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Comparative Studies on Three Anthropophilic Blackfly Species in Ecuador as the Vector of Human Onchocerciasis[1)]

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Abstract

In the Rio Cayapas (a hyperendemic focus of human onchocerciasis) and the Rio Santiago (one of hypoendemic foci) in Esmeraldas Province, Ecuador, the role of the 3 anthropophilic blackfly species as the vector of *Onchocerca volvulus* was examined by experimental and natural infection studies. In the hyperendemic focus, *Simulium exiguum* yielded a high rate of experimental infection (23.7% and 41.2% of flies fed on 2 infected persons with a microfilarial density (MfD) of 5 and 50, respectively, harboured third-stage larvae), and was shown to be the most dominant species with a natural infection rate of 8.2% for any stages of larvae. On the other hand, *S. quadrivittatum* fed on the same persons, showed a low experimental infection rate (3.5% and 0%), and no natural infection was found. *Simulium pseudoantillarum* was shown to be susceptible to experimental infection with *O. volvulus*, but was the least abundant species and had no natural infections. In the Rio Santiago, 27.3% of *S. exiguum* fed on an infected person with an MfD of 128 supported the larval development of *O. volvulus*, and its natural infection rate was 5.6%, although this species was far fewer in biting abundance than *S. quadrivittatum*. The experimental infection rate of *S. quadrivittatum* which fed on infected persons with an MfD of 5, 44 and 128, respectively, was very low (0%–1.1%), and no natural infection was found. *Simulium pseudoantillarum* was shown to be naturally infected with first-stage larvae indistinguishable from *O. volvulus*. However, this was a very rare species. Overall, it is suggested that *S. exiguum* is the main or the only important vector of *O. volvulus* in both the hyper- and hypoendemic foci of Ecuador.

Key words: *Onchocerca volvulus*, onchocerciasis, *Simulium*, transmission, vector, Ecuador

Introduction

The first focus of human onchocerciasis in Ecuador was reported on the upper region of the Rio Cayapas, in Esmeraldas Province (Arzube, 1982; Guderian *et al.*, 1982). Soon later, 10 other foci scattered in different river systems of the same province were discovered (Guderian *et al.*, 1983a). According to Guderian *et al.* (1983a, b), the localities on the middle

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and upper regions of the Rio Cayapas (ca. 100–270 km upriver) are hyperendemic (prevalence 51–85%, intensity 26.7 mf/mg skin), and all other foci are hypoendemic (prevalence 1–33%, intensity 10.9 mf/mg). Extensive investigations on clinical and epidemiological features of the disease have been carried out in these foci (Guderian *et al.*, 1983a, b, and 1984; Molea *et al.*, 1984).

In relation to the disease transmission, Shelley and Arzube (1985) demonstrated *Simulium exiguum* and *S. quadrivittatum* as the chief and secondary vectors of *Onchocerca volvulus*, respectively, in the hyperendemic focus along the Rio Cayapas. However, a detailed assessment of the vectorial capability of these 2 blackfly species was hampered by the high early mortality of flies in their experimental infection studies. Moreover, no works have been done as to vector incrimination in other hypoendemic foci.

We conducted natural and experimental infection studies on anthropophilic blackflies to clarify their roles in onchocerciasis transmission in both the hyper- and hypoendemic foci.

Materials and Methods

The experimental and natural infection studies were made in July and August of 1986 at 3 localities (Zapallo Grande, San Miguel, Agua Blanca; 100–140 km upriver) in the middle and upper regions of the Rio Cayapas where the disease was hyperendemic, and at 4 localities (Chanuscal, Palma Real, Angostura, San Juan) situated 70–150 km upriver in the Rio Santiago, one of the hypoendemic foci. General information on these localities, their infection rates and intensities has already been given by Guderian *et al.* (1983a).

Wild female blackflies were allowed to feed to repletion on the calves of the following 5 persons naturally infected with *O. volvulus*;

Volunteer A, a 20 year old, black male, MfD 5 (number of microfilariae in a single skin snip on the calf region taken by Holth-type corneoscleral punch).

Volunteer B, a 50 year old, black male, MfD 50.

Volunteer C, a 32 year old, black male, MfD 5.

Volunteer D, a 40 year old, black female, MfD 44.

Volunteer E, a 22 year old, black male, MfD 128.

The volunteers A and B were inhabitants of Zapallo Grande, and C, D and E were of Angostura.

