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TRANSMISSION OF *ONCHOCERCIASIS* IN NORTHWESTERN UGANDA

Transmission of *Onchocerca volvulus* Continues in Nyagak-Bondo Focus of Northwestern Uganda after 18 Years of a Single Dose of Annual Treatment with Ivermectin

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Abstract

The objective of the study was to determine whether annual ivermectin treatment in the Nyagak-Bondo onchocerciasis focus could safely be withdrawn. Baseline skin snip microfilariae (mf) and nodule prevalence data from six communities were compared with data collected in the 2011 follow-up in seven communities. Follow-up mf data in 607 adults and 145 children were compared with baseline (300 adults and 58 children). Flies collected in 2011were dissected, and poolscreen analysis was applied to ascertain transmission. Nodule prevalence in adults dropped from 81.7% to 11.0% ($P < 0.0001$), and mf prevalence dropped from 97.0% to 23.2% ($P < 0.0001$). In children, mf prevalence decreased from 79.3% to 14.1% ($P < 0.0001$). Parous and infection rates of 401 flies that were dissected were 52.9% and 1.5%, respectively, whereas the infective rate on flies examination by polymerase chain reaction (PCR) was 1.92% and annual transmission potential was 26.9. Stopping ivermectin treatment may result in onchocerciasis recrudescence.

INTRODUCTION

Onchocerciasis, a leading cause of blindness, is caused by infection with *Onchocerca volvulus*, a filarial nematode parasite. The female worms that live in the nodules produce microfilariae (mf), which inflame the skin. The mf may also enter the eyes, giving rise to inflammatory lesions that may eventually result in partial or complete blindness.[1] The parasite is transmitted by black flies of the genus *Simulium*, which breed in fast-flowing rivers and streams (hence, the name river blindness for the disease). The mf are picked up by female *Simulium* flies from an infected person during a blood meal, and within the fly, they develop into larval (L1, L2, and L3) stages. The L3 (infective) stage is passed on to other persons during subsequent bites.

Ivermectin (Mectizan), a safe and effective microfilaricide, has been donated by Merck & Co Inc. since 1987. Merck pledged to provide as much ivermectin as required for as long as necessary for mass treatment of onchocerciasis. Ivermectin kills the mf and reduces the risk of developing eye disease and severe skin lesions associated with the infection. Ivermectin also reduces the fecundity of adult worms and shortens their lifespan, although treatment must still be given for an undetermined length of time when taken one time in a year.[2,3]

The African Program for Onchocerciasis Control (APOC) was set up to establish, promote, and support sustainable community-directed treatment with ivermectin (CDTI) projects for the control of onchocerciasis in meso- and hyperendemic areas that had onchocercal nodule rates of $\geq$ 20% and $\geq$ 40%, respectively.[4–6] The goal was to target the most highly onchocerciasis-endemic communities with a single annual dose of Ivermectin through mass treatment using the CDTI strategy; therefore, the disease would no longer be a public health problem.[7–9] This goal was not defined but logically taken to be when prevalence is driven below the original baseline threshold required to launch the mass ivermectin treatment program, which is an onchocercal nodule rate of $\geq$ 20% or an mf rate of $\geq$ 40%.[6] However, achieving elimination of onchocerciasis as a public health problem defined at these levels did not necessarily indicate interruption of transmission. In the case that transmission has not been interrupted, halting mass treatment could result in disease recrudescence.[2,3]

