THE ROLE OF *S. METALLICUM* AS THE VECTOR OF ONCHOCERCIASIS IN GUATEMALA

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INTRODUCTION

Simulium ochraceum is considered to be the most important vector of human ochocerciasis in Guatemala, followed by *S. metallicum*. It has been reported that *S. ochraceum* is a compatible host, capable of supporting the development of the Guatemalan strain of *Onchocerca volvulus*, while *S. metallicum* is a poor host for this parasite, since the development of most parasitic larvae arrests and few reach the infective stage. In addition, high fatality in flies which ingested many microfilariae has often been observed in *S. metallicum*.

The Onchocerciasis Control Project in Guatemala was confronted with the decision, whether or not *S. metallicum* should be regarded as one of the control targets. Therefore, experimental infection studies were carried out using *S. metallicum* and *S. ochraceum*, to obtain further information on the role of *S. metallicum* in onchocerciasis transmission. The details of this study were published by Ito, Tanaka and Ochoa (1980) in Jap. J. Sanit. Zool., 31: 261-270.

MATERIALS AND METHODS

From March to June 1978, wild *S. metallicum* and *S. ochraceum* flies were caught after feeding on four microfilaria-carriers of *O. volvulus*. Fully engorged flies were captured one by one, each in a small plastic tube from upper torso, arms and lower legs of the carriers. Most flies were maintained in a dark incubator at 25°C, although some were dissected immediately after feeding, to count ingested microfilariae (Mf). The surviving flies were killed mainly on days 8 to 10, however a small number were also killed during the maintenance period. The killed flies, together with those found dead during the maintenance period were dissected.

Since it has been observed that the frequency distribution of the numbers of microfilariae or larvae found in the blackflies is roughly a logarithmic normal type, all counts were coverted to log.(n+1), and the geometric mean was calculated to compare the results.

RESULTS

Survival of the infected S. metallicum and S. ochraceum.

In the group of *S. metallicum*, which had ingested no or very few Mf, all flies survived the first 24 hours. However, in the groups with increased Mf intake, for example in those with more than 10.8 Mf per fly, some flies were found dead after 24 hours, but the survival rate of *S. ochraceum* after 24 hours was as high as 96% even in the higher Mf-intake groups.

In *S. ochraceum*, many Mf in the stomach were injured, presumably by the cibarial armatures. In contrast, most Mf ingested by *S. metallicum* were undamaged, due to the absence of such

armatures. This is probably the main reason why many *S. metallicum* heavily infected with Mf died within several hours after feeding. In fact, a number of Mf were often found in various organs of the fly.

In order to see the effects of microfilariae within the flies, mortality of *S. metallicum* exposed to different worm loads of *O. volvulus* was compared 5 to 10 hours after ingestion. Percent mortality of the flies was low when worm load was less than 99, but it reached 100% with worm laod higher than 400. The worm load for 50% survival of the fly was around 200.

Development of O. volvulus in the two fly species.

Flies of both species, that died during the maintenance period or killed after various days from the feeding were dissected to observe the development of *O. volvulus* larvae. The speed of larval development in *S. metallicum* was similar to that in *S. ochraceum*, and the third-stage larvae first appeared in thorax on day 7 and in head on day 8. Therefore, all surviving flies were killed on days 8 to 10, for dissection. In both *S. ochraceum* and *S. metallicum*, the infection rate was higher in the flies which had taken blood from the upper torso of the Mf-carriers than in those from arms or lower legs. No marked difference was found in the infection rates between the two species. However, larval development was orderly and synchronous in *S. ochraceum*, while it was asynchronous and often malformed or stunted larvae appeared in *S. metallicum*. Eight to 10 days after infection, more than 93% of the larvae detected in *S. ochraceum* reached the infective stage, among which 53% in head. In *S. metallicum*, only 54% of larvae matured, of which 30% were in head.

It should be mentioned, however, that although the development of many larvae is hampered in *S. metallicum*, the mean number (minimum – maximum) of the third-stage larvae per infected fly was 2.7 (0–27) in *S. metallicum*, and was even higher than 2.4 (1–19) of *S. ochraceum*.

Vectorial capacity of Simulium horacioi

In the Guatemalan study area, the presence of an unidentified balckfly species closely resembling *S. metallicum* but differing in larval characters was noticed in 1976. This was described later by Okazawa and Onishi (1980, Jap. J. Sanit. Zool., 31:167–179) as new species, *Simulium horacioi*. At the initial stage of this study, no information was available for distinguishing adult *S. horacioi* from that of *S. metallicum*. However, some female genitalia of the so-called "*S. metallicum*-complex" were preserved individually in 70% alcohol for future re-identification after they were experimentally infected with *O. volvulus* and dissected for the filarial infection. Later, it was elucidated that the two species could be differentiated also by the structure of female genitalia. In re-examination of 117 preserved genitalia of the *S. metallicum*-complex with knwon history of filarial infection, 17 (14.5%) was identified as *S. horacioi* and the rests as *S. metallicum* s. str. By checking with the previous records of dissection, it was shown that 1 (5.9%) of *S. horacioi* was harboring one infective larva of *O. volvulus*, and 5 (29.4%) among them were also infected with an "unknown filaria" presumably of animal origin. The rates observed with *S. metallicum* s. str. were 20.0% (20 of 100) and 1.0% (1 of 100), respectively. This and later studies of experimental infection with this blackfly species suggest that *S. horacioi* is also acting as a vector of human onchocerciasis, though further studies are needed before the role played by this species in the actual transmission of onchocerciasis could be fully evaluated.

DISCUSSION AND CONCLUSIONS

The results obtained suggested rather high transmission potential of *S. metallicum*. On the other hand, natural infection rate of the species in the study area is extremely low. The possible reasons for the above discrepancy might be; first, high biting preference of *S. metallicum* on animals, second, strong trend of biting lower part of human bodies in which Mf density is usually low in Guatemala, third, fly mortality increased as Mf ingestion increases, and fourth, larval development is

asynchronous and abnormal.

It should be pointed out that, theoretically, *S. metallicum* is not to be neglected from the vector control targets in Guatemala. Yet, *S. metallicum* control could be given only a lower priority in Guatemala, since natural infection rates of *S. metallicum* are very low. It should also be noted that vector control methodology must be altered if *S. metallicum* is included as one of the control targets, since the breeding sites of the species have a wider distribution than *S. ochraceum*.

It is suggested that the surveillance of *S. metallicum* is essential, particularly on monitoring the role of the species in transmission of the disease, especially when *S. ochraceum* is successfully controlled.

INVESTIGACIONES BIOLOGICAS CON *SIMULIUM OCHRACEUM* Y *S. METALLICUM* EN GUATEMALA

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Entre los estudios biológicos sobre oncocercosis mencionaremos:

1. Infección experimental de *S. ochraceum* y *S. metallicum* con microfilarias de *O. volvulus.*
   Resultados y Discusión:
   - 1.1 En condiciones de laboratorio la temperatura afecta directamente la sobrevivencia de ambas especies de simúlidos. A mayor temperatura menor es la sobrevivencia. A menor temperatura mayor sobrevivencia se observa. Figura 1.
   - 1.2 Se encontró una relación directa entre la sobrevivencia y la temperatura de mantenimiento en condiciones de laboratorio. Para *S. ochraceum* el coeficiente de correlacion $R = -1$, para *S. metallicum* $R = -0.9$.
     En ambos casos la distribución "F" nos indica que la hipótesis no debe ser rechazada a un nivel de significasión de 0.01.
   - 1.3 La temperatura afecta directamente el tiempo requerido para el desarrollo de la larva de *O. volvulus.* A mayor temperatura menor es el tiempo requerido para completar el desarrollo del nemátodo en ambas especies de simúlidos. Figura 2.
   - 1.4 El rango de temperatura en el que aparece la larva infectiva del tercer estadío de *O. volvulus* en ambas especies de simúlidos es de 18 a 30°C.
     La temperatura al afectar la sobrevivencia de la mosca y la velocidad de desarrollo del parásito, se convierte en un factor determinante en la transmisión de la oncocercosis. A la vez, podría explicar la distribución altitudinal de ésta enfermedad en Guatemala.
   - 1.5 La sobrevivencia de las moscas al día en que la larva infectiva de *O. volvulus* aparece en la cabeza del insecto es menor en los extremos del rango de temperatura.
     Para *S. ochraceum* aparentemente la temperatura óptima de transmisión es entre 22 y 24°C, debido a que en ésta se observa la mejor sobrevivencia al día en que la larva infectiva se encuentra en la cabeza y el mayor número de larvas de *O. volvulus* en dessarrollo.
   - 1.6 La morfología interna de las larvas en desarrollo de *O. volvulus* observadas en *S. ochraceum* y *S. metallicum* no difieren significativamente.

2. Ciclo gonadotrófico de *S. ochraceum* y *S. metallicum* a distintas temperaturas
   Resultados y Discusión:
   - 2.1 En condiciones de laboratorio la temperatura afecta directamente el desarrollo ovarial de las dos especies de simúlidos. A bajas temperaturas como 5 y 10°C y a altas temperaturas como 32°C o 34°C no se completa la formación del huevo.
   - 2.2 El tiempo requerido para completar la formacion de los huevos de ambos simúlidos está afectado por la temperatura. Existe una correlación negativa entre la temperatura y el tiempo requerido para la formación del huevo.
     Para ambas especies $R = -0.97$
   - 2.3 Aparentemente el tamaño del primer folículo ovárico en desarrollo, no varía en las diferentes temperaturas de mantenimiento.

2.4 En condiciones de laboratorio se requiere un mínimo de 48 horas despues de la ingurgitación con sangre, para que se complete la formación del huevo en ambas especies de simúlidos.

3. Oviposición de *S. ochraceum* en condiciones de laboratorio.
Resultados y Discusión:

3.1 Despues de probar various métodos de oviposición, la técnica que ofreció mejores resultados fue:
Utilizando un tubo plástico de mantenimiento y a la vez de oviposición que tuviera un agujero en el fondo, tapado con papel parafilm. El objeto del agujeor es permitir la entrada de agua por capilaridad, evitando así tener que llenar cada tubo. El objeto del papel parafilm es tener un sustrato de oviposición fácil de sacar del tubo y de mantener en el agua. El agua des-ionizada, previamente tratada para controlar el pH, cloro y dureza, se colocó en cajas de Petri grandes para luego sumergir los tubos, la mosca que queda flotando en el agua deja caer sus huevos, los cuales se adhieren al papel parafilm.

3.2 Con ésta técnica se obtienen huevos fértiles de simúlidos, habiendose logrado dos oviposiciones consecutivas.
El promedio de huevos ovipuestos por una hembra es de 92, existiendo un 22% de hembras con huevos residuales. Un 44% de los huevos llegaron a eclosionar en condiciones de laboratorio.

3.3 De las moscas a las que se les dió la oportunidad de oviponer 47 horas después de la ingurgitación, solamente las cultivadas a 26°C ovipusieron en un pequeñísimo porcentaje. A las 72 horas se observa un incremento notable en el porcentaje de oviposición, que se continúa a las 96 horas.
Posiblemente en el campo la oviposición tenga lugar principalmente a las 72 horas de la ingesta de sangre.

4. Marcaje, liberacion y recaptura de *S. ochraceum* en condiciones de campo.
Metodología:
Se trabajó durante la segunda semana de diciembre de 1979 y la primera semana de diciembre de1980. En base a la experiencia de 1979, se hicieron algunas modificationes para 1980. Los simúliodos se marcaron con polvos metálicos espolvoreados sobre el cuerpo del insecto con un atomizador de cirugía. Las hembras de *S. ochraceum* completamente ingurgitadas se dejaron en libertad para posteriormente ser capturadas y examinadas al esteréoscopio para buscar la marca; en la manipulación de los insectos se utilizaron agujas de disección.

Resultados:

4.1 La mayor frecuencia de moscas recapturadas se encontró en el período de 72 a 120 horas después de la ingesta con sangre. Tabla 2.

4.2 La temperatura promedio en ambas semanas de trabajo fué de 22°C. Según datos de laboratorio, se esperaba una mayor oviposición a las 72 horas de la ingesta de sangre, por lo que se encuentra una correspondencia entre los datos de laboratorio y los de campo.

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Table 1 Development of *O. volvulus* in *S. ochraceum* and *S. metallicum* at different temperatures

Temperature (°C)	Number of flies dissected		Mean number of third stage larvae					
			head		torax and abdomen		Total	
	So	Sm	So	Sm	So	Sm	So	Sm
18	9	6	0.3	0.5	4.8	3.0	5.1	3.5
22	11	22	2.7	0.5	2.6	1.6	4.4	2.1
24	50	21	2.7	0.6	5.4	1.1	7.2	1.7
28	19	20	2.4	0.8	3.2	5.4	5.5	6.2
30	13	19	1.6	0.1	2.6	0.4	3.2	0.5

So: *S. ochraceum;* Sm: *S. metallicum*

Table 2 Mark, release and recapture of *S. ochraceum* under field conditions 1979 and 1980

Year	Mean temperature (°C)	Number of flies released	Days after release	Number of flies recaptured	Recapture rate (%)
1979	22	898	1	0	0.00
			2	0	0.00
			3	15	1.67
			4	14	1.57
			5	2	0.22
	Total	898		31	3.45
1980	22.25	3904	1	0	0.00
			2	6	0.15
			3	40	1.02
			4	12	0.31
			5	7	0.18
	Total	3904		65	1.66

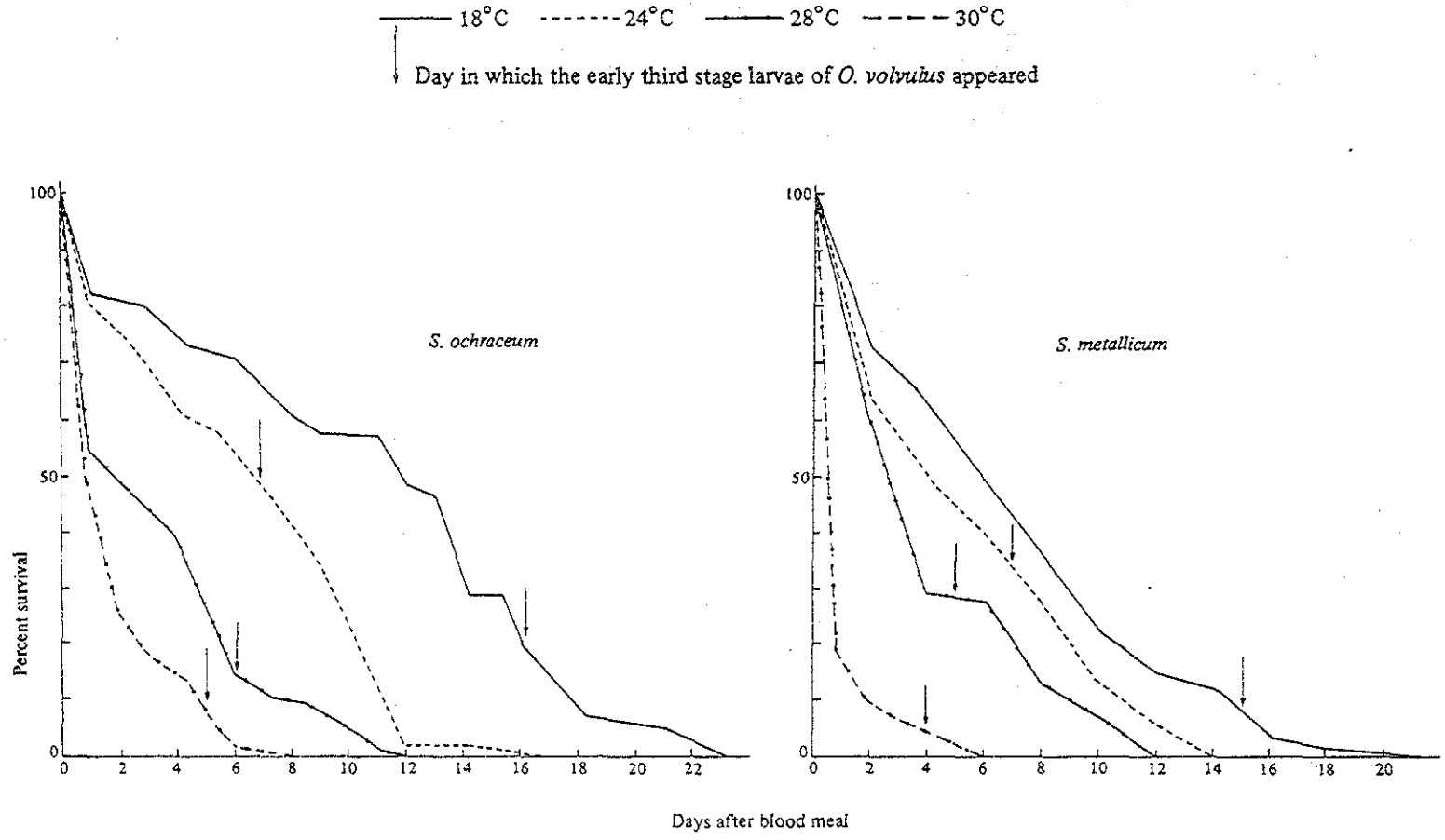

Figure 1 Survival rate of *S. ochraceum* and *S. metallicum* engorged with human blood at different temperatures

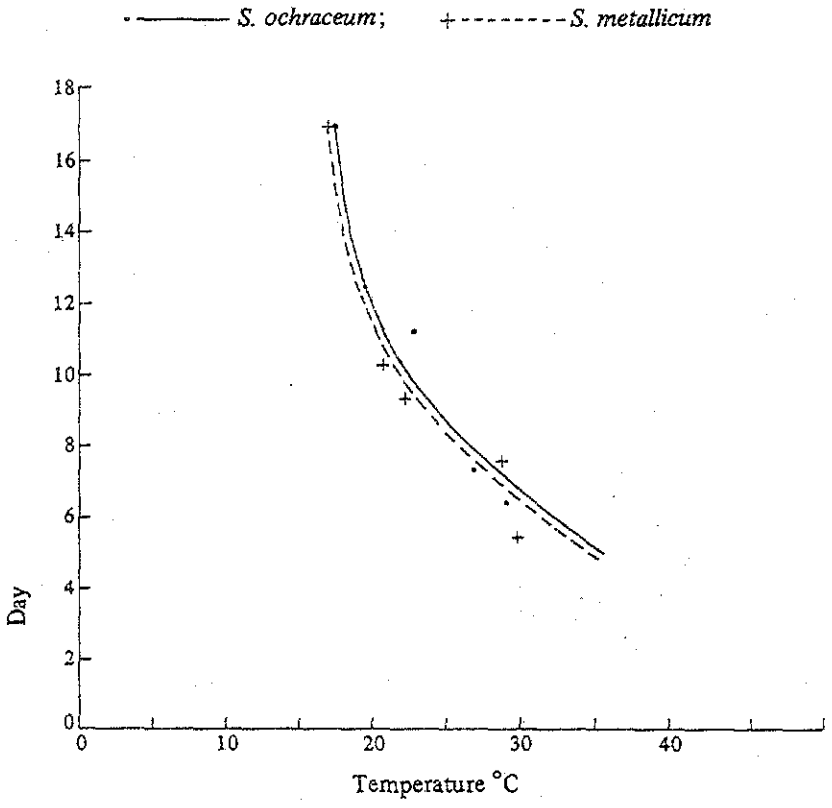

Figure 2 Day in which the early third stage larvae of *O. volvulus* appeared in the head of *S. ochraceum* and *S. metallicum* at different temperatures

RELATIONSHIP BETWEEN BLOOD SUCKING DURATION AND INTAKE OF *ONCHOCERCA VOLVULUS* MICROFILARIAE IN *SIMULIUM OCHRACEUM*

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In order to clarify the intake of microfilariae (Mf) of *Onchocerca volvulus* by *Simulium ochraceum* with the feeding time, the blackflies were caught by human bait collections after the flies fed on patients with *O. volvulus* microfilariae for a limited time. The captured flies were dissected individually and the number of Mf ingested were examined.

The initiation of intake of Mf was around 30 seconds after landing, when 3 out of 43 (7%) flies were found to have ingested one larva each. Thereafter, the number of Mf taken by flies increased as the feeding time became longer (Table 1).

After three to four minutes of landing, 69% of flies have finished taking their blood meal, and the intake of Mf was the highest. Thereafter, increase of feeding time did not increase the intake so much, and the variation in the number of ingested Mf increased as the Mf density of the donners became larger. With the volunteers of moderate density of Mf (55–166 Mf per 10 mm^2), the average number of Mf taken by the flies was correlated with the density of Mf in the human skin samples.

With the volunteer of very low density (1.8 Mf per 10 mm^2), however, an extraordinalily high intake of Mf was observed. This suggested the possibility that the flies are attracting or stimulating the Mf in the skin for being ingested with the blood meal, especially in the case of low density carriers (Table 2).

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Table 1 Duration of sucking time required for satiation with the blood meal in freely-fed flies and the numbers of microfilariae ingested while feeding on a donner

Item	Duration of sucking time							
	1'01" 2'00"	2'01" 3'00"	3'01" 4'00"	4'01" 5'00"	5'01" 6'00"	6'01" 7'00"	7'01" 8'00"	8'01" 9'00"
No. of flies satiated	2	5	18	6	7	4	2	1
Total No. of Mf taken	7	95	1,127	96	632	182	31	130
Mean No. of Mf per fly	3.5	19.0	62.6	16.0	90.3	45.5	15.5	130.0

Table 2 Density of microfilariae in the skin of volunteers, feeding time of *S. ochraceum* for satiation and number of microfilariae taken by the flies in free-feeding experiments

Volunteer No. Name	MfD/10mm³ (GM)	No. of flies tested	Feeding time for satiation		No. of Mf taken		Index*
			Mean	Range	Mean	Range	
1 M.R.C.	1.8	5	5'13"	4'15"–6'55"	31.4	10– 57	17.44
2 S.F.S.	42.1	7	4'22"	3'24"–5'53"	166.7	94–203	3.96
3 A.M.	55.3	8	4'26"	2'45"–7'56"	10.6	4– 22	0.19
4 I.R.	69.0	3	6'09"	4'00'–7'27"	14.0	6– 18	0.20
5 M.M.	77.2	9	3'10"	1'35"–5'00"	27.3	0– 54	0.35
6 F.S.	78.7	10	4'34"	3'10"–6'55"	39.6	3–127	0.50
7 D.S.	115.7	3	4'58"	2'25"–8'35"	69.0	10–130	0,60

* Index: (Mean of No. of Mf taken (MfD)/10mm³)

LONGEVITY AND INFECTIVITY OF *SIMULIUM OCHRACEUM* KEPT IN LOCALITIES WITH DIFFERENT ALTITUDES AFTER EXPERIMENTALLY INFECTED WITH *ONCHOCERCA VOLVULUS*

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Transmission dynamics of onchocerciasis is influenced by many factors, however ambient temperature appears to be dominant in regulating the development of *Onchocerca volvulus* in *Simulium ochraceum*, while affecting the vector's survival probabilities. This study was made to observe the vector's survival ability, in addition to the development of *O. volvulus* larvae within the principal Guatemalan vector, *S. ochraceum* within and outside of onchocerca endemic areas.

Materials and Methods

The three experiments were conducted in November and December 1979 and June 1980. Approximately 1000 female adult *S. ochraceum* were captured from 9–12 a.m. at coffee plantations, Monica Ivoné and Monte Quina, located on the southwestern slope of volcano Atitlán, Department of Suchitepequez. The flies were allowed to fully engorge with human blood from moderately infected microfilarial carriers. Flies were immediately collected after dislodging from the skin, in small 7 cm long plastic tubes, which were partially lined with filter paper and supplied with small cotton balls, soaked in saturated sugar solution, then squeezed-out. The tubes were placed horizontally in plastic boxes, wrapped in damp towels and in experiments I and II, placed in plastic bags. The flies were housed in meterological shelters, set at the four locations, Escuintla (350m), Maria Santisima (650m), Barretal (1250m), and Guatemala City (1500m), along the Central American Highway #9.

Each day thereafter, all sites were visited, recording the number of live flies and removing the dead, which were then temporarily stored in the freezer for later dissection. The flies were dissected in saline solution, using dissecting scope, then transfered to the microscope to observe the quantity and stage of larvae, according to size and morphology, as reported by Duke 1968 and Bain 1969. In addition, follicular development of the ovaries was examined to determine the length of the gonotrophic cycle at each location.

