3. Review Reports of the Guatemala-Japan Cooperative Project on Onchocerciasis Research and Control

2) A Guatemala-Japan Collaboration Project for Onchocerciasis Research Supported by JICA, Japan*

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On July 21 of 1975, an agreement was signed to start a collaboration project for the research of onchocerciasis. For this project, Japan decided to send researchers to Guatemala to aid Guatemalan staff of technicians in a research to combat a disastrous filariasis. This disease was discovered in this country by Dr. Rodolfo Robles in the year 1915 and that is why it is known as Robles disease. This disease exists widely in Africa, but the Guatemalan disease was considered different from the African onchocerciasis. The disease is caused by the same parasite *Onchocerca volvulus* and transmitted by different species of blackflies of the genus *Simulium*. In America, onchocerciasis has later been found in Mexico, Venezuela, Colombia, Ecuador and Brazil without having satisfactory solution of control to date.

**ONCHOCERCIASIS**

In Guatemala, onchocerciasis is confined to limited regions of the foothills of piedmonts and is the cause of eye injuries that can culminate in blindness and severe skin conditions. Onchocerciasis is manifested by subcutaneous signs, known as nodules or fibrous tumors, oncocercomas. That can appear in different parts of the body, especially in the head and chest.

The filaria *Onchocerca*, is a characteristic very thin 70 mm long nematode, from where the name “filaria” comes from. They disperse under the skin to the entire body to develop. The microfilariae invade into the anterior chamber of the eye and are responsible for the loss of the eye sight.

The endemic foci of onchocerciasis are located in five regions affecting severely coffee farms in the foothills of the Sierra Madre mountains. There are seven departments of Guatemala partially affected and none of the municipalities involved have entire onchocerciasis transmission. It is then a disease targeted for the particular circumstances.

The foci of oncocerciasis are located in the following departments:

- Huehuetenango,
- Suchitepequez,
- Solola,
- Chimaltenango,
- Escuintla and Santa Rosa. The total extension is 5,130 km² including about 390 localities with the population of 343,000 at risk inhabitants of which an estimated 30,000 are affected by the disease.

The Ministry of Health has maintained surveillance for many years by brigades that make monitoring visits to search oncocercomas among inhabitants and make surgical removal operations of palpable nodules although deeply located nodules are not palpable. However, it is clear that this measure provides only partial solution because the transmission of the disease is still viable.

Since the time of Dr. Rodolfo Robles, the Ministry of Public Health kept a section responsible for the monitoring of endemic foci of onchocerciasis located in five localities in the foothills of the Sierra Madre. This section was composed of technicians who were able to do efficiently the minor surgical operations to make nodules extraction after the palpation of the skin.

On May 18, 1976, this Section was transferred to National Service for Malaria Eradication (SNEM) and one of the brigade teams with two technicians was assigned to work directly with Japanese epidemiologists.

Dr. Horacio Figueroa Marroquin, a distinguished researcher of Guatemala had been in contact with Dr. Isao Tada of Kanazawa Medical University, Japan, and invited him to Guatemala in 1970 to promote research of onchocerciasis. Based on a field report of onchocerciasis in Guatemala proposed by Dr. Tada in 1973, Mr. Junzo Mori, the ambassador of Japan recommended to the Ministry of Foreign Affairs, Japan to start an ODA project for the research of oncocerciasis. The agreement of this project was signed in 1975 between Japanese International Cooperation Agency (JICA) and The Minister of Public Health and Welfare of Guatemala, Dr. Julio Castillo Sinibaldi.

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THE PILOT AREA

For the convenience of approach to the capital city, an endemic core was chosen and this core partially covers the municipalities of San Vicente Pacaya, Guanagazapa, Palin, Escuintla and Villa Canales. The internal roads of this focus are unpaved, but were passable by cars. Revenues in the Pilot Area are on the road to Escuintla, through Plantation El Salto, Escuintla and Santa Elena de Barillas Villa Canales.

