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Status of *Onchocerca volvulus* infection and transmission by blackflies after 15 years of ivermectin distribution at Adani, Nigeria

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Abstract. The nuisance bites of blackflies and transmission of *Onchocerca volvulus*, which causes onchocerciasis, constitutes a threat to public health and an impediment to food production in rural and riverine communities in Nigeria. The entomological profile of onchocerciasis at Adani, Nigeria, was investigated from August 2010 to January 2011 to determine the transmission of *O. volvulus* after 15 years of ivermectin distribution in the area. A total of 548 adult female blackflies of the *Simulium damnosum* complex were caught using human baits and dissected. Of this number, 248 flies were caught in the wet season (August to October), while 300 flies were caught in the dry season (November to January). The relative abundance of flies at Adani varied from 21 in December to 243 in January. The monthly catches between September and October and between December and January were significantly different. The monthly population density of the flies ranged from 0.5Flies/Man/Hour (FMH) in December to 5.5FMH in January. The diurnal biting pattern of the *S. damnosum* complex at the site showed a bimodal peak of activity with the evening peak being higher than the morning peak except in October when the morning peak was higher than the evening peak. The morning peaks were observed between 7.00 am and 10.00 am, whereas the evening peaks occurred between 4.00 pm and 6.00 pm. The morning and evening biting peaks in all the months were not significantly different. Nulliparous flies accounted for 75.7% of the total catch, whereas 24.3% of the flies caught were parous. The infection, infective bites and transmission of *O. volvulus* during the study period were zero. This study suggests that transmission of *O. volvulus* has been halted and the flies are presently more nuisance biters than disease vectors since no stage of *O. volvulus* was found in the flies dissected.

INTRODUCTION

Onchocerciasis is a disease caused by the filarial nematode *Onchocerca volvulus*. It is also known as river blindness and constitutes a public health problem and a serious obstacle to socio-economic development (Moyou-Somo *et al.*, 1993; Etyàale, 2001). Onchocerciasis is a vector-borne disease transmitted by black flies of the genus *Simulium*. It is one of the major causes of blindness in the world. The disease affects more than 15 million people worldwide (Cheke, 2017). Onchocerciasis is widespread and a major cause of blindness in most rural communities in Nigeria. In Nigeria, *O. volvulus* is transmitted primarily by the *S. damnosum* complex (Opara *et al.*, 2008).

The prevalence of onchocerciasis is directly related to the abundance and distribution of the vectors, species in the *S. damnosum* complex (Atting *et al.*, 2005; Opara *et al.*, 2005). In Nigeria, *S. damnosum* complex is responsible for transmission of *O. volvulus* that causes onchocerciasis. An understanding of occurrence and biting patterns of *S. damnosum* complex, and the transmission dynamics of onchocerciasis will be crucial in breaking the transmission of the disease in susceptible human populations.

Research on the biting patterns of *S. damnosum* complex in Nigeria has been extensive (Nwoke, 1988; Ubachukwu & Anya, 2001; Ekpo *et al*., 2009; Adeleke *et al*., 2010). However, few studies assessed the infectivity and transmission dynamics of *S. damnosum* complex in different parts of Nigeria (Okenu *et al*., 2005; Opara *et al*., 2008) despite mass distribution of ivermectin in many parts of the country.

Whether ivermectin distribution could control onchocerciasis and at the same time interrupt transmission of the parasite *O. volvulus* had been a question raised among public health scientists (Remme *et al*., 1990; Plaisier *et al*., 1995; Dadzie *et al*., 2003). However, mass administration of ivermectin once or twice a year has been shown to reduce or break transmission of *O. volvulus* that causes onchocerciasis (Remme *et al*., 1989; Borsboom *et al*., 2003). Ivermectin clears circulating microfilaria within 5 days (Ismail *et al*., 1991). In addition, Diawara *et al*. (2009) provided the first direct evidence that ivermectin can eliminate onchocerciasis as a public health problem in some endemic areas in Africa. In Ecuador, transmission of *O. volvulus* has also been interrupted in all the endemic areas by biannual distribution of ivermectin (Lovato *et al*., 2014; Guevara *et al*., 2018). Transmission of *O. volvulus* has also been successfully interrupted in other countries of the Americas (Rodriguez-Perez *et al*., 2015; Tekle *et al*., 2016) and also in some endemic foci in Africa (Traore *et al*., 2012; Lakwo *et al*., 2013; Zarroug *et al*., 2016) as a result of many years mass distribution of ivermectin.