Despite this information, a recent study in Mali and Senegal suggested that 15–17 years of annual treatment was sufficient to eliminate *O. volvulus* transmission.[10] This study called for additional studies in other endemic areas of Africa that have distributed single annual doses of ivermectin for onchocerciasis control over similar time frames to determine whether treatment could safely be withdrawn without the risk of disease recrudescence. It is possible that the amount of time necessary to eliminate transmission could vary considerably among foci in Africa depending on the initial prevalence of infection and the intensity of transmission.[11]
Epidemiological assessments done in 1993 had shown that the Nyagak-Bondo area of northwestern Uganda (Zombo district and parts of Arua and Nebbi districts) was endemic for onchocerciasis. The vectors in Nyagak-Bondo focus are members of the *S. neavei* complex, with larvae and pupae that live in a phoretic association on freshwater crabs of species *Potamonautes aloysiisabaudiae* and *P. niloticus*.[12] The River Blindness Foundation (RBF) -assisted community-based treatment with ivermectin (CBTI) for onchocerciasis control activities was carried out from 1993 to 1996 in the then Nebbi district (now Nebbi and Zombo districts). Subsequently, RBF operations were taken over by The Carter Center in 1996, which continued to assist the program to date. The Kuluva Missionary Hospital, funded by Christoffel Blinden Mission (CBM), assisted mass treatment activities in neighboring Arua district from 1993 to 1995. In 1996, APOC was established and

subsequently provided 5 years of financial assistance for implementation and establishment of mechanisms to sustain the CDTI program. APOC continued providing financial support to the program for 3 additional years to replace capital items and provide some training and advocacy. Subsequently, the CDTI project was expected to sustain annual mass treatment using only national resources. However, the national government did not take over the funding of CDTI activities.[13] Therefore, APOC continued to provide some assistance for specific activities alongside regular CDTI support from The Carter Center in Nebbi and Zombo districts and recently, Arua district by USAID funds for Neglected Tropical Diseases through RTI International. Overall, with the support of these agencies, annual ivermectin treatment has been provided to the at-risk population of Nyagak-Bondo for a period of 18 years. The objective of the present study was to determine whether annual mass treatment with ivermectin for 18 years could be withdrawn in Nyagak-Bondo onchocerciasis focus like in Mali and Senegal without the risk of disease recrudescence

MATERIALS AND METHODS

Study sites.

The study was carried out in Nyagak-Bondo focus of onchocerciasis, which covers areas of Arua, Nebbi, and Zombo districts of northwestern Uganda (Figure 1). The main river systems responsible for vector breeding are the Agoi, Nyagak, and Ora.[14] The Nyagak-Bondo focus covers an area of about 1,550 km^2, with a total of about 510,600 people.

Annual mass treatment with ivermectin for onchocerciasis control began in 1993 after six communities (Abilambe, Agweci, Jupa Ngali Upper, Nyadima, Patek-Athele, and Ukongo) were selected for sentinel evaluations, and assessed for microfilardermia and nodule prevalence. Three of these communities (Abilambe, Agweci, and Patek-Athele) were assessed again in 2011, and the results were compared with those results obtained in 1993. It was not possible to locate the other sentinel communities, because their structure and names had changed because of administrative changes since 1993. However, the Health Department wanted to know the situation in and outside the sentinel communities, and therefore, additional communities (Aguru, Kairo, Pachen, and Oloamura) were included in the follow-up survey.

Parasitological (nodule and mf prevalence) surveys.

Baseline parasitological surveys carried out in 1993 before mass treatment were followed up in 2011 (11 months after mass treatment).

Nodule assessments.

Every participant was examined in a well-lit private room. Trained district and Ministry of Health workers performed a palpation examination on the partially undressed participant, paying attention to bony prominences of the torso, iliac crests, and upper trochanter of the femurs. Onchocercal nodules were identified clinically as being firm, painless, and mobile.[15,16] In 1993, 180 adults ages 20 years and over who had lived in their respective communities for at least 10 years were assessed for nodule prevalence. In the seven communities in 2011, 607 adults were assessed, and the results were recorded on the study registration form as positive or negative. Nodule prevalence was expressed as a percentage, and follow-up data were compared with baseline data.

Mf assessments.

At baseline, 300 adults ages 20 years and over who had lived in their respective communities for at least 10 years and 58 children at 5 years of age from six baseline communities were assessed for mf prevalence. In the 2011 follow-up study, 607 resident adults and 145 resident children less than 10 years of age (born after mass treatment in all seven communities had started) were skin snipped. The procedure for skin snipping involved cleaning the site with an antiseptic; then, a piece of skin raised with the help of a disposable sterile dermal hook was carefully removed with a sterilized surgical blade. Two skin snip samples were taken from the posterior iliac crests of every selected person. Every person that was skin snipped had his or her own dermal hook and surgical blade, which were carefully disposed of after use.