The pilot area measured approximately 236 km² and had 5,700 inhabitants. The main crop of the region was coffee and subsidiary activities were for corn and beans. Following administrative districts are involved.

| Locality          | Population | House | Area (km²) |
|-------------------|------------|-------|------------|
| Department of Escuintla |
| San V. Pacaya     | 39         | 6,413 | 1,317      |
| Palin             | 9          | 403   | 2,015      |
| Guanagazapa       | 9          | 129   | 423        |
| Escuintla         | 5          | 79    | 303        |
| Department of Guatemala |
| Villa Canales     | 6          | 416   | 85         |

These five municipalities with endemic onchocerciasis are situated on the slope of Pacaya volcano, which has countless rivulets down to the Pacific slope, where the valley of Apatitlán and Canyon of Palin are located on the west, and the coffee areas are in the slopes of San Vicente Pacaya, Escuintla and Villa Canales to the south. The inside of Pilot Area was with intense human subsistence and commercial mobility and 49 locations received internal migration in the region.

| Locality          | Population | Rate |
|-------------------|------------|------|
| San Vicente Pacaya| 22         | 641  | 30.4% |
| Palin             | 21         | 1,110| 52.6% |
| Escuintla         | 1          | 23   | 1.2%  |
| Guanagazapa       | 3          | 36   | 1.7%  |
| Villa Canales     | 2          | 34   | 1.6%  |
| Total 5 municipalities | 49      | 1,844| 87.5% |

These data may vary in different years, but the above figure was a result of survey in the 1979 to establish references. During the four months from May to Aug. there was an average of 600 traveling people internally for the labor issues.

Within this area there is a large space of crops known as El Chilar which belongs to the Municipality of Palin and has unique conditions: It is the only communal farm in the region and is intended for the exclusive benefit of the indigenous population of ethnic Pocomam. It is a center of intense agricultural production and all the workers 1,250 farmers of both sexes live in the village of Palin and commute and round trip. This place, coffee farms, is the manifest origin of onchocerciasis in the municipality of Palin.

The plantations located in the pilot area are between 700 and 1,100 meters from the sea level. The local workforce is insufficient and accept laborers from whole the region, including troopers from other departments. In 26 localities they accept 353 laborers from nearby locations and 358 more from distant places. An unknown number of temporary crews come from distant regions of the Republic.

The people of the county of San Vicente Pacaya is the epicenter for the activities of the pilot area, located 1,500 meters above the sea level and from there the same 500 laborers go to work in the farms and 107 other travel through different places, including center of Escuintla and the capital city of Guatemala.

In the plantation San Jose Guachipilín located adjacent to Chilar they hire external laborers for cutting coffee. From November to January, the number of women increase in the activities of the coffee harvest and the rest of the year, only men work there.

The temporary laborers reach at the pilot area for coffee cultivation:

- 19% from Jan. to March for slashing and composting,
- 33% from Apr. to Nov. to clean seedbeds, reseeding and desuckering and 48% from Nov. to Jan. to cut the coffee.

In coffee workers, 59% are male adults, 20% are adult women, 13% are boys and 8% are girls. The origin of the laborers in 1977 was: Finca Hamburgo: 1,104 coming from Tutuapa, San Miguel, Tejutla, San Jose Ojotenán, Democracy, San Pedro Necta, Colotenango, Huehuetenango and Joyabaj Cubulco. Finca La Suiza: 609 laborers from Cubulco and Cobán; Finca San Nicolas: 453 laborers come from Tutuapa, San Miguel and Tejutla.

Other smaller farms like La Providencia, Terranova, Santa Eulalia, El Camaron, Puerta de Oro, Berlin, Coyolito, Santa Fe and Peña Blanca received 350 troopers from Jalapa, Ixhuatán, San Juan Sacatepéquez and surrounding areas. The recruiters are people trusted by the farmers and their remittances usually repeated every year. These contractors make commitments with several owners of other farms, so many of the same workers spend in different farms and in the activities of coffee, sugar cane and cotton. In reference sources, only Sibaja of Yepocapa is the
onchocerciasis area in all registered sources. About 15% of these workers will also work in the farms of Chiapas of Mexico.

