STATUS OF FOREST ONCHOCERCIASIS IN THE LOWER CROSS RIVER BASIN, NIGERIA: ENTOMOLOGIC PROFILE AFTER FIVE YEARS OF IVERMECTIN INTERVENTION

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Abstract. In the Lower Cross River basin in Nigeria, no pre-control entomologic profile of Onchocerca volvulus infection in the local Simulium damnosum population was available prior to the initiation of an ivermectin control program in 1995. A longitudinal entomologic study was therefore carried out over a 12-month period (January–December 2001) at the Agbokim waterfalls and Afì River, which are breeding sites of S. damnosum in the river basin. A total of 9,287 adult S. damnosum were caught on human bait; 9,048 (97.43%) were dissected, of which 313 (3.46%) were infected. Annual biting rates (ABRs) of 42,419 and 28,346 bites per persons per year were recorded at the Agbokim Waterfalls and Afì River, respectively. The annual transmission potential (ATP) was 419 infective larvae per person per year at the Agbokim Waterfalls and 427 at the Afì River. Monthly biting rate and monthly transmission potential varied significantly ($P < 0.05$) at the two sites. Transmission was highly seasonal from April to September, corresponding to the peak biting period of the vector. The high ATP and ABR values are a measure of the mesoendemicity of onchocerciasis in the river basin. There was a significant $F_r,05 (1, 10) (P < 0.05)$ variation in the relative fly abundance from both sites. It was observed that human activities such as farming, fishing, timber cutting, and hunting are done in the early morning and late afternoon, which corresponds to the peak diurnal biting period of the vector. Changes in these practices and attitudes may markedly affect the disease intensity and transmission.

INTRODUCTION

Onchocerciasis is both a public health hazard and a socioeconomic problem of considerable magnitude in Nigeria. The filarial worm Onchocerca volvulus is the causative agent of the disease. In Nigeria, O. volvulus is transmitted primarily by the Simulium damnosum complex.1–4 Prevalence of human onchocerciasis was shown to be directly related to the abundance of this blackfly.5 Many studies have been carried out in Nigeria on the transmission of O. volvulus by S. damnosum.6–8 On the Lower Cross River basin, S. damnosum s.l. is an important vector of onchocerciasis,5,9 and it breeds in rivers, streams, and waterfalls. An understanding of the transmission dynamics of onchocerciasis as in other forms of filariasis is important in advancing knowledge of how vector competence, behavior, and abundance influence the level of infection and disease in susceptible human population.10 Quantitative analysis of potential vectors and their level of infection may also provide cost-effective and non-invasive means of rapid assessment of the need for and success of various control measures.11 The capture and dissection of adult flies can be used as a means of following the dynamics of a Simulium population and vector infectivity and thus the level and magnitude of parasite transmission. Different infectivity rates can be found at different sites and it is recognized that different cytospecies of the S. damnosum complex living in different biotypes may carry heavier O. volvulus infective worm loads than others.12,13

In the Lower Cross River basin in Nigeria, ivermectin had been administered over a five-year period (1995–2000). Prior to the initiation of this control program, no pre-control entomologic profile of O. volvulus infection in the local S. damnosum population was available. This quantitative entomologic study was therefore conducted to obtain baseline data for future monitoring and evaluation of the trends of S. damnosum infection rates and for quantifying the effectiveness of an ivermectin control program.

MATERIALS AND METHODS

Study site. The Cross River basin encompasses an area of 70,000 km², of which 50,000 km² is in Cross River State, Nigeria, and the remaining 20,000 km² is in Cameroon. The entire Cross River basin is located within the evergreen rainforest belt, except for a small savanna belt located at 7°N and drained by a principal tributary, the Anyim River. There are two different climatic seasons in the area, the rainy season from March to October and the dry season from November to February. Year round rainfall of approximately 2,942–3,424 mm occurs, with maximum precipitation occurring from July to September. Based on physiogeographic, ecologic, and zoogeographic considerations, the Cameroon section of the Cross River basin is referred to as the Upper Cross River, while the Nigerian section is termed the Lower Cross River.14 The Cross River is formed from the numerous headwaters of the western slopes of the Cameroon highlands, which has two extensions in Nigeria, namely the Oban hills in the south and Obudu Plateau that is further north.15 The main channel of the Cross River is 6,000 km from its source to its mouth. The Cross River enters Nigeria at Ekok (a town on the border with Cameroon). Downstream of Ikom, the Cross River is joined by the Afì River (our study site), a northern tributary that drains the Oshie Ridge of the Sankwala Mountains. The basin is characterized by the absence of any well-developed flood plains and the presence of numerous ecologic and zoogeographically important high-gradient streams, rapids, and waterfalls, one of which is the Agbokim Water-
falls, another study site. The swifter upper reaches of the river basin often have a rocky or stony substratum. All these topographic features are conducive for the breeding of blackflies, especially in the rainy season.

