Tropical Medicine and Health Vol. 43 Supplement, 2015, 87–94
doi: 10.2149/tmh.2015-S13

4. Review Articles Related to the Cooperation Project (Republications)

6) Epidemiology and Control of Guatemalan Onchocerciasis†

Hiroyuki Takaoka[1]* and Takeshi Suzuki[2]

Abstract: Recent studies on the epidemiology and control of Guatemalan onchocerciasis, chiefly made by the Guatemala–Japan Cooperative Project on Onchocerciasis Research and Control, are reviewed. Epidemiological features of Guatemalan onchocerciasis are summarized as to characteristic altitudinal distribution of endemic areas, disease manifestation, vector taxonomy, biology and transmission dynamic of the disease. Extensive insecticide studies in the field and laboratory demonstrate that the characteristic situations of Guatemalan streams where *Simulium ochraceum*, the main vector of onchocerciasis, breeds require ingenious methods of larviciding. Finally, the feasibility of an area vector control is indicated by the successful control operation in the San Vicente Pacaya Pilot Area, in which a new fixed-dose larviciding method was applied.

INTRODUCTION

Human onchocerciasis, a disease caused by the filarial parasite *Onchocerca volvulus*, has been known in Africa, the Arabian peninsula, and Central and South America. In the New World it is sporadically distributed in localized areas of six countries: Mexico, Guatemala, Venezuela, Colombia, Brazil, and Ecuador. In Mesoamerica this disease is also known as "Robles' disease", in honor of Dr. Rodolfo Robles Valverde, who first discovered onchocerciasis in the Western Hemisphere from Guatemala in 1915.

Since the discovery of onchocerciasis in Guatemala, a great deal of work has been carried out on the bionomics and control of the vector black flies, as well as on clinical, epidemiological, pathological, and parasitological aspects of the disease. Excellent reviews of these studies were done by Dalmat [1], Hamon [2], and Sasa [3].

Beginning in 1970s, more advanced studies have been made through the participation of research workers from foreign countries (e.g., the United States, Germany, and Japan). One such joint project was carried out from 1975 to 1983 by the Guatemala–Japan Cooperative Project on Onchocerciasis Research and Control. Extensive studies on the disease itself and its vector conducted during an early stage of the project were briefly reviewed by Ogata [4]. At the end of the project the entire study was thoroughly reviewed by Suzuki [5].

In the early days, nodule excision, or nodulectomy, which was first recommended by Dr. Robles, was the only countermeasure against onchocerciasis, and it has been carried out as a nationwide campaign from 1935 to the present. A chemical control trial using DDT against black fly larvae was performed in the mid-1950s, with successful suppression of adult density for a short period, but that program was suspended for administrative reasons [6, 7].

A major problem in vector control of Guatemalan onchocerciasis is the difficulty in accessing all vector-breeding streams for periodic larviciding, because these numerous, small streams are located mostly in the rugged terrain of mountainous areas. By overcoming these difficulties through basic research on vector bionomics and insecticides, a control trial against larvae of the principal vector, *Simulium ochraceum*, in the San Vicente Pacaya Pilot Area by the Guatemala-Japan Project yielded good results. It has reduced the human biting density of the vector to a very low level, below which transmission may not take place.

In this article, recent studies on the epidemiology and control of Guatemalan onchocerciasis, chiefly made by the project, are reviewed. First, we describe the special epidemiological features of Guatemalan onchocerciasis in relation to vector control. Second, the results of insecticide studies are summarized. And finally, the area vector-control trial is briefly described and discussed.

---

[1] Division of Medical Zoology, Oita Medical College, Oita, Japan (Present address: Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, 50603 Malaysia)
[2] Guatemala–Japan Cooperative Project on Onchocerciasis Research and Control, Guatemala
*Corresponding author:
E-mail: takaoka@oita-u.ac.jp

†Reproduced from Chapter 27 in Kim KC and Merritt RW ed. (1987) "Black flies: Ecology, population management, and annotated world list". pp. 528, with permission from Pennsylvania University Press.