The Pacaya area is the second endemic focus of the country with epidemiological importance. From its gush many springs of inclined flanks fast flowing streams and tributaries flow into Lake Amatitlán or Michatoya River. The inhabitants of the area are mostly farmers of Ladino mestizos who are with the indigenous genetic Pocomam group including many people with the evidence of Negroid character.

In the valleys of this region, the sugar cane plantations which were properties of religious orders by Dominicans, Jesuits and Mercedians during the colonial era introduced African slaves in large numbers. Many of them fled to the mountains and began to live in the foothills of the Pacaya volcano particularly in Maroon villages and they evolved to the current populations of the region.

BEGINNING OF THE PROJECT

The Japanese mission started the project first with nine specialists who arrived at Guatemala in Apr. 1976. By the completion of the whole project in Sep. 1983 a total of 74 experts were involved in entomology, epidemiology, parasitology, immunology, ophthalmology and other specialties.

Formal activities of Guatemala-Japan onchocercosis research Project began on Jun. 9 of 1976. Among many works its first activity was the recognition of the geographical area: sketches, location of towns, houses, counting people, medical records, censuses, v immigration checks, etc.

In the central campus of the Malaria Division, the Central Laboratory building was inaugurated on Oct. 8, 1976 in the honor of Dr. Isao Tada.

An auxiliary laboratory was also constructed in San Vicente Pacaya with the name of Dr. Hiroshi Takahashi.

TEAM MEMBERS

Dr. Isao Tada, Parasitologist, Project Manager  Apr. 76
Dr. Hiroshi Takahashi, Team Leader  May 76–Sep. 80
Dr. Takeshi Suzuki, Team Leader  Oct. 80–83
There were processes of supervision by Dr. Jiro Yamamoto, the Chief of Medical Division, JICA, Mr. Akira Kamakura (JICA)  Sep. 76
Dr. Shigeo Hayashi and Mr. Touichi Hashimoto  Jun. 77
Dr. Koichi Nakazawa (JICA)

ENTOMOLOGY**
Dr. Kikuo Matsuo  May 76–May 77, Aug. 79–Dec.79
Dr. Takao Okazawa  Jul. 76–Oct. 78, Jan. 80–Sep. 80
Dr. Osamu Onishi  Jul. 76–Jul. 77
Dr. Ikuo Tanaka  May 77
Dr. Ikuo Tanaka  May 77–Jun. 78
Dr. Ogata Kazuki  Jun. 77–Jan. 78
Dr. Mamoru Watanabe  Oct. 77
Dr. Yuzuru Nakamura  Jan. 78–Jan. 79
Dr. Sumiyo Ito  Jan. 78–Jul. 78
Dr. Yuichiro Tabaru  Jul. 78–Oct. 78
Dr. Yoshito Wada  Sep. 78–Dec. 78
Dr. Atsuo Shimada  Sep. 78–Dec. 78
Dr. Masakazu Takahashi  Feb. 79–May 79
Dr. Junichi Hasegawa  Feb. 79–May 79
Dr. Masatoshi Takaoka  Oct. 78–Oct. 79
Dr. Takeshi Suzuki  Jun. 86–Jul. 80
Dr. Tokuko Umino
Dr. M. Yasuno

EPIDEMIOLOGY AND PARASITOLOGY**
Dr. Shigefusa Sato  Jul. 76–Oct. 76
Dr. Yoshihisa Hashiguchi  May 76–Aug. 77
Dr. Yoshiki Aoki  May 76–Aug. 77
Dr. Isao Tada  Jun. 77
Dr. Masato Kawabata  May 77–May 79
Dr. Akihisa Hasebe  Aug. 77
Dr. Hitoshi Kasuga  Sep. 77
Dr. Tsuguyoshi Suzuki  Sep. 77
Dr. Manabu Sasa
Dr. Akihisa Hasebe  Aug. 77–Sep. 77
Dr. Hitoshi Kasuga  Sep. 77
Dr. Hiroyuki Takaoka  Aug. 78–Jan. 80
Dr. Makoto Sakamoto  Apr. 79–Sep. 80
Dr. Yoichi Ito  Jun. 79–Sep. 80
Dr. Kaoru Kondo  May 80–Nov. 80
Dr. Kiyoshi Kamimura  Mar. 80–Sep. 80
Dr. Tohru Inaoka  Mar. 80–Sep. 80
Dr. Hideta Satou  Sep. 80
Dr. Kiichi Uemoto  Sep. 80
Dr. Tamotsu Sasayama
Dr. Tokuko Umino
Dr. Saito