Mass distribution of ivermectin started at Adani in 1996 (WHO, 1996; Ubachukwu, 2004) but to date no research has been conducted to ascertain the vector infectivity and thus the level and magnitude of parasite transmission in the area. The objectives of this study, therefore, were to assess the diurnal biting patterns of *S. damnosum* complex and the infectivity and transmission indices of onchocerciasis at Adani, Nigeria, after 15 years of mass distribution of ivermectin in the area.

MATERIALS AND METHODS

The study area

Adani is one of the communities in Uzo-Uwani Local Government Area of Enugu State, Nigeria. It lies between latitude 6° 44' and 6° 52' N and longitude 7° 1' and 7° 8' E (Fig. 1). The approximate population of Adani for a 7-km radius from this point is 17, 992. It belongs to the forest-savanna-mosaic zone of Nigeria. The area is traversed by many rivers and streams which belong to the Anambra River system. These rivers form the major breeding sites for the *Simulium* vector of human onchocerciasis (Crosskey, 1981). The area is bordered on the West by Ojjor, Igga, Ogurugu and Umueje. On the North are Igulala, Okwoengele, Anokwo, Ameke, Alakija and Akpatebe; on the East are Opandas, Ogbo-Uvuru, Adaba, Ukpabi-Nimbo, Ugwojoro, Nimbo, and Ngalakwu. On the south are Omash and Ifute. The mean annual temperature is 22.5°C. The people are primarily farmers being famous in rice and cassava production.

Blackfly catching method

The classical method of collecting blackflies for a period of 11 hours, using human bait, was employed in the study (Taye *et al*., 2000; Grillet *et al*., 2001; Opara *et al*., 2008). A sampling point approximately 300 metres from the Obina River at Adani was used as a fly collection site. The site was sampled 4 times a month from August 2010 to January 2011. This period corresponded with peak planting and harvesting of agricultural products in the region. Fly catching was conducted from 7.00 am to 6.00 pm by two fly collectors who worked alternately (Walsh *et al*., 1978; Opoku, 2000; Atting *et al*., 2005). The fly collectors dressed in knickers exposing the lower legs (Atting *et al*., 2005). Any fly that perched on the exposed parts was collected before it started feeding by inverting a small glass tube over it and replacing the cap immediately (Walsh *et al*., 1978). The tubes containing the flies were labelled to indicate time and date of collection and the total number of flies caught per day was recorded. The flies captured were packed in a cold box containing ice