Flies for experimental infection studies were collected from the 2 volunteers along the Rio Cayapas, and from the 3 along the Rio Santiago. A proportion of engorged flies were killed immediately and preserved in 80% ethanol to assess microfilarial intake. All other engorged flies captured were maintained in a polypropylene tube, under a temperature varying between 25°C and 30°C. The methods of fly maintenance followed those of Takaoka *et al.* (1982) with a slight modification of the diet of a 35%-sucrose solution provided at 24-hr intervals.

During the period of post-infection days (PID) 0–10, all the flies were checked every day for survival data, and dead flies were removed and preserved in 80% ethanol. On PID 10, all live flies were killed and preserved in 80% ethanol. Dissection of preserved flies for microfilarial intake was done using the technique of Nakamura (1964). All preserved flies were divided into head, thorax and abdomen and dissected in a drop of Giemsa solution on a glass slide under a binocular microscope. The number of larvae in each part of the body was counted, and their stages of development were determined by the size and morphological features, as defined by Duke (1968).

The vector efficiency of each blackfly species was assessed in terms of the proportion of flies positive for third-stage larva (L_3) among flies fed on an infected person, which were expressed as the product of the survival rate of flies until PID 6 and the rate of surviving flies with L_3 on PID 6–10.

Additional collections of unfed wild female flies on men were made between 8:00 and

14:00 at 7 localities mentioned above. Female flies landing on the body surface were collected by suction tube and preserved in 80% ethanol. Natural infection rate of each blackfly species with *O. volvulus* was obtained by dissecting these preserved flies in a similar manner, as mentioned above.

Simuliid species captured were identified by the keys of Ramírez-Pérez (1983) and Coscarón (1984).

Results

Anthropophilic blackfly species and their relative abundance

Three simuliid species (*S. exiguum, S. quadrivittatum* and *S. pseudoantillarum*) were captured in both the hyper- and hypoendemic foci during our survey period. The majority of the man-biting flies were composed of either *S. exiguum* or *S. quadrivittatum* depending on the localities (Table 1). At San Miguel and Agua Blanca, areas in the upper region of the Rio Cayapas, the predominant species was *S. exiguum,* while at Zapallo Grande, an area in the middle of the Rio Cayapas, and at all 4 localities on the Rio Santiago, *S. quadrivittatum* was most predominant.

Natural infection rate

Natural infection with larvae indistinguishable from *O. volvulus* was found in *S. exiguum* at all the 3 localities along the Rio Cayapas and at Palma Real along the Rio Santiago. Natural infection was also found in *S. pseudoantillarum* at Angostura along the Rio Santiago (Table 2). The larvae found were all in the first stage (L_1). The number of larvae per fly was 1 in 6 *S. exiguum,* 2 in 1 *S. exiguum* and 1 *S. pseudoantillarum,* and 3 in 1 *S exiguum.* None of the 70 and 204 *S. quadrivittatum* captured in the Rios Cayapas and Santiago, respectively, was found to be infected.

Microfilarial intake

The proportion of flies ingesting *O. volvulus* microfilariae and the mean number of ingested microfilariae per fly are shown in Table 3. In both *S. exiguum* and *S. quadrivittatum,* a higher proportion of flies was found to have ingested microfilariae and each had a greater microfilariae density when fed on the persons with a higher MfD. No microfilarial intake was observed in flies when fed on volunteer C. Most microfilariae ingested by *S. quadrivittatum* were injured while those ingested by *S. exiguum* were intact. Unfortunately, no comparison could be made between the 2 species due to the insufficient numbers of one of these species in the same river system. The small number of flies captured did not allow an assessment of the microfilarial intake by *S. pseudoantillarum.*

Table 1. Anthropophilic blackfly species and their relative abundance at different localities along the 2 rivers in Esmeraldas Province, Ecuador*

Localities (date)	No. flies collected	% composition by species		
		S. exiguum	*S. quadrivittatum*	*S. pseudoantillarum*
Rio Cayapas				
Zapallo Grande (Jul. 13)	126	18.2	79.4	2.4
San Miguel (Jul. 14)	84	83.3	7.2	9.5
Agua Blanca (Jul. 15)	121	92.6	4.9	2.5
Rio Santiago				
Chanuscal (Aug. 6)	32	6.2	90.7	3.1
Palma Real (Jul. 9)	138	8.0	92.0	0.0
Angostura (Jul. 8)	96	18.8	77.1	4.1
San Juan (Jul. 7)	64	17.2	82.8	0.0

*The collections were made between 8.00 and 14.00 hr, using 1–2 volunteers.