The skin samples were placed immediately in wells of microtiter plates containing a sterile normal saline solution; they were kept at room temperature for 12–24 hours and examined microscopically for mf.[17–19] The results were expressed for each individual as positive or negative and recorded in the study registration form. Mf prevalence was expressed as a percentage.[4] The follow-up results were compared with baseline mf prevalence data of 1993 from adults and children.

ENTOMOLOGICAL ASSESSMENT

Crab infestation.

Crab trapping was conducted in the months of July and December of 2010 and continued through the months of January, March to June, and September to December of 2011 at a number of sites on the Nyagak, Agoi, and Wariki river systems. The crabs carrying larval and pupal stages of *S. neavei* were counted, and infestation rate (number of crabs positive for young stages of the fly) was expressed as a percent of the total number of crabs captured.[20]

Fly collection and analysis.

Two *Simulium* fly collection sites were set up at selected breeding points on the Agoi and Wariki rivers. Organized full-day catches were conducted for 7 consecutive days, 1 month in June, and September to December of 2010. Human landing collections were carried out from 07:00 to 18:00 daily.[19] A total of 401 flies collected during the period were dissected to determine parity, infection, and infective rates.[21,22] Examination of the larval stages of *O. volvulus* involved dissection of the abdomens, thoraces, and heads of the collected *Simulium* flies. The numbers of flies with larval stages (L1, L2, and L3) were counted, and monthly infection and infective rates were calculated. The result was expressed as a percentage of the total number of flies dissected $\times$ 100.[4]

Additional *Simulium* flies were caught from routine fly collections in 2011 from two previous collection sites on the Agoi and Wariki rivers, with an additional site on the Nyagak river system over a period of 9 months (2 days per week every month). Human landing collections were carried out from 07:00 to 18:00 daily as previously described.[19] A total of 1,100 individual flies divided into 22 pools of 50 flies each was analyzed using polymerase chain reaction (PCR). Only the heads of these flies were subjected to PCR analysis to calculate the infective rate and annual transmission potential (ATP).[23–25] The ATP was estimated based on the number of flies collected and the estimated 7,766 exposure hours (i.e., daylight hours when flies could bite) at the site. This information implies 12 hours of daylight over 365 days in the year. Collection times in estimating the monthly and annual biting rate were based on 40 collection hours per month over the 9- month collection period.

History of mass treatment with ivermectin.

Annual mass treatment with ivermectin commenced in 1993, and it was carried out annually through 2011 in all three districts. The objective was to sustain annual treatment of at least 90% of the ultimate treatment goal (UTG). The UTG is the sum of all eligible persons for treatment (minus children < 5 years old) among the total number of people at risk living in all at-risk communities in the onchocerciasis-endemic area that the program ultimately has to treat.[26] Annual mass ivermectin treatment had been provided for 18 years in Arua, Nebbi, and Zombo districts. The reports since inception of the program were available, and from these reports, UTG treatment coverage for each district was computed.

Data analysis.

Parasitological and entomological data were entered and analyzed graphically in Microsoft Excel and Epi Info, Centers for Disease Control and Prevention, Atlanta GA, for χ^2 test of independence. The prevalence of flies carrying infective larvae and an associated 95% confidence surrounding this point estimate were calculated using Poolscreen v2.0 software.[27]

Ethical approval.

Collections of data from the baseline to the follow-up studies were considered routine program evaluation by the Government of Uganda and the Emory University Institutional Review Board (eIRB 11 438). Therefore, this study was considered non-research and routine program evaluation. Individuals assessed were educated and informed of the option to opt out without any repercussions.

RESULTS

Mass treatment.

Treatment coverage of the eligible population in Nebbi and Zombo districts was better than in the Arua district. It reached the desired level of 90% in Nebbi and Zombo districts in 1998 and remained above 90% for the next 13 years, whereas in Arua district, it was above 90% for 10 years during the 18-year period of CDTI implementation (Table 1).

