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A Cross-compatibility Study of Guatemalan and North Venezuelan *Onchocerca volvulus* to *Simulium metallicum* from Two Countries

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Introduction

In the American continents, onchocerciasis has been known to be distributing sporadically in the following 6 countries; Mexico, Guatemala, Venezuela, Brazil, Colombia and Ecuador. From the viewpoint of the geographical distribution and vector simuliid species, the endemic areas will be classified into 4 major foci; one in the Central-North America and the other three in South America. In the first focus, Mexico and Guatemala, the disease is transmitted by *Simulium ochraceum*, *S. metallicum* and *S. callidum*; the second in the northern coastal region of Venezuela, by *S. metallicum*; the third in the upper Orinoco-Amazon region of Venezuela-Brazil border, by *S. cuasisanguineum* and *S. pintoi*; and the fourth in the Pacific coastal region in Colombia and Ecuador, by *S. exiguum*, respectively. The clinical pictures of the disease in Central America differ from those of South America, as reviewed by Choyce (1964), Duke (1974) and Sasa (1976). The difference in the distribution of onchocercal nodules in the patients has long been considered to be a good parameter (Choyce, 1964; Duke, 1974; Tada *et al.*, 1974; Convit, 1974).

Among the various factors which would affect the features of the disease depending on the localities, several trials have ever been performed to find out the differences of parasite *per se* by using cross transmission experiments. The cross infections of the vector blackflies were carried out between Sudan savanna and the rain forest/Guinea savanna zones in West Africa (Duke *et al.*, 1966), and between West Africa and Latin America (De Leon and Duke, 1966; Duke *et al.*, 1967; Duke, 1970). Through this series of experiments, Duke (1974, 1976) concluded that there was a variety of strains in *Onchocerca volvulus*, each adapted to a proper species of *Simulium* in the transmission at different geo-topographic regions. Thus it may be considered that the difference of parasite strain has been involved in the character of the disease in individual foci. Although Duke (1970) pointed out the remoteness of *O. volvulus* between West Africa and

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two foci in the American continents, he did not compare *Onchocerca-Simulium* complex between Central and South America.

The present study aimed at the comparison of *Onchocerca* strains between Guatemala and northern Venezuela, by means of cross-infection of a vector blackfly species which was previously introduced by Duke *et al.* (1966).

Materials and Methods

We appreciated the participation of two volunteers for this investigation with full understanding of the principle of the experiment and agreement on the remuneration. The carrier of Guatemalan strain of *O. volvulus* was a 29-year old man, a native villager of Palin, Department of Escuintla, and that of Venezuelan strain was a 36-year old man, a native villager of Rio Chiquito in the Monagas State. Neither of them had ever been abroad. The microfilarial densities of the calf in Guatemalan and Venezuelan volunteers were 2 and 13 per snip, respectively, taken by a Holth-type corneo-scleral punch.

The experimental infection of *S. metallicum* in Guatemala was carried out in July, 1984 at a coffee plantation "Ceilan", an endemic focus in the Municipality of Pochuta, Department of Chimaltenango. Wild females of *S. metallicum* were arbitrarily fed to repletion on legs of the above 2 volunteers. All the blood-fed flies were captured and maintained individually each in a polypropylene tube using the method of Takaoka *et al.* (1982).

The first group of flies was dissected immediately after feeding and the second one, 24 hr post-feeding, in order to assess microfilarial intake and the larval movement to the thorax, respectively. All the other flies were kept at a constant temperature of 22°C and were checked daily for the survival. Dead flies thus found were removed daily and stored at −20°C in a freezer for later dissection. During the period between 8 and 16 days post-feeding, all the live flies were dissected to death in physiological saline under a dissecting microscope. The number of larvae in the thorax, abdomen and head was counted with the determination of the stage of development by size and morphological criteria by Duke (1968).

The experiment in Venezuela was performed in August, 1984, at Rio Chiquito, an endemic focus and home village of the Venezuelan volunteer based on the above mentioned manner, with minor modification in the incubation temperature (20-24°C). All the flies surviving through 8 to 16 days were preserved in 70 % ethanol for transportation and were later dissected in a drop of 2.5 % Giemsa's solution.

As the infection experiments were carried out simultaneously on two volunteers at the same site with a proper distance, the results obtained by domestic combinations of *Onchocerca-Simulium* were considered as the control for the cross combination.