Table 2. Natural infections of the 3 blackfly species with larvae indistinguishable from *Onchocerca volvulus* at different localities along the 2 rivers in Esmeraldas Province, Ecuador

Localities	*S. exiguum* No. pos./No. exam. (%)	*S. quadrivittatum* No. pos./No. exam. (%)	*S. pseudoantillarum* No. pos./No. exam. (%)
Rio Cayapas			
Zapallo Grande	2/12 (16.7)	0/ 60 (0)	— (—)
San Miguel	2/44 (4.6)	0/ 6 (0)	0/7 (0)
Agua Blanca	2/17 (11.8)	0/ 4 (0)	— (—)
Subtotal	6/73 (8.2)	0/ 70 (0)	0/7 (0)
Rio Santiago			
Chanuscal	0/ 2 (0)	0/ 29 (0)	0/1 (0)
Palma Real	2/12 (16.7)	0/118 (0)	— (—)
Angostura	0/18 (0)	0/ 54 (0)	1/4 (25)
San Juan	0/ 4 (0)	0/ 3 (0)	— (—)
Subtotal	2/36 (5.6)	0/204 (0)	1/5 (20)

Table 3. Number and percentage of *Simulium exiguum* and *S. quadrivittatum* ingesting *Onchocerca volvulus* microfilariae from calf region of infected volunteers

Volunteers	Skin MfD	*S. exiguum* No. flies examined	*S. exiguum* No. (%) flies positive	*S. exiguum* No. Mf* Mean (Range)	*S. quadrivittatum* No. flies examined	*S. quadrivittatum* No. (%) flies positive	*S. quadrivittatum* No. Mf* Mean (Range)
Rio Cayapas							
A	5	7	2(29)	4(1- 6)	—	—(—)	—(—)
B	50	8	6(75)	12(1-23)	—	—(—)	—(—)
Rio Santiago							
C	5	1	0(0)	—(—)	14	0(0)	—(—)
D	44	2	1(50)	1(1)	13	3(23)	1(1)
E	128	—	—(—)	—(—)	16	7(44)	3(1-7)

*No. of microfilariae per positive fly

Development of O. volvulus *microfilariae to the third-stage in flies experimentally infected*

In both the hyper- and hypoendemic foci, data for each blackfly species from each volunteer at one locality were limited due to the small number of flies captured at each locality and then pooled together.

The L_3 was first seen in *S. exiguum* on PID 6, while the development to L_3 was almost synchronous, as shown in Table 4. The infection rate of *S. exiguum* with L_3, on PID 6–10, was medium or high when fed on volunteers A, B and E. However, 7 *S. exiguum*, which fed on the volunteer C, were all negative for any stage of larvae (Table 6).

The number of L_3 per positive fly was 1–7 with an average of 3 in the fly group fed on the volunteer A, while that was 1–12 with an average of 4 in the fly group fed on the volunteer B. Three positive flies fed on the volunteer E harboured 1–5 L_3 with an average of 3 L_3. The proportion of flies harbouring L_3 in the head was 29% (2/7) (volunteer A), 62% (16/26) (B) and 0% (0/3) (E), while the proportion of L_3 reaching the cephalic region was 24% (5/21) (A), 41% (45/109) (B) and 0% (0/10) (E). The

Table 4. Development of *Onchocerca volvulus* larvae in *Simulium exiguum* fed on infected volunteer B and kept at a temperature varying between 25°C and 30°C

Days post-infection	No. flies examined	No. flies with larvae	Total no. larvae	No. larvae/positive fly	No. (%) stage of larvae*			
				Mean (Range)	Mf.	L_1	L_2	L_3
0	13	11	253	23(1-56)	232(92)	10(4)†	12(5)†	0(—)
1	4	3	62	21(11-29)	60(97)	2(3)†	0(—)	0(—)
2	3	1	2	2(2)	1(50)	1(50)	0(—)	0(—)
3	3	2	5	3(1-4)	1(25)	4(75)	0(—)	0(—)
4	3	2	8	4(4)	0(—)	6(75)	2(25)	0(—)
5	4	3	12	4(1-10)	0(—)	10(83)	2(17)	0(—)
6	5	3	18	6(2-10)	0(—)	0(—)	3(17)	15(83)
7	6	4	14	4(1-5)	0(—)	1(7)	0(—)	13(93)
8	9	9	27	3(1-8)	0(—)	2(7)	1(4)	24(89)
9	12	10	61	6(1-12)	0(—)	0(—)	9(15)	52(85)
10	4	3	6	2(1-4)	0(—)	1(17)	0(—)	5(83)
Total‡	36	26	109	4(1-12)				

