Japan. J. Trop. Med. Hyg., Vol. 12, No. 2, 1984, pp. 89–96

SUSCEPTIBILITY OF *SIMULIUM METALLICUM* TO INFECTION WITH *ONCHOCERCA VOLVULUS* IN VENEZUELA[1]

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Received 26 January 1984/Accepted 10 May 1984

Abstract: The degree of susceptibility of *Simulium metallicum* to infection with *Onchocerca volvulus* was studied in Venezuela. Wild flies which had fed on the infected volunteer were maintained at a temperature varying between 22°C and 28°C, and all flies dying every 24 hours were dissected for larval development. Third-stage larvae were first seen in the head of flies dying between five and six days. Few abnormal and deformed larvae were observed. The larval development was asynchronous; only 41 of 117 larvae recovered from 32 flies examined on days 6–11 were in the third stage, the remaining larvae being still in earlier stages. However, the proportion of third-stage larvae among all larvae recovered increased from 6 to 69 per cent, with the passage of time from days 6–8 to 9–11. Likewise, the percentage of third-stage larvae found in the head during the same period rose from 25 to 54 per cent. These results indicate that despite the asynchronous and retarded development, many larvae can develop to the infective stage in *S. metallicum* and the possibility of infective larvae capable of being eventually inoculated into man is high, when flies could survive more. It is suggested that the susceptibility of *S. metallicum* in Venezuela to the indigenous strain of *O. volvulus* is relatively high, as compared with that of the same species in Guatemala or Colombia.

INTRODUCTION

Peñalver (1961) regarded for the first time *Simulium metallicum* Bellardi as the principal vector of onchocerciasis in the northern foci of Venezuela. Lewis and Ibáñez de Aldecoa (1962) assessed the vectorial capacity of this species, as well as *S. exiguum* Roubaud, from their field observations. They concluded that *S. metallicum* was the only important vector in these regions. Duke (1970) made the experimental infection and compared the developmental potential of *Onchocerca volvulus* microfilariae

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ingested by these two blackfly species. Ramírez Pérez *et al.* (1976) described the morphological features of *O. volvulus* larvae developing in *S. metallicum*. However, no data were available on the degree of susceptibility of this blackfly species to infection with *O. volvulus* in Venezuela. Apparently such information is needed to compare the vectorial capacity of *S. metallicum* in Venezuela with that of the same species in Guatemala or Colombia, where this species has been reported to be less important as a vector (De León and Duke, 1966; Collins, 1979; Ito *et al.*, 1980; Tidwell *et al.*, 1980).

In the present experiment, the degree of susceptibility of the Venezuelan *S. metallicum* to the indigenous strain of *O. volvulus* was assessed, mainly by means of the proportion of third-stage larvae among all larvae recovered from the flies which survived beyond the time when larvae became infective, as well as that of surviving flies positive for third-stage larvae.

Materials and Methods

The experimental infection was carried out in November 1982, at Guanaguana in Monagas, one of the onchocerciasis-endemic foci in the north-eastern region of Venezuela. The general information on this village or its surrounding areas was previously reported by Lewis and Ibáñez de Aldecoa (1962). The predominant man-biting blackfly species during the present study was *S. metallicum*, comprising 95 per cent of the total catches.

Wild females of *S. metallicum* were allowed to feed on the lower half of the legs of a human volunteer with low microfilarial density — two microfilariae per skin snip from a calf, taken with corneoscleral punch (Holth type). Simultaneously, engorged blackfly females were captured from a control volunteer without microfilariae. All these blood-fed flies were collected and maintained individually in a polypropylene tube, using a method previously described (Takaoka *et al.*, 1982). All flies were kept at a temperature varying between 22°C and 28°C, dead flies being removed everyday thereafter and stored in 70 per cent ethanol solution for later dissection. The number of dead flies was recorded to compare daily probabilities of survival in the groups of flies which had fed on the infected and control persons, respectively. All preserved specimens were divided into head, thorax and abdomen and dissected in a drop of five per cent Giemsa solution on a glass slide under a binocular stereoscopic 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). To assess microfilarial intake, 20 flies which fed on the infected volunteer were killed immediately after feeding, and dissected using a technique by Nakamura (1964).