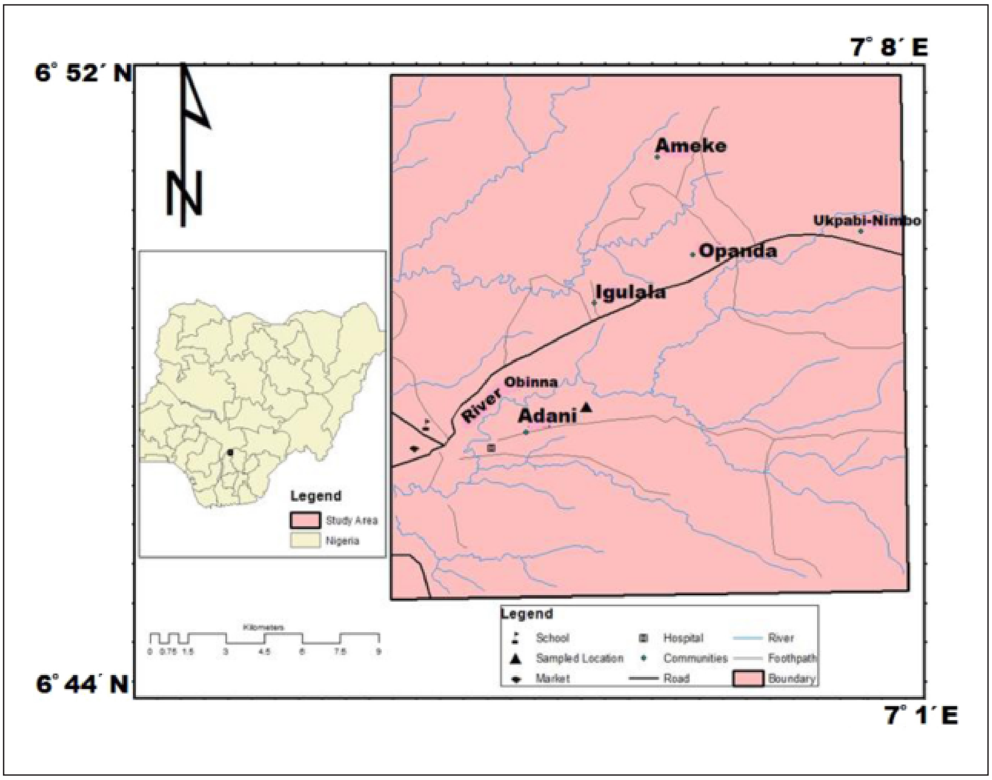

Figure 1. Map of Adani and its environs.

packs to stop further development of microfilariae in the flies, if present, and then transported to the Entomology Laboratory, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, for dissection.

Dissection of the blackflies

The flies captured were inactivated by refrigerating them at 4°C and then dissected fresh to determine their physiological age (parity) (Atting *et al.*, 2005). Before dissection for parity, each fly was placed on a glass slide in normal saline solution (Ibo & Braide, 1987; Renz & Wenk, 1987; Mafuyai *et al.*, 1997; Okon *et al.*, 2002). The flies were dissected in the laboratory using a stereo dissecting microscope. The ovaries of the dissected flies were stretched and classified as parous or nulliparous after observing the stretching characteristic and other characters, such as absence or presence of fat bodies and the colour of Malpighian tubules. Flies were recorded as nulliparous if they had tightly coiled tracheal systems and absence of follicular relics (corpora lutea). Nulliparous flies do not transmit *O. volvulus* and, hence, were discarded. On the other hand, flies were identified as parous, indicating that they had blood-fed and completed at least one gonotrophic cycle (Cupp & Collins, 1979; Morkry, 1980; Hoc & Wilkes, 1995). The parous flies were further dissected to search for larvae of *O. volvulus*. In the second stage of dissection, the parous flies were divided into head, thorax and abdomen. Each division was dissected by teasing it apart in a normal saline solution, using dissecting needles and stereo dissecting microscope, and searched for *O. volvulus* larvae (Nelson & Pester, 1962; Opoku, 2000; Opara *et al.*, 2008).

Data analysis

Monthly population density: The monthly population density, also called the biting density of flies (Opara *et al.*, 2005), was calculated with the fly per man hour (FMH) using the formula:

$$\text{FMH} = \frac{\text{Number of flies caught in a month}}{\text{Number of catching days} \times \text{time in hours}}$$

(Crosskey, 1958; Davies *et al.*, 1962; Gemade & Dipeolu, 1983).

Daily biting rate (DBR)
The Daily Biting Rate (DBR) was calculated by using the formula:

$$DBR = \frac{\text{Total number of flies caught in a month}}{\text{Number of catching days}}$$

The Daily Parous Biting Rate (DPBR) was calculated by using the formula:

$$DPBR = \frac{\text{Total number of parous flies caught in a month}}{\text{Number of catching days}}$$

They are expressed as flies per person per day.

Monthly biting rate (MBR)
The monthly biting rate (MBR_1) was computed by using the formula:

$$MBR_1 = \frac{\text{Number of flies caught in a month} \times \text{number of days in the month}}{\text{Number of catching days}}$$

(Walsh *et al.*, 1978; Grillet *et al.*, 2001; Py-Daniel & Medeiros, 2004).

The Monthly Parous Biting Rate (MPBR) was calculated using the formula:

$$MBR_2 = \frac{\text{Number of parous flies caught} \times \text{number of days in the month}}{\text{Number of catching days}}$$

(Py-Daniel & Medeiros, 2004). They are expressed as flies/person/month.

Monthly transmission potential (MTP)
This index estimates the number of L3 (infective stage) that can be transmitted to a person exposed to the vector during a one-month period (Duke, 1968; Py-Daniel & Medeiros, 2004). It is given by the formula:

$$MTP = \frac{\text{Number of days in the month} \times \text{number of infective larvae}}{\text{Number of days worked}} \; x \; \frac{\text{No. of flies caught}}{\text{No. of flies dissected}}$$

$$= \frac{MBR_1 \times \text{Number of L3 stage larvae by blackflies}}{\text{Total number of blackflies dissected}}$$

(Duke, 1968; Walsh *et al.*, 1978; Opoku, 2000; Py-Daniel & Medeiros, 2004). It is expressed as L3/person/month.