Epidemiological Features of Guatemalan Onchocerciasis in Relation to Vector Control

Distribution of Endemic Areas

The onchocerciasis-endemic areas in Guatemala are distributed in seven departments, which are divided into four zones: North-Western Zone, West-Central Zone, East-Central Zone, and Eastern Zone (Fig. 1). Approximately 10% of the 300,000 inhabitants in these endemic areas are supposedly infected [8]. Historically, no sign of enlargement of endemic foci was observed, despite frequent movements of inhabitants, especially by seasonal workers in coffee plantation areas. On the contrary, during the project of the nationwide nodulectomy campaign from 1935 to the present, the nodule rate has gradually decreased over entire endemic areas (Yamagata et al. unpublished data). In some areas, e.g., the Eastern Zone, the nodule rate has become almost zero. A recent epidemiological survey carried out in the Eastern Zone (i.e., Santa Rosa) showed that out of 2,257 persons examined, 20 (1.9%) were positive for microfilariae and/or nodules (Uchida et al. unpublished data). Among positives, 15 were 30 or more years old, while the youngest was a 10-year-old boy who had recently come from one of the other endemic areas. These data clearly indicate a marked decline or near disappearance of the endemicity in the Eastern Zone. It is, however, still uncertain whether the reduction was due to the direct effect of the nodulectomy campaign or to other factors, such as a change in the socio-economic condition of the people, or a decrease in the number of suitable streams for vector breeding by deforestation. The gross area endemic for onchocerciasis in Guatemala was calculated as 6,335 km^2 by Figueroa [8] or as 4,708 km^2 by Garcia-Manzo [9]. These figures were based on an administrative demarcation system and may overestimate the real range of the disease. Further reassessment should be made with regard to the recent analysis of the nodulectomy campaign.

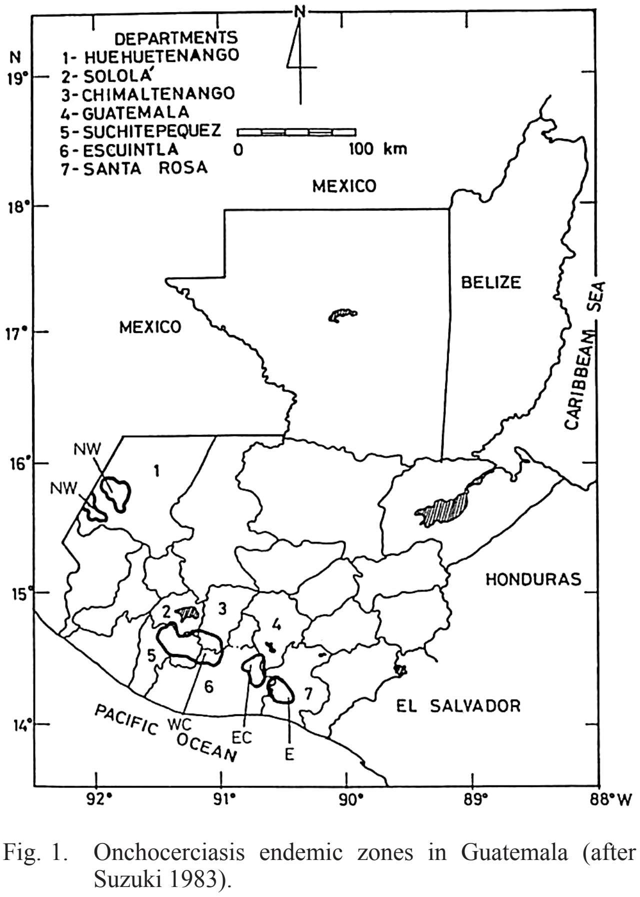

Fig. 1. Onchocerciasis endemic zones in Guatemala (after Suzuki 1983).

It is well known that the distribution of endemic foci is stable within altitudes from 500 to 1,500 meters above sea level, roughly coinciding with the distribution of *S. ochraceum* breeding sites. Based on experimental infection studies under various temperature conditions, Takaoka et al. [10] suggested that the distribution of onchocerciasis in this country may have been prevented from extending farther into the lowlands by the intolerance of adult *S. ochraceum* to high temperature, or into the uplands by the inability of the parasite to develop in the simuliid vector at low temperature. On the other hand, lateral borders of the disease distribution may be explained by environmental differences, such as topographical and geographical features [11].

Disease Manifestation

The most severe symptom of onchocerciasis is an ocular lesion that leads progressively to blindness. In Guatemala, the blindness rate seems generally lower than in West Africa. According to Yamada [12], the blindness rate was 0.5% or less in the San Vicente Pacaya Pilot Area, where, of 2,153 inhabitants examined, 30.8% were positive for the infection by the skin-snip method [13]. In the other Guatemalan endemic areas, the blindness rate was in the range of 1.5% to 4.6% [14]. It was suggested that the present nodulectomy campaign can be effective in at least suppressing or preventing ocular lesions [15].