Results and Discussion

As expected, the mean daily temperatures decreased as altitude rose. This was most apparent in the first two experiments performed in the dry season. At all locations, there was considerable range in temperatures throughout the day.

There was a tendency for reduced fly survival at lower altitudes, although this tendency was less noticeable during the rainy season. Low survival could be attributed to high midday temperatures. Since it was reported by Monroy (1979) and Takaoka (1981), that temperatures above 28°C were debilitating to *S. ochraceum*. Also, recent experimental infection work suggests that the maintenance method could be improved.

Among flies dissected from Escuintla, with highest mean temperatures, only late second stage larvae (K) developed on day 7 in exp. I and on day 6 in exp. II. If the flies' survival could have been extended, perhaps third stage larvae would have been encountered on the following day in each case. In exp. III, third stage larvae were observed on the 8th day. Although larval development seems to be accelerated at low altitudes, the flies survival is reduced.

In Maria Santisima, with moderate temperatures, third stage larvae developed on day 8 in exp. I, day 9 in exp. II, and day 10 in exp. III. It is interesting to note that the mean temperature range at Maria Santisima coincides with the optimum temperatures for the vector's infectivity with *O. volvulus* from previous laboratory experiments.

At higher altitudes, larval development was slow, although fly survival was somewhat extended. No larvae developed beyond late first stage (H) in Barretal. In Guatemala, only first stage larvae were encountered, except in a single fly, which died on day 16 in exp. I and harbored late second stage larvae (J & K). This could suggest that even at low temperatures, *O. volvulus* may eventually develop to the third stage, if the fly is capable of surviving the extended period.

Ovarian follicular development was least influenced by environmental temperatures at these varying altitudes. The length of the gonotrophic cycles was short, only three days, irrespective of location. In natural conditions at high altitudes, the expected fly survival may be reduced due to the increased risk of exposure to the hazards in nature, especially when the female oviposits and takes subsequent blood meals. Since more gonotrophic cycles might be repeated before the female becomes infective, the probability of transmission is reduced.

From these experiments, the great temperature variation within the endemic area, according to altitude is more pronounced during the dry season, while during the rainy season, May through October, temperature range is decreased. There is individual variation of *O. volvulus* larval development within each *S. ochraceum* fly in the groups of flies at the distinct locations. The vector's survival capabilities differ depending on altitude and the speed of *O. volvulus* larval development appears to be affected by temperature, as suggested by laboratory experiments.

Further epidemiological surveys on transmission studies in the field could clarify the relationships among the parasite, host, vector and environment. Improved understanding of various aspects of onchocerciasis transmission dynamics may reveal more effective and efficient control measures in the future.

Table Percentage survival and larval development at four locations

	Locality	Altitude	Temperature (°C)			Day when larvae first appear larval stage			Percentage survival days post-ingestion				
		(m)	mean	max.	min.	I	II	III	6	7	8	9	10
Exp I Nov.'79	Escuintla	350	27.2	32.2	21.8	2	7	–	0	0	0	0	0
	Ma. Santisima	650	22.8	30.8	17.0	3	6	8	14.8	8.6	4.9	4.9	2.5
	Barretal	1250	17.9	23.0	13.0	5	–	–	40.2	36.8	32.8	19.5	14.9
	Guatemala	1500	17.2	27.6	11.2	1	16	–	13.3	9.6	9.6	9.6	8.4
Exp II Dec. '79	Escuintla	350	27.1	35.0	21.0	1	6	–	0	0	0	0	0
	Ma. Santisima	650	24.2	31.6	17.0	2	7	9	6.3	4.7	4.7	1.6	1.6
	Guatemala	1500	16.8	26.2	8.6	1	–	–	25.8	21.2	12.2	7.5	7.5
Exp III Jun. '80	Escuintla	350	26.6	32.5	21.5	2	4	8	31.0	15.0	5.3	3.5	2.6
	Ma. Santisima	650	24.4	33.0	19.0	3	7	10	19.0	7.8	5.2	2.6	1.7
	Barretal	1250	20.6	29.0	15.5	3	–	–	36.3	20.4	8.8	2.7	0.9
	Guatemala	1500	20.2	28.5	15.0	3	–	–	29.5	9.8	7.4	4.9	3.3

Schiller

¿*Tiene algún efecto la altura del sitio de* recolección sobre el subsiguiente desarrollo larval en las diferentes alturas experimentales?

Hansen

El efecto de cambiar el medio ambiente de *S. ochraceum* infectado experimentalmente es un aspecto interesante, peor que no examinamos en nuestro trabajo.

Schiller

Does the elevation of the collection site have any effect on subsequent larval development at the different experimental altitudes?

Hansen

The affect of changing the environment of experimentally infected *S. ochraceum* is an interesting aspect, which we did not examine in our work.

INFECCION NATURAL DE LAS MOSCAS NEGRAS EN EL AREA PILOTO DE SAN VICENTE PACAYA, GUATEMALA

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INTRODUCCION

Especies del Género *Simulium ochraceum, metallicum* y *callidum* han sido reportados como los vectores de la *Onchocerca volvulus* en Guatemala. Las tres especies son capaces de experimentalmente soportar el desarrollo competo del parásito como lo demostró De León y Duke (1966). Estudios recientes Garms (1975), Garms y Ochoa (1979) y Collins (1979) han demostrado que el *S. ochraceum* es el principal y único vector significativo y dominante en el país. Solamente en esta especie la tasa de infección fué asociada con la población humana y ninguna infección fué encontrada en áreas sin oncocercosis. *S. metallicum* y *S. callidum* que tienen predominancia zoófilica aún queda incierta su importancia como vectores en ausencia de *S. ochraceum.* Investigaciones preliminares como lo reporta Garms y Ochoa (1979) revelan que tasas de infección natural de estas especies fueron muy bajas e independientes de aquellas de la población humana. La infección natural de *S. metallicum* en áreas sin oncocercosis humana sugiere que algunas o por lo menos las infecciones no fueron de *O. volvulus* y se originaron de huéspedes no humanos (Garms 1975).

MATERIAL Y METODOS

Las investigaciones fueron hechas en la Finca Peña Blanca, del municipio de San Vicente Pacaya del Departamento de Escuintla, situada en los declives del Volcán de Pacaya a una altura de 750 metros sobre el nivel del mar, con una población de 5 habitantes permanentes y 15 más que se reunen durante la cosecha del café de octubre a diciembre. El estudio se realizó durante un año consecutivo, en 1977.

Las colecciones de simúlidos se hicieron utilizando un cebo humano y las moscas que aterrizaron en el cebo, el cuál tenía las piernas y la parte superior del cuerpo desnudo, fueron capturados por dos colectores. Las colecciones se hicieron durante 12 horas consecutivas de las 0600 a las 1800 horas; dos o tres colecciones se hicieron por mes. Todos los simúlidos capturados en el cebo humano, sin picar fueron guardados separadamente en tubos individuales para contaje, clasificación y disección. La disección se hizo en solución salina, primero para determinar paridad y después para ver presencia de parásitos; la paridad fué determinada por la técnica de Garms (1975) utilizando microscopio estereo con magnificación de 50 X.

RESULTADOS

La tasa de hembras paríparas de *S. ochraceum* fué de 8.6 a 43% a través del año. El patrón de la tasa de paridad durante el día, resultó baja en la mañana y alta en la tarde. *S. metallicum* mostró un patrón distinto y reversivo comparado con *S. ochraceum.* La tasa de paridad mensual en *S. ochraceum* mostró que el pico aparece cada dos meses, con tendencia a subir en la época lluviosa.

Un total de 46 larvas metacíclicas infectantes de *Onchocerca volvulus* fueron obtenidas en 16 ejemplares de *S. ochraceum*. El largo de estas larvas montadas en glicerol variaron entre 540 y 570 micrones. Todas las larvas fueron encontradas en la cabeza. La tasa más alta de infección en *S. ochraceum* fué de 11% y la tasa de infectividad de 1.6%.

El patrón de la tasa de infección y la tasa de infectividad fué muy similar, es decir; el pico apareció casi cada dos mess con tendencias ascendente en la época seca. La tasa de infectividad durante el año fué inesperadamente baja 0.25 y 1.6%. Sin embargo esta tasa es similar a las encontradas en diferentes localidades de la zona endémica por Garms (1975), Garms y Ochoa (1979) y Collins (1979).

No se encontraron larvas de tercer estadio de *O. volvulus* en *S. metallicum* pero no obstante filarias de origen desconocido fueron encontradas en la cabeza de tres ejemplares cuyo largo varió entre 700 y 800 micrones. Se encontraron también otras tres larvas de filaria fácilmente distinguidas de las de *O. volvulus*, por la presencia de tres caudales protuberantes.

La presencia de ecto y endo-parásitos se observó en los ejemplares capturados. Las especies afectadas fueron *S. metallicum* (2.0%), *S. callidum* (3.7%) en las cuales se encontró nematodes de la familia Mermithidae en los tubos de Malpighi. La presencia de hongos en el ovario, posiblemente Phyconiycetos se encontró; dos de estos ya reportados por Garms (1975) para Guatemala y dos más aún por clasificar. También el 3.2% y el 3.4% de acaros fué observado en *S. ochraceum* y *S. metallicum*, respectivamente. Cuatro especies fueron colectadas sobre cebo humano: *S. ochraceum*, *S. metallicum*, *S. callidum* y *S. downsi*. El *S. ochraceum* fué la especie predominante, durante la estación seca.

CONCLUSIONES

El resultado del presente estudio está muy relacionado con los estudios realizados por Garms (1975) porque se confirmó las consideraciones epidemiológicas en la que se sugiere que *S. ochraceum* es la especie principal y predominante en Guatemala . La tasa de infectividad más alta fué de 1.6% y ocurrió en el mes de septiembre. La tasa promedio anual de hembras paras fué de 42.9%. Todas las larvas encontradas en *S. ochraceum* fueron morfologicamente in distinguibles de *O. volvulus*.

En contraste a *S. ochraceum* la tasa de infección en *S. metallicum* y *S. callidum* fué muy baja; la infección no se consideró de origen humano, debido a su marcada tendencia zoófilica y a la imposibilidad en clasificación. Ninguna larva infectiva fué encontrada en *S. metallicum* durante este estudio. Ya ha sido discutido por Dalmat (1955) que *S. metalliucm* probablemente sea un vector de *Onchocerca* spp. en caballos y ganado vacuno, de alta prevalencia en Guatemala (Gibson 1966). Hashiguchi informó en persona. En conclusión se ha comprobado que *S. ochraceum* es el único vector de la *O. volvulus* y que *S. metallicum* aún es incierta como vectora. Si esta última especie juega un papel en la transmisión de la *O. volvulus* sería muy relativo y de poca importancia epidemiológia, porque el factor que faculta su exclusión, es la tendencia zoófilica, que le permite picar antes a los animales, que al hombre.

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THE PRESENT SITUATION OF THE REINVASION BY *SIMULIUM DAMNOSUM* s.l. OF THE ONCHOCERCIASIS CONTROL PROGRAMME IN THE VOLTA RIVER BASIN AREA IN WEST AFRICA

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The Onchocerciasis Control Programme (OCP) started its aerial operations against the larval stages of the *Simulium damnosum* complex in February 1975 in Phase I (northern Ivory Coast, western Upper Volta, eastern Mali). Shortly later fly densities declined rapidly in the whole area. However, at the end of the dry season increasing numbers of *S. damnosum* s.l. females reappeared at some river valleys, particularly in the SW of Phase I. Intensive surveys, which were carried out at once, did not detect failures of treatments or breeding sites which had not been known before, which could account for the high number of biting flies. It was concluded that the flies were immigrants from sources outside the OCP (Walsh 1977). The reinvasion continued throughout the rainy season and occurred again during the wet season of the following years. When in 1976 the programme area was extended into northern Ghana and central Upper Volta (Phase II) and in 1977 into northwestern Ivory Coast and western Mali (Phase III West) these extensions had no influence on the reinvasion in Phase I, but a reappearance of flies was also now observed along some rivers in Mali. A similar phenomenon occurred in the east, in Togo and Benin, where control operations had started in the second half of 1977. According to the OCP evaluation report of 1978 less than 30% of the total area of the OCP were affected by reinvasions.

A detailed account of the studies carried out between 1975 and 1978 to explain and overcome the reinvasion of the south-western regions of the OCP was given by Garms et al. (1979) and Le Berre et al. (1979). The majority of flies caught in the reinvaded areas were parous females, and a high proportion of them carried 3rd stage filarial larvae indistinguishable from those of *Onchocerca volvulus*. Main components of the reinvading fly populations were the savanna cytospecies *S. damnosum* s. str. and *S. sirbanum*. Forest cytospecies *S. sanctipauli*, *S. soubrense* and *S. yahense* rarely occurred at catching sites deep inside the OCP area. The sources of the reinvading flies therefore had to be sought in areas where breeding sites of the savanna cytospecies were highly productive at the end of the dry and during the rainy season. Surveys carried out between 1976 and 1978 revealed that both cytospecies were widely distributed in river systems S and SW of the OCP. Following experimental treatments of some of these rivers which were thought to be potential sources biting densities declined significantly in Phase I. With the extension of the OCP in 1979 into the southern Ivory Coast, which covered all areas in this country where *S. damnosum* s. str. and *S. sirbanum* were known to breed, the reinvasion into Phase I ceased, but biting densities in Phase III West remained high (Walsh et al., in press). Presumably these flies are invaders from breeding sites west to the OCP where no control operations are carried out yet.

Results indicate that movements of flies are mainly in a SW-NE direction. It is assumed that the movements are wind-supported and are closely associated with the northward shift of the Intertropical Convergence Zone and the monsoon winds. High numbers of flies may appear at distance of up to 300 km from their sources and many may be carried much further.

Studies on the reinvasion in Togo and Benin were carried out in 1979 and 1980 (Garms, Cheke and Kerner, unpublished reports). The species composition of reinvading fly populations was different from those observed in the western areas of the OCP. While in Togo *S. squamosum* was by far the predominant cytospecies, the proportion of *S. damnosum* s. str. and *S. sirbanum*

within the invading fly populations increased further to the east, and the reinvasion into Benin was almost exclusively by the savanna cytospecies. Breeding sites of *S. squamosum* were found to be confined to mountainous regions and to rivers close to the hills to the south of the OCP boundary. It can be assumed that a treatment of these areas will suppress the reinvasion by *S. squamosum*. Since also in Togo and Benin the direction of the prevailing winds during the rainy season is from the SW to the NE the productive breeding sites of large rivers in the south of these countries are the potential sources of the reinvasion by *S. damnosum* s. str. and *S. sirbanum*.

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Schiller

¿Existen planes para extender el programa de control a Liberia o es que esta área no representa ninguna amenaza de re-invasión?

Garms

Se encontraron criaderos de *S. damnosum* en algunos ríos de Liberia. Por lo tanto, no se puede excluir que las moscas que invaden el área del OCP sean originarias de ese país. En la actualidad la OMS está llevando a cabo estudios en la cenca fluvial de Senegal para investigar la factibilidad de llevar a cabo programas de control en partes de Mali, el Senegal, Guinea, Sierra Leone y Guinea Bissau. Pero no se que exista ningún plan para el control de la oncocerciasis en Liberia. Sería deseable llevar a cabo un control de vectores por lo menos en aquellas partes de Liberia en que se reproducen las citoespecies de sabana.

Hayashi

Dr. Garms, piensa usted que sea posible descontinuar a aminorar las actividades de control en la parte central del área del OCP de poder resolver el problema de reinvación en las zonas fronterizas? Espero que de ser esto posible, puedan poner más recursos hu-

Schiller

Are there plans for extending the control program into Liberia or does this area not represent a threat for re-invasion?

Garms

Breeding sites of *Simulium damnosum* s. str. were found some rivers in Liberia. Therefore it cannot be excluded that flies invading the OCP area originate from this country. Studies are carried out at present by WHO in the Senegal basin to investigate the feasibility of control programmes in parts of Mali, Senegal, Guinea, Sierra Leone, and Guinea Bissaou. But I don't know of any plans to control onchocerciasis in Liberia. It would be desirable to carry out a vector control at least in those parts of Liberia, where savanna cytospecies are breeding.

Hayashi

Dr. Garms, do you think it is possible to discontinue or slow down the control activities in the central part of OCP area in so far as you can manage to solve the reinvasion problem in the boundary zones? I hope, if this is possible, you can put more man power and budget to the expansion of control areas. The second

manos y monetarios en la expansión de las áreas de control. La segunda pregunta es respecto al temor de rehabilitatión y/o repoblación del vector en la parte central del OCP una vez hayan cesado las actividades de control allí. ¿Cuál es opinión?

Garms

Debido a la gran habilidad migratoria de las citoespecies de sabana del complejo *S. damnosum* es de esperar que el vector volvería a poblar rápidamente el área del OCP después de la descontinuación de las operaciones de control. Por lo tanto, las medidas de control se tienen que continuar hasta que el parásito haya desaparecido. Sin embargo, la experiencia ha demostrado que el aumento inicial de las poblaciones de moscas podría ser lento. Así es que ya ha sido posible interrumpir o recortar las operaciones durante ciertos períodos, especialmente durante la estación seca, en las áreas centrales del OCP. Ampliaciones adicionales del programa serían extremadamente ventajosas ya que un área central mucho más grande estaría protegida de una rápida reinvasión y repoblación del vector. Esto permitiría una considerable reducción de las medias de control en estas áreas.

Ochoa

¿Cual fue la razón de invasión de áreas no tratadas hacia areas tratadas, fue aire o falta de alimentación en la zona?

Garms

La migración de las especies de sabana del complejo de *S. damnosum*, según se supone, es un comportamiento natural que les permites repoblar temporalmente los criaderos de la estación lluviosa a lo largo de los ríos estacionales de sabana en el Africa Occidental.

Undeen

Ud. mencionó que las aplicaciones de control se podrían reducir en el área del OCP después de que el parásito comience a desaparecer. Ayer, el Dr. Philippon, según me pareció, indicó que el objetivo era reducir la transmisión por debajo del nivel que causa daños oculares.

question is about the fear of rehabilitation and/or repopulation of vector in the central part of OCP when the control activities were stopped there. What is your opinion?

Garms

Owing to the great migratory ability of the savanna cytospecies of the *Simulium damnosum* complex we can expect that the vector would rapidly repopulate the OCP area after discontinuation of the control operations. Therefore, the control measures have to be continued until the parasite has disappeared. However, experience has shown that the initial build up of the fly populations may be very slow. So it was possible already to interrupt or to cut back the operations for certain periods, especially during the dry season, in central areas of the OCP. Further extensions of the programme would be extremely advantageous, because a much larger central area would be protected from rapid reinvasion and repopulation by the vector. This would allow a considerable reduction of control measures in these areas.

Ochoa

What was the reason for the invasion from non-treated areas to treated areas? Was it the air or lack of food in that area?

Garms

The migration of the savanna species of the *Simulium damnosum* complex is assumed to be a natural behaviour which enables them to repopulate temporary wet season breeding sites along the seasonal savanna rivers in West Africa.

Undeen

You mentioned that control applications could be reduced in the OCP area after the parasite begins to die out. Yesterday, Dr. Philippon seemed to say that the objective was to reduce the transmission below the level which causes occular damage. What is the program objective?

Garms

I said already that it can be expected

Cuál es el objectivo del programa?

Garms

Ya anteriormente dije que se puede esperar que el vector volvería a poblar el área del OCP después de una descontinuación de las operaciones de control. Por lo tanto, tenemos que continuar el control hasta que el parásito se extinga. Las cifras de 100 larvas infectivas o 1000 moscas por hombre/año mencionadas ayer por el Dr. Philippon son límites máximos definidos por un grupo Científico de Trabajo que se reunió en Ginebra para evaluar el Criterio Biomédico para un re-asentamiento en las áreas del OCP, sin que hubiese ningún peligro de desarrollar lesiones oculares serias e irreversibles. Una descontinuación del control del vector en esta etapa, ciertamente sería seguida de un aumento rápido de las tasas de infeción en la población humana.

that the vector would repopulate the OCP area after a discontinuation of the control operations. Therefore we have to continue the control unitl the parasite dies out. The figures of 100 infective larvae or 1000 flies per man per year mentioned yesterday by Dr. Philippon are maximum limits defined by a Scientific Working Group which met in Geneva to assess the Biomedical Criteria for a resettlement in the OCP area. It is assumed that at this level a human population could live in OCP areas without being endangered to develop serious or irreversible eye lesion. A discontinuation of the vector control at this stage certainly would be followed by a rapid increase of infection rates in the human population.

TRANSMISSION OF ONCHOCERCIASIS IN GUATEMALA: RECENT STUDIES AND THEIR IMPLICATIONS FOR VECTOR CONTROL

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Recent studies by serveral different research groups from Guatemala, Japan, Germany, France, and the Untied States have provide much new information about 2 different aspects of the transmission of *Onchocerca volvulus* in Guatemala. These aspects are the identification of the vector species and the vectorial capacity of the vectors.

The purpose of this paper is to review this new information and to suggest what it might mean for the changes of a successful onchocerciasis control program in Guatemala based on vector control.

Identification of the Vector

Some of this information is not new as it was presented at the onchocerciasis meeting here in Guatemala in October 1977. The general conclusion was that *Simulium ochraceum* is the major, but not the only vector species, and it is probably the only one that needs to be considered for purposes of control, at least in the areas studied so far. However, a close inspection of the published data sounds a word of caution. In the Pochuta-Yepocapa area, the number of *S. metallicum* collected from human attractants was about the same as *S. ochraceum* (Garms, R. 1975; Garms, R. and J. Ochoa 1979). However, on the southeastern slope of Volcan Atitlan at Fincas Los Tarrales-El Vesubio, 87% of all flies collected were *S. ochraceum* (Collins, R.C. 1979). It is also worthwhile to note that Garms and Garms and Ochoa made their collections during January–April or September–December while collections by Collins were made throughout the year. Thus, the composition of collections can vary considerably both with collection site and time of collection. All three authors reported substantial and consistant infection rates in *S. ochraceum* collected within endemic zones but also found infective larvae in *S. metallicum* and *S. callidum*. Similar results were presented by Dr. Porter in this meeting, i.e. consistant and relatively high infection rates in *S. ochraceum* with an occasional infective larvae found in *S. metallicum* and *S. callidum*. Infection rates in these latter 2 species particularly in *S. callidum* are alarming unless they are considered together with their respective human biting rates as shown in Table 1 (Collins, 1979). Here the total number of flies of 4 species are given together with the estimated number of parous, and infective flies in the populations and the theoretical number of infective larvae available for transmission. For *S. ochraceum* these are 25,622, 9,255, 79 and 174 respectively. Even though 0.14% of all *S. callidum* collected had infective larvae, there were so few individuals biting man at this location, that it is unlikely to be contributing significantly to transmission of *O. volvulus*. These infection rates in species other than *S. ochraceum* however, should put us on the alert for areas where populations of these species are more anthropophilic or abundant.

Vectorial Capacity

While *S. ochraceum* has been proven to be the major vector species in Guatemala, it is a poor host for *Onchocerca volvulus*, as compared to *S. damnosum* as shown in Table 2. These are

data from experimental feedings on infected volunteers done by DeLeón and Duke (1966). *Simulium ochraceum* ingested an average of 390 microfilariae (mf) and produced only average of 2.5 infective larvae for an average development of 0.65%. In other words, over 99% of the ingested mf were eliminated. With *S. damnosum* the number of mf ingested was only 11, but about half of them succeeded in developing to infective larvae.

Factors limiting the infection of *S. ochraceum* by *O. volvulus* were first studied by Bain, Desset and R. DeLeón (1974). These authors described the phenomena of proportionality where the number of mf penetrating the gut of the fly and reaching the haemocele is proportional to the total number ingested. Their studies also indicated that a minimum number of mf must be ingested before any mf will reach the haemocele and undergo development to infective larvae. Also, even when large numbers of mf are ingested by *S. ochraceum*, the proportion reaching the haemocele rarely exceeds 1.5% of the total ingested mf. Somehow *S. ochraceum* is able to eliminate the majority of the ingested mf. Omar and Garms (1975) explained this elimination of mf by showing that the ciberial armature of *S. ochraceum* kills many mf during the ingestion of the blood meal.

According to Bain (1976) an entirely different type of relationship exists between the savanna forms of *S. damnosum* and *O. volvulus*. Here, the proportion of ingested mf reaching the haemocele varies inversely with the total number of mf ingested. For example, she started that when the total mf intake is from 1–3, approximately 1/3 to 1/2 of these mf will reach the haemocele and develop to infective larvae, whereas if mf intakes reach 100–150 mf, only about 3.5% will reach the haemocele. Duke and Lewis (1964) also reported that with the forest strains of *S. damnosum* and *O. volvulus*, about 40% of all ingested mf will develop to infective larvae.