Natural infection in *S. metallicum* at the study areas was assessed by capturing blackflies which alighted on the unifected volunteers.

Results

In Guatemalan site of the experiment, the natural infection rate was 0 % (0/110) in the female *S. metallicum* captured, and that in Venezuela, 0.86 % (3/350). In the latter area, each of 3 flies harboured one first-stage larva indistinguishable from that of *O. volvulus*.

In both countries, *S. metallicum* ingested as many microfilariae of foreign *O. volvulus* as those of domestic ones, while the positivity and the average number of ingested microfilariae in Venezuelan flies were rather higher than those of Guatemalan flies (Table 1).

The migration of microfilariae of both strains to the thorax, 24 hr post-ingestion, was examined in flies from two countries (Table 2). The order of microfilarial number which moved to the thorax was almost the same, irrespective of the strain of *O. volvulus* in any *S. metallicum* populations. However, the rate of migration was slightly high in Venezuelan flies.

Table 3 shows the result of the dissection of infected flies which were killed between days 8 and 16. In the head of *S. metallicum*

Table 1 Intake of microfilariae of *O. volvulus* from Guatemala and northern Venezuela by *S. metallicum* from both countries

Locality of *S. metallicum*	No. of flies examined	No. of flies positive for mf* (%)	No. of mf Mean (range)
		Guatemalan *O. volvulus*	
Guatemala	15	6 (40)	2 (1 − 4)
Venezuela	20	13 (65)	3 (1 − 7)
		Venezuelan *O. volvulus*	
Guatemala	15	9 (60)	5 (1 −12)
Venezuela	16	13 (81)	9 (1 −25)

* microfilariae

Table 2 Thoracic migration of microfilariae of *O. volvulus* from Guatemala and northern Venezuela in *S. metallicum* from both countries, 24 hrs post-ingestion

Locality of *S. metallicum*	No. of flies examined	No. of flies positive for mf* (%)	No. of mf Mean (range)
		Guatemalan *O. volvulus*	
Guatemala	16	5 (31)	5 (1 −14)
Venezuela	21	10 (48)	4 (1 −21)
		Venezuelan *O. volvulus*	
Guatemala	21	8 (38)	4 (2 − 9)
Venezuela	19	12 (63)	5 (1 −16)

* microfilariae

Table 3 Larval development of *O. volvulus* from Guatemala and northern Venezuela in *S. metallicum* from both countries, 8–16 days post-ingestion

Locality of *S. metallicum*	No. of flies examined	No. of flies with any stage of larvae (%)	No. of flies with L_3* (%)	No. of L_3 Mean (range)
		Guatemalan *O. volvulus*		
Guatemala	58	10 (17)	3 (5)	1 (1)
Venezuela	48	7 (15)	4 (8)	1 (1 − 2)
		Venezuelan *O. volvulus*		
Guatemala	80	30 (38)	16 (20)	2 (1 − 6)
Venezuela	45	15 (33)	7 (16)	3 (1 − 5)

* third-stage larvae

examined in both countries, the third-stage larvae (L_3) from two strains appeared as early as day 8. There was essentially no difference in the proportions of the flies with L_3 and in the worm burden per positive fly, so far as the same strain of microfilariae was concerned. In Venezuelan flies previously fed with Guatemalan microfilariae, 15 % were with any stage of larvae and 8 %, with L_3. Likewise, nearly equal rates were obtained in the Guatemalan flies with Guatemalan microfilariae. In contrast, the above rates were 38 % and 20 %, respectively, when Guatemalan flies were fed with Venezuelan mi-

Table 4 Number of larvae of *O. volvulus* from Guatemala and northern Venezuela, by the stage of development, recovered from *S. metallicum* from both countries, dissected on days 8–16 post-ingestion

Locality of *S. metallicum*	No. of flies with larvae	No. of larvae recovered	No. (%) of larvae* of			
			mf	L_1	L_2	L_3
		Guatemalan *O. volvulus*				
Guatemala	10	17	1(6)	13(76)	0(0)	3(18)
Venezuela	7	13	0(0)	8(62)	0(0)	5(38)
		Venezuelan *O. volvulus*				
Guatemala	30	128	1(1)	74(58)	19(15)	34(26)
Venezuela	15	57	0(0)	32(56)	6(11)	19(33)