Results

Since wild flies were used in this study, it is possible that natural infections were mixed in the results. According to Lewis and Ibáñez de Aldecoa (1962), 3.9 per cent of wild-caught *S. metallicum* were naturally infected with *O. volvulus* larvae in the

Table 1 Larval development of *Onchocerca volvulus* in *Simulium metallicum* at 22–28°C

Days post-infection	No. flies with any stage of larvae/No. flies dissected (%)	No. larvae per positive fly	No. flies with 3rd-stage larvae/No. flies with any stage of larvae (%)	No. 3rd-stage larvae per positive fly	No. flies with 3rd-stage larvae in the head/No. flies with 3rd-stage larvae (%)	No. 3rd-stage larvae in the head per positive fly
		Mean (Range)		Mean (Range)		Mean (Range)
0	8/20 (40)*1	4.5 (1–10)	—	—	—	—
1–5	13/25 (52)	9.8 (1–59)	—	—	—	—
6–8	20/72 (28)	3.2 (1–19)	4/20 (20)	1 (1)	1/4 (25)	1 (1)
9–11	12/39 (31)	4.2 (1–12)	10/12 (83)	3.6 (1–11)	6/10 (60)	3.3 (1–7)
Total*2	32/111 (29)	3.4 (1–19)	14/32 (44)	2.9 (1–11)	7/14 (50)	3.3 (1–7)

*1 These flies were killed immediately after feeding to assess microfilarial intake.

*2 Total from days 6–11.

Table 2 Number and proportion of each developmental stage of *Onchocerca volvulus* larvae in *Simulium metallicum* which died on days 6–11 after ingestion of an infected blood-meal

Days post-infection	No. (%) larvae of				
	Any stage	Microfilarial stage	First stage	Second stage	Third stage
6–8	63	0 (0)	31 (49)	28 (45)	4 (6)
9–11	54	1 (2)	11 (20)	5 (9)	37 (69)
Total	117	1 (1)	42 (36)	33 (28)	41 (35)

Guanaguana area. Therefore, the rate of experimental infection shown in Table 1 was assumed to be slightly overestimated.

Under a temperature varying between 22°C and 28°C, third-stage larvae were first seen in the head of female *S. metallicum* dying between 5 and 6 days after ingestion of microfilariae. Few abnormal and deformed larvae were observed. The development of *O. volvulus* larvae in *S. metallicum* was asynchronous, as evidenced by the fact that third-stage larvae were found in 44 per cent (14/32) of the flies harboring any stages of larvae examined on days 6–11 (Table 1). In other words, only 41 of 117 larvae recovered during the same period were in the third stage, while the other larvae remained in earlier stages (Table 2).

However, as shown in Table 1, the proportion of flies with third-stage larvae among all flies positive for any stage of larvae increased from 20 to 83 per cent, with the passage of time elapsing from days 6–8 to 9–11. The proportion of third-stage larvae among all larvae recovered during the same period also increased from 6 to 69 per cent (Table 2).

Likewise, the proportion of flies with third-stage larvae in the head augmented from 25 to 60 per cent (Table 1); and the percentage of third-stage larvae found in the head rose from 25 to 54 per cent, as the time passed from days 6–8 to 9–11 (Table 3).

Table 3 Distribution of third-stage larvae of *Onchocerca volvulus* in the body of *Simulium metallicum* which died on days 6–11 after ingestion of an infected blood-meal

Days post-infection	Total no. third-stage larvae	No. (%) third-stage larvae found in		
		Head	Thorax	Abdomen
6–8	4	1 (25)	3 (75)	0 (0)
9–11	37	20 (54)	17 (46)	0 (0)
Total	41	21 (51)	20 (49)	0 (0)

The average length of four third-stage larvae measured was 474.2 μm (range, 462.7–498.9 μm), and the width, 18.3 μm (range, 16.6–19.2 μm). The relatively small size of the third-stage larvae is probably attributed to the high temperature under which larval development took place, and the fixation of samples.

The average number of larvae per positive fly examined on days 6–11 was 3.4 with the range of 1–19. And, those of third-stage larvae in any part of the body and also in the head per positive fly were 2.9 (range, 1–11) and 3.3 (range, 1–7), respectively (Table 1). These numbers were slightly smaller than the corresponding one observed for microfilarial intake (average, 4.5 and range, 1–10) (Table 1).

The daily probabilities of survival in the group of flies which fed on the infected volunteer are shown in Figure 1, together with those in the control group. Within 24 hours of feeding, no marked mortality was observed in both groups. This is probably due to the scantiness of ingested microfilariae. Moreover, there was no difference in the daily survival rates during days 2–11 between the two groups (Figure 1).