*Mf. = microfilarial stage, L_1 = first stage, L_2 = second stage, L_3 = third stage
†Probably originated from previous natural infection
‡Total only for L_3 during days 6–10

L_3 recovered from this species, which fed on volunteers A and B, averaged 489.7 μm (356.0–583.0) in length by 19.4 μm (17.3–23.0) in width (n=34).

On the contrary, development of *O. volvulus* microfilariae to the L_3 in *S. quadrivittatum* was not found except in 2 flies which fed on volunteers A and D respectively and died on PID 8 (Table 5). Each of these 2 flies harboured 1 L_3 in the head and thorax region. The body length and width of 1 L_3 found in the fly fed on the volunteer D were 620 μm and 19.7 μm. No other stages of larvae were recovered in any of other flies dissected on PID 1–10 (Table 5).

The 2 *S. pseudoantillarum*, which fed on the volunteer B, died on PID 8 and 10. Only 1 contained an L_3 in the abdomen, which measured 428.3 μm long by 18.6 μm wide.

Survival rate of flies

None of the 33, 7 and 11 *S. exiguum*, which fed on volunteers A, C and E, respectively, died within 24 hours of ingestion of infected blood meal. However, 17 (24.3%) of 70 *S. exiguum* fed on the volunteer B died within 24 hours. Thirteen of these died soon after blood ingestion, and dissection showed that 11 of these 13 dead flies harboured 1–56 microfilariae with a mean of 23 (Table 4). On the other hand, none of the 276 *S. quadrivittatum* and 2 *S. pseudoantillarum* died within 24 hours of blood ingestion.

The survival rate of all the 3 blackfly species until PID 6, when L_3 was first found, was medium or high (Table 6).

Vector efficiency

Table 6 shows the vector efficiency of these 3 blackfly species in relation to the MfD. There was a marked difference in vector efficiency between blackfly species. In the Rio Cayapas, the vector efficiency of *S. exiguum* was 23.7% and 41.2% when fed on volunteers A and B, respectively. On the other hand, the corresponding value of *S. quadrivittatum* was 3.5% and 0%, respectively.

In the Rio Santiago, the vector efficiency of *S. exiguum* was 27.3% when fed on the volun-

Table 5. Development of *Onchocerca volvulus* larvae in *Simulium quadrivittatum* fed on infected volunteers (A–E) and kept at a temperature varying between 25° C and 30° C

Days post infection	No. flies examined	No. flies with larvae	Total no. larvae	No. larvae/ positive fly		No. (%) stage of larvae*			
				Mean	(Range)	Mf.	L_1	L_2	L_3
Volunteer A									
0- 5	10	0	—	—	(—)	—(—)	—(—)	—(—)	—(—)
6-10	18	1	1	1	(1)	0(0)	0(0)	0(0)	1(100)†
Volunteer B									
0- 5	11	0	—	—	(—)	—(—)	—(—)	—(—)	—(—)
6-10	16	0	—	—	(—)	—(—)	—(—)	—(—)	—(—)
Volunteer C									
0- 5	32	0	—	—	(—)	—(—)	—(—)	—(—)	—(—)
6-10	42	0	—	—	(—)	—(—)	—(—)	—(—)	—(—)
Volunteer D									
0- 5	29	0	—	—	(—)	—(—)	—(—)	—(—)	—(—)
6-10	63	1	1	1	(1)	0(0)	0(0)	0(0)	1(100)‡
Volunteer E									
0- 5	13	0	—	—	(—)	—(—)	—(—)	—(—)	—(—)
6-10	45	0	—	—	(—)	—(—)	—(—)	—(—)	—(—)

*Mf. = microfilarial stage, L_1 = first stage, L_2 = second stage, L_3 = third stage
†Found in the head on PID 8
‡Found in the thorax on PID 8

Table 6. Comparison of vector efficiencies of the 3 anthropophilic blackfly species, in terms of the proportion of flies in which third-stage larvae (L_3) of *Onchocerca volvulus* were developed, among flies experimentally fed on infected persons