Selection of study sites. Agbokim Waterfalls was chosen because it is less than 500 meters from Agbokim, a rural community with a population of approximately 4,000 inhabitants. It is the only source of water for the community due to the absence of piped water. We had previously surveyed this community for onchocerciasis. The Afì River was selected based on its proximity to the first-line community (Orimekpong) with a population of 1,500, which was also surveyed for onchocerciasis. No other human settlement is located between Orimekpong and the river. All criteria for selection were based on those of Ngoumou and others. The two communities have been treated once a year over a five-year period (1995–2000) with ivermectin, with an average therapeutic coverage of 60% of the entire population.

Catching method. Biting, adult, female *S. damnosum* were caught using human baits at Agbokim Waterfalls and the Afì River from January to December 2001. Each station was sampled four times a month, and the stations were located near the bank of the waterfalls and the river. Flying catching was conducted between 7:00 AM and 6:00 PM by two fly collectors working alternately. The fly collectors sat or stood with their hands and legs exposed. Any fly perching on the exposed parts was caught before it fed by inverting a small glass tube over it. The caps of the tubes were then immediately replaced. All tubes containing flies were labeled to indicate time, date, and place of capture. Long hours of practice by the fly collectors made them more skilled and the flies were observed to be attracted by their exposed body extremities. Each fly was caught in a different tube. Hourly captures were pooled and labeled. All captured flies were packed in a cold box containing ice packs to stop further development of microfilariae in the flies before being transported to the laboratory. The process was repeated the next day at other stations using different fly collectors from the locality. The study protocol was reviewed and approved by the ethical committee of the Ministry of Health, Cross River State, Nigeria. Informed consent was obtained from the individuals and the communities involved.

Dissection method. Depending on the sample size, either all or a percentage (90%) of the blackflies in each catch period was dissected to distinguish nulliparous and parous flies. The nulliparous flies were distinguished from the parous flies by the presence of tightly coiled trachea systems and absence of follicular relics (corpora lutea). Flies were identified as parous by the presence of follicular relics below the maturing oocyte and a loosely stretched condition of the tracheal system.. Nulliparous flies were discarded because they do not transmit *O. volvulus*. Further dissection of the abdomen, thorax, and head was continued if a fly was found to be parous to check for the presence of filarial worms. The criteria of Porter and Collins were used to distinguish and characterize all larvae of *O. volvulus*. The number of sausage-shaped larvae (*L*₂), pre-infective (*L*₃), and infective (*L*₄) larvae of *O. volvulus* found in the abdomen, thorax, and head, respectively, were counted and their stages of development at these various sites were recorded.

Calculation of transmission indices. The fly density and level of transmission of onchocerciasis at a given location were quantified by two entomologic indices, the annual biting rate (ABR) and the annual transmission potential (ATP). The ABR is the theoretical total number of bites that would be received in one year by a person stationed at a capture point for 11 hours (7:00 AM to 6:00 PM) a day. It is calculated using the sum total of the monthly biting rate (MBR) established for each capture point. The annual transmission potential is the theoretical total number of infective larvae (third-stage larvae found in the heads of these blackflies) that would be received in one year by an individual stationed at a capture point for 11 hours a day. It is calculated using the sum total of the monthly transmission potential (MTP). Transmission indices were evaluated by established methods. 16

Statistical methods. The monthly relative abundance of *S. damnosum* from the two sites was evaluated using the two-way analysis of variance, and the difference in infection rate was assessed by the chi-square test.

RESULTS

Relative abundance. A total of 9,287 adult *S. damnosum* were caught, 5,575 at the Agbokim Waterfalls and 3,712 at the Afì River. The monthly variation in the relative abundance of flies at the two sites is shown in Figure 1. Fly catching from both sites started in January and increased gradually to a peak in September, which corresponds to the peak of rainfall. After September, fly abundance decreased. There was a significant variation in the relative fly abundance at the two sites: F₁,05 (1, 10) (P < 0.05).