Onchocercal nodules are frequently found in the head region of the Guatemalan patients. This characteristic distribution of nodules is probably related to the biting preference of *S. ochraceum* for upper-body regions. However, microfilarial density in skins from the head and arms is usually low [16]. In all age groups, males are more frequently nodule-positive than females, while in either sex the positive rate increases with age. There was a close correlation between nodule rate and microfilarial rate in the San Vicente Pacaya [13], and the onchocercal nodules serves as an important index of early infection.

Vector and Transmission

Confirmed and potential vectors

In Guatemala, eight black fly species are captured on human attractants in endemic areas. Among these, *S. ochraceum* is generally regarded as the principal vector because this species (1) often harbors infective larvae of *O. volvulus*, (2) is highly anthropophlic, and (3) exhibits a high human biting density. *Simulium metallicum* and *S. callidum* are the secondary vectors due to infrequent natural infections and zoophilic biting habit [1]. In addition, recent experimental infection studies indicate that *S. horacioi* (a newly described species belonging to the *S. metallicum* group), *S. colvini* (misidentified as *S. downsi*), and *S. haematopotum* are all capable of supporting the development of *O. volvulus* microfilariae to the third-stage larvae [17, 18]. There is a possibility that some of these species might take over the role in transmission if and when *S. ochracerum* density is reduced to an extremely low level by successful vector control. The epidemiological importance of two other species, *S. gonzalezi* (or *S. exiguum*) and *S. veracruzanum*, remains to be studied.

Vector taxonomy

It is worthwhile to note that *S. ochraceum* is a species complex, comprised of at least three cytoforms (Hirai, unpublished data). Each cytoform should be investigated for its distribution, biting habit and susceptibility of *O. volvulus* infection. Dr. Hirai (unpublished data) has also found that *S. metallicum* is divided into two sibling species, which are usually found sympatrically.

Vector biology

Larvae of *S. ochraceum* usually prefer small permanent streams in rugged mountainous terrain [1]. Cool and minute streams with water temperature of 16°C to 20°C and a discharge of 0.1 to 1.0 liter per sec are usually colonized by this species. Under such cool water conditions more than two weeks are required for the larvae to develop from hatching to pupation [5].

It was recently shown that an observed shifting of larval sites of *S. ochraceum* in some localities was related to the pronounced wet and dry seasons in the foothills along the Pacific slope of the Sierra Madre [19]. In the late dry season (February to April), larval sites were restricted to perennial streams in intermediate altitudes, but during the rainy season preimaginal sites of this species extended to the upper reaches of the numerous small, newly emergent, temporary streams that often form subterranean sections along their stretches. Accordingly, seasonal fluctuation of the adult population of *S. ochraceum* varies, depending on the characteristics of the water systems of the localities, although the human biting density of this species generally peaked during the dry season [19].

Vector efficiency

Simulium ochraceum is characterized by its inefficiency in transmission competence, in terms of the percentage of ingested microfilariae that develop to infective larvae [20]. This is chiefly because most microfilariae ingested by *S. ochraceum* are destroyed by the cibarial armature before they pass to the stomach, and consequently only a few microfilariae reach the thorax and develop further [21].

Recent natural infection studies also indicate that only a small proportion of wild-caught *S. ochraceum* had infective larvae of *O. volvulus*, ranging from 0.02% to 3% [22–26].

Time of transmission

A year-long collection and dissection of *S. ochraceum* adult females carried out at two localities in endemic areas revealed that the most suitable time for transmission of the disease might be the dry season (November to March), although lower transmission might take place in the rainy season [25]. A similar study [27] also showed that the highest infective rates were observed during the period from late February through March.

Critical annual biting rate

The data accumulated in the Guatemala-Japan Project were theoretically analyzed using Muench's simple catalytic model [28]. The critical ABR (the number of biting flies per man per year, below which transmission is not maintained) was calculated as 7,665. This tentative value is very close to the value of 8,700 empirically obtained in a village with no cases of eye lesions despite 33% skin biopsy positivity [29].

Insecticide Studies for Vector Control

Larvicide Agents

Temephos is well known to be effective against black fly larvae, with comparatively low adverse effects on non-target organisms, as well as low toxicity to man. Trough tests revealed that this chemical is effective against larvae of *S. ochraceum*, with a minimum concentration of 3 ppm/min for a 95% mortality [30].