Parasitology

Nodule prevalence.

There was a highly significant reduction in baseline nodule prevalence among adults from 81.7% in 180 persons assessed (range = 73.3–96.7%) to 11.0% in 607 persons (range = 2.3–20%) in the follow-up survey ($P < 0.0001$) (Table 2). However, nodule prevalence in Kairo community remained at 20%, the threshold where mass treatment is recommended. All other communities were still positive for nodules. *Mf prevalence.* Baseline mf prevalence in 1993 was 97% in 300 adult skin snips, with a range of 90– 100% (Table 3). In the follow-up survey of 2011, mf prevalence had been reduced to 23.2% in 607 persons examined, with a range of 3.4–40% ($P <$ 0.0001). However, mf prevalence in six of seven communities in 2011 was at least 19%. In children, mf prevalence had reduced from 79.3% (with a range of 36.4–100% in 1993) to 14.1% (with a range of 0% to 36.8%, $P < 0.0001$) (Table 4).

Entomology.

In the Nyagak-Bondo onchocerciasis focus, crab infestation rate was 21.4% (N = 3,245), with a monthly range of 8.3–43.9% in an intermittent 11-month survey from July of 2010 to December of 2011 (Figure 2). The *S. neavei* parous rate investigated from June and September to December (a period of 5 months) was 52.9% (N = 401), with a monthly range of 0–67.8%. The infection rate (L1, L2, and L3 larval stages) was 1.50%, with a monthly range of 0–3.85%, whereas the infective rate was zero (Table 5). Pool screening of 22 head pools (1,100 flies) resulted in a single confirmed positive pool, which revealed a prevalence of infective flies of 1.92% (95% upper limit = 7.4/2,000 flies). In addition, the ATP was estimated at 26.9 (95% confidence interval = 0–66) third-stage (L3) larvae per person per year.

DISCUSSION

The results showed that, after 18 years of annual mass treatment with ivermectin, there was a significant reduction in infection. However, the children born well after ivermectin distribution commenced were still getting infected with *O. volvulus*, and a substantial proportion of adults were still positive for mf. This finding was not surprising, because breeding of *S. neavei* was ongoing, with mean crab infestation at 21.4%, a fly parous rate of 52.9%, infection rate of 1.50%, and poolscreen infective rate of 1.92% with the ATP of 26.9.

Nodule and mf rates.

The reduction in nodule rate was significant in most communities; however, one community remained at 20%, which under the APOC policy for controlling onchocerciasis as a public health problem, was at the threshold for mass treatment. It is, however, possible that, at low nodule prevalence, there are likely confounding factors, such as ganglia and *Taenia solium*-related nodules, that may artificially increase the calculated nodule rate.[6] Despite this information, the data showed consistency of high microfildermia prevalences, implying a continuing high force of transmission. These data suggest that, if annual mass treatment was halted, it is likely that recrudescence of onchocerciasis would occur.[28]

It is interesting to note that, in the Patek-Athele community, the mf rate was brought to zero from 100%, whereas in other communities, it was not. Because the vector of onchocerciasis is *S. neavei*, it is possible that there could have been ecological changes resulting into unfavorable breeding conditions of the vector in the vicinity of Patek- Athele. Such conditions could result in interruption or suppression of transmission.[29,30] However, no *Simulium* fly collection and crab trapping were done in the vicinity of Patek-Athele. We recommend entomological investigations in the streams close to this community to understand the reason for the dramatic change. Alternatively, given that skin snip (microscopy) has low sensitivity at low endemicity levels, the results obtained may not reflect the actual endemicity levels.[31] If this result is true, undetected microfiladermic individuals could still pose serious threat to disease recrudescence.[31,32] Additional studies are needed to understand the roles of ecological changes, low endemicity in disease transmission, and possible recrudescence.

Continuing transmission.