Vector transmission indices. The monthly entomologic indices of transmission at Agbokim Waterfalls is shown in Table 1. Of the 5,452 (97.79%) flies dissected, 4,555 (83.54%) were parous, while 897 (16.45%) were nulliparous. A total of 159 (2.92%) of the parous flies were infected (containing *L*₁, *L*₂, and *L*₃ larvae) with *O. volvulus*, while 53 (0.97%) were infective (containing *L*₂ only). The peak MBR was recorded in August with 7,354 bites per person per month, while the lowest MBR was recorded in January with 217 bites per person per month. The peak ATP was 42,419 bites per person per year. There was no transmission from November to March. The ATP was 419 *L*₃ per person per year. There was no significant difference in the monthly infection rates in this site (χ² = 19.12, P > 0.05).
The monthly entomologic indices of transmission at the Afi River is shown in Table 2. A total of 3,596 (96.87%) flies were dissected, of which 3,061 (84.89%) were parous and 535 (14.54%) were nulliparous; 154 (4.28%) of the parous flies were infected (containing L₂, L₃, and L₄ larvae) and 54 (1.50%) harbored infective (L₂) larvae of *O. volvulus*. The peak MBR was recorded in September with 6,893 bites per person per month, while the lowest MBR was observed in January with 46.5 bites per person per month. The ABR was 28,346 bites per person per year. The peak MTP was recorded in July with 127.95 L₄ per person per month. The lowest MTP was observed in April with 15.9 L₄ per person per month. An ATP of 427 L₄ per person per year was recorded at this site. There was a significant difference in the monthly infection rates at this site ($\chi^2 = 39.36, P < 0.05$).

**Infection rate.** A summary of the result of catches and dissection of *S. damnosum* at Agbokim Waterfalls and the Afi River is shown in Table 3. A total of 9,287 flies were caught and 9,048 (97.43%) were dissected, of which 7,616 (84.17%) were parous and 1,432 (15.83%) were nulliparous; 313 (3.46%) were infected while 107 (1.18%) were infective. The mean annual biting rate was 35,381 bites per person per year, while the mean annual transmission potential was 432 L₄ per person per year. There was a significant difference between the infection rates at the two sites ($\chi^2 = 12.1, P < 0.05$).

**Diurnal biting rates of parous flies.** The diurnal biting rate of parous flies from both sites is shown in Figure 2. The biting cycle showed a bimodal peak of activity. There was a small peak between 10:00 AM and noon and a more pronounced evening peak between 4:00 PM and 6:00 PM. The two peaks were separated by hours of low biting intensity (1:00 PM to 3:00 PM). There was a significant difference in the diurnal biting rates of parous flies at the two sites ($\chi^2 = 99.62, P < 0.01$).

**DISCUSSION**

We have used a longitudinal study to measure the degree of transmission of *O. volvulus* in a forested river basin located in southern Nigeria by evaluating the entomologic indices of transmission after a five-year period of intervention with ivermectin. There was significant difference in the monthly catches of *S. damnosum* at Agbokim Waterfalls and the Afi River in the Lower Cross River basin. The lowest number of flies was caught during the dry months of November to February. There was a direct relationship between monthly rainfall and relative fly abundance because most of the flies were separated by hours of low biting intensity (1:00 PM to 3:00 PM). The rainy season has been reported to enhance development, which results in increased amounts of nutrients and availability of pre-imaginal sites, all of which enhance pre-imaginal development, which results in an increase in the adult fly population during the rainy season (March–September). The rainy season has been reported to enhance development, which results in increased amounts of nutrients and availability of pre-imaginal sites, all of which enhance pre-imaginal development, which results in an increase in the adult fly population during the rainy season (March–September). The rainy season has been reported to enhancements in the breeding sites may be accompanied by increased amounts of nutrients and availability of pre-imaginal sites, all of which enhance pre-imaginal development, which results in an increase in the adult fly population during the rainy season (March–September).
be accompanied by flooding and phytoplankton (detritus, bacteria, diatoms, and filamentous algae) blooms. These phytoplankton constitute the food required by Simulium larvae for its development. It is plausible that the high abundance of blackflies during this period is due to the phytoplankton present in the river basin. However, this contrasts with the results of some reports, in which the number of Simulium were reduced during the heavy storms of the rainy season. The stormy weather may be a factor because it may have washed away most of the breeding sites, thus resulting in a smaller fly population. More flies were caught at the Agbokim Waterfalls than at the Afi River. This might be due to the decreased volume, but faster flow, of the waterfalls.