Proportion of parous flies
This formula represents the percentage of parous flies among the total dissected. It is given by the formula:

$$\frac{\text{Number of parous flies}}{\text{Total number of flies dissected}} \times 100$$

Statistical analysis
The data collected were subjected to one-way analysis of variance (ANOVA) using SPSS version 21.0 (SPSS INC. Chicago, IL. USA). Fisher's Least Significant Difference (FLSD) test was used to compare the biting peaks for the different months and also to establish if there were significant differences among the transmission indices for the different months. A paired t-test was used to compare the parity rates within and between the months. The analysis was set at P = 0.05.

RESULTS

Diurnal biting patterns of *Simulium damnosum* complex at Adani
Two peaks of diurnal biting were observed for *S. damnosum* complex at Adani. The peaks occurred in the morning from 7.00 am to 10.00 am and in the evening from 4.00 pm to 6.00 pm (Figs. 2 and 3). The evening peaks were more pronounced than the morning peak, with the exception of October when the morning peak was higher than the evening peak. There was no significant

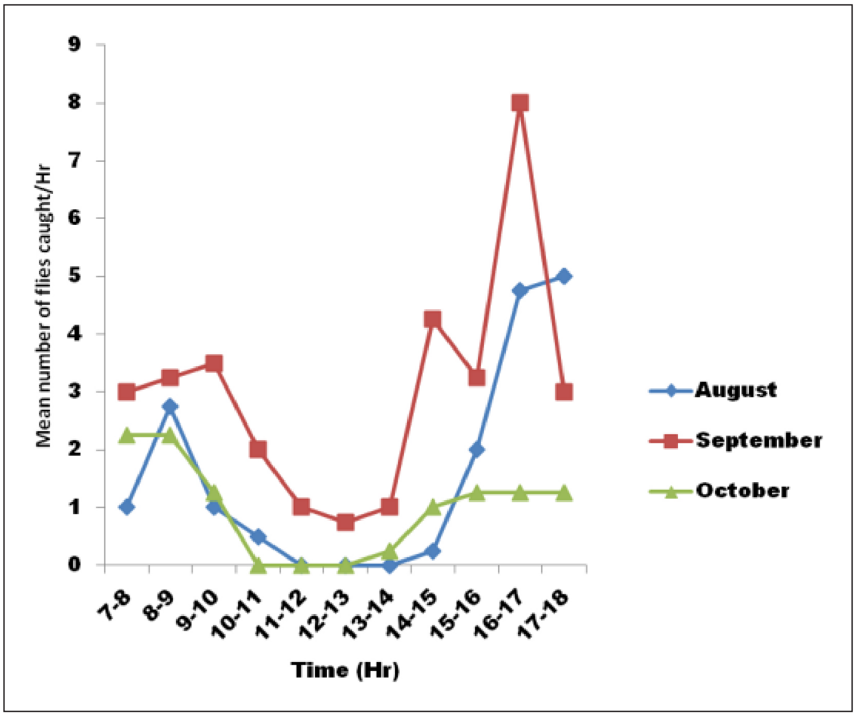

Figure 2. Diurnal biting patterns of *S. damnosum* at Adani from August to October 2010.