A bacterial insecticide, *Bacillus thuringiensis* var. *israelensis*, is also known to be ingested by black fly larvae and to produce mortality at adequately low concentrations, with low toxicity to non-target organisms. The efficacy of *B.t.i.* against the larvae of *S. ochraceum* was tested [31] and it was shown that a one-minute treatment with initial concentration of 2×10^5 spores/ml resulted in up to 100% mortality, but that downstream carry was poor.

Larval Susceptibility Level to Insecticides

Insecticide susceptibility tests were carried out with the test kits supplied by WHO (Mizutani, unpublished data). At least in 1983, there existed no sign of temephos resistance in *S. ochraceum* larvae. The baseline susceptibility level of this species in the Rincon area of Guatemala was estimated to be as follows: LC-50 of temephos 0.055 ppm, chlorpyrifos-methyl 0.040 ppm, chlorphoxim 0.0079 ppm, and DDT 0.044 ppm. The possibility of resistance developing in the future control cannot be disregarded.

Short Carry of Temephos

A surprising report was made by Umino et al. [32] that the carry of temephos was only 25 meters with an application of 2 ppm/10 min in a minute stream, and it could not be increased even with an extremely high dose application of 200 ppm/10 min. Further studies in the laboratory revealed the highly adsorptive nature of temephos [33], which was later confirmed in the field by Mizutani (unpublished data).

While Umino et al. [30] showed that a dose of more than 3 ppm/min is necessary to expect 95% mortality at a site immediately downstream, some researchers reported that a dose far smaller than 3 ppm/min temephos was effective for a very long distance under field conditions. In the Onchocerciasis Control Programme in West Africa (OCP), application of temephos emulsion at a dose of 0.05–0.1 ppm/10 min (= 0.5–1.0 ppm/min) by aircraft has been the standard control measure, which is reportedly giving excellent control [34, 35]. Helson and West [36] reported particulated temephos at a dose of 0.1 ppm/15 min (=1.5 ppm/min) was effective 175–960 meters downstream.

Taking into consideration the highly adsorbent nature of temephos, the above discrepancy might be the result of the effect of particulates as carriers of the toxicant in streams, as suggested by Fredeen et al. [37, 38] in the cases of DDT and methoxychlor. Once the insecticide is adsorbed, either artificially or naturally, onto particulates of suitable size for ingestion by black fly larvae, the availability of the toxicant could be enhanced and eventually result in a high mortality effect for a long distance downstream.

In Guatemala, small target streams are usually distributed in the uppermost part of each channel network, close to the headsprings. Therefore, a short carry of temephos in these streams might be explained by (1) a high probability of adsorption to static soil or substrates on the streambed due to the small size of the stream; or (2) no or only negligible adsorption to mobile particulates suspended in the streams due to the clarity of the stream water [33]. Extensive stream tests revealed that the concentration of temephos had no relationship to its carry, but it was apparent that the larger the water discharge, the longer the carry [39], which was also reported for DDT by Lea and Dalmat [7].

Formulation of Larvicide and Method of Application

Trough tests failed to find any marked difference in efficacy between emulsifiable concentrate (EC) and water-dispersible powders (wdp) of temephos [30]. Furthermore, extensive stream tests of temephos did not indicate any distinct difference in efficacy among four formulations: wdp, EC, oil solution, and solid [39]. In addition, laboratory tests of adsorption of temephos to sands did not show any difference between the wdp and EC [33]. These results all suggest no practical difference in efficacy between the formulations, most likely due to the particular conditions of the Guatemalan streams. Improved larvicide formulations are badly needed to provide maximum carry in the small streams of Guatemala.

No difference was observed in efficacy between the two application methods, i.e., applying during 10 minute and applying instantaneously, with wdp or EC [39]. The instantaneous application is particularly efficient in Guatemala, where periodic visits to numerous dosing sites located in the mountainous terrain are needed.

Effects of Temephos on Non-target Organisms

The target streams in Guatemala are rather poor in fish and insect faunas. The temephos application in such streams did not have a serious impact on non-target organisms, except a slight effect on Chironomidae [40]. However, the long-term effect of insecticide in downstream areas should be monitored.

AREA VECTOR-CONTROL TRIAL

Following the basic studies on vector bionomics and insecticides, an area vector-control trial for *S. ochraceum* was carried out in the San Vicente Pacaya Pilot Area (236 km^2, in the East-Central Zone, Fig. 1) by the Guatemala-Japan Cooperative Project. This consisted of biweekly application of temephos into breeding streams, and its objective was to suppress the biting-population density to a level low enough to interrupt the transmission of the disease organism (i.e., below the provisional critical ABR of ca. 8,000). This control trial began in 1979 in a small valley of Lavaderos, then gradually expanded to other neighboring valleys, with a final coverage of about 90 km^2 in

January 1984. An outline of the control operation and the results of entomological evaluations have already been reported [41, 42]. Details of the entire program were reported by Yamagata et al. [43] and are excerpted below.