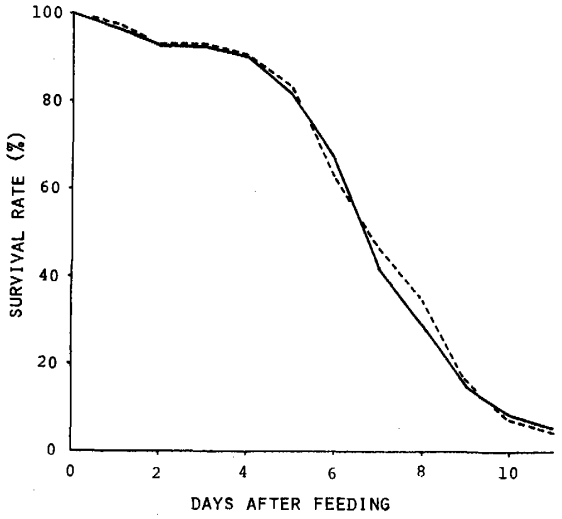

Figure 1 Survivorship curves of *Simulium metallicum* which fed on the infected (solid line) and control (dotted line) persons, respectively, and were kept at a temperature varying between 22°C and 28°C.

Discussion

Duke (1970) has pointed out that *S. metallicum* in Venezuela differently behaves as a vector of onchocerciasis, as compared with the same species in Guatemala. In fact, the apparent difference has been observed with regard to the preimaginal site and the host preference. In Venezuela, *S. metallicum* breeds in small streams, and shows a strong anthropophily (Lewis and Ibáñez de Aldecoa, 1962), while this species in Guatemala utilizes a wide range of watercourses as a larval site, and prefers animals to man for feeding (Dalmat, 1955). Further, there seems to be a slight difference in biting preference for the site on human body. According to Duke (1970), *S. metallicum* in Venezuela has a strong predilection for the lower parts of the body (96.5% of the total catches), whereas the same species in Guatemala bites the upper parts as well as the lower ones (26% vs. 74%). However, it remained unverified whether there is any difference in susceptibility of this blackfly species to infection with the indigenous strain of *O. volvulus* between the two countries. This was mainly due to the lack of data on the degree of susceptibility of *S. metallicum* in Venezuela, although experimental infection studies have been made by Duke (1970) and also by Ramírez Pérez *et al.* (1976).

In this context, the present experiment was performed, and it demonstrated that the development of *O. volvulus* larvae was asynchronous, as reported in *S. metallicum* in Guatemala (De León and Duke, 1966; Collins, 1979; Ito *et al.*, 1980). Namely, an overall proportion of the third-stage larvae among all larvae recovered throughout days 6–11 was 35 per cent, the remaining larvae being still in earlier stages (Table 2).

It is, however, noteworthy that although retarded in development, 69 per cent of the larvae found on days 9–11 had already completed their maturation to the infective stage (Table 2). The proportion of third-stage larvae among all larvae

recovered on days 8–10 in the Guatemalan *S. metallicum* was 26.3 per cent by Collins (1979) and 53.6 or 64.0 per cent by Ito *et al.* (1980). The low rate reported by the former author was probably due to the low temperature condition fluctuating between 16°C and 25.5°C under which flies were maintained. However, it is interesting that even the relatively high rates reported by Ito *et al.* (1980) are slightly lower than 69 per cent observed in the present study, in spite of the fact that the maintaining temperature (constant 25°C) used by Ito *et al.* (1980) was more stable and suitable for larval development than the condition used in our experiment. Moreover, the rate of third-stage larvae moving to the head (29.5%) remarked by Ito *et al.* (1980) seemed to be very low, as compared with 54 per cent in our result (Table 3), suggesting that the probability of infective larvae eventually invading man is higher with the Venezuelan *S. metallicum*, than with the Guatemalan one. When the difference in the temperature condition in experiments is taken into account, this could be a reflection of the difference in degree of susceptibility of *S. metallicum* to the indigenous strain of *O. volvulus* between the two areas.

Further, abnormal and deformed larvae were rarely seen in the present experiment. This is in marked contrast to the observations made in the Guatemalan *S. metallicum*, in which such larvae were frequently encountered (De León and Duke, 1966; Collins, 1979; Ito *et al.*, 1980).