* mf, microfilariae; L_1, first-stage larvae; L_2, second-stage larvae; L_3, third-stage larvae

Table 5 Distribution of third-stage larvae of *O. volvulus* from Guatemala and northern Venezuela recovered from *S. metallicum* from both countries, dissected during days 8–16 post-ingestion

Locality of *S. metallicum*	Total No. of L_3*	No. of L_3 in		
		Head	Thorax	Abdomen
	Guatemalan *O. volvulus*			
Guatemala	3	1	2	0
Venezuela	5	1	2†	2
	Venezuelan *O. volvulus*			
Guatemala	34	16	16	2
Venezuela	19	10	5	4

* third-stage larvae,. † one of these 2 larvae was found in a halter

crofilariae. Slightly higher rates were seen in this combination in comparison with those of Venezuelan flies fed on the Venezuelan volunteer.

Larval development of two strains of *O. volvulus* in the fly throughout the observation period (8-16 days post-ingestion) was asynchronous in any combination (Table 4). A high proportion of the larvae stayed at immature stages in both the domestic and cross combinations.

The L_3 of Guatemalan *O. volvulus* obtained in *S. metallicum* of any country were less in number in comparison with Venezuelan parasite and only a single L_3 each attained the head portion of the fly from any country (Table 5). On the other hand, in case of Venezuelan strain of the parasite, 34 L_3 were found even in the Guatemalan flies, 47 % (16/34) of which were in the head. The correspondent rate in Venezuelan parasite-Venezuelan fly combination was almost indistinguishable, 53 % (10/19).

The measurements of body lenght of L_3 recovered from head/thorax are given in Table 6. The average length and its range of Venezuelan L_3 recovered in the flies from two conutries was almost the same. The scarcity of larval samples, 1 in Guatemalan fly and 2 in Venezuelan flies, did not enable us to compare precisely the lenght of Guatemalan larvae with that of Venezuelan ones.

Discussion

Duke *et al.* (1966) showed that microfilariae of Sudan savanna strain of *O. volvulus* fully developed in *S. damnosum* from the identical zone, but not or poorly developed in flies from the rain-forest/Guinea savanna, and *vice versa*. Their study proposed a hypothe-

Table 6 Body length of the third-stage larvae of *O. volvulus* from Guatemala and northern Venezuela found in *S. metallicum* from both countries, dissected during days 8-16 post-ingestion

Locality of *S. metallicum*	Body portion where L_3* were found	No. of L_3 measured	Body length (μm) Mean (range)
	Guatemalan *O. volvulus*		
Guatemala	Thorax	1	350
Venezuela	Head	1	496
	Thorax	1	500
	Venezuelan *O. volvulus*		
Guatemala	Head	15	423 (350−520)
Venezuela	Head	5	466 (380−513)

* third-stage larvae

sis that in West Africa, there existed 2 strains of *O. volvulus*, each adapted to the physiological strains of *S. damnosum*, thus forming a distinct "*Onchocerca-Simulium* complex" in each topographic zone. By using the above-mentioned cross transmission technique, similar studies were performed between West Africa and Guatemala (De Leon and Duke, 1966; Duke *et al.*, 1967) and West Africa and Venezuela (Duke, 1970). They concluded that the "*Onchocerca-Simulium* complexes" in American continents were far removed from those in West Africa, judging from the incompatibility of the parasite to the flies of different foci.

In this context, a similar cross infection technique was adopted in our study to differentiate *O. volvulus* between Guatemala and northern Venezuela. The study showed that there was no difference in the microfilarial intake and the subsequent larval movement to the thorax in *S. metallicum* in any *Onchocerca-S. metallicum* combinations, domestic and cross. A successful development to L_3 was also seen in both combinations, too.

The proportion of Venezuelan *S. metallicum* which harboured L_3 of Guatemalan strain was low, 8 %, while this was rather higher than the corresponding rate, 5 %, in Guatemalan *S. metallicum* fed on Guatemalan carrier. The natural infection rate, 0.86 %, will not be enough to explain the gap. In contrast, that of Guatemalan fly with Venezelan L_3 was high, 20 %, and that of Venezuelan fly with Guatemalan L_3, 16 %, respectively. This evidence shows that there is no mutual incompatibility of *O. volvulus* to *S. metallicum* between Venezuela and Guatemala at cross combinations.

