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Experimental infections of three Guatemalan blackfly species with north Venezuelan *Onchocerca volvulus*[1)]

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Abstract: The larval development of the north Venezuelan *Onchocerca volvulus* in the 3 Guatemalan simuliid species, *i.e. Simulium callidum, S. ochraceum* and *S. haematopotum* was experimentally studied, as compared with that in *S. metallicum,* the natural vector in the northern Venezuela. All these 3 species ingested as many microfilariae as did the Venezuelan *S. metallicum,* while feeding on the infected volunteer. However, most microfilariae ingested by *S. ochraceum* and *S. haematopotum* were found damaged probably due to the buccopharyngeal armature. The subsequent migration of the microfilariae occurred in 20% *S. callidum* but very rarely in the other 2 species, while it took place in 76% *S. metallicum.* At 22°C, third-stage larvae were found in 8% *S. callidum* and 3% *S. haematopotum,* but not in all the 51 *S. ochraceum* examined 9-14 days post-feeding. In contrast, they were found in 16 or 29% of *S. metallicum* from Venezuela. Overall, it was indicated that beside *S. metallicum,* the 2 other Guatemalan blackfly species, *S. callidum* and *S. haematopotum,* can, despite the low rates, support larval development of the north Venezuelan *O. volvulus* to the third stage. Our data do not seem to support the assumption that there is mutual incompatibility of *O. volvulus* to the vectors between Guatemala and north Venezuela.

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Duke (1970) pointed out the remoteness of *Onchocerca volvulus* strains between Latin America (both Guatemala and Venezuela) and West Africa. This was based on the results of cross transmission experiments, *i.e.* no or less compatibility of *O. volvulus* strains from West Africa was seen to Guatemalan or north Venezuelan vector blackflies, and *vice versa* (De Leon and Duke, 1966; Duke *et al.*, 1966; Duke, 1970). However, there is no such a cross transmission experiment to study the interrelation of the parasite strains between individual endemic regions in the American continents, which are now classified into 4 major endemic foci (1 in Central America and the other 3 in South America) depending on the clinical pictures, the geographical distribution and the vector simuliid species (Takaoka *et al.*, 1986).

We made a comparative study on the *O. volvulus* strains between Guatemala and northern Venezuela, by means of the cross infection experiments using *Simulium metallicum* as the vector. And, it was indicated that both the Guatemalan and north Venezuelan *O. volvulus* were equally compatible to *S. metallicum* at any combinations (domestic or cross), and thus these 2 strains of the same parasite were at least very close to each other, despite the difference in the clinical pictures of the disease between the 2 countries (Takaoka *et al.*, 1986).

Consequently, we performed experimental infections of 3 Guatemalan blackfly species other than *S. metallicum,* with the north Venezuelan *O. volvulus* larvae. This paper reports on the intake, migration and development of the north Venezuelan *O. volvulus* microfilariae in the 3 Guatemalan blackflies, *S. callidum, S. haematopotum* and *S. ochraceum.*

Materials and Methods

The carrier of the north Venezuelan *O. volvulus,* who took part in this study, was a 36-year-old man, a native villager of Río Chiquito, in the Municipality of Guanaguana, Monagas State. He had never been abroad. The number of microfilariae in the volunteer's iliac and calf skin regions were 39 and 13 per snip taken with a corneoscleral punch (Holth type), respectively.

The experimental infections of 3 Guatemalan blackfly species were carried out in July 1984 at the following localities: for *S. callidum* at a coffee plantation "Ceilan"; for *S. ochraceum* at "Los Andes," both endemic foci in the Municipality of Pochuta; for *S. haematopotum* at "Ruina de Mixco Viejo," outside of the onchocerciasis areas, all the 3 localities in Department of Chimaltenango.

Wild females of these 3 simuliid species were allowed to feed to repletion on legs and/or back of the volunteer. All the blood-fed flies were captured and maintained individually in a polypropylene tube, using the method of Takaoka *et al.* (1982).

The first group of flies were dissected immediately after feeding and the second group, 24 hr post-feeding, in order to assess both the microfilarial intake and the larval migration to the fly's thorax. All the other flies were kept at a constant temperature of 22°C and were checked daily for their mortality. During the period between the 9th and 16th days post-feeding, all the live flies were dissected in 0.9% saline solution under a dissecting microscope. The number of third-stage larvae in the head, thorax and abdomen was counted.

