.06–0.97)</td>
<td>4.9 (0.6–10.1)</td>
</tr>
<tr>
<td>LasGolondrinas2004</td>
<td>93,815 (91,945–95,723)</td>
<td>0.94 (0.06–2.1)</td>
<td>0.31 (0.0003–0.81)</td>
<td>14.5 (0.01–38.0)</td>
</tr>
<tr>
<td>LasGolondrinas2006</td>
<td>57,857 (51,476–65,007)</td>
<td>0 UL:0.34</td>
<td>0 UL:0.34</td>
<td>0 UL:0.8</td>
</tr>
<tr>
<td>Las NubesII/2004</td>
<td>26,429 (22,921–30,442)</td>
<td>0.48 (0.0005–1.25)</td>
<td>0.8 (0.05–1.8)</td>
<td>10.6 (0.5–23.8)</td>
</tr>
<tr>
<td>Las NubesII/2006</td>
<td>22,667 (20,720–24,782)</td>
<td>0.72 (0.0007–1.88)</td>
<td>0 UL:0.84</td>
<td>0 UL:9.5</td>
</tr>
</tbody>
</table>

* From 1993 to 2003, 39 hyperendemic communities from Southern Chiapas had been treated with Mectizan, two times per year. Since 2003, 50 hyperendemic communities including Las Golondrinas and Las Nubes II had been treated with Mectizan four times per year.

The bold numbers represent the point estimate for the value in question, and the numbers surrounding the point estimate represent the 95% CI.

UL = 95% upper limit.
detect parasite DNA is more sensitive than the skin biopsy technique, it was also used to test skin snips from a sample of individuals examined by skin biopsy in 2006. All skin snips (from 129 individuals that were negative by biopsy) were also PCR negative.

A similar pattern was found in Las Nubes II. Here, the prevalence of skin mf was 1.8% in 2004, and no evidence of skin microfilaremia was seen in 2006 in 37 individuals tested. All snips collected in 2006 were also negative by PCR.

During the pre-treatment evaluation in Las Golondrinas, the prevalence of corneal opacities (punctate keratitis) was 33.3% (95% CI = 26.7–40.4%). In 2004 and 2006, after 13 and 15 years of treatment with Mectizan, the prevalence of corneal opacities was significantly reduced ($P < 0.05$) to 1.6% and 0%, respectively (Table 2). A similar reduction was noted in Las Nubes II, where the prevalence of corneal opacities was also 0% in 2006 (Table 2). Furthermore, the prevalence of mf in the anterior chamber of the eye in Las Golondrinas during the pre-treatment was 30.4% (95% CI = 23.9–37.4%). In 2004 and 2006, it was 0.5% and 0%, respectively (Table 2), whereas that of Las Nubes II in 2006 was 0%.

### DISCUSSION

This study represents the latest entomologic and epidemiologic evaluation of the effectiveness of Mectizan (ivermectin) treatment of *O. volvulus* in the main endemic focus for onchocerciasis of Mexico. The data suggest that the Mectizan distribution-based program in Mexico has succeeded by apparently suppressing transmission, as well as dramatically reducing skin microfilaremia, corneal morbidity, and mf in the anterior chamber of the eye. Of the two communities examined, no evidence for ongoing transmission was detectable, and patient infection in the community (as assessed by the presence of mf in the skin) had disappeared when the studies were reported completed in 2006. In Las Golondrinas, where pre-control entomologic data were available, 15 years of Mectizan treatment had reduced transmission by greater than 99% when compared with the seasonal transmission potential of 19.5 L3s per person per year that existed prior to initiating Mectizan treatment, meeting the criterion proposed by WHO for a “near absence” of transmission for areas where pre-treatment data on transmission rates exist.