It is noteworthy that microfilariae of Venezuelan *O. volvulus* fully developed to L_3 in some of Guatemalan blackflies, *S. callidum* and *S. haematopotum*, when they were fed on the identical Venezulan volunteer (Takaoka *et al.*, unpublished data). This finding indicates the compatibility of Venezuelan *O. volvulus* to a wide range of unfamiliar blackfly species. In that study, however, it was unable to feed Guatemalan microfilariae to Venezuelan flies other than *S. metallicum*.

Summarizing the present study, it is unlikely that there exist 2 distinct strains of *O. volvulus* between Guatemala and northern Venezuela, despite the fact that clinical features of the disease differed between two continents (Choyce, 1964; Duke, 1974, 1976). However, apart from the similarity of the parasite in the compatibility to the blackfly from different continent, a possibility can not be ruled out that the pathogenicity of the parasite differs between 2 countries. Further, racial factor of the human populations affected should also be taken into consideration to elucidate the difference of the disease.

With regard to the origin of Guatemalan onchocerciasis, De Leon and Duke (1966) considered that the close adaptation of Guatemalan parasite to transmission by *S. ochra-*

ceum is unlikely to have evolved within the relatively short period of some 400 years since the earliest possible arrival of the first African slaves. On the other hand, South American onchocerciasis has been often considered as that imported by slave trade, because of its clinical features, geographic distribution and affected race of African origin. Although the present study does not lead to a direct proof on the origin of Central American disease (Robles' disease), it strongly suggested the biological identity or close similarity of *O. volvulus* between Guatemala and northern Venezuela. Thus, we tend to speculate that Central American onchocerciasis was also introduced through the slave trade.

Summary

Guatemalan and Venezuelan *Onchocerca volvulus* were compared by means of the cross infection technique using *Simulium metallicum* as the vector. The average intake of Guatemalan microfilariae and percentage of positive flies were 2 microfilariae and 40 %, in Guatemalan flies, and 3 microfilariae and 65 % in Venezuelan flies, respectively. The subsequent migration of ingested microfilariae to the thorax at 24 hours post-feeding was seen in 31 % of Guatemalan flies and 48 % of Venezuelan flies. The development of microfilariae to the third-stage larvae (L_3) took place as early as day 8 at about 22°C in any combinations, accompanying asynchronous nature in the growth. When the flies were fed on Guatemalan volunteer, only 8 % of Venezuelan flies surviving through 8-16 days were positive for L_3, which was slightly higher than the corresponding rate, 5 %, in Guatemalan flies. When the flies were fed on Venezuelan volunteer, both the Guatemalan and Venezuelan flies revealed resembling but much higher rates, 20 and 16 % each. Probably the difference in the microfilarial density of two volunteers will be attributable to the discrepancy between above results. This study indicated that both of Guatemalan and north Venezuelan *O. volvulus* were equally compatible to a blackfly species, *S. metallicum*, at any combinations (domestic/cross) and thus these two strains of the same parasite were at least very close to each other.

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グァテマラとベネズエラ北部における *Onchocerca volvulus* の *Simulium metallicum* に対する交叉適合性の検討

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オンコセルカ症の臨床像や伝搬ブユ種は地域によっては一律でない．臨床像を異にする各々の流行地には系統の異った *Onchocerca volvulus* が分布していると一般に考えられている．今回中米のグァテマラと南米のベネズエラ北部の流行地間で，*O. volvulus* の系統による違いの有無を，交叉感染実験によって検討した．実験では，グァテマラとベネズエラ北部の本症流行地に居住する *O. volvulus* ミクロフィラリア（mf）保有者成人男子各1名を感染源とした．この保虫者を吸血源とし，自国および相手国において媒介ブユ *Simulium metallicum* に感染実験を試み，mf とりこみ，mf の胸筋への移行および第Ⅲ期幼虫への発育に関して比較検討した．

その結果，両地域の *O. volvulus* は，自国の *S. metallicum* に対すると同程度に相手国の同種ブユに親和性を示すことが分った．これらの結果から，両地域の *O. volvulus* は，媒介ブユ *S. metallicum* に対する伝搬性に関する限り，同じか，または非常に近縁の系統と思われ，両地域に2つの異った "*Onchocerca-Simulium*" 複合体の存在を考えることには無理があると思われる．