Tactics for Larvicide Application

The control operation was divided chronologically into three phases. In phase 1, 10% temephos briquettes were applied with a dose rate of 0.1 ppm/60 min to all the streams with a discharge range of 0.1 to 50 liters per sec. In phase 2, 50% temephos wdp was applied at 2 ppm/10 min into streams of less than 1 liter/sec discharge. In phase 3, a new system of fixed dose application was introduced; 24 grams of 5% temephos wdp in a packet was diluted with stream water and poured into a stream instantaneously every 50 to 100 meters, irrespective of the water discharge at each dosing site. The target streams were those with less than 50 liters/sec discharge. The fixed-dose larviciding system in phase 3 could compensate, at least partially, for the inconsistent carry of temephos.

Under this system, temephos concentration is high in smaller streams and low in larger streams. But from the operational viewpoint, this system had the great advantage that no measurement of water discharge was required at any dosing site or time. This improvement was particularly helpful during the rainy season, when water discharge is extremely variable. The tactics applied in phase 3 of the operation are currently being used.

ABRs Pre- and Post-control

Entomological evaluation for this control trial was made every two weeks by collections of adult *S. ochraceum* using human attractants at seven stations (four inside the study area and three outside). The data obtained were summarized using the criterion of the ABR. As a result, the phase 1 and 3 operations, and especially the latter, were effective in suppressing adult density, whereas phase 2 was not satisfactory, presumably because of the neglect of large streams with more than 1 liter/sec discharge. Before the control operation was initiated, the ABRs of *S. ochraceum* were high at any collecting station, ranging from about 50,000 to 300,000 (Table 1). In phase 3, the values decreased to level of 500 to 7,500, which was considered below the provisional permissible value.

Table 1. Annual Biting Rate (ABR) in the controlled and uncontrolled areas in relation to the control phases (from Suzuki 1983)

Station	Item	1978–1979	1979–1980	1980–1981	1981–1982	1982–1983
Lavaderos	Phase	0	1	1	2	3
	Period	Aug–Mar[a]	June–May	June–May	June–May	June–May
	ABR	315,740	9,315	3,480	3,274	556
Barretal	Phase	—	1	1	2	3
	Period		Aug–July	Aug–July	Aug–July	Aug–May[b]
	ABR		26,852	19,063	47,810	2,263
Peña Blanca	Phase	0	0	—	2	3
	Period	Oct–Sept	Oct–Sept		June–May	June–May
	ABR	84,090	142,371		103,093	7,536
Guachipilín-23	Phase	0	0	—	2	3
	Period	Oct–Sept	Oct–Sept		June–May	June–May
	ABR	120,697	72,720		35,146	865
Rodeo	Phase	0	0	0	0	—
	Period	Aug–July	Aug–July	Aug–July	Aug–July	
	ABR	48,849	48,448	23,068	36,814	
Tarral	Phase	0	0	0	—	—
	Period	Aug–July	Aug–July	Aug–July		
	ABR	21,995	27,796	22,227		
Rincón	Phase	0	0	0	0	—
	Period	Sept–Aug	Sept–Aug	Sept–Aug	Sept–Aug	
	ABR	179,440	103,234	155,011	150,836	

Note: Phase 0: Precontrol phase.

[a] Based on 8 months data.

[b] Based on 10 months data.

Consumption of Larvicide and Manpower

Consumptions of 5% temephos wdp in the phase 3 operation was 488g/km^2 in a biweekly application cycle, and annual consumption of 5% temephos was 12.7kg/km^2, or 634 grams temephos active ingredient. In phase 3, the mean area covered by one field operator in a biweekly cycle (10 working days) was 4.6 km^2. These figures should be valuable from a cost perspective for future programming of a large scale control operation.

Fly Infiltration

Infiltration of vector species into the treated area from the surrounding untreated areas is one of the most serious problems in many cases of vector control. Gradual expansion of the area under control in this operation made it possible to estimate the extent of infiltration of *S. ochraceum*. Using the relationship of fly densities at each catching station with various distances to the border of the untreated area, it was indicated that infiltration might not occur beyond 3 or 4 kilometers. This distance is much smaller than the flight range (6.3 miles or 10.1 kilometers) estimated by mark-release-recapture experiments [44].