### Table 2
Transmission indices of Simulium damnosum at the Afi River in Nigeria

<table>
<thead>
<tr>
<th>Month</th>
<th>Persons day worked</th>
<th>Total flies caught</th>
<th>Average daily catch/person</th>
<th>Total no. (%) of flies dissected</th>
<th>No. (%) of parous flies</th>
<th>No. (%) of nulliparous flies</th>
<th>Total no. (%) of flies infected</th>
<th>Flies (%) infected with L₁</th>
<th>Flies (%) infected with L₂</th>
<th>Flies (%) infected with L₃</th>
</tr>
</thead>
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<tr>
<td>Jan</td>
<td>48</td>
<td>44</td>
<td>1.5</td>
<td>0.4</td>
<td>106.0</td>
<td>897</td>
<td>206</td>
<td>100</td>
<td>6</td>
<td>54</td>
</tr>
<tr>
<td>Feb</td>
<td>48</td>
<td>44</td>
<td>1.5</td>
<td>0.4</td>
<td>106.0</td>
<td>897</td>
<td>206</td>
<td>100</td>
<td>6</td>
<td>54</td>
</tr>
<tr>
<td>Mar</td>
<td>48</td>
<td>44</td>
<td>1.5</td>
<td>0.4</td>
<td>106.0</td>
<td>897</td>
<td>206</td>
<td>100</td>
<td>6</td>
<td>54</td>
</tr>
<tr>
<td>Apr</td>
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<td>44</td>
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<td>0.4</td>
<td>106.0</td>
<td>897</td>
<td>206</td>
<td>100</td>
<td>6</td>
<td>54</td>
</tr>
<tr>
<td>May</td>
<td>48</td>
<td>44</td>
<td>1.5</td>
<td>0.4</td>
<td>106.0</td>
<td>897</td>
<td>206</td>
<td>100</td>
<td>6</td>
<td>54</td>
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<tr>
<td>Jun</td>
<td>48</td>
<td>44</td>
<td>1.5</td>
<td>0.4</td>
<td>106.0</td>
<td>897</td>
<td>206</td>
<td>100</td>
<td>6</td>
<td>54</td>
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<tr>
<td>Jul</td>
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<td>44</td>
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<td>0.4</td>
<td>106.0</td>
<td>897</td>
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<tr>
<td>Aug</td>
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<td>0.4</td>
<td>106.0</td>
<td>897</td>
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<td>100</td>
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<tr>
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<td>44</td>
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<td>106.0</td>
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<td>Dec</td>
<td>48</td>
<td>44</td>
<td>1.5</td>
<td>0.4</td>
<td>106.0</td>
<td>897</td>
<td>206</td>
<td>100</td>
<td>6</td>
<td>54</td>
</tr>
</tbody>
</table>

### Table 3
Summary of annual vector transmission indices of Agbokim Waterfalls and the Afi River in Nigeria

<table>
<thead>
<tr>
<th></th>
<th>Agbokim Waterfalls</th>
<th>Afi River</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persons day worked</td>
<td>48</td>
<td>48</td>
<td>96</td>
</tr>
<tr>
<td>Total flies caught</td>
<td>5,575</td>
<td>3,712</td>
<td>9,287</td>
</tr>
<tr>
<td>Average daily catch/person</td>
<td>116.15</td>
<td>73.0</td>
<td>189.45</td>
</tr>
<tr>
<td>No. (%) of flies dissected</td>
<td>4,552</td>
<td>3,596</td>
<td>8,148</td>
</tr>
<tr>
<td>No. (%) of parous flies</td>
<td>(97.79)</td>
<td>(96.87)</td>
<td>(97.43)</td>
</tr>
<tr>
<td>No. (%) of nulliparous flies</td>
<td>(83.54)</td>
<td>(84.89)</td>
<td>(84.17)</td>
</tr>
<tr>
<td>Total no. (%) of flies infected</td>
<td>897</td>
<td>535</td>
<td>1,432</td>
</tr>
<tr>
<td>No. (%) of flies with L₁ and L₂</td>
<td>(16.45)</td>
<td>(14.53)</td>
<td>(15.83)</td>
</tr>
<tr>
<td>No. (%) of flies with L₃</td>
<td>(2.91)</td>
<td>(4.28)</td>
<td>(3.46)</td>
</tr>
<tr>
<td>No. (%) of flies with L₁ and L₂</td>
<td>(1.96)</td>
<td>(2.78)</td>
<td>(2.28)</td>
</tr>
<tr>
<td>No. (%) of flies with L₃</td>
<td>53</td>
<td>54</td>
<td>107</td>
</tr>
<tr>
<td>Biting density FMH</td>
<td>126.74</td>
<td>84.5</td>
<td>211.24</td>
</tr>
<tr>
<td>Annual biting rate</td>
<td>42,418.8</td>
<td>28,345.8</td>
<td>70,763</td>
</tr>
<tr>
<td>Annual transmission potential</td>
<td>419.3</td>
<td>426.74</td>
<td>846.04</td>
</tr>
</tbody>
</table>

* Annual mean.