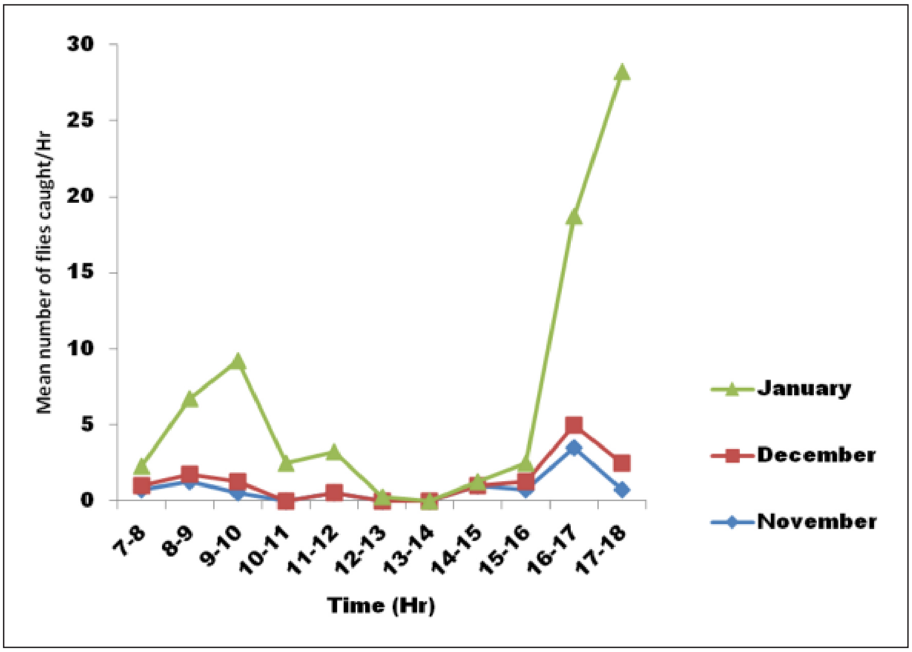

Figure 3. Diurnal biting patterns of *S. damnosum* at Adani from November 2010 to January 2011.

difference (P > 0.05) in mean morning and evening biting peaks in all the months.

Transmission indices of onchocerciasis at Adani

The Daily Biting Rate (DBR) varied from 5.3 flies per man per day in December to 60.8 flies per man per day in January. The Monthly Biting Rate (MBR) was highest in January with 1883.3 flies per man per month, whereas the least MBR was recorded in December with 162.8 bites per man per month. Parous flies accounted for 24.3% (133 flies), whereas the nulliparous flies constituted 75.7% (415) of the total catch during the study period. The infection and infectivity rates during the study were zero (Table 1). The biting/population densities, biting rates, infection and infectivity rate, proportion of parous flies and transmission potential of *O. volvulus* by *S. damnosum* during the study are shown in Table 1. Flies caught in August and September were all nulliparous. A paired t-test of the parity rates showed that there were significantly more parous flies than nulliparous flies in October and November (P < 0.05) but no significant differences in December and January (P > 0.05). None of the flies was infected with *O. volvulus* larvae; hence, transmission of *O. volvulus* at Adani during the study period was zero.

DISCUSSION

The diurnal biting patterns of *S. damnosum* complex at Adani showed a bimodal peak of activity – a morning peak and an evening peak in all the months. These findings are in consonance with those of Opoku (2000), Ubachukwu & Anya (2001), Opara *et al.* (2005) and Opara *et al.* (2008) but contradict those of Adeleke *et al.* (2010), who observed three biting peaks at two different sampling points in southwestern Nigeria, and Barbiero & Trpis (1984) who observed a unimodal biting peak activity pattern in Liberia. The cause of biting activity peaks is still poorly understood, but it has been suggested that an innate clock rhythm may be involved (Crosskey, 1990). Blackflies do not suck blood daily; hence, the biting cycle may be described as a circadian rhythm, which entails a biological rhythm on a one-day periodicity (Lincoln *et al.*, 1982). Diurnal variations in the biting density have been related to variations in the temperature (Leberre, 1966) and humidity (Hausermann, 1969) or to the intensity of light (Kaneko *et al.*, 1973). The variation in the biting activity of the flies from morning to evening delimits the hours of maximum or minimum danger of transmission of onchocerciasis (Renz, 1987) since the flies are more active in the morning from 7.00 am to 10.00 am, and the evening from 4.00 pm to 6.00 pm. These times also correspond to peak human outdoor activities. However, Nwoke, (1988), Porter & Collins (1988), Adewale *et al.* (1999) and Ubachukwu & Anya (2001) reported that the biting activities of *S. damnosum s. l.* are greatly influenced by illumination and temperature. The diurnal biting cycle of the flies may have epidemiologic implications since their biting peaks correspond to periods of peak human outdoor activities.

The biting density of flies at Adani ranged from 0.5 FMH in December to 5.5 FMH in January. The daily biting rate ranged from 5.3flies/man/day in December to 60.8flies/man/day in January. The monthly biting rate of the flies ranged from 162.8flies/man/month in December to 1883.3flies/man/month in January. The biting density of flies recorded at Adani is far below the maximum range (Crosskey, 1990).