Pre-treatment data for the level of transmission do not exist for Las Nubes II. However, in order for transmission to be significant from an epidemiologic perspective, it must occur at a level that maintains the effective reproductive ratio at a value above 1.0. If a control program can succeed in bringing transmission to a level that results in a reduction of the reproductive ratio below 1, and can maintain this level until a point where the parasite population is no longer able to increase transmission to a level where the reproductive ratio equals or surpasses 1 when control ends, the parasite population will eventually become extinct in the area under control. The reproductive ratio will be determined by the force of infection, which may be measured by the ATP. Unfortunately, the exact relationship between the ATP and the reproduction rate is not precisely known, and the “threshold ATP” necessary to maintain the reproductive rate below 1.0 is controversial. However, previous deterministic modeling

<table>
<thead>
<tr>
<th>Community/period of evaluation</th>
<th>Prevalence of skin microfilariae, corneal opacities, and mf in the anterior chamber in two communities of the Southern Chiapas endemic focus for onchocerciasis in Mexico</th>
<th>Prevalence (%) of skin microfilariae (by biopsy)</th>
<th>Prevalence (%) of corneal opacities and mf in the anterior chamber of the eye</th>
<th>Prevalence (%) of infected skin (by DNA test)</th>
<th>Prevalence (%) of infected skin (by biopsy)</th>
<th>Prevalence (%) of infected skin (by DNA test)</th>
<th>Prevalence (%) of infected skin (by biopsy)</th>
</tr>
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<tbody>
<tr>
<td>Las Golondrinas/before treatment, May 1991‡</td>
<td>78 (72.9–82.6)</td>
<td>34.0 (27.4–41.2)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Las Golondrinas/after 13 years of treatment, May 2004</td>
<td>73 (61.8–84.5)</td>
<td>26.7 (20.5–33.9)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Las Golondrinas/after 15 years of treatment, May 2006</td>
<td>65 (53.3–76.6)</td>
<td>18.2 (12.6–23.9)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Las Nubes II/after 13 years of treatment, May 2004</td>
<td>129 (101–156)</td>
<td>31.3 (23.7–38.7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Las Nubes II/after 15 years of treatment, May 2006</td>
<td>124 (101–151)</td>
<td>17.3 (12.8–21.7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>The bold numbers represent the point estimate for the value in question, and the numbers surrounding the point estimate represent the 95% CI.</td>
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<td>* Indicates that pre-treatment data are available.</td>
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<td>† No pre-treatment data are available.</td>
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</table>
studies using data derived from West Africa have suggested that threshold likely lies somewhere between 5 and 20 L3s per person per year. Because no infective flies were found in Las Nubes II in 2006, the point estimate for the seasonal transmission potential in this year was zero. Even taking the product of the upper bounds for the 95% CIs for both the prevalence of infective flies and the biting rate, the maximal possible transmission potential for Las Nubes II is just 9.5 L3s per person per year (a figure similar to that for the upper bound in Las Golondrinas of 9.8 L3s per person per year). Considering this worst-case estimate of the seasonal transmission potentials, our findings for ATP are shown to be within that range referred to as the “threshold transmission potential,” where the parasite population is likely on the path to elimination. These data, when taken together therefore suggest that transmission may have been suppressed in both formerly hyperendemic communities by the time that this study was completed in 2006.

Mectizan treatment also had a significant impact on the prevalence of skin mf and ocular pathology. These results are similar to those observed in other endemic areas. In 2004, very few individuals harbored skin mf, had ocular opacities in anterior segment, and mf in the anterior chamber of the eye in the two communities under study after 13 years of semi-annual ivermectin treatment. Furthermore, in 2006 in Las Golondrinas, after two years of four ivermectin treatment rounds per year, none of the 216 individuals examined exhibited ocular opacities and just one individual harbored skin mf (all 129 individuals examined by DNA test were negative). In Las Nubes II in 2006, there were no individuals presenting with ocular opacities, with mf in the anterior chamber of the eye, or with skin mf (detectable by conventional microscopic examination of the skin snip or by PCR). These results are in accordance with other studies that demonstrate that anterior segment changes (i.e., punctuate keratities) are onchocerciasis-specific and resolve completely after chemotherapy, thus preventing later severe ocular pathologies.

The data presented above on the effects of quarterly treatment lend support to recent studies that suggest repeated Mectizan treatments may have long term effects on the survival and fecundity of adult female worms. The data also suggests that increasing the frequency of Mectizan treatments may be an important tool for rapidly reducing O. volvulus transmission in problematic areas where transmission is persisting.

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