The control experiment with *S. metallicum,* the principal vector in the northern Venezuela, was performed in August 1984 at Río Chiquito, an endemic focus and home village of our volunteer, based on the above mentioned manner, with minor difference in the incubation temperature (20–24°C).

Results

All the 3 Guatemalan simuliid species examined ingested as many microfilariae of north Venezuelan *O. volvulus* as did the Venezuelan *S. metallicum,* when they were fed on the volunteer's legs (Table 1). Also, microfilarial intake from skins of his back by *S. ochraceum* and *S. haematopotum* was almost the same, in terms of the fly's positive rate and the mean number of microfilariae ingested, as that by *S. metallicum,* except that the mean intake of *S. ochraceum* was almost one-fourth as low as that of *S. metallicum.* Most microfilariae ingested by *S.*

Table 1 Intake of north Venezuelan *O. volvulus* microfilariae by three Guatemalan blackfly species.

Blackfly species	Body area bitten	No. flies dissected	No. (%) flies positive	No. Mf./fly	
				Mean	(Range)
S. callidum	Legs	7	6 (86)	8	(4- 14)
S. ochraceum	Legs	15	13 (87)	10	(1- 30)
	Back	21	21 (100)	13	(1- 79)
S. haematopotum	Legs	20	17 (85)	11	(1- 49)
	Back	14	14 (100)	45	(1-160)
S. metallicum (Venezuela)	Legs	16	13 (81)	9	(1- 25)
	Back	20	20 (100)	48	(7-129)

Table 2 Thoracic movement of north Venezuelan *O. volvulus* microfilariae in three Guatemalan blackfly species 24 hr after ingestion of blood meal.

Blackfly species	Body area bitten	No. flies dissected	No. (%) flies positive	No. Mf./fly	
				Mean	(Range)
S. callidum	Legs	10	2 (20)	1	(1)
S. ochraceum	Legs	14	1 (7)	1	(1)
	Back	15	0 (0)	—	(—)
S. haematopotum	Legs	15	0 (0)	—	(—)
	Back	15	1 (7)	1	(1)
S. metallicum (Venezuela)	Legs	19	12 (63)	5	(1-16)
	Back	10	10 (100)	14	(2-30)

Table 3 Development of north Venezuelan *O. volvulus* microfilariae to the third stage in three Guatemalan blackfly species, 9-16 days after ingestion of infected blood meal.

Blackfly species	Body area bitten	No. flies dissected	No. (%) flies positive	No. L_3/fly	
				Mean	(Range)
S. callidum	Legs	53	4 (8)	3	(1-7)
S. ochraceum	Back	51	0 (0)	—	(—)
S. haematopotum	Legs	93	0 (0)	—	(—)
	Back	61	2 (3)	1	(1)
S. metallicum (Venezuela)	Legs	45	7 (16)	3	(1-5)
	Back	7	2 (29)	3	(2-4)

ochraceum and *S. haematopotum* were cut off and very few were intact and alive.

The migration of microfilariae to the thorax during the first 24 hr of feeding occurred very rarely in all 3 Guatemalan blackfly species, as shown in Table 2. Only 1 microfilaria each was found in the thorax of 2 *S. callidum*, 1 *S. ochraceum* and 1 *S. haematopotum*. This was in marked contrast to the results obtained in *S. metallicum*, in which 1-30 microfilariae per fly were recognized in 22 of 29 flies (Table 2).

The results of dissection of the flies surviving through 9 to 16 days post-feeding are shown in Table 3. Four (8%) of 53 *S. callidum* which fed on the lower extremities harboured a mean of 3 third-stage larvae, while 2 (3%) of 61 *S. haematopotum* (fed on back) had 1 larva of third stage. However, no third-stage larvae were found

in 51 *S. ochraceum* and 93 *S. haematopotum* which fed on the back and legs, respectively. On the other hand, 7 (16%) and 2 (29%) *S. metallicum* which fed on legs and back, respectively had a mean of 3 third-stage larvae.

All the 11 third-stage larvae found in *S. callidum* were mobile, and their lengths were 500–650 μm (mean 549 μm). Among these, 2 larvae each were recovered from the head and abdomen, while others were from the thoracic region. In *S. haematopotum,* only 2 third-stage larvae, each, were found from the head and thorax. These looked normal in appearance, but not measured. On the other hand, of the 25 third-stage larvae found in the Venezuelan *S. metallicum,* 12, 9 and 4 were recovered from the head, thorax and abdomen. The body lengths of these larvae were 380–650 μm (mean 517 μm).