Remarks

Epidemiological evaluation for this vector-control operation is now underway, so its ultimate effect on the human population cannot be assessed at present. The pre-control baseline data for this project were already reported by Yamagata et al. [43]. However, from the results obtained so far, it is suggested that larval vector control to suppress female density of the vector to a level low enough to stop the transmission of the disease is feasible if realistic planning is made and the staff is devoted in its efforts. Even in a small, limited area, a successful vector-control operation might be achieved because of the limited flight range of adult *S. ochraceum*.

In any future vector control of Guatemalan onchocerciasis in which a larvicide is applied, one of the key factors is to cover all the vector-breeding sites without omission. In order to find all the preimaginal habitats of *S. ochraceum* in the rugged terrain of mountainous areas, the necessity and importance of proper and precise mapping of the target area prior to larviciding cannot be overemphasized. The macrodistribution of *S. ochraceum* larvae may be efficiently delimited by understanding the topographical or geological features of the target areas [11].

Conclusion and Future Research Needs

Since the mid-1970s, much new information on Guatemalan onchocerciasis has been accumulated. In relation to vector control, some special epidemiological features of the disease were clarified. Furthermore, extensive insecticide studies in the field and laboratory demonstrated that the characteristic situations of Guatemalan streams where *S. ochraceum* breeds require ingenious methods of larviciding. And finally, the feasibility of an area vector control was indicated by the successful control operation in the San Vicente Pacaya Pilot Area, in which a new fixed-dose larviciding method was applied.

Apart from vector control, there remain two other control measures against Guatemalan onchocerciasis. Chemotherapeutic control may be effectively carried out in some low endemic areas with a low vector density. Likewise, it appears that a nodulectomy campaign can be significant in lowering the risk of severe eye lesions caused by invasion of microfilariae, probably from head nodules.

In conclusion, it is emphasized that further research on onchocerciasis and its control in Guatemala should continue in order to improve control measures and its evaluation. As already pointed out by Suzuki [5], this research should include the followings.

Vector Biology and Control

(a) Cytotaxonomy of vector species, (b) search for resting black flies, (c) laboratory rearing of *S. ochraceum*, (d) further studies on critical ABR value, (e) role of transmission by minor potential vectors, (f) transmission potential of component cytoforms of *S. ochraceum* complex, (g) effect of reduced microfilarial density in DEC-treated patients on transmission by vector black flies, (h) trial of intermittent vector control, (i) further studies on the time interval between two successive larvicide applications, (j) efficacy of insecticides and formulations against black fly larvae in Guatemala, (k) dispersal range of *S. ochraceum* in large basins.

Epidemiology

(a) Epidemiological evaluation of vector-control operations in the San Vicente Pacaya area, (b) relationship between the prevalence or eye lesions in human population and vector density, (c) human movements between endemic and non-endemic areas, (d) animal distribution and movements in the endemic areas, (e) elucidation of the reasons for sharp decline in endemicity in the Eastern Zone.

Parasitology

(a) Longevity of adult worms of *O. volvulus*, (b) differentiation of *O. volvulus* larvae from other filariae, (c) existence and distribution of impalpable nodules in human body.

Immunology

(a) Improvement of immunodiagnoses.

Chemotherapy

(a) Treatment of patients with DEC and Suramin, (b) trial of chemotherapeutic control by new chemicals, (c) effect of DEC treatments on eye lesions.

ACKNOWLEDGMENTS

We are indebted to Steven Tessler, The Pennsylvania State University, for reading, editing and retyping the manuscript, and whose efforts helped to clarify many points in our initial draft.