Two other studies on the phenomenon of proportionality are of epidemiological significance. Collins *et al.* (1977) showed that people with low levels of mf in the skin were not effective reservoirs of infection for *S. ochraceum*. Flies fed on lightly infected persons ingested an average of 1.7–12.5 mf/fly but 95–98% of flies surviving to the infective age did not have infective larvae and the mean number of infective larvae per surviving fly ranged from 0.02–0.04. Campbell *et al.* (1981) repeated this study in 10 patients and showed that the minimum mf intake required to produce infective larva was provided by people with at least 14 mf/mg of skin. These results indicate that chemotherapy, perhaps DEC to reduce the number of skin mf should also reduce transmission.

Finally, studies by Dr. Porter have shown that the annual biting density of *S. ochraceum* can reach 8700 flies/man in an area where prevalence of skin infection is 33% but where careful ophthalmologic examination has shown that there is virtually no eye disease due to onchocerciasis. This suggests that a reduction in the number of *S. ochraceum*, rather than eradication of the fly could reduce transmission below levels causing onchocercal eye disease. This same study has shown distinct seasonal variation in infection rates in *S. ochraceum* with highest rates occurring during the late dry season when fly populations are at their lower levels. This too, increases the chance for a successful control program, as larvacides could be applied to have their greatest effectiveness when the number of streams producing *S. ochraceum* is at the minimum.

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Table 1 **Biting densities and transmission potentials of *Onchocerca volvulus* by four species of black flies at Los Tarrales, Guatemala, during 52 man/day of collection from October 1976 to November 1977**

Data on	*Simulium*			
	ochraceum	*metallicum*	*callidum*	*downsi*
Number of flies				
Dissected	4,851	1,855	702	503
Parous	1,752	396	166	86
With 1st or 2nd stage larvae	69	23	3	1
With infective larvae*				
O. volvulus	15(2.2)	0	1 (1.0)	0
Other filariae	0	4(7.5)	9	2(3.0)
Rate (percent)				
Parous	36.12	21.35	23.65	17.10
With 1st or 2nd stage larvae	1.42	1.24	0.43	0.20
Infective (*O. volvulus*)	0.31	0	0.14	0
Biting density†				
Total	25,622	2.486	786	566
Parous	9,255	531	186	97
Infective (*O. volvulus*)	79	0	1	0
Transmission potential‡	174	0	1	0

* Average number of infective larvae per infective fly in parentheses

† Estimate equals total flies landing during 52 man/day of collection

‡ Total number of infective larvae available for transmission, rounded off

Table 2 **Desarrollo de *Onchocerca volvulus* en *Simulium ochraceum* y en *Simulium damnosum* (sensu latu)[1]**

Cepa de *O. volvulus*	Mf por Mg piel	Promedio de Mf Ingeridas	Promedio de larvas Metaciclicas	% de Mf desarrollo a larvas Metacilicas
		Simulium ochraceum		
Guatemala	183	390	2.53	0.65%
		Simulium damnosum		
Selva de Africa	150	11	5.12	46.55%

[1] Tomado de DeLeon, R.& B.O.L. Duke, 1966

Martinez

Usted mecionó que el *S. ochraceum* no desarrolló larvas infectivas cuando ingirió las mismas de pacientes con menos de 14 mf por biopsia de piel? Si ese umbral se mantienne por debajo en una comunidad oncocercosa, se pudiera cortar la tramsmisión on dicha localidad?

Collins

En los 10 pacientes que estudiamos, personas con menos de 14 mf/mg de piel en biopsias no produjeron *S. ochraceum* con larvas infectivas creo no podemos interpretal el número 14 tan estrecho o exacto. Lo que podemos decir es que por lo general, personas con menos mf en la piel no son reservorios eficientes para la infección de *S. och.* y medidas para reducir el nivel de mf. va a reducir la transmisión. Tenemos que llevar a cabo más estudios para definir conmás precisión el umbral mínimo de mf en la piel para la infección de *S. ochraceum.*

Collins

Dr. Hayashi, su tasa crítica anual de picadura calculada en aproximadamente 8000 *S. ochraceum* por hombre por año concuerdan con la tasa anual de picadura observada por el Dr. Porter en la Finca El Jardín de 8700, donde el 33% de los residentes permanentes muestran microfilarias en biopsias pero donde casi no existen enfermedades oculares debido al *O. voluvulus.* Es muy alentador ver esta concordancia.

Martinez

You mentioned that *S. ochraceum* did not develop infective larvae when it ingested some from patients with less than 14 mf per skin biopsy. If that threshold could be maintained at that low level in an onchocercotic community, would it be possible to stop transmission in that place?

Collins

In the 10 patients studied, those with less than 14 mf/mg skin in biopsies did not produce *S. ochraceum* with infective larvae. I believe that we cannot interpret this number of 14 mf in such a narrow or exact manner. What we can say is that, in general, people with less mf in the skin are not efficient reservoirs for infection of *S. ochraceum,* and that measures to reduce the level of mf will reduce transmission. More studies have to be carried out to define with greater precision the minimum threshold of mf in the skin for *S. ochraceum* infection.

Collins

Dr. Hayashi, your calculated critical annual biting rate of approximately 8000 *S. ochraceum* per man per year is in close agreement with Dr. Porter's observed annual biting rate of 8700 at Finca El Jardin where 33% of the permanent residents have microfilariae in biopsies, but where there is almost no eye disease due to *Onchocerca volvulus.* It is very encouraging to see this agreement.

TRANSMISSION DYNAMICS OF ONCHOCERCIASIS BY *S. OCHRACEUM* IN THE PILOT CONTROL AREA IN GUATEMALA

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The Onchocerciasis Control Project in Guatemala was started in 1975, and many basic data have been accumulated. However, the transmission of onchocerciasis is affected by many complicated factors, therefore the dynamic aspects of the epidemiology are usually difficult to be explained. In such a case, the theoretical approach is often very useful for better understanding of the epidemiology. For this reason, the data collected by the Project was theoretically analyzed.

Firstly, the simple catalytic model (Muench, 1959) was applied to the age distribution of microfilaria positive rate in each village to obtain the force of infection. If we assume that the turning rate of persons negative for microfilariae to a positive state is constant irrespective of ages,

$$dy/dt = r(1 - y)$$

holds true, where y is microfilaria positive rate, t is age and r is the contstant showing the force of infection. It can be expressed in another form,

$$y = 1 - \exp(-rt).$$

The values of r in the catalytic model in individual villages were calculated from the values of positive rate with the age groups. It was found that the values of r thus obtained were very clearly related to the microfilaria positive rates of individual villages, and the relation can well be expressed by a theoretical curve.

$Y_0 = 1 - (1 - \exp(-70r))/70r$, where Y_0 is the microfilaria positive rate of a village. Therefore, from the above formula, we can estimate the force of infection in terms of r for a village, if the microfilaria positive rate is obtained.

Next, the difference in microfilaria positive rates between males and females was examined. The value of r was estimated from the microfilaria positive rate for each sex in a village, then the ratio of r in males to r in females was calculated. If the ratio is nearly equal to one, it is indicated that males and females are receiving approximately the same intensity of infection, and if the ratio is much greater in the former, higher intensity of infection in males than in females is suggested. It was indicated that in the periphery of the Pilot Area, there existed villages with higher intensity of infection in the males, while in villages in the central part the males and the females were found to be receiving nearly equal intensity of infection.

The difference in the infection intensity between the sexes can well be understood by the mode of distribution of the vector, *Simulium ochraceum*. In the peripheral villages, the males usually go more frequently to the mountainous area with high density of *ochraceum* bites to take care of coffee trees. Therefore, the higher infection rate in males can be explained by more frequent exposure to infection. On the other hand, in the central part of the Area, the *ochraceum* density is high within the villages and the exposure to infection is estimated to be nearly equal in both sexes. It seems that by comparing the infection rates between the sexes in a village, we can estimate the site where the infection is actually taken place.

In the earlier sections, the relation between microfilaria positive rate and the force of infection in a village was mentioned. It is presumed that this force of infection is proportional to the density of vectors as well as to the positive rate of microfilariae. Based on this assumption, we can calculate the vector density index, if the value of r is given. It was shown, from the relation between the vector density index and the microfilaria positive rate in individual villages, that in villages with high endemicity the microfilaria positive rate is expected to become abruptly low with the fall of

vector density index. From these results, a value of 0.0286 was obtained as the vector density index for non-existence of onchocerciasis. This value of the critical vector density may be modified through further studies, but the evidence of the presence of a critical density for non-existence of onchocerciasis would be useful in the understanding of epidemiology of this disease.

Other available data have shown that the mean number of *ochraceum* attracted to a man in a day was 52.3 in a village, where the vector density index was estimated to be 0.0716. Therefore, we can convert the vector density index to the actual number of vectors being attracted to a man by multiplying 730, a factor obtained by dividing 52.3 with 0.0716. It is thus assumed that onchocerciasis does not exist when the density of *ochraceum*/man/day is less than 21.

From the critical vector density for non-existence of onchocerciasis, 21/man/day, we can calculate the critical annual biting rate, ABR (Walsh et al., 1978). The ABR is defined as the total number of vectors coming to bite one man in a year. The critical ABR was obtained as 7665 by multiplying 21 by 365, the number of days in a year. This critical ABR in Guatemala is much greater than that of 1000 being estimated by OCP in West Africa. The greater value of the critical ABR in Guatemala suggests that the transmission efficiency is lower in *S. ochraceum* in Guatemala than in *S. damnosum* in West Africa.

Another criterion used for vector control in OCP is the annual transmission potential, ATP (Duke, 1968), which is defined as the total number of infective larvae of *Onchocerca volvulus* carried by the vectors biting per man per year, from which the ABR is calculated. The data for natural infections of *ochraceum* in the Pilot Area of Guatemala are still insufficient, but it was reported that the infective rate of vectors was 0.0018 and the mean number of infective larvae per vector was 3.5. By using these values, we obtain 48 as the critical ATP for non-existnece of onchocerciasis. This value is not very much different from 100 that is estimated as the critical ATP in OCP of West Africa.

I would like to express my sincere thanks to Dr. Juan José Castillo Orellana, Dr. Hiroshi Takahashi and all other Guatemalan and Japanese members of the Project, who helped this study in various ways.

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TRANSMISSION OF *O. VOLVULUS* BY *S. OCHRACEUM* – SPATIAL, SEASONAL AND DIEL PATTERNS

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Since the discovery of onchocerciasis in Guatemala, black flies have been implicated in the transmission of the disease. A number of recent studies have shown that the natural infection rate of *Onchocerca volvulus* (Leukart) is substantially greater in *Simulium ochraceum* Walker than in any of the other common anthropophilic species.

We have recently investigated the relationship between the biting density of *S. ochraceum* and the transmission of *Onchocerca volvulus* at four localities with differing levels of onchocercal infection and eye disease. For the four communities selected, the percent of residents infected and the percent with eye lesions were, respectively, Santa Isabel 90%, 7.3%; Los Tarrales 67%, 5.4%; El Jardin 32%, 0.7%, Panimaquib 13.8%, 0.0%. Semimonthly catches of black flies using human attractants were carried out at the four localities over a 14-month period. Depending upon sample size, either all or a percentage of the black flies taken in each catch were dissected for parity determination. Parous specimens were then further dissected for the presence of filaria larvae. Over 42,000 females of *S. ochraceum* were captured and 84% were taken at Santa Isabel. More than 14,000 specimens of *S. ochraceum* from this locality were dissected, and infective larvae indistinguishable from *O. volvulus* were found in 38 (0.25%). A significantly higher percentage of those specimens taken in the housing area (0.31%) than in the coffee plantings (0.11%) had infective larvae. Although the density of *S. ochraceum* at Los Tarrales was about one-sixth of that at Santa Isabel, the percentage of flies with infective larvae (0.23%) was very similar to that observed at the latter finca. At Panimaquib and El Jardin, the density of *S. ochraceum* was only 1% and 2%, respectively, of that at Santa Isabel. Infective stage larvae indistinguishable from *O. volvulus* were found in *S. ochraceum* on one occasion at El Jardin but were never encountered at Panimaquib.

At Santa Isabel there was a distinct seasonal fluctuation in the percentage of flies with infective larvae. The highest rates occurred during the period from late February through March in both 1979 and 1980. This period coincides with the dry season and a rapidly declining density of adult *S. ochraceum.*

Very little fluctuation was observed between approximately 07:00 and 16:00 hours in the magnitude of biting activity by parous females of *S. ochraceum.* Likewise, infected females were encountered at a rather constant rate throughout this time interval.

A small percentage of transmission may be attributed to *S. metallicum sensu lato* as first, second, and infective stage larvae indistinguishable from *O. volvulus* were observed in this species on a few occasions.

GENERAL VIEW OF THE ONCHOCERCIASIS VECTOR CONTROL TRIAL IN THE PILOT AREA OF GUATEMALA – PLAN AND SCHEDULE –

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In 1915, Dr. Robles made the first report on the American onchocerciasis from Guatemala. He assumed the vectors as being blackflies. Since his discovery, both basic research and pilot experiments in the treatment and control of this disease have been carried out in this country. However, due to the difficulty of vector control under the local conditions, there has been no organized campaign of onchocerciasis control in this country, except denodulization.

In March 1975, Japan sent a mission to Guatemala to study the feasibility of technical cooperation with onchocerciasis control project, at the request of Guatemalan Government. After several times of negotiations of the protocol for a cooperative program, the present project was launched in October, 1975. The aim of this project is to determine the feasibility of the vector control in Guatemala. A pilot project area was established in San Vicente Pacaya, in a part of the endemic areas. In selecting this area, special consideration was given to the following criteria: (1) The area represents a typical feature of endemic zones of Guatemala; (2) exhibits moderate onchocerciasis endemicity; (3) has a topographic barrier; (4) be isolated and able to avoid vector invasion from surroundings; (5) includes important coffee plantations; (6) be easily accessible; (7) has permanent inhabitants, (8) be located not distant from Guatemala City; (9) be at least 50 km^2; etc.

The project was initially scheduled as a five-year plan, including the preparatory, the attack and the evaluation phases. The entomological activities in each phase were designed as follows. *Preparatory Phase:* In the first year (1976), the first priority was given to determination of the vector, which involved, survey of the blackfly fauna, investigation of the anthropophilic species, studies of the natural incidence of *Onchocerca volvulus* in adult blackflies, experimental infection of *O. volvulus* and so on. Several anthropophilic blackfly species had already been recorded from the pilot area. It was thus crucial to successfully ascertain which of these was responsible for onchocerciasis transmission. In the second year (1977), biological studies were carried out, in order to accumulate basic information in vector control operation, which included; mapping of larval habitats, observations of the seasonal prevalence of adults and immature stages, investigations of the gonotrophic cycle, transmission dynamics, methods for evaluating the effects of vector control operations. In the third year (1978), it was aimed to determine the control measures to be adopted in the attack phase. Particular attention was paid to the following items; selection and formulation of insecticide, determination of dosage, decision on the frequency of larvicide applications, determination of the optimum season for larviciding, selection of treatment points, transmission analysis, investigation of the environmental impact of larviciding, survey of predators, parasites and pathogens of blackflies and operational planning including organization and training of personnel, etc. *Attack Phase* (1979): This phase of the five-year plan envisaged intensive larviciding, covering the entire pilot area. *Evaluation Phase* (1980): There would be a comprehensive program for evaluation of procedures used and results achieved. Thus, it was aimed that a standard vector control methodology would be developed for the country-wide implementation.

The preparatory phase went fairly satisfactory, but the larviciding operation had to be retarded, due to the difficulty of total coverage. The rugged terrain, the highly complicated water-

courses and the very small breeding streams of the vector, *Simulium ochraceum*, required tremendous man power and time for the mapping and larviciding operation. In 1980, the project was decided to be extended for three more years. The larviciding operations will be extended and continued until 1983, aiming that the vector density be suppressed in the entire pilot area, so that the effect of the vector control would be evaluated also from human side.

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Time schedule of eight year plan of the onchocerciasis control pilot project in Guatemala

Phase	Entomological activities
Preparatory Phase	
First Year 1976	Vector determination
Second Year 1977	Vector biology studies
Third Year 1978	Control measure studies
Attack Phase 1979–1982	Vector control operation
Evaluation Phase 1983	Evaluation of the project

EFFECT OF LARVICIDES ON ONCHOCERCIASIS VECTORS IN GUATEMALA

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Since current vector control trials of onchocerciasis in Guatemala are entirely based on insecticide application to *S. ochraceum* breeding streams, insecticide study is one of the essential components of all activities. At the first stage of the studies, comparative effectiveness tests using several insecticides were conducted in the laboratory. Field-collected blackfly larvae were exposed to the insecticide solution for 10 or 60 minutes, and then maintained in clean, aerated water for 20 hours at 15°C, when mortality count was taken. The most effective chemical was chlorphoxim, followed by chlorphyrifos-methyl. Temephos, or Abate, was the third, and fenitrothion and diazinon were least effective. In the pilot control area, it was decided to adopt temephos as the larvicidal agent, since it has been used for blackfly larval control in the other parts of the world giving satisfactory results, in addition considerable information has already been accumulated on its safety to humans and its low toxicity to non-target organisms.

Vector breeding streams in Guatemala are extremely small, usually within the flow range of 0.1 to 10 litre per second, being scattered throughout rugged mountainous terrain. Under such conditions, one of the most important points to be considered is easy transport and instantaneous application of the chemical, which would save man-power tremendously. To fulfill such requesites, a solid type formulation was devised. It consisted of 8% temephos technical grade, 33% polyvinyl alcohol, 33% fat, 8% Tween 20 and 17% water. In laboratory tests, the solid was confirmed to dissolve in running water from ten minutes to two hours.

In stream test, using the solids with the dose of 1 ppm per 10 minutes flow, all larvae disappeared within 3 hours, up to 160 m downstream from the application sites. On the 5th day still no larvae were found, however small larvae reappeared 7 days after the application. No marked difference was observed in the species configuration of the blackfly larvae between the pre-control and the post-control populations. Similar tests were also done with fenitrothion solids, but they were less effective than temephos.

This type of temephos solid was used successfully in the vector control of the Barretal River basin, since its initiation in 1979. However it was found later that more time was required for the

solids to completely dissolve in stream water than previously expected. Even after one week, a fragment of the solid was sometimes found still remaining in the wire-cage set in the streams.

In 1979, trial and error based studies to find out improved formulations of the briquettes revealed that those consisting of 62% temephos 5% wdp, 19% of Tween 20 and 19% of water were more effective and appropriate for the Guatemalan conditions. In the Barretal River basin, the briquettes replaced the old type of the solids in April 1980.

In 1980, extensive stream tests were conducted in Guachipilin, to compare the effectiveness of various formulations of temephos, using a total of more than 400 streams or rivulets. In these tests, instantaneous application of kerosene solution proved to be most effective, although the oily smell of water caused by its presence hampered its wide use. In addition, heavy weight of the solution was another operational disadvantage of the formulation.

The emulsion was fairly effective, and a suspension prepared from water dispersable powder (wdp) proved to be even more effective than briquettes. Also from on operational point of view, wdp was preferred to the briquettes, because it is lighter and easier to carry. In the overall control program of the Guachipilin River basin, which will soon be launched, the temephos water dispersable powder is to be adopted.

Throughout the stream testing in Guachipilin, it was experienced that the effective distance was markedly affected by the stream conditions. Larvicide was most effective in the rapid-flowing streams than in the slow streams. It was also recently found that larvicide was less effective in the dry season, compared to the rainy season. In the dry season, the flow speed is slower and the streams are usually often covered with fallen leaves and debris. These might be the reasons for reduced effectiveness in the dry season.

Le Berre

1. Ustedes están usando 10 veces más de insecticida.
2. Deberían probar las formulaciones del Africa y Europa.

Suzuki

Si, estamos usando 10 veces más insecticidas que en el Africa. Esto se debe a las condiciones de los riachuelos en Guatemala. Es común que se necesite más insecticidas en los riachuelos pequeños, y los riachuelos ocon criaderos del vector son extremadamente pequeños en Guatemala.

Le Berre

1. You are using 10 times more insecticide.
2. You should try African and European formulations.

Suzuki

Yes, we are using 10 times more insecticides than in Africa. This is due to stream conditions in Guatemala. It is common that more insecticides are needed in smaller streams, and the vector breeding streams in Guatemala are extremely small.

LARVICIDING IN THE PILOT CONTROL AREA IN GUATEMALA: OPERATION AND RESULTS

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The Barretal River basin in the San Vicente Pacaya Pilot Area, formed by three branch streams; Lavaderos, Barretal and Zapote, was selected for the vector control trial using temephos as the larvicide. The area is 7.6 km^2, and fairly isolated from outside *S. ochraceum* breeding areas, except to the south. In this area, *S. ochraceum* was found abundantly in the tributaries of the streams.

Control operation of *S. ochraceum* was launched in 1979. During the first year, solid formulations with 8% temephos a.i. was used, but later it was replaced by briquettes which contained 5% temephos a.i. Fortnight application strategy was established by the studies of observing the reappearance of larvae after flushing-out all the larvae with high-dosage larvicide application.

In order to locate all the application sites, detailed mapping was made throughout the target area. All the watercourses with discharge between 0.1 and 50 litres per second were considered to be target streams. The target concentration of temephos in the running water was 0.1 ppm for 60 minutes. In practice, a unit quantity of insecticide of 2 g was applied for every 0.5 litre of discharge per second. The solids enclosed in a wire-net were set in the stream water.

Entomological evaluation was based on fortnight collection of adult female blackflies on human bait for three hours, from 9 a.m. to noon, and fortnight collections of immatures in the treated streams.

After initiation of the larviciding, particularly during the first year, overlooked *S. ochraceum* breeding sites were often found in the area under control. For example, a rivulet harbouring *S. ochraceum* larvae was found above the main branch stream of Zapote. The water of the rivulet once goes underground, then joins the main stream. This type of isolated or discontinued rivulet raises one of the most difficult problems in the survey and mapping, since it was undetected by the ordinary survey method going upstream from the main stream.

In the control operation of *S. ochraceum* lasting 19–22 months in the Barretal River basin, a total of 3,317 g of temephos technical grade was consumed. Monthly average consumption was 164 g, to cover the area of 7.6 km^2. Throughout the entire operation, only one team consisting of two members was enough to cover the area. This has encouraged the feasibility of future control activities on a wider scale.

Larval evaluation was made fortnightly, one week after each application. About 40 check sites, located 10–20 m downstream from the application sites were registered, and larvae and pupae were searched for at each site for 10 minutes. Collected immatures were brought back to the laboratory and identified. When positive sites were detected, careful checking was made, and new application sites were established before the following application.

In addition to the regular fortnight larval checking, a through checking of the breeding sites was made in March 1980. Thereafter, none of the positive sites with *S. ochraceum* were found, except one in Zapote in June 1980.

For evaluation of the female density, nine stations were registered, of which three stations, i.e. Lavaderos, Entrada de Lavaderos and Barretal were situated just inside the control area. Four other stations were located outside the control area which act as the uncontrolled check stations, while the remaining two were situated near the border line of the controlled and uncontrolled areas.

The results are illustrated in Fig. 1. In Lavaderos, monthly average density of *S. ochraceum* before control ranged from 25 to 383 flies per three hour collection. The first insecticide application was made on 27 March 1979. The average density of *S. ochraceum* was reduced to 92.5 in April and further to 22.0 in May. Thereafter, the density maintained the level below 10, except during the September–November period. Particularly after March 1980, the density was reduced to 1.0 or lower. At the two stations, Entrada de Lavaderos and Barretal, some females were collected during the period from July to December 1979, but it was reduced thereafter, maintaining the low level of 10 or less. Since the Barretal station is situated 2 km from the southern border of the control area, we suspect that the flies might have invaded from the uncontrolled southern area.

In the four stations situated in the uncontrolled check area, there existed a tendency of high density in the dry season from September/October to March, and low density in the rainy season from April to August.

It is encouraging that control operations in the Barretal River basin were successful, using only a small amount of insecticide and man-power. It should be stressed, however, that for future control activities on a wider scale, it is essential to establish more efficient methods or pre-control survey and mapping and to improve insecticide formulations, in order to reduce the number of application and/or to prolong application intervals.

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Schiller

¿Se han hecho estudios sobre los efectos de estos insecticidas en el medio ambiente?