Discussion

In the previous paper (Takaoka *et al.,* 1986), we reported for the first time that microfilariae of the north Venezuelan *O. volvulus* successfully developed to the third-stage larvae in *S. metallicum* from Guatemala. The present study also indicates the complete development of the same strain of the parasite to the third stage in the other 2 Guatemalan blackfly species, *i.e. S. callidum* and *S. haematopotum.* However, the proportions of the flies with third-stage larvae of the north Venezuelan *O. volvulus* were only 8% for *S. callidum* and 3% for *S. haematopotum.* These rates were half and one-tenth as low as the rates obtained in *S. metallicum* from Venezuela, respectively (Table 3). Further, no larval development was observed in *S. ochraceum.*

In the present study, a great difference was observed in the microfilarial migration to the thorax between the 3 Guatemalan species and the Venezuelan *S. metallicum,* although the ingestion of microfilariae was almost at the same level. It is likely that the absence or low rate of larval development in *S. ochraceum* and *S. haematopotum* is ascribed to the low rates of the thoracic migration of microfilariae, rather than to the lack of compatibility of the parasite to these 2 simuliids. In fact, most microfilariae of the north Venezuelan *O. volvulus* ingested by *S. ochraceum* and *S. haematopotum* were found injured, probably by the toothed buccopharyngeal armature, and very few of them could reach the thorax. Similar results were also reported with the same simuliid species which fed on the Guatemalan carrier with a relatively low density of skin microfilariae (Takaoka *et al.,* 1984). Therefore, substantial larval development in the thorax might have been achieved even in these 2 blackfly species, if the skin microfilarial density of the Venezuelan volunteer were high enough to allow the microfilariae to reach the developmental site. On the other hand, the relatively low migration rate observed with *S. callidum,* which lacks such a destructive armature, might have been caused by the less compatibility of the Venezuelan *O. volvulus* to this blackfly, but it is not conclusive due to the limited number of fly samples examined.

It is now clarified that the north Venezuelan *O. volvulus* is capable of developing to the third-stage larvae at least in 3 Guatemalan blackfly species, *i.e. S. metallicum, S. callidum* and *S. haematopotum.* Moreover, microfilariae of the Guatemalan *O. volvulus* could complete their larval development in *S. metallicum* from Venezuela (Takaoka *et al.,* 1986) and in *S. quadrivittatum* from Panama (Schiller *et al.,* 1984).

All these data do not seem to support the assumption that there is mutual incompatibility of *O. volvulus* to the vector blackflies between Guatemala and north Venezuela. Thus, it is not probable, unlike in West Africa, that there exist 2 different *"Onchocerca-Simulium"* complexes, each in Guatemala and in northern Venezuela, despite the fact that the clinical pictures of onchocerciasis differ between the 2 countries (Choyce, 1964; Duke, 1974, 1976).

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摘　要

ベネズエラ産 *Onchocerca volvulus* のグァテマラ産ブユ3種への感染実験

西半球のオンコセルカ症各流行地間の *Onchocerca volvulus* 株の相異を調べる目的で，ベネズエラ北部流行地の *O. volvulus* 保有者をグァテマラのブユ3種 (*Simulium callidum, S. haematopotum, S. ochraceum*) に吸血させ，仔虫のとりこみ，胸筋への移行，感染幼虫への発育等を検討した．仔虫のとりこみは，3種すべてにみられたが，*S. haematopotum* と *S. ochraceum* では，咽頭部の歯状突起で傷害をうけほとんどが死亡していた．その結果，これら2種では仔虫の胸筋への移行もきわめて少なかった．第3期幼虫への発育は *S. ochraceum* ではみられなかったが，*S. callidum* と *S. haematopotum* ではおのおの，8% と 3% のブユに認められた．これらの結果から，ベネズエラ北部の *O. volvulus* は，先に報告した *S. metallicum* と合わせて，グァテマラ産の少なくとも3種のブユ体内で発育することが明らかとなった．また，同じく先に報告したように，グァテマラの *O. volvulus* もベネズエラの *S. metallicum* で第3期幼虫へ発育する．したがって，従来の考え方に反してグァテマラとベネズエラ両国間では，本症の臨床像に差が認められるものの，両国の *O. volvulus* はブユへの感染能に関してはほとんど差がないものと思われる．