REFERENCES

1. Dalmat HT. The black flies (Diptera, Simuliidae) of Guatemala and their role as vectors of onchocerciasis. Smithsonian Misc Coll 1955; 125: 1–425.
2. Hamon J. The onchocerciasis vectors in the Western Hemisphere. Vector biology and vector parasite relationship. In: Pan American Health Organization, Proc Int Symp On Research and Control of Onchocerciasis in the Western Hemisphere. Washington, D.C.: PAHO Scientific Publication; 1974. 298: 58–68.
3. Sasa M. Human filariasis. Tokyo: University of Tokyo Press; 1976.
4. Ogata K. Preliminary report of Japan-Guatemala onchocerciasis control pilot project. In: M. Laird, ed. Blackflies: the future for biological methods in integrated control. New York and London: Academic Press; 1981. pp 105–115.
5. Suzuki T. A guidebook for Guatemalan onchocerciasis (Robles disease), with special reference to vector control. Guatemala: Guatemala–Japan Cooperative Project on Onchocerciasis Research and Control; 1983. 155 pp.
6. Lea AO, Dalmat HT. A pilot study of area larval control of black flies in Guatemala. J Econ Entomol 1955; 48: 378–388.
7. Lea AO, Dalmat HT. Field studies on larval control of black flies in Guatemala. J Econ Entomol 1955; 48: 274–278.
8. Figueroa MH. Robles' disease (American onchocerciasis) in Guatemala. In: Pan American Health Organization, Proc Int Symp On Research and Control of Onchocerciasis in the Western Hemisphere. Washington, D.C.: PAHO Scentific Publication; 1974. 298: 100–104.
9. Garcia-Manzo GA. Geographical distribution of Robles disease in Guatemala. In: Proc. Guatemala–Japan Joint Conf. on Onchocerciasis Research and Control. Tokyo: Japan International Cooperation Agency; 1981. pp 52–59.
10. Takaoka H, Ochoa AJO, Juarez EL, et al. Effects of temperature on development of *Onchocerca volvulus* in *Simulium ochraceum* and longevity of the simuliid vector. J Parasitol 1982; 68: 478–483.
11. Yamagata Y, Okazawa T, Molina PA. Geologic and geomorphologic studies on distribution of *Simulium ochraceum* (Diptera: Simuliidae) larvae in Guatemala. Jpn J Sanit Zool 1984; 35: 95–102.
12. Yamada H. Onchocerciasis (Robles disease, River blindness) in Guatemala and Ghana. Clinical features and epidemiological research. Floia Ophthalmol Jpn 1978; 29: 1817–1837.
13. Tada I, Aoki Y, Rimola CE, et al. Onchocerciasis in San Vicente Pacaya, Guatemala. Am J Trop Med Hyg 1979; 28: 67–71.
14. Brandling-Bennett AD, Anderson J, Fuglsang H, et al. Onchocerciasis in Guatemala: Epidemiology in fincas with various intensities of infection. Am J Trop Med Hyg 1981; 30: 970–981.
15. Yamada H, Oikawa T. Ocular onchocerciasis in heavily endemic focus in Guatemala. Floia Ophthalmol Jpn 1980; 31: 1637–1647.
16. Kawabata M, Hashiguchi Y, Zea FG. Distribution pattern of microfilariae in relation to sex and age in Guatemalan onchocerciasis. Trans R Soc Trop Med Hyg 1983; 77: 215–216.
17. Ito S, Tanaka I, Ochoa AJO. Comparative studies on the affinities of two blackflies, *Simulium metallicum* and *S. ochraceum* for the larvae of *Onchocerca volvulus* in Guatemala. Jpn J Sanit Zool 1980; 31: 261–270.
18. Takaoka H, Suzuki H, Noda S, et al. The intake, migration and development of *Onchocerca volvulus* microfilariae in *Simulium haematopotum* in Guatemala. Jpn J Sanit Zool 1984; 35: 121–127.
19. Takaoka H. Seasonal occurrence of *Simulium ochraceum*, the principal vector of *Onchocerca volvulus* in the southeastern endemic area of Guatemala. Am J Trop Med Hyg 1981; 30: 1121–1132.
20. DeLeon JR, Duke BOL. Experimental studies on the transmission of Guatemalan and West African strains of *Onchocerca volvulus* by *Simulium ochraceum*, *S. metallicum* and *S. callidum*. Trans R Soc Trop Med Hyg 1966; 60: 735–752.
21. Omar MS, Garms R. The fate and migration of microfilariae of a Guatemalan strain of *Onchocerca volvulus* in *Simulium ochraceum* and *S. metallicum*, and the role of the buccopharyngeal armature in the destruction of microfilariae. Tropenmed Parasitol 1975; 26: 183–190.
22. Garms R. Observations on filarial infections and parous rates of anthropophilic blackflies in Guatemala, with reference to the transmission of *Onchocerca volvulus*. Tropenmed Paasitol 1975; 26: 169–182.
23. Garms R, Ochoa AJO. Further studies on the relative importance of Guatemalan blackfly species as vectors of *Onchocerca volvulus*. Tropenmed Paasitol 1979; 30: 120–128.
24. Collins RC. Onchocerciasis transmission potentials of four species of Guatemalan Simuliidae. Am J Trop Med Hyg 1979; 28: 72–75.
25. Ochoa AJO. Studies on the anthropophilic blackfly spe-

cies in Guatemala, with special reference to the transmission of onchocerciasis in the southeastern endemic area. Jpn J Sanit Zool 1982; 33: 129–138.