Sasa

Estos son pequeños riachuelos que no son usados por la gente como fuente de agua potable. Casi no hay efectos tóxicos para otros organismos, como está dicho en el papel R-4 de los papeles adicionales.

Schiller

What environmental studies of these insecticides have been made?

Sasa

These are small rivers which people don't use for drinking. There have been almost no toxic effects on non-target organisms, as stated in resource paper R-4.

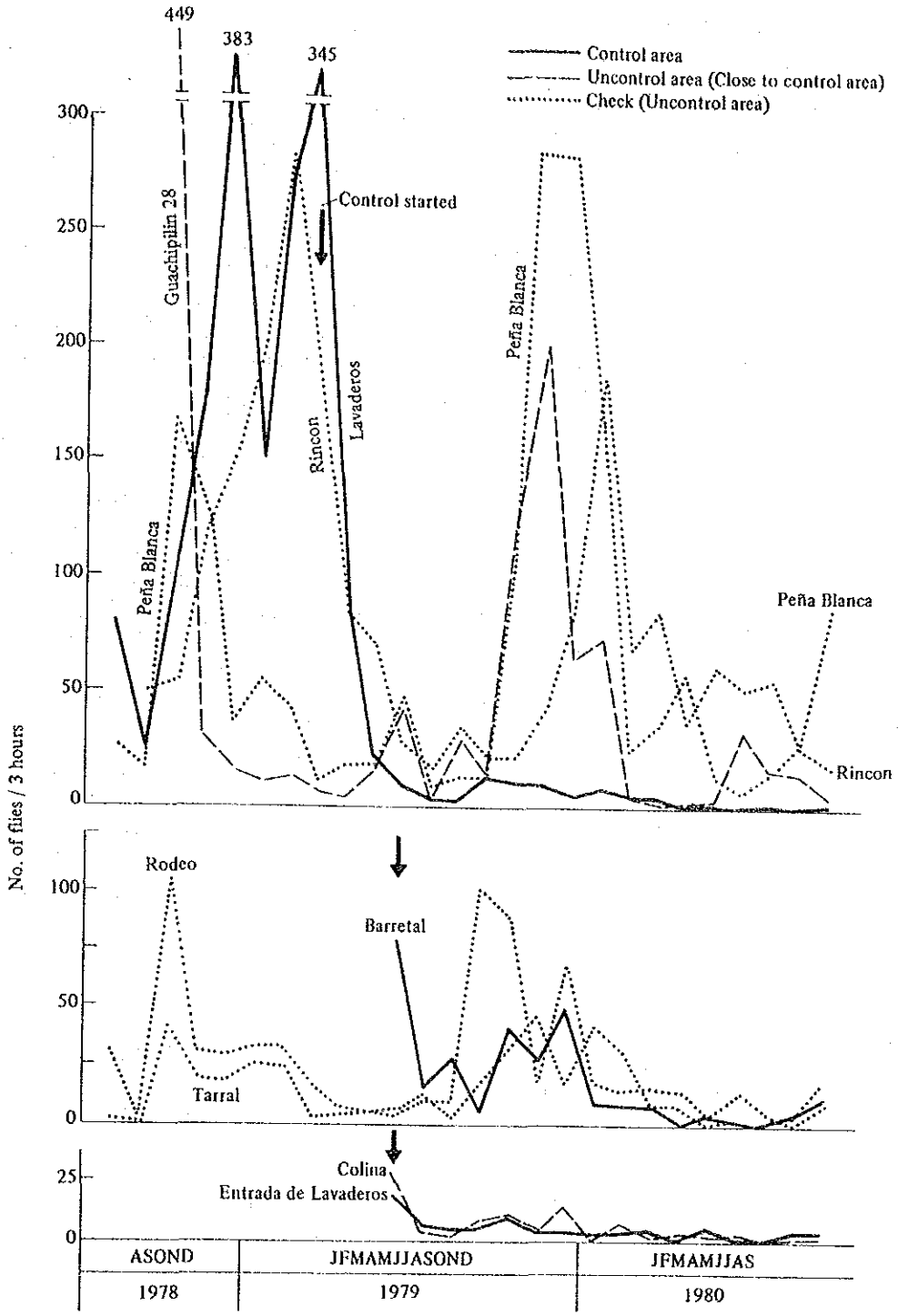

Fig. 1 Monthly average density of *S. ochraceum* before and after the initiation of larviciding

BIOLOGICAL CONTROL OF BLACK FLIES

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Simuliidae are hosts for many pathogens which play a part in the natural regulation of their populations. The common fungal pathogens are *Coelomycidium simulii, Entomophthmora* species, and an ovarian phycomycete. Protozoa are represented by a Tetrahymena-like ciliate and the microsporidia. The most common metazoan pathogens are the mermithid nematodes. Cytoplasmic polyhedrosis virus, a densonucleus virus, and irridescent viruses occur in black flies. All of these, except the viruses, have been reported from Guatemala and some of the microsporidia in Guatemala are unique.

Coelomycidium simulii, the microsporidia and the mermithids, all cause larval mortality. The ovarian phycomycete causes sterility in the female adult, while *Entomophthmora* and probably the ovarian phycomycete, cause adult mortality. The mermithid kills either the larval or adult simuliid when it emerges from the host. The effect of the ciliate pathogen, found in both larvae and adults, is unknown. The viruses cause mortality, but unpredictable stress factors appear to be involved. Of all these pathogens only the mermithid nematode and the cytoplasmic polyhedrosis virus have been experimentally transmitted. Life cycles of the microsporidia, ovarian phycomycete and *C. simulii* are incompletely understood. The zoospores of *C. simulii* and the spores of the microsporidia, logically the infective stages, do not infect black fly larvae in the laboratory. None of these pathogens with the possible exception of *Entomophthmora* can be reared *in vitro* and, because black fly larvae are expensive to maintain in the laboratory, *in vivo* culture is impractical.

The introduction of exotic black fly pathogens or the augmentation of endemic ones would, hopefully, result in higher levels of continuous control and dispersal of the pathogens to untreated larval sites. Dispersal and just maintaining itself in the same breeding site against the continuous downstream movement requires an intimate relationship between the pathogen and the adult stage of the host. There is evidence of transovarian transmission of the microsporidia and viruses of simuliidae. The ovarian phycomycete dispersal stage is deposited by and mermithid post-parasitic larva emerges from the adult at the oviposition site. Efficient dispersal of the pathogens is an especially important criterion in Guatemala where the most important vector species breeds in small and temporary streams.

Until transmission of black fly pathogens is understood and their mass culture is possible, pathogens of other insects might be used as biological insecticides. *Neoaplectana carpocapsae* is a terrestrial entomophilic nematode which is able to infect simuliid larvae but many infective larvae are required for a significant level of control (Malloy, personal communication) and its low host specificity might make it environmentally unacceptable. *Bacillus thuringiensis* var. *israelensis*, a mosquito pathogen is, however, a quite promising simuliid microbial insecticide.

Testing of *B. thuringiensis* var. *israelensis* is continuing against black flies in Newfoundland (Undeen and Nagel 1978, Undeen and Colbo 1980) and *Simulium damnosum* in West Africa (Guillet and de Barjac 1979). Industrial firms are developing formualtions for both mosquito and black fly control. The best formulation so far is an aqueous suspension of spores and crystals. Drying the bacteria appears to drastically reduce simuliid toxicity. Non-target organisms were unaffected in both the Newfoundland (Colbo and Undeen 1981) and the African tests.

In November and December 1979 *B. thuringiensis* var. *israelensis* was applied to three Guatemalan *Simulium ochraceum* breeding sites to test efficacy and carry. Estimations of larva reduction were by two methods; pre- and posttreatment in-place counts of larvae on 5 x 10 cm white

plastic film anchored to the stream bottom and pre- and posttreatment counts of larvae collected from natural substrates with a pair of forceps, in 5 minutes. Stream discharge was measured by diverting the entire flow into a large plastic bag. Streams were treated with aqueous suspensions of bacteria grown on tryptose blood agar base or SAN 402 WDC (Sandoz, Inc.), a liquid formulation.

Mortality was high on the artificial substrates but carry was limited (Table 1). Five minute collections from natural substrates revealed the same pattern. The increased dosage and treatment time at Guachipilin did not cause a commensurate increase in carry. By lab and field tests in Newfoundland, SAN 402 WDC has the same efficacy and carry as the other aqueous suspension used.

Four treatments of 1 x 10^5 spores/ml at 100 m intervals in a lower section of the Nimaya stream (840 liters/min) resulted in a mean mortality of 87% (range, 80–93%) throughout the entire 400 meter sampling area, by the 5 minute collection count method. Species present were *S. callidum*, *S. metallicum*, *S. samboni*, *S. paynei* and *S. horacioi*.

Downstream carry appears, from available data, to be more a function of stream size than dosage or treatment time. The particulate active ingredients are probably filtered from the water at all of the liquid-solid interfaces and so the greater the surface/volume ratio, the less the carry. A practical application in Guatemala will probably involve closely spaced treatment points and a formulation, either a paste or thick liquid, which would disperse over one to five minutes.

Table 1 Larval mortality on artificial substrates 24 hours after treatment with *B. thuringiensis* var. *israelensis*

Site	Medio Monte[a)]	Nimaya	Guachipilin
Meters below treatment site			
0–25	94	87	100
25–50	-	82	13
50–75	-	51	0
75–100	-	21	-
Species in order of abundance[b)]	*S. ochraceum* *S. metallicum* *S. callidum*	*S. metallicum* *S. callidum* *S. ochraceum* *S. samboni*	*Simulium* sp. *S. metallicum* *S. ochraceum*
Discharge liters/min	10	210	156
Dosage spores/min	2 x 10^{5} c)	2 x 10^{5} c)	1 x 10^{6} d)
Treatment time	1 min	1 min	10 min

a) Test site 14 meters long
b) From 5 min. pretreatment collections
c) Spores grown on tryptose blood agar base
d) SAN 402 WDC

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STUDIES ON PATHOGENS OF BLACKFLY LARVAE IN GUATEMALA AND THEIR INFLUENCE ON NATURAL POPULATIONS OF THREE SPECIES OF ONCHOCERCIASIS VECTORS

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In 1934, Bequarert found undetermined protozoa and nematodes in adult blackflies, including *Simulium ochraceum, callidum,* and *metallicum,* while searching for the developmental stages of *Onchocerca volvulus* in wild flies. Dalmat (1955) also reported the occurrence of nematode larvae in adults of *S. metallicum* and *S. ochraceum*, as well as microsporidians in undetermined *Simulium* larvae. Recently, in 1975, unidentified fungi, as well as nematode larvae were found by Garms in adults of the same three vector species. However, information on the pathogens and parasites of larval blackflies in this country is otherwise lacking.

A search for blackfly larvae pathogens and parasites was carried out from August 1978 through January 1980, within and outside of onchocerciasis endemic zones, with altitudes ranging from 200 to 3,000 m. The seasonal influence of pathogens on natural populations of the three vector species was observed in the south-eastern endemic area.

Freshly collected blackfly larvae were searched microscopically for mermithid, microsporidian, fungal and viral infections. Pathogens from infected larvae were provisionally identified using Weiser and Briggs and Weiser keys. Post-parasitic mermithid larvae emerging from blackfly larvae were held in the incubator until they molted to the adult stage, then sent to Dr. Poinar for identification. Microsporidian, fungal, and viral materials were sent to Dr. Undeen for reconfirmation.

About 70,000 blackfly larvae from 65 streams were examined. Twenty of the 26 species collected, harbored various kinds of pathogens and parasites.

Five mermithid species were found to parasitize 17 species of blackflies. In contrast, 10 microsporidian species were found in 15 blackfly species. Fungal infections encountered in fly larvae were caused by *Coelomycidium* species. Iridescent virus were found in four and Cytoplasmic Polyhedrosis virus were found in one species of blackfly.

Thirteen species of pathogens were related to the larvae of the three vector species (Table 1). *Simulium ochraceum,* the principal vector of onchocerciasis in Guatemala has six, while *S. metallicum* and *S. callidum* have ten and seven, respectively. *S. ochraceum* exhibited only sporatic parasitism in comparison to *S. metallicum* and *S. callidum.* Infection due to any kind of pathogen was found in 13.6% of the 44 streams where *S. ochraceum* larvae were collected. Furthermore, this species seems unique in lacking mermithid and viral infections. On the other hand, larvae of *S. metallicum* and *S. callidum* were most frequently found to harbor mermithids and microsporidians, whereas fungalal and viral infections were sporatic. *S. ochraceum* exhibited low infection rate, in comparison to the other two vectors. Average infection rate with mermithids in the larval populations of *S. metallicum* and *S. callidum* were 8.9% and 4.9%, respectively. The iridescent viral infeciton rate in *S. callidum* larvae was 7.6%. However, infections with microsporidians and *Coelomycidium* sp. were all at low levels.

Temporal variation of infection levels of the various kinds of pathogens was observed in four different types of streams in the upper regions of the Rio Verde and Guachipilin Rivers and in Finca Rincon. Type-1 is a mid or lower part of the permanent stream, over 500 m downstream from the headwaters, with a width greater than 0.5 m. Type-2 is also a permanent stream, but much smaller

than type-1. It is within 100 meters from the source of the water. Type-3 is a temporary stream which emerges during the rains and has neighboring permanent streams. Type-4 is also a temporary stream, about 3 kilometers from the nearest permanent streams. All four stream types can contain preimaginal habitats of the three vector species. Types-1, 3 and 4 were preferred mainly by *S. metallicum*, while *S. ochraceum* inhabited type-2.

In all stream types, *S. ochraceum* never harbored mermithids, even in type-1 streams, where the other two vector species were heavily infected with mermithid parsites. Mermithid infections of *S. metallicum* and *S. callidum* differed considerably depending on the stream type inhabited. The level of microsporidian infections of *S. ochracum* varied in accordance with different types of habitats. This species exhibited very low infections in type-1 and 2 streams, but was free from infections in temporary streams. In contrast, *S. metallicum* and *S. callidum* were equally parasitized by microsporidians in all types of streams, although infection rates were very low except in 40% of *S. callidum* in one type-1 stream. Fungal infections were found in *S. ochraceum* and *S. metallicum* in permanent streams, in Finca Rincon, although low infection level.

There appears to be a distinct seasonal pattern in mermithid infections of *S. metallicum* and *S. callidum*, with increased infection rates during the dry season, from November to April, then lowered during the rest of the year. This suggests that between the two peaks in seasonal abundance of larval *S. metallicum* populations, there are more mermithids in the dry season.

To conclude, although the present data were based on this short-term investigation, it seems likely that mermithids play the major role in suppressing the natural populations of *S. metallicum* and *S. callidum* in the mid or lower regions of perennial streams, especially during the dry season, but they hardly affect these blackfly larval populations in the upper reaches of permanent streams or in temporary ones. As of the moment, other groups of pathogens are considered to be of negligible importance as natual population regulatory factors for three vector species in any type of stream.

Table 1 Pathogens found in larvae of three blackfly vectors of onchocerciasis in Guatemala

Pathogens	Vector species		
	S. och.	*S. met.*	*S. cal.*
Isomermis benevolus	–	+	+
Mesomermis sp-1	–	+	–
M. sp-2	–	–	+
Thelohania bracteata	+	+	–
T. varians	–	+	+
T. fibrata	+	+	–
T. sp-1	+	+	–
T. sp-2	+	+	–
Pleistophora multispora	+	+	+
P. debaisieuxi	–	–	+
P. sp-1	–	–	+
Coelomycidium	+	+	–
Iridescent virus	–	+	+
Total	6	10	7

INVESTIGATIONS ON THE USE OF NON-CHEMICAL, NON-BIOLOGICAL CONTROL OF BLACK FLIES IN GUATEMALA

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The idea of using water management to control vectors is not new. Regulation of water flow by cleaning irrigation canals, flushing, river training and damming was suggested by Svensson (1943). The Tennessee Valley Authority (1947) employed water level management extensively to control vector of malaria in U.S.A.

The use of these techniques to control the vector of onchocerciasis in Guatemala has not been tried, although the size and character of the breeding streams lend themselves to their utilization. This report relates results of preliminary trials using the flushing technique.

TRIAL 1

The first trial utilized a barrel siphon. It was constructed by modifying a 55-gallon steel drum. A central metal, water-tight plate was installed to within 12.8 cm of the top, dividing the inside space in half. On the upstream side of the drum an intake hole about 14.7 cm in diameter was cut along the lower edge. On the downstream side, a reservoir siphon seal was made. A one-inch plastic pipe penetrated the inner chamber near the top of the drum and was connected to a similar discharge pipe erected on the outside of the drum. This pipe system acted to vent air when the drum was filling, to initiate discharge of water and allow air to enter the drum after discharge to reseal the siphon. This barrel was installed as part of a small dam, which impounded about 800 liters of water. When the siphon was operating it cycled every 12 minutes, alternately flooding and drying downstream for 300 meters.

To evaluate its effect, 90 pretreatment random substrate samples were collected and 535 black fly larvae recovered. After the cycle started, 80 samples were collected up stream from the dam site, yielding 166 larvae, while a similar number of samples for 300 meters downstream were negative.

It operated automatically without attention for 6 weeks.

TRIAL 2

The second type of automatic flushing device was the counter-balanced tilting bucket. It consisted of a water-tight front plate constructed of wood, 137.2 cm long and 122 cm high. In the middle of the plate a hole was cut 91.6 cm high and 45.8 cm wide. Attached to the hole is a moveable, hinged gate. The action of the gate was regulated by a counter-balanced weight.

This device was installed in a small stream as part of a dam. When filled with water, pressure of water on the gate overcame the counter balance and the gate opened releasing the water. The flushing cycle was about 7 minutes.

Although the efficacy of the device was not extensively studied, its performance was followed for six months and found to reduced numbers of black fly for 300 meters downstream. The final samples yielded 5 *S. metallicum* in 20 leaves examined downstream from the bucket, while in nearby similar, control stream, 20 leaves produced 107 larvae, of which 69 were *S. ochraceum* and 39 *S. metallicum.*

TRIAL 3

A permanent, concrete dam was used in the third trial. It contained a sliding door 55.9 cm wide and 91.6 cm high. The flow of the stream was also partly directed at the same place to a secondary tank which supplied water to the village, and also had a door that could be opened to release the water. The dam and tank had a combined capacity of 16 cubic meters of water. To flush, both doors were opened and impounded water was released in two minutes. To refill required 31 minutes. Each stream treatment consisted of 5 such cycles of flushing. The same schedule of flushing was followed weekly for 6 weeks.

To assess the system, four sampling stations were established above, and four below the dam site. At each, translucent plastic strips, 10.1 x 50.8 cm were placed and allowed to remain for one week. The number of larvae attached to the strip at the end of one week constituted the sample.

The population of larvae were markedly depressed by the treatment. The average number of larvae of all collections per station was 26.9 in the treated and 125.2 in the untreated stations. The average number of the vector, *S. ochraceum*, per sample per station in treated portion was 8.0 and in untreated, 37.1. There is obviously a reducing effect by the flushing. However, in this test the vector was not completely eliminated by the strong flow.

TRIAL 4

This test differed from the others in that a concrete tank had been constructed inset into the river bed. The tank measures 4 m x 4 m x 1 m deep and has a 17.5 cu.m. capacity. On the downstream side is a sliding door 1 m high and 0.45 m wide. It emptied in 2 minutes after opening the door which required about 30 minutes to refill. A treatment, which was executed weekly for 6 weeks, consisted of 5 consecutive flushings. They were completed during the rainy season. Appraisal of the effect was gained by situating four sampling sites in the treated stream and a similar number in another branch of the same stream with similar characteristics. Plastic strips could not be used successfully in this case because the stream was too small. Therefore a sample consisted of 20 leaves from plants trailing in the water. Balck fly larvae were removed, counted and identified.

Again there was a general reduction of the larval breeding. The average per treated station was 15.8 per sample, as compared to 45.2 in the untreated stations. That amounts to a 65 percent reduction.

During the period, August 13–October 9, a total of 864.5 mm of rain fell, or a daily average of 14.6 mm. The daily maximum was 148.8 mm on September 23. Especially the heavy rains had an effect on reducing the populations in both treated and untreated stations.

In all of these trials no attempt was made to measure downstream drift which may have occurred during the flushing, not destroying the larvae but merely displacing them downstream. Likewise no attempt was made to measure the effect of the trials on the adult populations present in the fincas.

It can be concluded that flushing could possibly be a valuable adjunct to a main control method but requires further evaluation. Many fincas in the endemic zones of Guatemala have water control structures already constructed. The only requirement to initiating their use is to instruct the fincas on the method of operation.

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PROBLEMS IN VECTOR CONTROL OF ONCHOCERCIASIS IN GUATEMALA

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1. BASIC STRATEGY FOR VECTOR CONTROL

1.1 Vector eradication

(1) The vector could be eradicated, only if the species is inflexible in its breeding and biting behaviours, and if its distribution is limited.

(2) In Guatemala, the vector distribution areas seem to be wider than the endemic foci of the disease.

Research Need:

Distribution and density of vectors both inside and immediately outside an endemic focus.

1.2 Vector control

(1) Vector density can be suppressed to a very low level by larvicide applications.

(2) The low density could be maintained only by repeated larvicide applications throughout the year.

Research Need:

(1) Determination of the threshold level of the vector density, below which transmission can not be maintained.

(2) Improvement of control measures.

1.3 Integrated control with vector control measures and chemotherapeutic measures

Research Need:

(1) Experimental infection studies with Mf carriers, before and after the drug administration, with reduced Mf density.

2. METHODOLOGY OF VECTOR CONTROL

2.1 Environmental or mechanical control

Advantage: No or minimum disturbance of the environment.

Disadvantage: No effective measures for wide application are available at the present.

2.2 Biological control

Advantage: No or minimum disturbance of the environment.

Disadvantage: No effective agents for wide practical control are available at the present.

Research Need: Basic studies on biological control agents.

2.3 Chemical control

a. Aerial application of insecticide for adult control, eg. ULV application

Advantage: Capability to cover a wide area in short time, with small man-power.

Disadvantage:

(1) High operational cost involved.

(2) Necessity of repeated applications.

b. Ground application of insecticide for adult control, eg. ULV application

Advantage:

Capability to cover specific target areas only, in a short time and with small man-power.

Disadvantage:

(1) Necessity of repeated applications.
(2) Difficulty in transportation of equipment to the roadless sites.

Research Need:

Field control trial, with special reference to effectiveness and its duration.

c. Aerial application of insecticide for larval control, eg. granule application

Advantage:

Capability to cover a wide area in a short time and with a small man-power.

Disadvantage:

(1) High operational cost involved.
(2) Necessity of repeated applications.
(3) Loss of insecticide which does not reach breeding streams, and resulting environmental contamination.

Research Need:

(1) Devise adequate formulations for such control.
(2) Field control trial.

d. Ground application of insecticide for larval control

Advantage:

(1) Low insecticide cost involved.
(2) Slight contamination of environment.

Disadvantage:

(1) Necessity of large man-power, causing high operational cost.
(2) Difficulty in locating all breeding sites.
(3) Difficulty in approaching certain breeding sites.

Research Need:

(See next chapter)

3. PROBLEMS IN VECTOR CONTROL BY GROUND LARVICIDE APPLICATION

3.1 *Strategy now being assigned*

(1) Insecticide: Temephos (Abate)
(2) Formulations: Briquette or wdp
(3) Dose: 1–2 ppm per 10 minute flow
(4) Effective distance: 50–150 m
(5) Application interval: 2 weeks
(6) Application sites: All the breeding streams in the area
(7) Application period: All the year round

3.2 Future strategy to be considered

Due to very large man-power involved in the above-mentioned control measures, the following improvements should be considered.

a. Limitation of larvicide application to permanent breeding streams only

Research Need:

(1) Classification of breeding streams into permanent and temporary ones.
(2) Field control trial in permanent breeding streams only.

b. Limitation of larvicide application to a specific period of the year
Research Need:
(1) Studies on transmission potential of vectors throughout the year.
(2) Field control trial during a specific period of the year.

c. Limitation of larvicide application to the areas only and/or in the period only with high vector density
Research Need:
(1) Determination of tentative threshold level of vector density, beyond which control will be carried out.
(2) Field control trial only in the high density area during the high density period.