FIGURE 2. Diurnal biting activity of adult Simulium damnosum at the Agbokim Waterfalls and Afi River in Nigeria, showing the bimodal peaks.
during the dry season, since these flies are known to breed better in fast-flowing waters. The Afi River is reduced to almost a trickle during the dry season and as such, could only support breeding of blackflies in large numbers during the rainy season with increased water level and a faster flow of the river.

The monthly relative abundance also shows that more parous flies were caught than the nulliparous flies (Table 3). This observation is similar to that of a previously published report. A high proportion of nulliparous females indicates incomplete vector control, probably resulting from deficiencies in larviciding or the colonization of untreated tributaries, while a high proportion of parous females indicates ageing of the local blackfly populations, which may reflect successful larviciding or the presence of migratory female flies. Since vector control has not been implemented in this river basin, it is possible that the high proportion of parous flies observed in this study might be due to migrations from the neighboring Cameroon, a country with endemic onchocerciasis. This is possible because \textit{S. damnosum} are reportedly long-distance migrants.

The transmission of onchocerciasis varies with location and season, and may also be influenced by the longevity of the fly and its ability to support the development of \textit{O. volvulus}. The fly-to-human ratio and the availability of microfilariae reservoirs in the human population may also affect infectivity rates. There was a significant difference in the monthly infection rate, with relatively more flies being infected during the rainy season than at the peak of the dry season. Thus, there were a greater chance of receiving a bite from an infected fly during the rainy season than in the dry season. Fly density and infectivity were high during the rainy season, in agreement with the findings of Renz, but in contrast to those of Okonkwo and others.

There was a seasonal variation in both the MBR and MTP with no transmission from November to March at both sites. This important finding suggests that transmission may be abolished in the foci by concentrated larviciding or other control measures during the peak biting months from June to September. In west Africa, the Onchocerciasis Control Program considers an annual biting rate of 1,000 and an annual transmission potential of 100 infective larvae per year as tolerable levels for a person living in a hyperendemic zone and receiving treatment. The ABR and ATP values of our sites are greater than these values, which indicates active transmission of onchocerciasis, despite the ongoing mass treatment with ivermectin. We could not compare the impact of drug treatment on disease transmission since no data on the entomologic aspects of the disease prior to treatment in 1995 were available. However, since this study was conducted as part of routine monitoring of ongoing ivermectin treatment to control onchocerciasis in Nigeria, these baseline data would be very important for future evaluation of the infection rate of \textit{S. damnosum} and assessment of the benefits of the ivermectin campaign in the river basin. The high ABR and ATP values may be a measure of the mesoendemicity of onchocerciasis in the river basin within the period of treatment. Similar findings have been documented in the Vina Valley River basin of Cameroon, where an ABR of 10,000–70,000 and an ATP of 1,030–2,171 were reported.

Blackflies often bite continuously from dawn to dusk, but seldom at the same sustained level throughout the day. The result of this study shows that biting activity manifested itself in a bimodal pattern with an early morning peak (10:00 AM to noon) and a late afternoon peak (4:00 PM to 6:00 PM) that is more pronounced. This finding is consistent with previous reports. The cause of biting activity peaks is still poorly understood, but it has been suggested that an innate clock rhythm may be involved. Since blackflies do not suck blood daily, the biting cycle may be loosely described as a circadian rhythm, which by definition entails a biologic rhythm on a one-day periodicity. The apparent diurnal variation in numbers of adult flies may have epidemiologic implications, since their biting corresponds to periods of peak human outdoor activities. It was observed in the study area that farming, fishing, hunting, cutting of timber, collection of wild fruit, and small-scale trading are done in the early morning and late afternoon, which corresponds to the peak biting period of the vector. Consequently, changes in such practices and attitudes would markedly affect the disease intensity and transmission dynamics. In view of the high abundance of blackflies and high ABR and ATP values, coupled with the concomitant nuisance resulting from their bites, it is hereby suggested that vector control should be complemented with the ongoing distribution of ivermectin. Such an integrated approach would increase the success of onchocerciasis control programs in the river basin. This strategy would facilitate the rapid disappearance of microfilariae from the skin, since repeated treatment with ivermectin is believed to be deleterious to the fecundity of the female adult \textit{O. volvulus} worms.

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