Interruption of transmission is considered to have been attained if the calculated upper 95% confidence interval of the ATP is less than 5–20 L3/person or the prevalence of flies carrying infective larvae is less than ½,000 in the overall fly population (< 0.05%).[33] The point estimate of the ATP in Nyagak-Bondo was still 26.9, with a point estimate of the prevalence of infective flies of 1.92/2,000 flies. The upper bound of the 95% confidence interval for both metrics was considerably higher than the accepted cutoffs indicative of transmission suppression. These findings are consistent with those findings obtained from northern Cameroon, which showed that 17 years of annual mass treatment did result in a significant decrease of skin mf and nodules but did not interrupt transmission.[11] When contrasted with recently published studies from Senegal, Mali, and Nigeria, which show successful interruption of transmission after annual Ivermectin treatment, this study points out the importance of local variations in the ecology of transmission of onchocerciasis in determining the ultimate success of an elimination program.[11] Local variations in vector density, biting rate, and vector capacity will all affect the potential for the success of an elimination program and by extension, the strategic plan adopted by the program to interrupt transmission. For example, *S. neavei*, although present in relatively low densities, is known to be a highly anthropophilic and competent vector for *O. volvulus*.[34] Annual treatment may not be sufficient to interrupt transmission in areas where such an efficient vector is present. In such areas, it may be necessary to increase the frequency of ivermectin treatments to two times per year.

Efficiency of diagnostic methods in entomology.

During dissection of freshly collected flies, larval stages L1 and L2 were observed, but none of the infective larval (L3) stages were observed. However, during poolscreen, L3 stages were observed. However, only 401 flies were dissected, which contrasts the 1,100 flies that were poolscreened. Dissections of flies may not be necessarily less efficient with well-trained and experienced personnel: the rate of infected flies and numbers of larvae (L1, L2, and L3) can rapidly be assessed, and immediate conclusions on the fly populations and *O. volvulus* can be made. However, when interruption of transmission is the objective, poolscreen should be applied, because it is 100% sensitive and 100% specific in detecting *O. volvulus* DNA.[35,36] Therefore, we recommend poolscreen in flies where interruption of transmission is launched without vector elimination.

Risk of emergence of resistance.

Anguru and Kairo communities had mf rates of 38% and 40%, respectively. The difference in infection between them and the rest of the sentinel communities was highly significant ($P < 0.0001$). This result could be because of either low treatment coverage or the possibility of risk of drug resistance caused by long-term mass drug administration.[11,37] However, there are validated reports of consistent and good annual treatment coverage with a significant reduction of mf rates from baseline to follow-up survey. Therefore, it is doubtful that treatment coverage could be the reason for high follow-up mf rate; alternatively, the high force of infection may be responsible.[3] However, studies are required to show whether resistance to ivermectin could be a threat to the long-term success of elimination programs in this region.

CONCLUSION

Although an annual dose of ivermectin given over a period of 18 years has reduced onchocerciasis infection significantly, it has not interrupted the transmission of *O. volvulus*. Although the reports from Senegal and Mali show that, in some areas, onchocerciasis can be eliminated with 15–17 years of annual treatment, the Nyagak- Bondo focus of northwestern Uganda showed that it was not possible. This finding alludes to the fact that local variations in the ecology of transmission of onchocerciasis could determine the ultimate success of an elimination program. Hence, it is imperative to focus on new innovative and flexible approaches to meet the needs of varying ecological conditions that determine the level of onchocerciasis transmission if onchocerciasis elimination becomes the goal throughout Africa.

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TABLE 1 - Population and treatment coverage of the eligible population from 1993 to 2010

TABLE 2 - Comparing data on baseline nodule prevalence of 1993 with data from the follow-up survey of 2011 among adults

TABLE 3 - Comparing data on baseline mf from 1993 with data from the follow-up survey of 2011 among adults

TABLE 4 - Comparing data on baseline mf of 1993 with data from the follow-up survey of 2011 among children

TABLE 5 - Monthly parous and infection (microscopy) rate of S. neavei spp. in Nyagak-Bondo onchocerciasis focus during 2010

FIGURE 1. Nyagak-Bondo onchocerciasis focus of northwest Uganda.

FIGURE 2. Percent monthly infestation of crabs (N = 3,245) captured in Nyagak-Bondo onchocerciasis focus in 2010 and 2011.