3.3 Technical problems in the present strategy

a. Pre-control survey and mapping
(1) Difficulty in registering certain breeding sites
The breeding sites located upstreams from subterranean flow are very difficult to locate by ordinary tracing methods from downstream, while those located in very rugged terrain are sometimes unapproachable.
(2) Inadequacy of ordinary mapping method
It is sometimes difficult to trace breeding sites by maps.
Research Need:
(1) Basic and systematic geographical studies to classify the area, in order to predict vector breeding streams.
(2) Improvement and standardization of mapping methods for easy location of breeding streams by field workers.

b. Control methodology
(1) Shortage in effective distance
Research Need:
(1) Field studies on new insecticides, as well as on new formulations of insecticides including temephos.
(2) Basic studies on the dispersal and adsorption of insecticide released in stream water.
(3) Affect of cleaning streams on effective distance of insecticide.
(2) Necessity of repetitive applications with a short interval
Research Need:
Further studies on reappearance of vector pupae and larvae after flushing-out under various stream conditions.

3.4 Operational problems and countermeasures
(1) Cost analysis of control operation
(2) Organization of nation-wide control operation
(3) Standardization and preparation of the manual entitled "OPERATION MANUAL OF ONCHOCERCIASIS CONTROL IN GUATEMALA".
(4) Staff training

EPIDEMIOLOGICAL FINDINGS IN SAN VICENTE PACAYA, PILOT AREA, GUATEMALA

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San vicente Pacaya county is located along with the southern slope of volcano Pacaya and its area is 236 km^2. There are abundant small streams draining the whole areas where a total of about 5,700 inhabitants are living. Out of 2,153 examinees from plantations and villages, 30.8% were positive in 1976 for *Onchocerca volvulus* microfilariae by single skin biopsy. The altitude dependent endemicity of the disease was seen. The higher prevalence was seen in the areas situated at altitudes between 700 and 1,300 m above sea level, suggesting that the rransmission foci are distributed in this particular topography.

Microfilarial rate in male rose in proportion to age, and the highest rate (65%) occurred in the 40--49 year age group. In all age groups, the rate in females was about one-half that in males. A similar evidence was also seen in the microfilarial density; when the MFD at 50% was compared between 2 sexes, the MFD_{50} was 0.9 for female positive and 3.2 for male ones.

There was a close correlation between the microfilarial rate obtained by single skin biopsy and the nodule rate in the 18 areas. A 50% microfilarial rate corresponded approximately to a 35% nodule rate. This value is higher than that of 16% in Guinea reported by Buck (1974). This evidence suggests that the nodules are more easily produced in man in Guatemala in spite of a long-term campaign of denodulization.

The history of nodulectomy among the inhabitants in the endemic areas was investigated by interview study. In highly endemic areas, 72.1% of the inhabitants had the experience of operations at 2.4 times in average, while in low endemic areas the rates were 16.2% and 1.4 times, respectively (Table 1). Among a total of 1,170 extirpated nodules examined since 1976, 1,077 (92.05%) were confirmed as of onchocercal origin. Further it was confirmed that the larger the size of the nodule, the higher was the microfilarial rates of the patients; the microfilaria-positive rate was only 15% in those with nodule of 5 mm in diameter, but the rate was 95% at 15 mm-nodule. This result is consistent with the observation that the percentage of nodule with only the female worms decreased

inversely as the nodule size. These findings indicates the importance of onchocercal nodule as the source of microfilariae and the necessity of nodulectomy campaing in this country.

In a survey in 1976, both eyes of 1,217 persons were examined with a slit lamp. The survey revealed that 6.2% of the examinees had microfilariae in their anterior chambers. The detection rate of microfilaria increased in proportion to the microfilarial density in the left shoulder. Namely, only 0.7% were positive for MFAC of 73 microfilarial negatives, while 35.1% were positive out of 37 microfilarial negatives whose MFD was 301 or more per snip (Table 2).

Based on an idea to establish a specific and stable immunologic test for the epidemiological analysis of the endemicity, we adopted an indirect hemagglutination test (IHA) using dried blood taken on filter paper from ear lobe as the test material. This technique showed a high correlation (r=0.96) with the test using serum, and showed high specificity. High correlation was seen between the IHA titer or its positive rate and the microfilarial density in the areas surveyed in the present study.

High prevalence between 60–100% of *O. gutturosa* infection in cattle and that of *O. cervicalis* in equine was shown within S.V.P. area. These results suggest the importance of future studies in the comparison between the transmission of human and animal onchocerciasis in this region.

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Schiller

¿Existe alguna correlación entre el tamaño del nódulo y la edad (etapa de desarrollo) del verme dentro del mismo?

Tada

No se exáctamente cuál es el tiempo de maduración del parásito, pero el crecimiento generalmente es rápido en Guatemala.

Schiller

Is there any correlation between the size of the nodule and the age (stage of development) of the worm within it?

Tada

I don't know exactly the time for the maturation of parasite, but the growth is usually rapid in Guatemala.

Table 1 An interview study of nodulectomy among the inhabitants in San Vicente Pacaya

Endemicity*	No. of residents in total	No. of residents with the history of nodulectomy (%)	Average frequency of nodulectomy	No. of village and plantations involved
High	204	147 (72.1)	2.41	5 plantations
Medium	779	325 (41.5)	1.80	4 plantations 1 village
Low	401	65 (16.2)	1.40	2 plantations 2 villages

* Microfilarial rate: high, 67% or more; medium, 34–66%; low, 33% or less

Table 2 Relation between microfilarial rate in anterior chamber and microfilarial density (MFD*) in the skin

MFD	No. of the examined	No. of microfilarial positives in anterior chamber	Positive rate (%)
0	732	5	0.7
1 – 9	166	5	3.0
11 – 50	151	23	15.2
51 – 100	63	13	20.6
101 – 200	52	13	25.0
201 – 300	16	4	25.0
301 –	37	13	35.1
Total	1,217	76	6.2

* MFD: No. of microfilariae per single skin snip

DATA RECORDING AND ANALYSIS SYSTEM FOR THE EPIDEMIOLOGICAL SURVEYS IN GUATEMALA

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Introduction

Epidemiological surveys on onchocerciasis have been carried out since 1976 in San Vicente Pacaya, Guatemala. This study aims to evaluate the effect of the larvicide application from the epidemiological point of view, and to clarify the epidemiological features of the disease in Guatemala. In order to attain this goal, data obtained through field survey should be stored properly and analyzed carefully from various points of view.

The data processing system in the present project was intended to centralize and process all of the data obtained in order to analyze the data more effectively, and to register all of the examinees in order to see the secular change of the individuals.

Data Processing System

Data obtained in the field have been recorded on the individual data sheets which were designed to contain the information for individual identification, life history, parasitological and immunological diagnosis, and ophthalmological and dermatological findings. The data sheets have been collected and checked whether the information was filled out properly.

In the Epidemiology Section, the following procedures are operated routinely.

1) Reception of the examinees, filling out the information for individual identification on the reception notebook.
2) Assignment of registry number, checking the identification (ID) lists prepared periodically by the system.
3) Coding for the ID and data cards on the coding sheets, referring to the coding manual prepared in advance.
4) Punching IBM cards (ID cards and data cards).
5) Completing the ID and data lists periodically using the punch cards.

In order to identify the examinees, the information of complete name (usually two family names and two given names), sex, date of birth and address is requested. These items are confirmed by official identification documents at the time of the census, when available. In Guatemala, full name and date of birth (day and month) are relatively reliable indices for individual identification. Therefore, in the system, two types of ID lists, i.e. name list by alphabetical order and the list by date of birth, have been utilized. Then, one examinee has been given his or her own registration number regardless of frequency of individual examination.

Finally, two series of the IBM cards punch are compiled, ID cards for individual identification and data cards for individual data the disease.

Data Analysis

As of now, data for 5,767 examinees, including re-examinees, from September 1977 through October 1979 have been stored. These cards are ready to be retrieved and analyzed, using a sorter with a cross tabulator installed at the laboratory in Guatemala City. This allows us to make longitudi-

nal type analysis (incident type), such as to estimate the incidence rates of the disease, besides cross sectional ones (prevalent type).

However, data analysis clearly depends on the purpose of the individual studies. Therefore it would be difficult to formalize the methods for analyses. In order to analyze the data at hand easily and effectively, the researchers should 1) make clear the study goal, 2) specify the data cards to be analyzed, and 3) demonstrate the table format required in which the data obtained through the machine are filled in.

An Example of the Data obtained through the System

It was intended to estimate the incidence rate of onchocerciasis as one of the indices for evaluation of the vector control operated by the project. Since registry of examinees has been established, it is rather easy to observe changes of individual signs in certain periods. Subjects adopted for the incidence had been examined more than twice and had negative biopsy results at the first examination. An incident case is defined as a person whose skin snip result has changed from negative at the first examination, to positive in a later examination. Population at risk was obtained by the person-month method, taking the observation periods into consideration. As shown in Table 1, 18 males and 13 females among the subjects chosen were defined as incident cases during two years. Based on these figures, the overall annual incidence rate for male and female was 0.08 and 0.04, respectively. The same analysis was already made in order to estimate the rate of the nodule formation.

REFERENCE

Yoshimura T., et al. Prevalence and incidence of onchocerciasis as baseline data for evaluation of vector control in San Vicente Pacaya, Guatemala. (submitted for journal publication).

Table 1 Incidence rate due to 2 skin snip biopsies in San Vicente Pacaya, Guatemala (1977–1979)

Age	No. of person*	Person-month	Incident case	Incidence rate/year	No. of person*	Person-month	Incident case	Incidence rate/year
	MALE				FEMALE			
0– 9	100	1,368	4	0.035	120	1,372	2	0.017
10–19	71	880	7	0.095	101	1,354	3	0.027
20–49	28	311	4	0.154	95	1,131	7	0.074
50–	12	138	3	0.261	26	292	1	0.041
Total	211	2,697	18	0.080	342	4,149	13	0.038

* negative at the first examination

METHODS FOR EVALUATING THE EFFECTS OF VECTOR CONTROL ON HUMAN POPULATION IN SAN VICENTE PACAYA, GUATEMALA

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The onchocerciasis research and control program was initiated in a pilot area, San Vicente Pacaya, in October, 1975. Following the preparatory phase a systematic application of larvicide to the breeding sites of *S. ochraceum* was launched in a small-stream area called Quebrada Los Lavaderos, in March of 1979. The areas to be treated are expected to be gradually expanded southwards to include other streams.

The aim of the vector control in San Vicente Pacaya Area is to suppress the biting population of *S. ochraceum*, the main vector in the area, to a level low enough to interrupt the transmission of the disease. Therefore, the effect of control should be evaluated in its influence on the human population.

The epidemiological surveys were completed with nearly all inhabitants in the pilot area during the preparatory phase. The records of examinations for each individual were stored in the computer data files and the epidemiological features have been analyzed in regard to the prevalence of mf positives, nodules, ophthalmological and dermatological findings in addition to the mf densities, results of skin test and other immunodiagnostic tests. These are expected to provide the base-line data to be compared with the post-control findings. However, the infection of onchocerca is considered to last for long years, if untreated, and therefore it is expected to take a long period before the effect of control which is based only on the suppression of blackflies would become remarkable in regard to the prevalence of the disease.

The more sensitive methods are required which make it possible to evaluate the effect of control in a short term after or even during the operation of the control. The observations of the incidence of mf positives and nodules during a given period are expected to be suitable for the purpose of determining the intensity of transmission in a given area. The usefulness of observing incidences of other findings as ophthalmic and dermal symptoms and positivities in immunological tests is still under examination.

For this purpose in the pilot area of San Vicente Pacaya, 6 villages are specially selected. They are, namely, Canton La Cruz, los Rios, Patrocinio, Guachipilin and Berlin, which are located in or near the on-going and planned control operation streams, and one control village, Hamburgo, which is far distant outside of the operation area. Two epidemiological surveys, as a set of pair, were conducted from 1978 to 1980 in each of the above mentioned 6 villages respectively at an interval of one year by the same technic and procedures. The results gave figures of incidence of the disease which had taken place during one year period before initiation of the control operation, and are considered to serve as the base-line data in relation to the incidences.

Canton La Cruz, the nearest village to Lavaderos in which the control operation started in 1979, was visited in 1978 and revisited one year later in 1979. There were 159 inhabitants who received the examinations both two times. The mf positive cases found by skin biopsies were 19 (11.95%) at first visit, but one year later 2 persons out of 140 negatives turned to mf positive, so a rough estimate of incidence rate of mf positive was 1.43% per year ($0.58\% \leqq p \leqq 4.32\%$, 90% confidence limits). Nodule prevalence rate was 16/159 or 10.06% in 1978. All nodule positive cases were denodulized but one year later 4 of 159 were observed to be nodule positive. The positive turnover rate was 2.52% ($1.22\% \leqslant p \leqslant 6.02\%$, 90% conf. limit). The nodules reappeared in 3 persons or 18.75% ($8.44\% \leqslant p \leqslant 39.55\%$, 90 conf. limits), of 16 who were nodule positive but denodulized one year before. It would be possible that the remaining free worms appeared later to the surface and formed the nodules.

Therefore, one must be careful in interpreting the result as the incidence reflecting the intensity of transmission during that period. Out of 143 who were nodule free at the first examination, a single case or 0.70% ($0.24\% \leq p \leq 3.25\%$, 90% conf. limits) turned nodule positive one year later, this might better reflex the transmission situation in the area. In the same village 110 people received two skin tests, first in March, and second four months later, in July of 1979. The first examination revealed 105 or 95.45% skin positives and all the rest 5 negatives turned to positive after 4 months, showing 100% ($60.73\% \leq p \leq 100\%$, 90% conf. limits) of positive turnover. However, FST antigen which was used here was a purified skin test antigen prepared from *D. immitis*, so it was not clear whether the positives had been sensitized through transmission of *O. volvulus* or through other animal species of onchocerca and/or filariids. Development of antigen from *O. volvulus* for the use in skin test, IHA and other immunological tests are in progress.

It is still early to make any epidemiological evaluation in San Vicente Pacaya because it is only one and a half year since the first control operation was initiated. However, the base-line data are already obtained, and it is planned to repeat the epidemiological surveys once a year at an interval of one year in order to find the changes in the intensity of transmission along with the progress of control.

Maselli

¿Usaron alguna prueba de control en pacientes negativos?

Hayashi

No tuvimos suficientes casos para hacerlo.

Maselli

¿En cuanto tiempo se verá el resultado de la campaña?

Hayashi

Tomará un largo tiempo

Le Berre

1. En el área del OCP se vió una reducción en la frecuencia y severidad de las lesiones tan sólo 4–5 años después, y aún hubo áreas en que la transmisión fue completamente interrumpida.

Esto significa que la actual campaña de control en San Vicente Pacaya se tendrá que continuar durante muchos años si es que se desean ver los resultados.

2. El área es pequeña y es posible que vuelva a ser reinvadida por moscas o que la gente se vaya temporalmente a otras áreas no tratadas.

Eso introducirá un sesgo negativo en la evaluación médica.

3. La adición de la nodulectomía al control

Maselli

Did you use any control test in negative patients?

Hayashi

We did not have a sufficient number of cases to be able to do it.

Maselli

How long will it take for the campaign results to be seen?

Hayashi

It will take a long time.

Le Berre

1. In the OCP area, a reduction in the prevalence and severity of lesions was seen only after 4–5 years, even in areas where the transmission was completely interrupted. That implies that the present Pacaya control campaign will have to be pursued for many years if one wants to see some results.

2. The area is small and – either reinvasion by flies or – people are going to leave it temporarily for non-treated areas, that will introduce a bias in the medical evaluation.

3. The addition of nodulectomy to vector control is also a cause of misinterpretation.

4. Why this criteria, in paper 21 (table 1,

de vectores también es causa de una mala interpretación.

4. ¿Porqué este criterio, en el trabajo 21 (tabla 1, No. 5) dice: "La zona deberá presentar una endemicidad moderada"?

Hayashi

1. Estamos conscientes de que pasarán muchos años de que veamos cambios en cuanto a la "frecuencia". Tenemos que desarrollar métodos para evaluar el efecto del control a corto plazo aún durante el curso de la campaña de control, para poder ver si el control contra el *ochraceum* se ha llevado a cabo en forma eficaz, o si aparecen otros vectores que conviertan el control en un fracazo aún cuando el *ochraceum* haya sido erradicado.

2. Sólamente se encontró una reinvasión a pequeña escala en el lindero occidental del área actual de operaciones. El problema será resuelto pronto por medio de la expansión del área de control en esa dirección, tal como se ha planificado. Los datos que presenté aquí se basan en observaciones hechas sólamente en una pequeña aldea, por lo que la población era escasa. En la actualidad se está haciendo el análisis de los datos de 6 aldeas, y esperamos reportar más adelante los resultados en base a una población más grande. Sin embargo, aquí lo que quería hacer era tan solo mostrarles como obtener los datos y la información de las "incidencias" de una variedad de señales de la enfermedad, que reflejarían en mejor forma la intensidad de la transmisión al momento de la observación, y la forma en que se interpretan los resultados.

3. "Endemicidad moderada" se usó en términos de promedio. De hecho, el área piloto seleccionada, que tiene un promedio de tasa positiva de mf del 30%, está compuesta de más de 40 comunidades cuya tasa positiva fluctúa de cero a más de 90%, lo cual nos permitió investigar la enfermedad bajo diversas endemicidades y bajo gran variedad de condiciones.

4. No creo que la denodulización afectará demasiado la situación de la transmisión. Podría ser eficaz en la prevención de la ceguera, pero tiene muy poco efecto en alterar la transmisión.

No. 5): "The zone should present a moderate endemicity"? (page 103)

Hayashi

1. We are aware that it will be many years before we see any changes as far as we look at the "prevalence". We need to develop methods to evaluate the effect of control in a short-term, even during the course of control campaign so that we may see the control against *ochraceum* has effectively been carried out or any alternative vectors come out and make the control failure even when *ochraceum* was eradicated.

2. Reinvasion was found on a small scale only in the western boundary of on-going operation area. The problem will be solved soon by the planned expansion of control area into this direction. The data I presented here were based on the observations only in one small village, so the size of population was small. The analysis on the data of 6 entre villages is now underway. I hope we can report later the results based on larger popualtion. However, here I wanted just to show how to obtain the data to get the information of "incidences" of a variety of signs of the disease which would better reflect the intensity of transmission at the time of observation and how to interpret the results.

3. "Moderate endemicity" was used in a term of average. As a matter of fact the selected pilot area, having around 30% mf positve rate on the average, is composed of more than 40 communities varying from zero to more than 90% in mf positive rate and enabled us to investigate the disease in a variety of endemicity and under a variety of conditions.

4. I don't think the denodulazation would effect the situation of the transmission so much. It might be effective in prevention of blindness, but has little effect in altering the transmission situation.

LA MIGRACION HUMANA EN EL AREA ONCOCERCOSA DEL PACAYA, GUATEMALA

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INTRODUCCION

Originalmente toda la zona dominada por el grupo Pocomán, y se sabe que en las montañas cercanas al inmediato lago de Amatitlán hubo adoratorios precolombinos. Todo el valle fue ocupado por diferentes encomiendas coloniales, sobresaliendo el Ingenio de la Compañía, propiedad de los padres de la Compañía de Jesús como una floreciente hacienda que en 1606 a 1767 cultivó caña de azúcar con abundancia de esclavos africanos. Esclavos prófugos de la Finca La Compañía, internados en los montes, fundaron un chocerío que dio origen al actual pueblo de San Vicente Pacaya; se sabe que hubo otros asentamientos de ellos en diversos lugares del actual área oncocercosa, de donde aún perduran los nombres de "Montaña del Negro", "Rincón de los Negros", etc. Desconocemos si alguno de estos esclavos llegó a la zona poseyendo la *Oncocerca volvulus* en su cuerpo, aunque si hay evidencias de su procedencia de zonas oncocercosas de Africa; sin querer adelantar juicios, esta fue una de las pocas ocasiones que pudieron haberse presentado, en un mismo lugar, africanos y *S. ochraceum.*

Los actuales habitantes del área del Pacaya son, en su mayoría, pequeños agricultores que se ven obligados a complementar sus ingresos empleándose eventualmente como jornaleros en las fincas de la zona o desplazándose a tierras de cultivo alejadas de sus viviendas.

Esta zona comprende, parcialmente, cinco municipios de dos departamentos, así:

Departamento	Municipio	Locs.	Casas	Habts.	km²
Guatemala	Villa Canales	6	85	416	22
Escuintla	Sn. V. Pacaya	39	1,317	6,413	142
Escuintla	Escuintla	5	79	307	18
Escuintla	Guanagazapa	9	129	423	33
Escuintla	Palín	9	403	2,015	33
2	5	68	2,013	9,574	248

El principal cultivo comercial del área es el café y los cultivos de subsistencia son el maíz y el frijol. Hay facilidades de comunicación con las poblaciones vecinas (Amatitlán, Escuintla, Palín y Villa Canales).

LA MIGRACION INTERNA

En su gran mayoría, los habitantes del área, son agricultores y se desplazan a trabajar en terrenos propios o como jornaleros de las fincas con permanencia de corto tiempo, pues generalmente, vuelven a sus casas al finalizar la semana, muchos regresan el mismo día. 49 localidades reciben el 87.5% de este caudal de migración interna, distribuido en la forma siguiente:

Localidades	Municipios	Inmigrantes	%
22	San Vicente Pacaya	641	30.4
21	Palín	1,110	52.6
1	Escuintla	23	1.2
3	Guanagazapa	36	1.7
2	Villa Canales	34	1.6
49	5	1,844	87.5

En el segundo cuatrimestre del año, de mayo a agosto, se intensifica la migración interna que llega a un promedio de 900 personas, cuando el resto del año sólo hay un promedio de 600 personas viajando para asuntos laborales.

SECTOR "A" PALIN-CHILAR

Los terrenos de la Finca El Chilar son de beneficio comunal, para la población indígena de palín. Es el centro de actividad agrícola de la mayor parte de los campesionos que viven en el pueblo y lugares aledaños y la que más inmigrantes aporta al área oncocercosa, con un número aproximado de 1,280 personas.

Estos terrenos ahora se identifican con multitud de nombres de las parcelas, muchas de las cuales no tienen habitantes permanentes ya que la gran mayoría llegan y regresan diariamente a Palín. Las actividades agrícolas se inician muy temprano del día y muchos viajan entre las cuatro y cinco de la mañana a sus labranzas. No recibe la afluencia de emigrantes de otros municipios, salvo la inmediata finca de San José Guachipilín, que contrata a algunos cuadrilleros en la época de corte de café.

Durante los meses de noviembre a enero se incrementa el número de mujeres en las actividades de corte del café, el resto del año los trabajos son atendidos principalmente por hombres.

SECTOR "B" LAS FINCAS

Las principales fincas cafetaleras de la zona están situadas al sur del área del Pacaya, entre 700 y 1,100 metros sobre el nivel del mar. La mano de obra local no es suficiente y requiere el concurso de los trabajadores de las localidades de la parte alta de la zona y de cuadrilleros de otras partes de la República que llegan por temporada. Entre 26 localidades de este sector, se movilizan 353 trabajadores, más 358 que llegan de las localidades vecinas y un número de cuadrillas de otras partes de la República. La inmigración interna constituye el 32% del total del área piloto.

Los caminos son de tierra, muy irregulares pero transitables en todo tiempo; tienen acceso por el lado de la ciudad de Escuintla, vía El Salto y por el lado de Santa Elena Barillas, vía Los Pocitos. Hay muchas corrientes de agua (ríos, quebradas, riachuelos, etc.).

SECTOR "C" LA PARTE ALTA DE SAN VICENTE

Aquí están comprendidas todas las localidades situadas a inmediaciones de la costa de los 1,500 metros sobre el nivel del mar. Son las localidades más populosas del municipio de San Vicente Pacaya. Aproximadamente 500 personas de este sector viajan a las fincas bajas para trabajar por jornal. Un número de 107 personas se movilizan entre las diferentes localidades del sector y un número reducido que no pasa de 26 personas en el año, son las que emigran al sector.

MIGRACION DEL AREA PILOTO A OTRAS LOCALIDADES DE LA REPUBLICA

Con base en los datos de 1977, se estima un total de 576 personas (el 6%) los que viajan del área piloto a otras localidades de la República, abarcando un total de 42 municipios en 17 departa-

mentos. La mayor parte de ellos viajan a los Deptos. de Guatemala y Escuintla (el 8% de ellos) y los lugares más atrayentes son: La ciudad Capital y las fincas y terrenos de labranzas de Amatitlán.