Volunteers (MfD)	*S. exiguum*				*S. quadrivittatum*				*S. pseudoantillarum*			
	a	b	c	d	a	b	c	d	a	b	c	d
Rio Cayapas												
A (5)	33	57.6	41.2	23.7	28	64.3	5.5	3.5	—	—	—	—
B (50)	70	57.1	72.2	41.2	27	59.2	0.0	0.0	2	100	50	50
Rio Santiago												
C (5)	7	85.7	0.0	0.0	76	69.1	0.0	0.0	—	—	—	—
D (44)	—	—	—	—	92	68.5	1.6	1.1	—	—	—	—
E (128)	11	90.9	30.0	27.3	61	77.0	0.0	0.0	—	—	—	—

a, No. flies fed on volunteer; b, proportion (%) of flies surviving until PID 6; c, proportion (%) of flies with L_3 among flies examined on PID 6–10; d, vector efficiency (%), calculated by b x c/100

teer E, and 0% when fed on volunteer C. On the contrary, the efficiency of *S. quadrivittatum* was very low (0–1.1%) irrespective of the volunteers.

Discussion

The present study shows that *S. exiguum* is a very efficient experimental vector in the hyperendemic focus, as shown by Shelley and Arzube (1985). It is noted that 57.1% of flies fed on volunteer B with an MfD of 50 could survive until PID 6, although 24.3% died within 24 hours post infection, probably due to the lethal effect of increased number of ingested microfilariae.

On the other hand, *S. quadrivittatum* was shown to be a very poor experimental vector so far as the 2 volunteers with an MfD of 5 and 50, respectively, were concerned. Only 1 (3.5%) of the 28 flies which fed on the volunteer A contained 1 L_3, and none of flies fed on volunteer B had L_3. Moreover, it is remarkable that all flies which died before PID 6 were negative for any stage of larvae. It is most probable that very few microfilariae moved from the stomach to the thorax region in *S. quadrivittatum.* This is supported by our observation that most of microfilariae ingested by this blackfly species were injured, probably due to the armed buccopharyngeal structure which is present in this species but is absent in *S. exiguum.* Similarly, Shelley and Arzube (1985) obtained a very low infection rate (1.4% with L_3) for *S. quadrivittatum* which fed on 2 infected persons, one of whom had 70 mf/mg of skin snip of calf region. It remains unknown whether the vector efficiency of this species is also low or becomes higher, when it feeds on persons with a much higher MfD. However, such cases may happen infrequently, because there are very few persons having high MfD (i.e., over 100 per mg of skin snip), according to Guderian *et al.* (1983b).

It is noteworthy that *S. pseudoantillarum* supports experimentally larval development of *O. volvulus* to L_3, although the number of flies examined was very small.

The relative abundance and natural infection data in the hyperendemic focus during the study period suggest that *S. exiguum* is the primary and the only important vector of *O. volvulus,* and *S. quadrivittatum* and *S. pseudoantillarum* are minor ones. However, the importance of *S. quadrivittatum* as the vector in nature remains to be studied, because 4 (2.2%) of 178 females captured in May and June at San Miguel contained *O. volvulus* larvae (Shelley and Arzube, 1985).

In the Rio Santiago, a hypoendemic focus, the results obtained for *S. exiguum* in the experimental infections were a little different from those obtained in the Rio Cayapas, although this species was proved to support the larval development of *O. volvulus* to L_3. It is, however, uncertain whether this difference indicates the low susceptibility of *S. exiguum* in the Rio Santiago, as compared to that in the Rio Cayapas. Future experimental studies using the same infection source may answer to this question.

Simulium quadrivittatum was shown to be a very poor experimental vector, as observed in the Rio Cayapas.

The data for natural infections, together with the results obtained in the experimental infection studies, suggest that *S. exiguum* plays a main role in the disease transmission in the Rio Santiago as well, although this is not a predominant species.

Guderian *et al.* (1983a, b) thought that the different frequency of the man-vector contact may explain the clear difference in the prevalence and intensity of the onchocerciasis infection between the hyper- and hypoendemic foci in the Esmeraldas Province. Our data seem to support their hypothesis, if *S. exiguum* is considered as the only vector species. Further investigation throughout the year may clarify this point, as well as the vector status of the 3 anthropophilic species.

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