Flies caught during the dry season had higher parous rate than those caught in the wet season. This is in agreement with Opara *et al.* (2005), Opara *et al.* (2008) and Adeleke *et al.* (2010) who recorded greater percentages of parous flies in the dry season. The high percentage of parous flies in the dry season indicates that the risk of getting infected with *O. volvulus* is higher in the dry season because only parous flies transmit *O. volvulus*. The result of the dissection showed that the majority of the flies caught were nulliparous and they accounted for 75.7% of the total. This observation is consistent with the findings of Mafiana (1988), and Adeleke *et al.* (2010) who recorded 53.9%, 57.86% and 59.58% nulliparous flies

Table 1. Population density and transmission indices of *S. damnosum* complex at Adani, Uzo-Uwani Local Government Area of Enugu State

	August 2010	September 2010	October 2010	November 2010	December 2010	January 2011
Person days worked	4	4	4	4	4	4
Total number of flies caught and dissected	69	136	43	36	21	243
No (%) of parous flies	0 (0)	0 (0)	13 (30.2)	9 (25)	10 (47.6)	101 (41.6)
No (%) of nulliparous flies	69 (100)	136 (100)	30 (69.8)	27 (75)	11 (52.4)	142 (58.4)
Total No (%) of flies infected with *O. volvulus*	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Flies (%) with L1 and L2 of *O. volvulus*	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Flies (%) with L3 of *O. volvulus*	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Biting density of flies	1.6	3.1	1.0	0.8	0.5	5.5
Daily Biting Rate (DBR)	17.3	34	10.8	9	5.3	60.8
Daily Parous Biting Rate (DPBR)	0	0	3.3	2.3	2.5	25.3
Monthly Biting Rate (MBR1)	534.8	1020	333.3	270	162.8	1883.3
Monthly Parous Biting Rate (MBR2)	0	0	100.8	67.5	77.5	1100.5
Monthly Transmission Potential (MTP)	0	0	0	0	0	0

in three communities along Osun River in southwestern Nigeria, and Atting *et al.* (2005) who recorded 96.83% nulliparous flies in a forest area of Cross River State, Nigeria, but contrary to the reports of Usip *et al.* (1999) and Okolo *et al.* (2004). The high proportion of nulliparous flies at Adani may be indicative of local production of flies at the Obinna River.

The six months of study did not reveal any *O. volvulus* infection in the flies dissected. Hence, the infectivity rates and transmission of *O. volvulus* parasites at Adani was zero during the study period. This finding is in consonance with that of Adeleke *et al.* (2010) but contradicts that of Katabarwa *et al.* (2013) who conducted parasitological and entomological profiles and revealed that annual mass distribution of ivermectin for 15 years has not interrupted transmission of *O. volvulus* in parts of Cameroon. This sort of observation at Adani, according to Remme *et al.* (1990), is expected in areas where the distribution of ivermectin is being carried out. Recent reports from endemic communities in Mali and Senegal have indicated the possibility of elimination of onchocerciasis after several treatments with ivermectin (Diawara *et al.*, 2009). In Cameroon, continuous distribution of ivermectin for 6 years significantly reduced onchocerciasis from hyper-endemicity to hypo-endemicity (Kamga *et al.*, 2011). Ivermectin treatment has been very successful in eliminating onchocerciasis as a public health problem. At Adani, there have been many years (1996 – present) of uninterrupted mass distribution of ivermectin. Thus, transmission must have been interrupted in the area, which could be the reason for the absence of *O. volvulus* in the flies dissected. The absence of microfilaria in parous flies dissected at Adani could be a result of the mass distribution of ivermectin in the area since 1996.

In addition, the relatively high number of nulliparous flies among the flies dissected in the present study could also be a contributory factor because only aged flies have chances of transmitting the parasite. Thus, now the flies at Adani are more of a biting nuisance than disease vectors. During the study, actual man-fly contact was very low. This is because the flies have a patchy distribution at Adani, being encountered only in some farms and near farmlands that are far from dwellings. In addition, most households do not go to the river to fetch water because they have alternative water sources (wells), but a few who live near the river do otherwise. However, cattle graze in the area and also go to the river to drink water. It might also be that the flies feed more on the cattle, which they encounter in the forest and near the river, than on humans in the area.

CONCLUSIONS

Although old infections (individuals harbouring adult *O. volvulus*) might still be found at Adani, the transmission appears to have been temporarily halted by ivermectin distribution, and the flies are mere nuisance biters. Hence, no new infection has been recorded at Adani at present. However, a more extensive sampling, spanning a whole year and including other communities around Adani in Uzo Uwani LGA, are recommended to confirm that mass distribution of ivermectin has halted the transmission of *O. volvulus* at Adani.

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