TRABAJADORES TEMPORALES QUE LLEGAN AL AREA PILOTO PROCEDENTES DE OTROS LUGARES

La llegada de las cuadrillas de trabajadores temporales está condicionada a las actividades del cultivo del café, así:

%	Fechas	Actividades
19	Enero a marzo	Chapeo y abono de cafetales
33	Abril – noviembre	Limpia de cafetales, almácigos, siembra de cafetales, deshije . . .
48	Noviembre a enero	Corte de café

Los principales grupos de trabajadores de cuadrillas, en el área del Pacaya, se localizan en:

Fca. Hamburgo
1,104 personas (44%) proceden de: Tutuapa, San Miguel Ixtahuacán, Tejutla, San José Ojotenán, La Democracia, San Pedro Necta, Colotenango, Huehuetenango, Cubulco y Joyabaj.

Fca. La Suiza
609 personas (24%) procedentes de Cubulco y Cobán.

Fca. San Nicolás
453 personas (18%) procedentes de Tutuapa, San Miguel Ixtahuacán y Tejutla.

El 14% estante (350 personas aproximadamente), se distribuyen en las fincas: Santa Eulalia, La Providencia, Terranova, San José Guachipilín, El Camarón, Puerta de Oro, Berlín, Coyolito, Santa Fe y Peña Blanca, así como las aldeas Bejucal y Los Ríos. Todos ellos proceden de Jalapa, Santa María Ixhuatán, San Juan Sacatepéquez, y de los pueblos aledaños.

Más de la mitad (59%) de los trabajadores temporales, son hombres adultos (mayores de 15 años), el 20% son mujeres mayores de 15 años, el 13% son niños hombres y el 8% son niñas mujeres. En números absolutos, para el año 1977, las cifras fueron las siguientes:

Hombres adultos:	1,482
Mujeres adultas:	502
Niños varones:	327
Niñas mujeres	201
S U M A	2,512

Procedencia de los trabajadores temporales que llegan a la zona de San Vicente Pacaya:

Del Mpio. de:	Del Depto. de:	Locs.	Personas	%
Cubulco	Baja Verapaz	17	1,069	42.55
Concepción Tutuapa	San Marcos	14	773	30.77
San Miguel Ixtahuacán	San Marcos	4	200	7.96
La Democracia	Huehuetenango	1	119	4.74
Joyabaj	El Quiché	3	114	4.54
Tejutla	San Marcos	5	97	3.86
San Pedro Necta	Huehuetenango	2	51	2.03
Jalapa	Jalapa	1	18	0.72
Huehuetenango	Huehuetenango	1	17	0.68
Colotenango	Huehuetenango	1	17	0.68
Cobán	Alta Verapaz	1	13	0.52
San Juan Sac.	Guatemala	2	13	0.51
San José Ojotenán	San Marcos	1	8	0.32
Ixhuatán	Santa Rosa	1	3	0.12
14	8	54	2,512	100.00

Las mismas personas que van al área del Pacaya, reportan permanencia en otras fincas, ya sean cafetaleras, algodoneras o cañeras, en su constante peregrinar hacia las fuentes de trabajo:

Del Depto. de	Escuintla en cuatro municipios	8 Locds.
	San Marcos en dos municipios	4 Locds.
	Sta. Rosa en dos municipios	2 Locds.
	Retalhuleu en dos municipios	3 Locds.
	Chimaltenango en un municipio	1 Locd.
	Suchitepéquez en un municipio	2 Locds.
	Guatemala en un municipio	1 Locd.

De las 23 localidades que estas personas visitan, sólo Sibajá del Mpio. de Yepocapa, está en zona oncocercosa.

Por lo menos el 1.5% de los trabajadores temporales que llegan a la zona del Pacaya, acostumbran trabajar en diversas fincas algodoneras y cafetaleras en el estado de Chiapas, México.

CONCLUSIONES

a. La economía familiar de los habitantes del área del Pacaya está condicionada a los ingresos que obtienen en los trabajos de las fincas cafetaleras, unos, y en los labrantíos de los cultivos de subsistencia. En ambos casos esta necesidad los acerca a los lugares de reproducción de *Simulium ochraceum,* lo que parece determinante en la mecánica de la transmisión.

b. La migración interna justifica la alta densidad parasitaria de la zona, aún en localidades donde no se ha comprobado la posibilidad de la transmisión, como Patrocinio, pueblo de Palín, etc. Aún no se han medido las consecuencias de la migración externa.

c. Aún los pequeños propietarios residentes en las localidades más populosas del área, se ven necesitados de vender su fuerza de trabajo a los productores mayoritarios y son parte de la migración interna, de la cual también participan las mujeres y los niños, que en gran cantidad asumen responsabilidades en los trabajos agrícolas.

d. Llama la atención los estables límites de las zonas de la enfermedad de donde se puede deducir que: El SIMULIDO NO LLEVA LA ENFERMEDAD MAS ALLA DE SUS

DOMINIOS, ES EL HOMBRE QUIEN LA VA A TRAER. De allí que se hace necesaria una investigación más concienzuda de la conducta del principal elemento de la transmisión: EL HOMBRE.

Yamagata

La gran migración diaria en SVP es una característica muy singular entre las zonas oncocercósas de Guatemala. Es una desventaja en términos de entendimiento epidemiológico, pero por otra parte, es una gran ventaja en el control larvicida ya que hay una buena red de ensayos allí debido al frecuente tráfico.

Además, en relación con la pregunta del Dr. Le Barre en relación a la última presentación, *Cantón La Cruz es el único lugar dentro* de las municipalidades de SVP. La transmisión más severa ocurrió en el valle de Los Lavaderos, ya que los habitantes de las municipalidades de SVP van allí a lavar su ropa, a traer agua, a sus cultivos o para llegar a la carretera. Estas son migraciones diarias o semanales. El Sr. Molina mencionó especialmente las migraciones estacionales. Existe aún otra clase de migración, aquella debida a cambios de vida, tal como matrimonios y asistencia escolar. Desde este punto de vista, los Sectores B y C pertenecen a la misma población.

Yamagata

The great daily migration in SVP is a very unique feature among the onchocerciasis zones in Guatemala. It is a disadvantage in terms of epidemiological understanding, but on the other hand, is a great advantage in larvicidal control, because a good network of trials is available there, owing to a frequent traffic.

Also in relation to Dr. Le Berré's question to the last speech, Canton La Cruz is the only place among SVP townships. Most severe transmission occurred in Los Lavaderos valley, when the inhabitants of SVP townships go there for washing clothes, fetching water, cultivation or to approach highway. These are daily and weekly migrations. Sr. Molina mentioned mainly seasonal migration. There is still another kind of migration; migration due to life phase (like marriage and schooling). From this point of view, Sectors B and C belong to the same population.

NUEVO METODO PARA IDENTIFICAR MICROFILARIAS DE ONCOCERCOSIS EN BIOPSIA DE PIEL

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Esta técnica, sencilla, práctica y efectiva permite que personal adiestrado en Cuidados Primarios de Salud y en Cuidados Primarios del ojo; hagan el diagnóstico de positividad de Oncocercosis en una biopsia de piel; sin recurrir al microscopio; éste método podrá utilizarse en Campañas masivas para determinar la prevalencia y la incidencia en una área o región determinada en el menor tiempo posible, en gran escala y en forma más económica.

Técnica:

1. La biopsia de piel se toma del hombro u otra región; utilizando una hoja de razurar "Guillet", o con otros instrumentos ya descritos.
2. La biopsia se coloca sobre una laminilla porta-objetos; donde previamente se ha puesto una gota de solución salina normal; se agita la biopsia así colocada con un palillo de dientes, en forma suave.
3. Se utiliza una lámpara (Packet Lamp) con baterias de 1.5 volts y bulbo de 2.5 o baterias de 4.5 volts y bulbos de 3.8; cuyo diamétro de luz sea de unos 10 cms. o más sobre esta lámpara encendida se coloca el porta objetos con la biopsia.
4. La identificación de las microfilarias, se hará por medio de una lupa de +20 dioptrias; que estará colocada entre la biopsia y el observador a unos 5 a 8 cms. de la primera.
5. En vez de la lupa puede utilizarse oftalmoscopio corriente con +10 a +12.
6. Las microfilarias se verán brillantes, refringentes, con sus movimientos normales, facilmente identificables.

Figueroa M.

Yo no veo como se puede hacer DX.

Rivas-Alcalá

Creo que este método es bastante rudo.

¿Se puede hacer DX si la densidad es baja?

L.N. Figueroa

Se debe usar cuando no se tienen otros recursos disponibles.

Castillo

Felicitación por el trabajo.

Lugo

Creo difícil observar Mf con oftalmoscopio E + 10.

Aguilar

Sólo debemos cambiar el título del trabajo.

Figueroa M.

I don't see how DX can be carried out.

Rivas-Alcalá

I think this method is quite rough.

Can you do DX if the density is low?

L.N. Figueroa

It must be used when there are no other resources available.

Castillo

Congratulations for the paper.

Lugo

I think it is very difficult to see Mf with an E + 10 ophthalmoscope.

Aguilar

We should only change the title of the paper.

EXTRACCION Y MANTENIMIENTO *IN VITRO* DE FILARIAS ADULTAS VIVAS DE *ONCHOCERCA VOLVULUS* PROVENIENTES DE NODULOS (ONCOCERCOMAS) HUMANOS

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La primera parte de este estudio fue para comparar las diversas concentraciones de colagenasa y combinaciones de medios de cultivo de tejido NCTC 135 y NCTC 199 con sueros humanos para la digestión de los nódulos y para el mantenimiento *in vitro* de los gusanos adultos.

Se obtuvieron y examinaron 121 nódulos provenientes de la cabeza y el cuerpo de 108 personas, niños y adultos, residentes en un área endémica de oncocercosis cerca de Patulul, Scuchitepéquez, Guatemala. A todas las personas se les hizo dos biopsias de la piel (hombro y cadera) y el número total de microfilarias se determinó una hora después de la biopsia, en solución salina fisiológica. Los nódulos fueron extraídos bajo condiciones asépticas y con anestesia local, y lavados en solución salina fisiológica boferada estéril conteniendo 200 U.I. de penicilina, 200 mcgm de estreptomicina y 0.5 mcgm de fungizone por mililitro, para lavar cualquier exceso de sangre y mantener la contaminación al mínimo; luego se midieron a su largo y ancho al milímetro más cercano con una escala Vernier. Cada nódulo se colocó en un Erlenmeyer de 50 ml conteniendo 10 ml de una solución de medio de cultivo NCTC 135 o medio 199 con sales de Earle con L-glutamina y amortiguador de carbonatos a un pH final de 7.2, conteniendo los antimicrobianos por mililitro ya descritos, y adicionado con 0%, 5% y 10% (v/v) de suero humano normal. En cada uno de los medios de cultivo se variaron las concentraciones finales de colagensa a 3, 4 o 5 mg/ml. En un promedio de 2 a 3 horas después de extirpación del nódulo, en lo que se transportaron al laboratorio, se colocaron los Erlenmeyer en un baño de agua con agitación contínua a temperatura constante de 35 ±1°C. Cada tres horas se inspeccionó el grado de digestión del nódulo, se desmenuzó cuidadosamente el tejido fibroso del huésped con estiletes y se lavó con una pipeta de Pasteur. No hubo una diferencia aparente en el tiempo de digestión de nódulos en el medio 199 y NCTC 135 y con los diferentes suplementos de suero humano normal para un tamaño promedio de nódulo. Los nódulos pequeños (9 x 7 mm) se digerieron en 4 a 7 horas; los medianos (12 x 9 mm) en 6 a 14 horas; los grandes (13 x 18 mm) en 16 a 27 hors. Sin embargo, el tiempo promedio de digestión fue ligeramente menor con la concentración de 5 mg/ml de colagenasa que las otras usadas, auque varias filarias, especialmente hembras, estaban rotas y su cutícula delgada.

El mantenimiento *in vitro* de las filarias extraídas se hizo en 10 ml de medio de cultivo NCTC 135 o medio 199 como descrito anteriormente, pero sin colagenasa y suplemento con 10% o 20% de suero humano normal en frascos para cultivo de tejidos e incubadas a 35 ±1°C. El medio fue cambiado cada dos o tres días. Las filarias se observaron para manifestación de cualquier tipo de movimiento contorsional o a las microfilarias *in utero* en filarias hembras.

No se encontró una diferencia significativa entre el tiempo de mantenimiento en NCTC 135 o medio 199 y con los suplementos de suero humano, oscilando el promedio en 5.7 a 7.6 días ±1.1 a 3 días; el menor tiempo para machos fue de 5 días y el mayor de 9; el menor para hembras de 4 y el mayor de 12. Una hembra producía, aún después de 12 días de cultivo, microfilarias vivas.

En conclusión, el uso de 3 mg/ml de colagenasa en NCTC 199 es preferible al de las otras combinaciones debido a que es menos costoso y proporciona un efecto igualmente rápido en la digestión de los nódulos y la viabilidad de los gusanos. Sueros en concentraciones de 10% o 20% son necesarios para el mantenimiento *in vitro* de los gusanos pero no para la digestión de los nódulos.

En la segunda parte del estudio, se removieron nódulos de 36 personas que indicaban biopsias negativas y se pusieron a digerir en colagenasa. Los gusanos adultos fueron montados en glicerina y se examinaron para determinar su sexo y si las hembras estaban produciendo microfilarias. La mayoría de la hembras (81%) no lo estaban junto con el macho en el nódulo y la proporción de hembras y de machos fue de 4.3 a 1. Solamente el 31% de las hembras (15/48) examinadas, contenían microfilarias *in utero*. Denlas 15 hembras con microfilarias, 11 contenían un macho en el nódulo. La prueba Mazzotti le fue administrada a 16 pacientes con biopsias negativas y con nódulos. Solamente una tuvo una reacción positiva y el nódulo removido de este paciente contenía 1 gusano macho y 1 hembra produciendo microfilarias.

Estos resultados sugieren las siguientes concluciones:

1) Los nódulos se forman solamente alrededor de los gusanos hembras.
2) La producción de microfilarias por parte de la hembra no es esencial para la formación del nódulo.
3) El apareamiento ocurre antes o al principio de la formación del nódulo.
4) La prueba de Mazzotti no detecta a las personas con nódulos solamente, por lo que no puede esperarse que detecte a los gusanos adultos en tejidos profundos a menos que estos estén produciendo microfilarias.

Lugo

¿Qué téncicas usaron para ver las filarias por la enzima?

Luján

Se utilizó microscopía corriente.

Lugo

¿Qué dosis de Hetrazan se usa para Mazzoti?

Figueroa M.

50–100 mg.

Martinez

¿A qué hora se leyo Mazzoti?

Figueroa M.

Puede ser rapido o lenta se debe esperar hasta el día siguiente para decir que es negativa.

Kawabata

¿Usó suero de pacientes con oncocerciásis en su sistema de cultivos?

Luján

No. El suero usado fue de sujetos humanos normales de los E.U.

Lugo

What techniques were used to see filariae by the enzyme?

Luján

We used the usual microscopy technique.

Lugo

What dosages of Hetrazan is used for the Mazzotti test?

Figueroa M.

50–100 mg.

Martinez

How long after was the Mazzotti read?

Figueroa M.

It can be fast or slow. One should wait until next day to say that it is negative.

Kawabata

Did you use the serum of patients with onchocerciasis in your culture system?

Luján

No, the serum used was from normal human subjects from the U.S.

PARASITOLOGICAL SYMPTOMS OF AT-RISK-PATIENTS IN ONCHOCERCIASIS

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In foci of onchocerciasis the infection is usually prevalent with rates of 60% and more. This means that more or less 100% of the population older than 20 harbour the parasite. Eye-lesions leading to blindness are the most serious and disabilitating effects of the disease. Although they develop with advancing age in an ever growing proportion of the population not every patient will be affected. Schemes to control onchocerciasis are installed to prevent these adverse results of the infection. Unfortunately however chemotherapeutical treatment is accompanied by a number of severe side-reactions rendering mass-treatment almost impossible, denodulization does not eliminate all parasites from the patient, vector control is very costly and not feasible in mountainous, densely forested areas.

The selected care for those patients who are most endangered to loose their eyesight can be a compromise when mass-treatment of all persons infected is impossible. It may enhance the results of vector control.

In the absence of large and well planned long-term-studies, at-risk-patients can only be identified as those who have already developed eye-lesions like sclerozing keratitis, iritis, chorio-retinitis and optic nerve disease. This requires the help of an ophthalmologist who is difficult to find in most areas in which onchocerciasis occurs.

A study of 1,577 persons in the Onchocerciasis Control Programme in the Volta River Basin Area (OCP) has shown that certain parasitological signs correlate with the presence of eye-lesions. The single most important sign was the presence of 5 and more nodules. 40% of these persons had eye-lesions. More than 100 microfilariae per skin snip, microfilaruria, the presence of microfilariae in biopsies taken at the outer canthus are further criteria that alone or in combination allow to select subgroups from a 100% infected population containing high proportions of those with eye-lesions and thus at-risk to become blind.

Examinations of 1,403 persons in the area of Pochuta, Chimaltenango have shown that 17.1% had 100 and more microfilariae in skin snips from the iliac crest, 12.1% in skin snips at the shoulders. Microfilaruria existed in 6.9%, nodules - despite regular denodulization - in 11.5%.

Yamada

Estoy de acuerdo con usted en que la alta densidad de microfilarias en la piel y el número de nódulos parecen estar correlacionados con las lesiones oculares. En mi experiencia en Guatemala, un caso con microfilarias en la cámara anterior pero sin microfilarias en la piel fue imposible detectar las clases de microfilaria debido a que no había forma de extirparlo.

Yamada

I agree with you, that the high density of microfilariae in the skin and number of nodules seem to be correlated to ocular lesions. In my experience in Guatemala, I had one case with microfilaria in the anterior chamber with no microfilaria in the skin and nodule. It was impossible to detect the kind of microfilaria because there was no way to extirpate them.

Yoshimura

Los factores de riesgo deberían ser los factores que preceden la lesión ocular. Por lo tanto, se deberá tomar en cuenta el factor tiempo para identificar los factores de riesgo

Yoshimura

Risk factors should be the factors which proceed the eye lesion. Therefore time factor should be considered to identify the risk factors for eye lesions. I would like to recommend

para lesiones oculares. Quisiera recomendar que lleven a cabo un estudio de tipo longitudinal para determinar los factores de riesgo. Afortunada o desafortunadamente, en Guatemala no tenemos suficientes pacientes con lesiones oculares. Por lo tanto, sólamente en Africa sería posible aclarar los factores de riesgo para lesiones oculares, y así prevenir la ceguera debido a la oncocerciasis.

George Diaz

En la última tabla presentada habían columnas (±) indicando la presencia o la falta de parámetros sintomáticos que ustedes usan para determinar la probabilidad de lesiones oculares.

De 562 individuos, todos con signos negativos en las columnas, el 21.8% tenía lesiones. En la última línea todos los síntomas estaban presentes (+), pero sólamente 4.8% tenía lesiones oculares.

Por favor comentar.

El % se relaciona al numero total examinado. 21% de todos los pacientes con lesiones oculares no mostró señales parasitológicas tal como se han definido.

Por otra parte, aunque el 90% con síntomas presentes tenían lesiones oculares, sólamente cubren el 4% de todos los pacientes en la población examinada.

that you carry out a longitudinal type study to find the risk factors. Fortunately or unfortunately, in Guatemala we do not have enough patients with eye lesions. Therefore, only in Africa it would be possible to clarify the risk factors for eye lesions in order to prevent blindness due to onchocerciasis.

George Diaz

In the last table presented, there were columns (±) indicating the presence or lack of symptomatic parameters you use to determine the probability of eye lesions.

Out of 562 individuals, all with negative signs in the columns, 21.8% had lesions. In the last line all symptoms were present (+), but only 4.8% had eye lesions.

Please comment.

The % relates to the total number examined. 21% of all patients with eye-lesions did not show parasitological signs as defined.

On the other hand, although 90% of those with all criteria present had eye lesions, they are only 4% of all patients in the population examined.

ULTRAMORFOLOGIA DE *ONCHOCERCA VOLVULUS*

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A fin de clarificar la ultramorfología de *Onchocerca volvulus en situ*, nódulos con *O. volvulus* extirpados en Guatemala durante una campaña de nodulectomía de rutina fueron fijados y preparados por estudios con microscopio electrónico de transmisión y de rostro.

VERME HEMBRA

Cutícula. La cutícula de la hembra adulta, de un grosor aproximado de 8 μm, tiene seis capas. La capa superficial es una esterilla con apariencia de musgo de un grosor de 0.2 a 0.5 μm formada por los numerosos pliegues de las extensiones membranosas de dicha capa.

La masa de la cutícula está compuesta de una capa externa fibrosa de unos 4 μm, que está separada por una capa gruesa y homogénea de 0.2 a 0.4 μm de la capa media, fibrosa y gruesa, de 0.6 a 0.8 μm. Las fibrillas que contienen estas dos capas están alineadas a lo largo del eje del verme. Las fibras de la membrana interna, fibrosa y de 3 a 4.5 μm de espesor, parecen estar orientadas en sentido transversal. La membrana basal, homogénea y de 0.1 a 0.6 μm de espesor, está situada entre la capa fibrilar interna y la hipodermis. Dentro de la capa basal se observan grupos de estructuras electrónicamente densas, recubiertas por una membrana y que miden de 0.15 a 0.25 x 0.3 a 1.0 μm. Las numerosas proyecciones que salen de la superficie interna de la capa basal sirven como puntos de unión con las membranas hipodérmicas, y otra serie de membranas paralelas pueden extenderse hacia la superficie perientérica de las cuerdas laterales.

Cuerdas laterales. A diferencia de otros filáridos, la masa de la pared del cuerpo de *O. volvulus* está compuesta por la cuerda lateral, y las bandas musculares sólo ocupan de 1/3 a 1/4 de la circunferencia total. La membrana de la cuerda lateral que limita con la cavidad perientérica está perforada por numerosos poros que parecen ser las aberturas de un sistema canalicular, el cual se extiende dentro del citoplasma de la cuerda. Fuera de los poros se ve una capa que parece un filtro, de 0.3 μm de espesor, compuesta de un material homogéneo y amorfo; en algunos casos, este material se observa dentro de aberturas más grandes. Muy probablemente, estos canales sirvan de conductos a los nutrientes provenientes de la cavidad perientérica, así como para aumentar el área de la superficie absorbente de la cuerda lateral.

El citoplasma normal de la cuerda lateral contiene agregados de glicógeno, gotitas de lípidos, cuerpos densos, muchos aparatos de Golgi, mitocondrios pequeños y densos, y manojos de filamentos y membranas. Los microorganismos tipo *Chlamydia* que se encuentran con frecuencia dentro del citoplasma de las cuerdas laterales y de otros órganos de la hembra, han sido descritos en detalle en otra publicación (Kozek y Figueroa, 1977).

El conducto excretor está localizado dentro de la cuerda lateral. El tejido citoplasmático que recubre la superficie interna del conducto contiene un sistema de canalículos similar al que se observa en el costado de la cuerda lateral que limita la cavidad perientérica. Sin embargo, parece flatar la densa capa homogénea que recubre los poros.

Musculatura. Las células musculares de las hembras adultas de *O. volvulus* parecen estar atrofiadas; ocupan aproximadamente de 1/4 a 1/3 de la circunferencia de la pared del cuerpo. Se observan pequeños manojos de microfilamentos delgados y gruesos separados por grandes áreas que contienen un material granuloso que podría ser glicógeno o ribosomas. En la porción metabólica de

los músculos de dos hembras se encontraron partículas con características de virus.

Aparato reproductor. El tejido que recubre la parte interna del útero está modificado en una serie de gruesos pliegues alineados paralelamente al eje del verme. La superficie citoplasmática de estos pliegues contiene un sistema canalicular semejante al que se observa en las cuerdas laterales. El útero contiene huevos en todos los estadíos de desarrollo y microfilarias maduras. En las cuerdas laterales de las microfilarias en desarrollo dentro del útero se encuentra tambien los microorganismos tipo *Chlamydia*.

Aparato digestivo. Hasta el momento no hemos podido obtener buenos cortes ni de la porción muscular ni de la glandular del esófago del verme adulto, ya fuera macho o hembra.

El intestino es un simple tubo de un diámetro aproximado de 20 μm. El tejido que recubre la parte externa de intestino es una capa homogénea de aproximadamente 1 μm de espesor, a la cual están adheridas las células intestinales. El epitelio intestinal está formado por células piramidales simples. Los núcleos están situados en la porción basal de la célula. El citoplasma contiene pequeños mitocondrios oscuros, cuerpos densos y numerosas inclusiones globulosas de hasta 0.3 μm de diámetro, que parecen ser depósitos minerales. De la porción apical, cónica, de la célula, emergen pequeños microvellos que se proyectan dentro del lumen.