26. Takaoka H. Observations on the bionomics of larval and man-biting female populations of *Simulium horacioi*, a new potential vector of *Onchocerca volvulus* in Guatemala. Jpn J Trop Med Hyg 1982; 10: 49–62.
27. Collins RC, Merino ME, Cupp EW. Seasonal trends and diurnal patterns of man-biting activity for four species of Guatemalan black flies (Simuliidae). Am J Trop Med Hyg 1981; 30: 728–733.
28. Wada Y. Theoretical approach to the epidemiology of onchocerciasis in Guatemala. Jpn J Med Sci Biol 1982; 35: 183–196.
29. Collins RC. Transmission of onchocerciasis in Guatemala: recent studies and their implications for vector control. In: Proc. Guatemala-Japan Joint Conf. on Onchocerciasis Research and Control. Tokyo: Japan International Cooperation Agency; 1981. pp 98–101.
30. Umino T, Suzuki T, Juarez OEL. Insecticide studies in vector control of Guatemalan onchocerciasis. 2. Efficacy of larvicides assessed by simulated trough tests. Jpn J Sanit Zool 1983; 34: 269–277.
31. Undeen A, Takaoka H, Hansen K. A test of *Bacillus thuringiensis* var. *israelensis* De Barjac as a larvicide for *Simulium ochraceum*, the Central American vector of onchocerciasis. Mosquito News 1981; 41: 37–40.
32. Umino T, Suzuki T, Ochoa AJO. Insecticide studies in vector control of Guatemalan onchocerciasis. 1. Short carry of temephos in minute streamlets. Jpn J Sanit Zool 1983; 34: 213–219.
33. Umino T, Suzuki T. Insecticide studies in vector control of Guatemalan onchocerciasis. 3. Laboratory tests on absorption of larvicides to soil. Jpn J Sanit Zoo 1984; 35: 1–6.
34. Davies JB, Le Berre R, Walsh JF, et al. Onchocerciasis and *Simulium* control in the Volta River Basin. Mosquito News 1978; 38: 466–472.
35. Walsh JF, Davies JB, Cliff B. World Health Organization Onchocerciasis Control Programme in the Volta River Basin. In: Laird M, ed. Blackflies: the future for biological methods in integrated control. New York and London: Academic Press; 1981. pp 85–103.
36. Helson BW, West AS. Particulate formulations of Abate and methoxychlor as blackfly larvicides: their selective effects on stream fauna. Can Entomol 1978; 110: 591–602.
37. Fredeen FJH, Arnason AP, Berck B. Adsorption of DDT on suspended solids in river water and its role in black fly control. Nature 1953; 171: 700–701.
38. Fredeen FJH, Saha JG, Balba MH. Residues of methoxychlor and other chlorinated hydrocarbon in water, sand, and selected fauna following injections of methoxychlor black fly larvicide into Saskachewan River, 1972. Pestic Monit J 1975; 8: 241–246.
39. Kamimura K, Suzuki T, Okazawa T, et al. Effect of temephos against the blackfly larvae in stream tests in Guatemala. Jpn J Sanit Zool 1985; 36: 189–195.
40. Hasegawa J, Yasuno M, Sasa M. Effects of insecticide treatment of streams for the control of black fly larvae on the non-target organisms. In: Proc. Guatemala-Japan Joint Conf. on Onchocerciasis Research and Control. Tokyo: Japan International Cooperation Agency; 1981. pp 187–190.
41. Nakamura Y, Yamagata Y, Takaoka H, et al. A control trial of the vector of onchocerciasis, *Simulium ochraceum* (Diptera: Simuliidae) in the Lavaderos River Valley, Guatemala. Jpn J Sanit Zool 1981; 32: 51–58.
42. Takaoka H, Ochoa AJO, Takahashi M, et al. Evaluation of temephos as a larvicide against *Simulium ochraceum* (Diptera: Simuliidae) in Guatemala. J Med Entomol 1981; 18: 145–152.
43. Yamagata Y, Ochoa JO, Molina PA, et al. Chemical control of *Simulium ochraceum* Walker (Diptera: Simuliidae) larvae in an onchocerciasis endemic area of Guatemala. Trop Med Parasitol 1987; 38(3): 205–210.
44. Dalmat HT, Gibson CL. A study of flight range and longevity of blackflies (Diptera: Simuliidae) infected with *Onchocerca volvulus*. Ann Entomol Soc Am 1952; 45: 605–612.