In our study, the decrease in number of the microfilariae ingested by *S. metallicum* during the migratory and developmental phases was inconspicuous. The average number of third-stage larvae in any part of the body and in the head per positive fly (i.e. each 2.9 and 3.3) were only slightly smaller than that of microfilariae ingested (i.e. 4.5). This may reveal a good adaptation of the parasite to the Venezuelan vector.

It is suspected that flies which had ingested more microfilariae may have died earlier than the other, as the infection rate, as well as the average number of larvae per positive fly during days 1–5 (Table 1), was somewhat higher than those attained during later periods. However, it seems to be the rule that survival of female *S. metallicum* is scarcely affected by the presence of the parasite therein, if small in number. As many as 11 third-stage larvae were found in one fly examined on day 10.

From the findings mentioned above, it is likely that apart from the difference in the host feeding preference and the preimaginal habitat, the Venezuelan *S. metallicum* somewhat differs from the Guatemalan one even in susceptibility to the indigenous strain of *O. volvulus*.

On the other hand, Tidwell *et al.* (1980), from the experimental infection study in Colombia, reported that out of 206 larvae in *S. metallicum* examined on days 7–13, 25 per cent were abnormal, 69 per cent had not developed beyond the first stage and only 10 per cent had reached the third stage. Engorged flies were kept at a temperature varying between 22°C and 27°C, being almost the same condition as used in our experiment. Their results suggest that the susceptibility of *S. metallicum* in Colombia to the indigenous strain of *O. volvulus* remarkably differs from that of the same species in Venezuela.

In conclusion, the relatively high susceptibility remarked in our experiment, in combination with the predominancy and the high anthropophily (Lewis and

Ibáñez de Aldecoa, 1962), may explain why *S. metallicum* plays a major role in transmission of onchocerciasis in the northern areas of Venezuela, unlike in Guatemala and Colombia.

ACKNOWLEGEMENTS

The authors wish to thank Messrs. B. Diaz, L. Castellani, and F. Mota, Servicio Dermatologia, Monagas, E. Lopez and J. M. Colmenares, Servicio Dermatologia, Miranda, for their help in the field works.

Thanks are due to Dr. P. Figallo, Director, Officina Subregional de Salud Publica, Monagas, who supported our study and provided us with a jeep during our field survey in Monagas.

Finally, the senior author would like to appreciate the kind assistance in the dissection of flies given by Dr. M. Baba, Miss C. Aoki and Miss K. Ogata, Division of Medical Zoology, Medical College of Oita.

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ベネズエラにおける *Simulium metallicum* の *Onchocerca volvulus* に対する感受性について[1]

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ベネズエラ北部のオンコセルカ症伝搬ブユ種である *Simulium metallicum* の *Onchocerca volvulus* に対する感受性の程度を実験感染により検討した。実験には，*O. volvulus* ミクロフィラリア保有者の脚から充分吸血した野外の雌成虫を用いた。これらの吸血個体を 22–28°C の温度条件下で個別に飼育し，毎日死亡した個体を解剖することにより幼虫の発育を調べた。その結果，吸血後5日と6日の間に死亡したブユから最初の第 III 期幼虫が見いだされた。幼虫発育は不揃いであったが異常形の幼虫は見られなかった。ブユ体内で見いだされた幼虫のうち第 III 期幼虫の占める割合は吸血後6–8日に剖検したブユ体内で6％であったが，9–11日に調べたブユでは69％と日数の経過とともに増加した。同期間におけるブユの頭部に見いだされた第 III 期幼虫の割合も25％から54％へ上昇した。すなわち，*S. metallicum* 体内での *O. volvulus* 幼虫の発育は不揃いであるが，ブユが長く生存すれば，とりこまれたミクロフィラリアの多くは第 III 期幼虫まで発育し，人へ感染する機会が多いことが示された。これらの結果と既に報告されているグアテマラとコロンビアでの実験感染の結果を比較すると，ベネズエラの *S. metallicum* は上記2国の同一ブユ種より，*O. volvulus* に対して高い感受性をもっていることが示唆された。

1 本研究は，文部省科学研究費助成による海外学術調査（No. 57041041 及び 58043041）の一環として実施した 2 大分医科大学医動物学教室　3 長崎大学熱帯医学研究所ウイルス学部門　4 鹿児島大学医学部医動物学教室　5 熊本大学医学部寄生虫病学教室　6 ベネズエラ国立皮膚科学研究所モナガス研究室　7 ベネズエラ国立皮膚科学研究所ミランダ研究室　8 ベネズエラ国立皮膚科学研究所