VERME MACHO

En el macho las cuerdas laterales son mucho más pequeñas; las células musculares, que tienen porciones contráctiles y metabólicas muy claras, ocupan casi todo el espacio de la pared del cuerpo. La cutícula es más delgada que la de la hembra y carece de la capa superficial en forma de esterilla que se observa en la (superficie de la) hembra.

La pared del testículo es semejante a la del útero, y tiene células epiteliformes y células musculares externas. Los espermatocitos están adheridos al raquis central en la misma forma en que lo están los oocitos.

MICROFILARIA

Las microfilarias muestran la organización interna típica del estadío: cutícula, hipodermis, bandas musculares dorsales y ventrales, y la columna nuclear. La vesícula excretora y la anal, parecen ser más pequeñas y la célula excretora y las células R menos prominentes que en las microfilarias de otras especies. Por otro lado, las aberturas de los canales anfidiales parecen ser más prominentes que los de otras microfilarias.

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FINE STRUCTURE OF THE CUTICLE OF ADULT *ONCHOCERCA VOLVULUS*

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The particular morphology of the cuticle of female *Onchocerca volvulus* was revealed first by Deas et al. (1974). Recently, Franz (1980) demonstrated by SEM studies that the surface fine structures of male and female *O. volvulus* differed considerably. Therefore, a TEM study of male and female worms of different stages of development was undertaken.

The cuticle in the midbody region of the male worm is composed of a surface membrane and seven layers underneath, which can be differentiated by the direction of their fibrils and their density in the electron microscope (Fig. 1). The surface structures of this region consist of ridges, which are separated only by a narrow intermediate space, and of a fine honeycomb like surface relief.

The surface structures of the first 500 μm of the anterior regions of the male and female worm look very similar. Both of them show fine annulations. In the female worm the cuticle of this region is composed of the same layers which are known from the male worm's cuticle.

In the midbody region, however, the cuticle of the female worm shows an other structure and a different surface morphology than that of the male worm. Only four layers are revealed by electron microscopy. The two innermost layers remain constant in the direction of their fibrils and in their electron density, whereas the composition of the external cuticle is simplified. The characteristic ridges with a wide intermediate space are formed by the outermost cuticular layer. The surface membrane of the cuticle forms irregular folds. Usually there is an electron dense coat separating the worm from the host tissue. The folds of the surface membrane penetrate far into the coat, whereas they are much smaller where a coat is lacking.

Obvious changes take place during the development from the immature to the old female worm. There are the same cuticular layers in the midbody region of an immature female worm, which is only about three centimeters long, as in the adult worm (Fig. 2). But in the immature worm the surface membrane is folded less highly, the external cuticular layers are thinner and the ridges are much closer togehter. Therefore, the growth of the cuticle must take place by deposition of cuticular material into the different layers. In a degenerating worm the thickening of the cuticle is formed differently (Fig. 3). An accumulation of additional cuticular material is found only at the internal border of the cuticle.

The motile male worm and the motile anterior region of the female worm show a very similar surface consisting of folds and honey-comb like patterns. The cuticle also demonstrates a corresponding structure with seven layers. On the contrary the extremely elongated midbody region of the female worm lies firmly enclosed in the host tissue. We suppose that the simplification of the cuticular structure and the extreme folding of the surface membrane are adaptations to the particular situation of this region of the worm.

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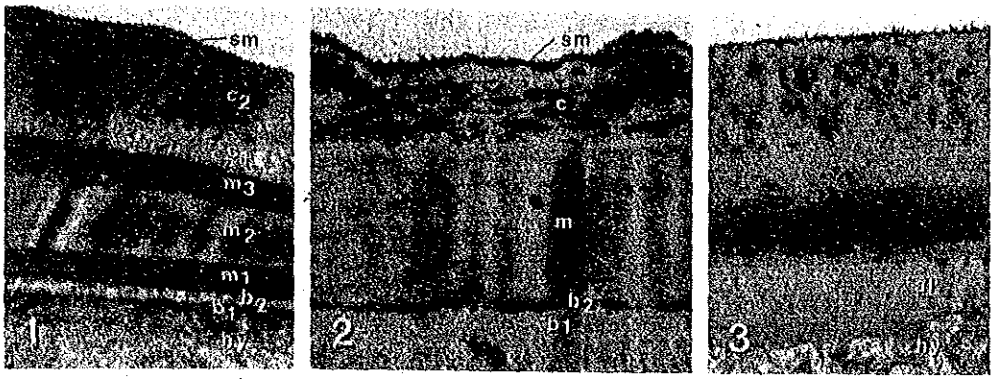

Figures 1–3.

Fig. 1: Transverse section of the cuticle in the midbody region of a male worm. hy = hypodermis, b_1, b_2 = two basal sublayers, m_1, m_2, m_3 = three median sublayers, c_1, c_2 = two cortical sublayers, sm = surface membrane (1 : 7,000)

Fig. 2: Longitudinal section of the cuticle of an immature female worm. b_1, b_2 = basal sublayers, m = median layer, c = cortical layer, sm = surface membrane (1 : 8,000)

Fig. 3: Transverse section of the cuticle in the midbody region of a female worm with an additional layer inside of the commonly observed layers. al = additional layer, hy = hypodermis (1 : 6,000)

PARASITOLOGICAL DIAGNOSIS OF ONCHOCERCIASIS IN GUATEMALA

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From the diagnostic point of view it is essential to establish a reliable method of skin biopsy with an excellent detecting capacity. In addition, simplicity in manipulation is required for the use in field epidemiological surveys. In order to establish a reliable and practical method of skin biopsy on Guatemalan patients with onchocerciasis, several skin snipping instruments were comparatively tested and the most suitable portion for skin snipping with highest microfilarial density (MFD) was searched. Three sorts of instrument (Holth type and Walser type sclerocorneal punch and disporsable scalpel) having been commonly used for skin biopsy were tested and the usefulness was evaluated in an endemic forcus in Guatemala. The disporsable scalpel technique gave the highest detecting rate of 61.1%. However this technique has disadvantages, such as painfulness, bleeding and occasional infection. So long as the convenience in the field survey is concerned, sclerocorneal punch seemed to be more practical, and Holth type punch was recommended as the more appropriate skin snipping instrument because a higher detection rate (55.6%) was obtained than with Walser type (51.9%).

A new line study of microfilarial distribution and the quantitative examinations for MFD were carried out. Thirteen skin biopsies were made from each patient at various regions of body surface. The iliac crest and scapula region were found to be the portion with the highest frequency of detection of microfilariae, while on the extremities and head portion microfilariae were distributed to lesser densities. However, the cases heavily infected with microfilariae in the anterior segment of the eye had also significantly higher densities of microfilariae on the outer canthus and retroauricular reigon. Among the male patients, the average MFD on the iliac crest was 2.97/mm^2 and greater than 1.46/m^2 on the scapula site and the difference of MFD between them was statistically significant in hypoendemic area or in young age groups. In the females, on the other hand, the scapula showed greater MFD (1.38/mm^2) than the iliac crest site (0.90/mm^2). From these results, a decision was made to take routine biopsy samples one from the iliac crest and another from the scapula region in the case of male, and each one sample from both sides of scapula region in female.

ATTEMPTS TO ESTABLISH *O. VOLVULUS* INFECTION IN PRIMATES AND OTHER LABORATORY ANIMALS

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Much of our progress in the basic and clinical studies on onchocerciasis has been hampered by the lack of a practical laboratory animal host. Natural infections of animals with *O. volvulus* have been reported, to date, only in the gorilla by Van der Berghe et al (1964) and in a golden spider monkey by Caballero and Barrera (1958). Duke (1962) was the first investigator to obtain an experimentally induced, patent infections in the chimpanzee. Other animals which were tested but proved to be refractory to the infection were: the mandril, the mangaby, Preuss monkey and the goat (Duke, 1962). Suswillo et al (1977) were unable to obtain patent infections in homsters and jirds.

Our study was established in 1975 with the purpose of screening other species of primates and small laboratory animals for their susceptibility to *O. volvulus* with the aim of identifying an animal which would serve as a practical animal host that could be available to numerous investigators.

The vectors, *Simulium ochraceum*, were collected in the onchocerca endemic zones of Guatemala, after feeding on volunteers with naturally required *O. volvulus* infection. The flies were maintained during the post-pradial period of 8 to 10 days according to the method of Figueroa et al (1977), until the ingested micrfilariae of *O. volvulus* developed into the infective stage. These larvae, recovered from the dissected flies, were inoculated either subcutaneously or intraperitoneally into the following animals:

4	Rhesus monkeys
4	Bonnet monkeys
6	Golden spider monkeys
6	Black spider monkeys
4	Galagos
2	Opossums
10	Newborn Swiss mice
10	Adult Swiss mice
12	Male jirds
13	Multimammate rats, normal and splenectomized
3	Calves
1	Kinkajou
1	Cebus monkey

The inoculated animals were observed for up to two years. They were palpated monthly to determine whether nodules were forming. Beginning at 6 months after inoculation skin snips were taken each month from each animal and examined for microfilariae. Some of the mice were sacrificed during the 6 month period after inoculation and were examined for developing stages of *O. volvulus*. Any animals which died was carefully examined during necropsy for *O. volvulus* larvae. All animals alive 2 years after inoculation were sacrificed and carefully autopsied.

The calves, multimammate rats, the kinkajou and the Cebus monkey are still under observation, but all other animals tested were unsuitable for the development of *O. volvulus* since palpation,

skin biopsies, and thorough necropsies failed to reveal any signs of infection.

Our results indicate that identification of a small and practical laboratory animal host for onchocerciasis may be a difficult task. The report of Caballero and Barrera (1958) prompted us to use a large group of spider monkeys during these studies. However, we were unable to infect neither the golden nor the black spider moneky with *O. volvulus*. Although we cannot exclude the possibility that the host strain differences might have been responsible for the refractoriness of the spider monkeys, it appears that further studies are warrented, using both the monkeys and the parasite from the same geographical area, to determine whether the golden spider monkeys are susceptible to *O. volvulus*.

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ANTICUERPOS CONTRA *O. VOLVULUS* EN LAGRIMAS DE ONCOCERCOSIS

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INTRODUCCION

La oncocercosis es una enfermedad endémica en Guatemala, se ha considerado que en la actualidad, esta enfermedad afecta del 20 al 30% de habitantes de las zonas o focos oncocercosos, en los Departamentos de: Huehuetenango, Suchitepéquez, Sololá, Chimaltenango, Santa Rosa, Guatemala (en el Municipio de Villa Canales) y Escuintla. En este último departamento los municipios afectados son: Santa Lucía Cotzumalguapa, Siquinalá, Palín, Guanagazapa y San Vicente Pacaya (1). Esta enfermedad tiene importancia clínica y epidemiológica en nuestro medio, debido principalmente a sus manifestaciones que, se ha calculado, están presentes en el 10 al 60% de los enfermos oncocercosos (1), de este grupo el 2 al 5% y a veces hasta el 30%, desarrollan ceguera en las zonas endémicas (1). La relación huésped parásito en el caso de la oncocercosis, ha sido previamente investigado.

Se ha investigado la presencia de inmunidad específica a este parásito por medio de varias pruebas: a) Intradémicas: empleando antígenos de extracto de Vermes *Onchocerca gutturosa* (2), de *Onchocerca volvulus* y de *Dirofilaria immites* (3), encontrando anticuerpos aproximadamente en el 70% de los casos; la positividad de estas pruebas ha sido manifiesta en casos comprobados parasitológicamente, y negativas en casos seleccionados, provenientes de regiones en donde no existe la enfermedad. Antes de efectuar este tipo de pruebas, es necesario hacer evaluaciónes cuidadosas sobre sensibilidad, especificidad, posibilidad de reacciones cruzadas con otras parasitosis, relaciones con estados alérgicos, para aceptar o nó este método para aplicación clínica. b) Determinaciones de anticuerpos circulantes por diversos métodos: Por inmunofluorescencia se han reportado títulos elevados (4). Por hemaglutinación, indirecta, se han encontrado títulos en el 100% de los pacientes (5). Por precipitación en Gel Agar se han reportado títulos en el 60% de los investigados (6) (7). Y por la determinación de anticuerpos reagínicos en el 70 al 100% de los examinados (5), (8). Los resultados de éstas pruebas en general han puesto en evidencia la presencia de anticuerpos contra mezclas de antígenos de *Onchocerca volvulus* y otros parásitos. La función de éstos anticuerpos en la fisiopatología de la oncocercosis no ha sido establecida. El propósito del presente trabajo fués el de investigar anticuerpos contra *Onchocerca volvulus* en las lágrimas de pacientes oncocercosis con o sin lesiones oculares

MATERIAL Y METODOS

Se escogieron tres grupos de pacientes los cuales se muestran a continuación:

GRUPO I: Quince personas, pertenecientes a la Finca Berlín, del Municipio de San Vicente Pacaya del Departamento de Escuintla -Guatemala, con manifestaciones clínicas de oncocercosis; nódulos y/o filarias en cámara anterior y/o lesiones oculares debidas a oncocercosis.

GRUPO II: Treinta y una personas de edad y sexo comparables, del área oncocercosa de San Vicente Pacaya (Cabecera), no infectadas con oncocercosis. Grupo control del área oncocercosa.

GRUPO III: Diez personas de edad y sexo comparables, de la capital, normales (grupo control completamente sano).

A estas personas se les efectuó: interrogatorio para verificar si habían padecido o padecían oncocercosis, para buscar filarias en cámara anterior y/o detectar lesiones oculares. El examen ocular se practicó utilizando carteles de agudeza visual, lámpara de hendidura, oftalmoscopio directo e indirecto. Después de efectuado el interrogatorio y examen físico, a cada una de las personas se les tomó una muestra de su secreción lagrimal, del saco conjuntival, por medio de un tubo capilar sin heparina. Las muestras se conservaron a -4°C y fueron utilizadas para investigar en ellas la presencia de anticuerpos contra *O. volvulus*, con la técnica de Hemaglutinación Pasiva, para la que se utilizaron glóbulos rojos de carnero formolizados con extracto de *Onchocerca volvulus*.

FORMOLIZACION DE GLOBULOS ROJOS DE CARNERO

Método de Butler (9), modificado por Masselli, para este estudio. Se preparó sangre fresca de carnero en una solución al 50% en Alsever. Luego se diluyó formalina de 35% hasta 3% con solución Tamponada de Fosfatos (STF) pH 7.2, ajustando el pH a 7.4 NaOH 1 N. Después se reasizaron los siguientes pasos:

1. Los glóbulos rojos de carnero (GRC), en Alsever se lavaron 3 veces con 20 volúmenes de STF pH 7.2.(por centrifugación a -7°C a 2,000 rev. por minuto, durante 10 minutos por cada lavada).
2. Después de la última lavada, los GRC se resuspendieron al 8% en STF pH 7.2.
3. A un volúmen de lo anterior se agregó el mismo volúmen de formalina (ya preparada al 3%) agitando suavemente sin producir espuma.
4. La suspensión, se incubó durante 18 horas a una temperatura de 37°C, con constantes agitaciones suaves, en un recipiente tapado con papel de aluminio para que no se evapore la solución.
5. Al cumplir el tiempo, se lavaron los GRC-Formolizados (en frío a -7°C a 2,000 rev. por minuto), con 20 volúmenes de agua destilada 5 veces.
6. Y de nuevo se volvieron a lavar los GRC-F, 5 veces más con STF pH 7.2 (en 20 volúmenes también). Resuspendiéndose al 10% en STF pH 7.2 para guardarse a -4°C.

SENSIBILIZACION DE GRC-FORMOLIZADOS

A. Dilusión del Antígeno:
El antígeno utilizado para la sensibilización de los GRC-F, fue extracto de *Onchocerca volvulus*, obtenido por extracción con STF de *Onchocerca volvulus*, (obtenido por extracción con STF) de *Onchocerca volvulus* homogenizada. Diluyéndolo al 1:2 en STF pH 6.4, para utilizarlo en el siguiente paso.

B. Sensibilización:
A 0.5 del antígeno diluído al 1:2 se agregó 0.5 ml. de GRC-F al 10%, en un tubo de vidrio, incubando esta solución a 37°C en un baño María, durante 2 horas, agitándo suavemente en forma ocasional. Al cumplir este tiempo los GRC-F ahora sensibilizados (GRC-F), se lavaron 3 veces en 1 cc. de STF pH 7.2 (A temperatura ambiental en una centrífuga mediana, 5 minutos por cada lavada). Después los GRC-F-S, se resuspendieron al 1% en STF pH 7.2 conteniendo suero de conejo normal al 1%. Estos glóbulos se pudieron utilizar únicamente por 3 a 4 días.

HEMAGLUTINACION PASIVA:
Se practicó usando cámaras de microtítulos de acuerdo con la técnica convencional.

RESULTADOS

GRUPO I:

Este grupo lo formaron 15 personas de la finca Berlín de San Vicente Pacaya, infectadas con oncocercosis. De estas 8 (53.33%) eran del sexo masculino y 7 (46.67%) del sexo femenino. Cuatro (26%) tenían un año de evolución de las manifestaciones de oncocercosis, el resto un tiempo de evolución de 5 a más de 10 años. Trece personas (86.66%), presentaron como manifestaciones clínicas de oncocercosis, nódulos, predominando éstos a nivel de la cabeza.

De todo el grupo 4 (26.66%) personas presentaron síntomas oculares como: presencia de puntos móviles en su campo visual y agudeza visual disminuída. Al examen oftalmológico a 2 de ellas se les detectaron filarias en cámara anterior vivas y móviles, una persona con un número de más de 50 y otra sólo una; la tercera persona presentó leucomas múltiples en la córnea del ojo derecho porque en el pasado presentó microfilarias en cámara anterior.

De las 15 personas investigadas, 6 (40%) tuvieron títulos de anticuerpos contra *Onchocerca volvulus* presentes en las lágrimas, al investigarse éstas por medio de la Hemaglutinación Pasiva; notándose en su mayoría diluciones de 1:8 y 1:16. Sólo una persona tuvo una dilución de 1:4,046, ésta de 9 años de edad, con un año de presentar las manifestaciones clínicas de oncocercosis, pero al examen oftalmológico no se encontrón hallazgos anormales, (Ver gráfica No.1). Además dentro de este grupo con anticuerpos estaban: las 2 personas que habían presentado microfilarios en la cámara anterior, también 3 personas sin hallazgos oftalmológicos y la persona que presentó leucomas múltiples en la córnea, (Ver Table 1).

El resto, o sea 9 (60%) personas no presentaron títulos de anticuerpos en sus lágrimas, contra *O.v.*

GRUPO II:

Las 31 personas de este grupo pertenecían al área oncocercosa de San Vicente Pacaya y no estaban infectadas con oncocercosis. Encontrándose que 24 (77%) de ellas pertenecían al sexo femenino y 7 (23%) al masculino. De todo el grupo 9 (29.3%) personas presentaron síntomas oculares aparentemente no relacionados con oncocercosis, sucediendo igual con los hallazgos oftalmológicos.

Las lágrimas de éstas personas al ser investigadas por medio de la Hemaglutinación Pasiva, no presentaron títulos de anticuerpos contra *O. volvulus* (Ver table 1).

GRUPO III:

Este grupo control consistente en un número de 10 personas no infectadas con oncocercosis y con residencia en la ciudad Capital de Guatemala, tampoco presentó títulos de anticuerpos contra *O. volvulus* en sus lágrimas.

DISCUSION

Los resultados obtenidos en este estudio proporcionan cierta evidencia, de que la presencia de microfilarias de *Onchocerca volvulus*, en las lesiones oculares probablemente constituye un estímulo antigénico a nivel local. Pues se sabe que corrientemente las lágrimas contienen anticuerpos IgG, IgE y además IgA secretorios (10); éstos últimos se sintetizan a nivel local en las células epiteliales de las mucosas y células plasmáticas, su secresión se produce en respuesta al estímulo de la infección o administración de antígenos a las membranas mucosas donde ocurre la síntesis (11). Podría ser posible que los anticuerpos detectados en las lágrimas de los pacientes *oncocercosos*, pertenecieran al grupo IgA secretorios y que su síntesis fuese estimulada por la inflamación aguda o crónica (12), que se produce en las lesiones oculares, a diferentes niveles, a nivel del segmento anterior

donde se presentan: opacidades puntiformes lanosas (12), opacidades longitudinales (1), queratitis esclerosante (12), atrofia del iris (1), e iridociclitis (1). Como también en lesiones a nivel del segmento posterior donde se presentan: atrofia, retinopatía pigmentaria, junto a arbonización y degeneración retineal del epitelio pigmentario o junto a hiperplasia focal de esta misma capa (12), (13), (14); además se puede presentar atrofia de la coroides, edema de la coroides, del epitelio pigmentario y retineal posterior a uveítis; fibrosis subretineal con atrofia esparcida delepitelio pigmentario retineal y coroides (14), (15), (16); atrofia del disco óptico e isquemia del nervio óptico (14).

Los anticuerpos detectados podría ser que perteneciesen al grupo IgA secretorios, pues se sabe que hay lesiones inflamatorias locales a nivel ocular en oncocercosis. En la actualidad, realmente aún no se ha establecido la fisiopatología de éstas lesiones inflamatorias, algunos creen que se deben a irritación puramente mecánica (1), otros piensan que resultan de la acción de las toxinas liberadas por las microfilarias muertas (1). (12), y otros que se trata de una reacción de tipo alégica mediada por IgE (12).

El método utilizado en esta investigación no permite establecer a que clase de inmunoglobulinas pertenecen los anticuerpos detectados en las lágrimas de oncocercosos. Tampoco se pudo establecer el papel de estos anti-cuerpos en el proceso inflamatorio. Además no se descubrió un patrón definido de la presencia de anticuerpos en los diferentes estadios clínicos de la oncocercosis. Seía interesante investigar la posibilidad de que en la etiología de las lesiones oculares participarán mecanismos inmunológicos.

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J.R. Cruz

1. ¿Han utilizado Vds, métodos más sensibles que HAP para el diagnósitico de anticuerpos en lagrimas?
2. ¿Cómo fueron las muestras almacenadas después de su recolección? Se ha demostrado que el almacenamiento en recipientes de vidrio. Polietileno esta asociado a pérdida de actividad de SIGA.

Maselli

1. Para la investigación de anticuerpos contra *O. volvulus* se practicó unicamente por hemoaglutinación. Es probable que con técnicas más sensibles como ELISA la proporción de anticuerpos en estos pacientes sea mayor.
2. Las lagrimas fueron colectadas en capilares de vidrio y conservadas a -70°C.

J.R. Cruz

1. Have you used more sensitive methods than HAP for the diagnosis of antibodies in tears?
2. How were the samples stored after collection? It has been shown that storage in glass and polyethylene containers is associated with the loss of activity of SIGA.

Maselli

1. For the research on antibodies against *O. volvulus*, we only used the hemaglutination technique. It is possible that with more sensitive techniques, such as ELISA, the proportion of antibodies found in these patients will be greater.
2. The tears were collected in glass capillaries and kept at 70°C.

THE INTRADERMAL REACTIVITY OF EXCRETORY AND SECRETORY PRODUCTS OF ONCHOCERCAL MICROFILARIAE

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The search for a reliable intradermal test for the immunodiagnosis of onchocerciasis has been a continuing endeavor since the 1930's. The antigens employed in this test usually have been somatic extracts of parasites phylogenetically related to *Onchocerca volvulus*. The immediate hypersensitivity type reactions induced by these products appear to be due to a group of antigens shared by several species of filarial worms. The excretory-secretory (ES) products of helminths are believed to be less complex, antigenically, than substances extracted from the somatic tissues of these organisms and it has been established that antigens present in the excretions and secretions of living worms evoke a considerably higher level of protective immunity in laboratory animals than do somatic antigens derived from the whole parasite.[1,2] Thus, the use of ES antigens may eliminate some of the cross-reactivity that results from antigens that are shared by several species and may increase the sensitivity of such tests. Some preliminary results of skin tests performed with ESA harvested from chemically defined fluids in which viable onchocercal microfilariae had been incubated were reported recently.[3] The ESA is capable of inducing the immediate-type skin reaction in onchocerciasis infected subjects (Table 1). ESA was similarly prepared from *O. gutturosa*, except that it was passed through an affinity column composed of rabbit anti-bovine serum coupled to cyanogen-bromide activated Sephorose -4B to remove any contaminating bovine serum proteins. Data comparing the intradermal reactivity of ESA from *O. gutturosa* with that *O. volvulus* ESA are shown in Table II. Some data on the specificity of the *O. volvulus* ESA are shown in Table III. In an attempt to identify the proteins of parasite origin, rabbits were hyperimmunized with ESA. The anti-sera gave multiple precipitin bands against the ESA when tested by crossed immunoelectrophoresis (CIE), (Figure 1). Human albumin was the major contaminant of ESA. Antibodies to human serum proteins (hsp) were detected in the rabbit anti-ESA by gel diffusion and CIE. Absorption of this antiserum with human serum removed anti-HSP antibodies. CIE analysis of the ESA with absorbed anti-ESA revealed two antigens believed to be of parasite origin. For the purification of these antigens, affinity column chromatography consisting of the IgG-fraction of goat-anti human serum (at a ratio of 9.3 mg of protein/1.0 ml packed gel) was used. These purified antigens were analyzed with CIE against absorbed rabbit anti-serum to ESA. (Figure 2). Even though these proteins are different antigenically, they were not separable on the basis of molecular weight using SDS-PAGE (7.5% Acrylamide, 1% SDS and 1% B-mercaptoethanol). The molecular weight of each of these proteins is estimated at 84,000 daltons. These antigens appear to be biochemically "pure" proteins, inasmuch as they failed to stain with PAS. Moreover, they did not form a preciptin band in concanavalin-A incorporated agarose in crossed immunoelectrophoresis. The intradermal reactivity of the purified component, hereafter designated as ESP, was compared with that of the ESA in 47 Guatemalans known to have onchocerciasis (Table IV). Although the wheals resulting from the injection of ESP were smaller than those elicited with ESA, when compared on the basis of μg of antigen per wheal size, the potency of ESP is significantly greater than that of ESA. Patients infected with *O. volvulus* possess circulating antibodies to ESA of both and *O. gutturosa* as demonstrated by counter im-

munoelectrophoresis.

The biochemical purity and antigenic potency of the purified antigen suggest, not only that it is highly sensitive diagnostically, but also that it could be the basis for the development of a protective vaccine.

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Table 1

Intradermal reactivity of excretory-secretory antigens (ESA) of microfilariae of *Onchocerca volvulus*

Subjects	Number	Percent with postive skin test	Mean size of wheal ($mm^2 \pm SD$)	Percent with positive skin biopsy
Guatemalans[1]	11	100	142.8 (6.2)	82[3]
Nigerians	2	100	72.0 (8.0)	0
North Americans[2]	6	0		not done
Liberians[1]	35	83	129.3 (5.8)	57[3]

1 = Known to be infected with *O. volvulus*
2 = Uninfected controls
3 = $P < 0.05$ (Chi-square test)

Table 2

Comparative intradermal reactivity of excretory-secretory antigens (ESA) of microfilariae of *Onchocera volvulus* and *O. gutturosa* in 59 Guatemalans with onchocerciasis[1]

Antigen	Percent with positive skin test	Mean size of wheal ($mm^2 \pm SD$)
ESA of *O. volvulus*	100	127.0 (7.9)
ESA of *O. gutturosa*	70	55.7 (6.2)[2]
Saline control	0.0	0.0

1 = A control group of 8 Guatemalans who never had been in an onchocerciasis-endemic area showed no reaction to either antigen

2 = $P < 0.001$ (Student's pooled t-test)

Table 3

Comparative intradermal reactivity of excretory-secretory antigens (ESA) of microfilariae of *Onchocerca volvulus* in subjects with some other filarial infections

Filaria	Subjects	Number	Percent with positive test
O. volvulus	Cameroonians	4	100
Loa loa	Cameroonians	5	40[1]
L. loa	Nigerian	1	100[1]
Dirofilaria immitis	Dogs	17	0
Dipetalonema reconditum	Dogs	1	100[1]
D. immitis and *D. reconditum*	Dogs	5	60[1]

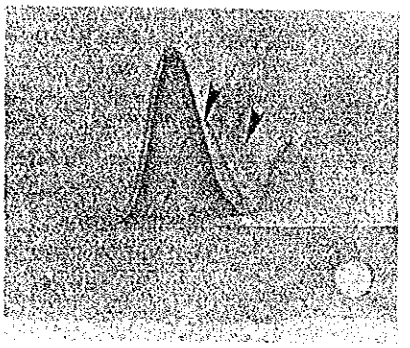
1 = Mean wheal size, as compared with that in African subjects with onchocerciasis, is significantly smaller ($P<0.005$)

Table 4

Comparative intradermal relativity of crude and purified excretory-secretory antigens (ESA) of microfilariae of *Onchocerca volvulus* in 47 Guatemalans with onchocerciasis[1]

Antigen	Protein content per inoculum (μg)	Percent with positive skin test	Mean size of wheal ($mm^2 \pm SD$)	Percent with positive skin biopsy
Crude ESA	75.0	100	180.6 (15.0)	53
Purified ESA	2.5	100	104.4 (8.1)	53
Saline control	0.0	0.0	0.0	53

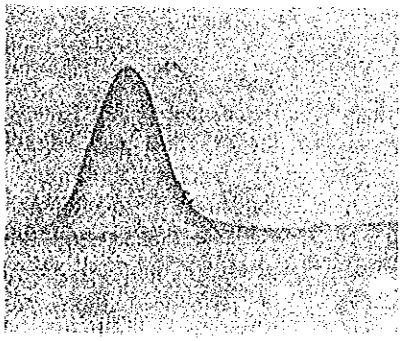
1= A control group of 9 Guatemalans who never had been in an onchocerciasis-endemic area showed no reaction to either antigen



2.5% Rabbit anti-ESA
in
1% Agarose

3 μg ESA

Fig. 1



2% Rabbit anti-ESA
(Adsorbed),
in
1% Agarose

2 μg ESA

Fig. 2

Rivas-Alcalá

¿Chequeó o hizo pruebas cutáneas en pacientes dentro de las áreas endémicas pero con resultado negativo en cortes cutáneos? Creo que la hipersensibilidad inmediata por la presencia de IgE específicos en células cebadas y basótilas.

Schiller

Se hicieron pruebas cutáneas con ESA en pacientes con resultados negativos a microfilarias en cortes cutáneos, pero que tenían nódulos que se podían palpar o una historia de nodulectomía anterior. Estos sujetos reaccionaron positivamente (tipo inmediato), pero la reacción Arthus no se puede excluir concluyentemente.

Maselli

1. ¿Ha usted investigado si estos pacientes tienen reacción positiva 48 a 72 horas más tarde?
2. Se ha utilizado esta prueba en individuos no oncocercosos con otros parásitos intestinales?
   Creo que por el tiempo de la reacción el anticuerpo involucrado es IgE.

Schiller

1. Si. No se observaron reacciones del tipo retrasado.
2. Se probaron antígenos de otros parásitos intestnales, v.g. *Ascaris lumbricoides* (somático) y de huevos de *Schistosoma mansoni* en inmunoelectrofóresis cruzada contra antígenos de conejo contra ESA, con resultados negativos.

Sasa

Creo que todo el mundo ha quedado muy impresionado con los bellos y definitivos resultados mostrados por el Dr. Schiller y en relación a los mismos tengo una pregunta fundamental. En pacientes con biopsia cutánea positiva, hay microfilarias presentes en la piel, y se supone que producen grandes cantidades de antígenos. ¿Porqué es que una inyección adicional de una pequeña catidad del mismo antígeno podría causar tal reacción tan sorprendente?

Rivas-Alcalá

Did you check or perform skin tests in patients within the endemic areas but with negative skin snip? I believe that the immediate hypersensitivity is given by the presence of specific IgE on mast cells and basophils.

Schiller

Skin tests with ESA were performed on patients with skin snips negative for microfilariae, but who had palpable nodules or a record of previous nodulectomy. These subjects reacted positively (immediate type), but the Arthus reaction cannot be excluded conclusively.

Maselli

1. Have you seen whether these patients have a positive reaction 48 to 72 hours later?
2. Has this test been used on individuals who do not have onchocerciasis, but with other intestinal parasites?
   I believe that because of the reaction time, the antibody involved is IgE.

Schiller

1. Yes. No reactions of the delayed-type were observed.
2. Antigens of other intestinal parasites, e.g. *Ascaris lumbricoides* (somatic) and from eggs of *Schistosoma mansoni* were tested in crossed immunoelectrophoresis against rabbit-anti-ESA with negative results.

Sasa

I think everybody is very much impressed by the beautiful and definitive results shown by Dr. Schiller. In this connection, I have a fundamental question. In patients with positive skin biopsy, the microfilariae are present in the skin, and they are presumed to be producing large amounts of antigens; why does additional injection of a small amount of the same antigen cause such a remarkable reaction?

Schiller

Se cree que antígenos metabólicos están siendo contínuamente liberados por las microfilarias *in situ*, y que el hospedero concurrentemente está respondiendo por medio de la producción de anticuerpos que eliminan a algunas de éstas, en tanto que forma complejos inmunes con otros. La inoculación adicional de un antígeno ESA altamente concentrado probablemente causa una reacción con cualquier anticuerpo liberado, resultando en la reacción intradérmica observada en nuestros estudios.

Schiller

It is believed that metabolic antigens are continuously being released by the microfilariae *in situ*, and that the host concurrently is responding by producing antibodies which eliminate some of them, while forming immune complexes with others. The inoculation of additional highly concentrated ES antigen probably causes a reaction with any free antibody, resulting in the intradermal reaction observed in our studies.

THE IMMUNE RESPONSE IN MEXICAN ONCHOCERCIASIS: IMMUNODIAGNOSIS AND PROMINENT ANTIGENS

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In a recent international meeting sponsored by the WHO on the immunology of filariasis, most participants agreed on the lack of correlation between the results of serologic analysis and the clinical state of the disease. Particularly lacking was an ability to detect prepatent cases, and dissatisfaction was expressed over the specificity of serologic analysis. Investigators were urged to identify the most pertinent antigens for diagnosis and, eventually, for vaccination.

In this paper we present some results of serologic investigations among onchocerotic patients in Mexico that relate to the above mentioned problems.

MATERIAL AND METHODS

Design

A crude antigen preparation was extracted from adult *O. volvulus* that had been dissected from glycerol-preserved human onchocercal nodules. The preparation was reacted in counterimmunoelectrophoresis (CIEP) against the sera of humans suffering to different extents from onchocerciasis, the sera of apparently healthy people residing in the endemic area, and the sera of patients with other parasitoses or healthy individuals, neither of which resided in the endemic area. The sera were scored as positive if they showed at least one precipitation band with the crude antigen extract. The total number of CIEP precipitating bands in each serum was also registered and their sum over all sera belonging to the same clinical category of onchocerciasis was examined by regression analysis against clinical categories. Likewise, the correlation between, microfilarial burden and number of CIEP precipitation bands was studied. Immunoelectrophoresis (IEP) of the crude antigen extract against precipitating antisera was employed to identify the antigen of *O. volvulus* most frequently involved in the immune response of man.

The total of 452 sera were classified in five categories according to the following characteristics: a) whether or not they had at least one microfilaria in two shoulder skin snips at the time the serum sample was taken (MF^+ or MF^-), b) whether a nodule was palpable or not (N^+ or N^-), c) whether the patient had a history suggestive of onchocerciasis* (H^+ or H^-), and d) if they resided or not in the onchocercotic area (R^+, R^-)

* *On this aspect the questionaire inquired about history of mal morado, a Mazzoti reaction, positive skin snips and of nodulectomy.*

Antigen of *O. volvulus*

Subcutaneous nodules were collected and preserved in glycerol (66% in water) at room temperature from 15 to 90 days, while in the field. The crude antigen extract was adjusted to 1 mg of protein as estimated by Lowry's method, per milliliter of a PBS containing 0.02% of sodium azide.

Estimation of Serologic Reactivity

All 452 sera were tested in CIEP against the crude antigen extract, and scored as positive if they showed at least one precipitation band. The total number of CIEP bands in each serum was also registered. CIEP was performed by conventional methods using strips of cellulose acetate (Beck-

man) and veronal buffer (pH 8.6, ionic strength 0.975) keeping the voltage constant at 250 volts for 40 minutes. One µl of undilute serum was placed in the anodic end of each strip and 1 µl of the crude antigen extract (1 mg/ml) was placed in the cathodic end, 6 mm from the position of the serum. After electrophoresis the strips were washed twice in saline solution (0.15 M NaCl) for 20 minutes each wash, and later stained with Ponceau Red (0.2% in trichloroacetic and sulfosalicilic acids, both at 3% in water). The strips were then destained in acetic acid (5%), air dried and examined in a negatoscope.

Identification of the Most Prominent Antigen

Twenty-one CIEP-positive human sera that belonged to Category IV were examined by immunoelectrophoresis (IEP) by the method of Scheidegger. After electrophoresis each through was emptied of gel and filled with 75 µl of undiluted serum. By juxtaposing all immunoelectrophoretic patterns a standard was constructed to aid in band identification of the individual sera. The total number of precipitation bands in each serum was registered, as well as the electrophoretic identity of the band as classified by the standard pattern.

RESULTS

Serology

Table 1 shows the percent of CIEP-positive sera in all clinical categories. The most striking result is that CIEP can detect precipitating antibodies in 80% of the confirmed onchocercotics (Category IV), which also defines a population of onchocercotics, about 20%, without detectable circulating antibodies. On the other hand, CIEP detects antibodies in 25% of apparently healthy people residing in the endemic area (Category I). Low proportions, 4% and 6%, of seemingly false positive results were encountered in the sera of patients with amoebioasis or malaria respectively, but by and large it was practically negative in people residing outside the endemic area (Category 0).

At the community level, a very interesting and potentially useful positive correlation was found by plotting, against clinical category, the quotient of the sum of all CIEP bands in all sera belonging to a given category, divided by the number of sera in that category. This quotient increases 53 fold from Category 0 to Category IV, but the increase is not constant from category to category, rather it is abrupt at first, and then tends to diminish. It increases by a factor of ten from Category 0 to I, of about two from Category I to II and from II to III, and of one and a half from Category III to IV.

The Most Prominent Antigen

In search of the most relevant antigens in the response of humans to *O. volvulus* twenty-one precipitating sera were examined in immunoelectrophoresis (IEP) against the crude antigen extract of *O. volvulus*. As in CIEP, the precipitating patterns in IEP were quite diverse. Figure 5 illustrates some of this heterogeneity as well as the standard pattern, as it was designed from the sum of all individual sera used for band classification in IEP. Upon classification, a most encouraging finding was that, in spite of the heterogeneity, there is one antigen -band #4- that is most conspicuous immunologically. It was found in 85.7% of the tested sera (Fig. 5C). Band 4 moves towards the anode at pH 8.6 and precipitates prominently, suggesting quite a high partial concentration in the antigen mixture.

DISCUSSION

These results satisfy some of the needs that CIES has identified for the advancement of its research program on onchocerciasis. Complementarily they also further some of the objectives of the WHO in that CIEP is identified as a potent serological tool in the epidemiologic evaluation of

onchocerciasis, and in that the detection of a very prominent antigen of *O. volvulus* heralds finer diagnostic tools and perhaps even immunization experiments.

CIEP detected 80% of the microfilaria-carrying individuals with a minimal of about 3% false positive cases. In our view these are quite satisfactory results since other, more sensitive methods, like hemagglutination are in general more cumbersome, variable, and yield even more false positive reactions.

As for the 25% of positive CIEP serology in apparently healthy people residing in the endemic area, there are a number of considerations. Firstly, these positive cases may represent pre-patent onchocerciasis. A second possibility is that the host was exposed to antigens from animal filariae, which do not of course establish, but may induce the synthesis of cross-reacting antibodies. Finally, these cases may represent successful confrontations with the disease which would allow some optimism on the possible benefice of vaccination in onchocerciasis.

Further, CIEP results did correlate in some respects with the state of the disease, which is an unusual finding in most serologic tests that detect antibodies. In individual cases, the number of CIEP bands per serum tended to increase somewhat with the number of skin dwelling microfilaria. The increase, however, was so erratic that it could not be considered statistically significant. In contrast, at the community level the total number of CIEP bands in a set of sera from within a clinical category of the disease did correlate with the state of the disease in the community, which could be potentially useful in the evaluation of the overall state of the disease in that community. That the main increment (ten fold) of this serologic index occurs from category 0 to 1 indicates the value of CIEP in serological monitoring of newly established transmission areas. However, there is still an increment of about five fold in CIEP index from category I to IV, an increment potentially valuable in assessing the effects of medical interventions upon the disease.

PREPARACION DE ANTIGENOS HOMOLOGOS PARA DETERMINACION DE ANTICUERPOS A *ONCHOCERCA VOLVULUS* EN SUEROS HUMANOS, POR LA TECNICA INMUNO-ENZIMATICA DE ELISA

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En este estudio se obtuvieron filarias adultas de *O. volvulus* de oncocercomas por digestión enzimática con colagenasa (5 mg/ml) a 35°C (Schulz-Key *et al.*, 1977, Tropenmed. Parasitol. *28*: 428-430). Las filarias libres fueron lavadas tres veces en solución salina boferada con fosfatos (pH 7.2) y luego congeladas a -20°C. La obtención de antígeno somático crudo soluble se hizo macerando las filarias en un macerador Ten Broeck y dejándolo reposar por la noche a 4°C. El extracto fue sonicado por 2 minutos a 200-watts, con intervalos de 30 segundos, en un sonicador Branson modelo W-350, y centrifugado a 10,000xG por 30 minutos en una centrífuga refrigerada. Se tomó el sobrenadante y se determinó la concentración de proteína (6mg/ml) por el método de Wadell, W., (1956, J. Lab. Clin. Med. *48*: 311-314).

El procedimiento de ELISA es esencialmente la técnica de microdilución descrita por Ruitenberg *et al.*, (1975, Medicon Nederland *4*: 30-31). Se sensibilizaron cajas de microdilución de poliestireno con 96 pozos de fondo plano (Dynatech) con 0.2 ml por pozo, de antígeno diluído (1:2000) en bofer de carbonatos 0.06M, pH 9.5, a 37°C por 3 horas en un baño de agua. Las cajas se lavaron tres veces con una solución salina boferada con fosfatos (pH 7.2) y Tween (PBS/T). Se prepararon diluciones seriadas del suero en prueba en PBS/T quedando de 1:80 a 1:2560, y se agregó 0.2 ml por pozo. Las cajas se agitaron brevemente e incubaron en un baño de agua a 37°C por 30 minutos. Luego se lavaron tres veces en PBS/T. Se agregó 0.2 ml de conjugado (conejo anti-IgG humano, conjugado con peroxidasa) a una dilución de 1:2000 a cada pozo. Se volvieron a incubar a 37°C por 30 minutos y se lavaron tres veces con PBS/T. A cada pozo se le agregó 0.2 ml de substrato (o-fenilenediamino, 1 ml en 99 ml de agua destilada con 0.1 ml de peróxido de hidrógeno al 3%) y las cajas se incubaron a temperatura ambiente en la oscuridad por 30 minutos. La reacción enzimática se detuvo agregando a cada pozo 0.025 ml de una solución de H_2SO_4 8N. La absorbencia a 492 nm de los contenidos de cada pozo se determinó en una máquina Titertek Multiscan (Flow Laboratories). Una absorbencia de 0.500 o más se consideró positiva.

En este estudio se evaluaron 450 sueros provenientes de personas de un área endémica de oncocercosis en la región de Patulul, Guatemala, 144 de personas con infecciones por otros nemátodos provenientes de El Salvador, y 50 sueros de norteamericanos sin ninguna infección parasitaria. Los sueros fueron obtenidos por punción del dedo y recolectados en un tubo capilar heparinizado con capacidad para 70 µl. La sangre fue absorbida en un papel-filtro y guardada de esta forma a -20°C. El punto de separación para un suero positivo por ELISA fue el recíproco del título de 1:160. La sensitividad general de la prueba para todas las edades fue de 88.2%, la especificidad de 65.3%. Se comparó ELISA con inmunofluorescencia indirecta (IFA) utilizando *O. volvulus* como antígeno (Collins, W.E. *et al.*, 1980, Am. J. Trop. Med. Hyg. *29*: 1220-1222): ELISA mostró mayor sensitividad y especificidad que IFA, 88.2 vs 79.7% y 65.3 vs 43.3%, respectivamente (Cuadro I). El número de falsos negativos se redujo al 11.8% con ELISA

comparado con 20.3% de falsos negativos con IFA. El 78% de los falsos positivos estuvo en el grupo de personas jóvenes (1 a 20 años) indicando infecciones tempranas no detectables por diagnóstico parasitológico (Cuadros 2, 3). Las respuestas serológicas a *O. volvulus* aumentaron con la edad hasta los 5 años, así como también la positividad de microfilarias, pero no se observó una diferencia estadísticamente significativa entre las medias geométricas de los títulos de las personas de las diferentes edades que respondieron a 1:160 o más. Se encontraron respuestas serológicas a *O. volvulus* diferentes entre individuos con y sin microfilarias en la piel, aunque no se observó una diferencia estadísticamente significativa entre las medias geométricas de los títulos de personas con differentes densidades de microfilarias en su piel (1 a +100) que respondieron a 1:160 o más. En el control negativo de oncocerca de suero de El Salvador 11 de 144 personas (7.6%) fueron positivas por la prueba de ELISA y 5.9% por IFA, sugiriendo algunas reacciones cruzadas con otros antígenos de nemátodos. Ninguno de los sueros de personas de Estados Unidos fue positivo.

CUADRO 1

RESULTADOS DE INMUNO FLUORESCENCIA INDIRECTA (IFA) Y DE LA TECNICA INMUNO-ENZIMATICA (ELISA) PARA EL DIAGNOSTICO DE ONCOCERCOSIS FINCA LOS TARRALES, GUATEMALA, 1976

Prueba	No. Examinado	Porcentaje			
		Sensibilidad	Falsos Negativos	Especificidad	Falsos Positivos
IFA	454	79.9	20.1	48.6	51.4
ELISA	450*	88.2	11.8	65.3	34.7

*ELISA no realizada en cuatro sueros.

CUADRO 2

DISTRIBUCION EN PORCENTAJE DE LOS FALSOS POSITIVOS Y FALSOS NEGATIVOS POR EDAD EN CADA PRUEBA, COMPARADO CON BIOPSIA DE LA PIEL FINCA LOS TARRALES, GUATEMALA, 1976

Edad (años)	IFA		ELISA	
	Falsos Positivos	Falsos Negativos	Falsos Positivos	Falsos Negativos
1-10	58.6(44)*	21.0(13)	40.0(20)	25.0(9)
11-20	25.4(19)	27.4(17)	38.0(19)	25.0(9)
21	16.0(12)	51.6(32)	22.0(11)	50.0(18)
TOTAL	75	62	50	36

*Número examinado en cada grupo

CUADRO 3

NUMERO TOTAL DE FALSOS POSITIVOS POR ELISA EN PERSONAS CON BIOPSIA NEGATIVA FINCA LOS TARRALES, GUATEMALA, 1979

Edad (años)	No. Biopsia Negativo	No. Falsos Positivos	No. Nódulo	
			Positivo	Negativo
1-5	99	8	0	8
6-10	66	13	5	8
11-15	30	10	3	7
TOTAL	195	31	8	23
PORCENTAJE		15.9	4.1	11.8

Fernández
Debe seguirse usando la reacción de Mazzotti para comparar la sensibilidad de cualquier prueba diagnóstica que se proponga, puesto que es la que más se correlaciona con los niveles de parasitación (en la comunidad).

Cruz
¿Qué pasaría si se utilizaran conjugado anti Ig G, IgM e IgA en lugar del conjugado a IgG solamente?

Luján
Todavía no se ha usado aunque si ya se probaron cada uno de los conjugados a las otras clases de inmunoglobulinas además de IgG, i.e, IgM, IgA e IgE. Sin embargo estos resultados aún no han sido evaluados.
El usar una mezcla de conjugados probablemente aumente la sensibilidad de la prueba de ELISA.

Fernández
The Mazzotti test must continue to be used to compare the sensitivity of any diagnostic test proposed, as it is the one which correlates the most with the parasitic levels (in the community).

Cruz
What would happen if an anti Ig-G and IgA conjugate is used instead of the IgG conjugate only?

Luján
That still has not been used, although each one of the conjugates of the other types of immunoglobulines besides IgG has been used , v.g. IgM, IgA and IgE. However, these results have not yet been evaluated. Using a mixture of conjugates will probably increase the sensitivity of the ELISA test.