IMMUNOLOGY**
Dr. Teruaki Ikeda  May 76–May 77
Dr. Masato Kawabata  May 77

DERMATOLOGY**
Dr. Shigeo Nonaka  Aug. 77–Oct. 77, Jul. 78–Oct. 78, Oct. 79–Dec. 79
During the initial five years, 43 Japanese experts participated and 28 Guatemalans were the counterpart. Seventy three Japanese Professional experts participated in total. Japanese team was assigned to attend national counterparts from SNEM**

In the first step the necessary research activities were planned due to the requirements to obtain the evidence of entomological and parasitological baseline. For the second stage the use of insecticides and control of insects responsible for the transmission of onchocerciasis at small scale.

The inhabited localities in the endemic foci are predominantly coffee plantations and so following to the geographical features of this culture, the filaria-transmitting insects are known as “coffee flies”.

With the method of palpation, the body distribution of oncocercomas was as follows: 58.8% in the head, especially in the occipital and parietal location, 23.2% and 22.6%, respectively. And 41.2% of all the nodules were found in the body trunk, mainly located in the iliac region 12.4% of the total. The probing and removal of nodules continued in the pilot area in the eight years of the research project.

In 1978 there were 10 Japanese and 25 national experts working in the project. The Japanese professionals succeeded over eight years. All the information and results of surveys obtained in 1977 were recorded in approximately 11,600 punched IBM cards.

The initial agreement of the five year project was planned to start in Oct. 1975 and close in Sep. 1980. In May 1980, the additional three-year prolongation agreement was signed which would close in Sep. 1983.

Dr. Hiroshi Takahashi served as the chief of Japanese team and Dr. Juan José Castillo was the Director of the Division of Malaria for the project until 1979. Then Dr. Barnabas Batres and later in Jan. 1980 Dr. Hector Godoy succeeded the position.

**ENTOMOLOGY**

The entomological research emphasized the following themes.

a) The taxonomy.
b) To define the vector blackfly species and to assess the minimum level required for the transmission.
c) Measurement of the larval densities and frequency of individual adult blackfly species.
d) Measurement of larval and adult blackfly populations.
e) Measurement of flight ranges of vectors.
f) Measurement of the time span of larval development.
g) Survey of the geographical distributions of blackflies.
h) Assessment of the flight range of adult blackflies. This was essential for the larviciding and calculation of costs, human displacement and etc.

i) Collection of blackflies by using human bait from all parts of the body which are exposed without garments.

j) It was decided to give priority to study the flight range and longevity of *S. ochraceum* even in the outside of endemic areas.

k) Studies of vector distribution and biting rates.

The feature of the endemic area is the proliferation of small river tributaries that encourage development of the larvae of various species of blackflies. Recognition and hydrological mapping of the Pilot Area resulted in 263,864 linear meters of these streams, as follows:

| Name              | tributary | sub-tributary | length (m) |
|-------------------|-----------|---------------|------------|
| Rio Barretal      | 7         | 3             | 8,011      |
| Queb. Lavaderos   | 13        | 3             | 6,245      |
| Rio Pajal         | 12        | 16,365        |
| Rio Marinalá      | 3         | 1             | 23,970     |
| Queb. Camaron     | 1         | 2,420         |
| Rio Camalote      | 1         | 1,480         |
| Rio Verde         | 4         | 12,090        |
| Rio Jazmines      | 10        | 12,895        |
| Queb. El Rodeo    | 3         | 4             | 14,630     |
| Rio Metapa        | 38        | 25            | 40,275     |
| Queb. Hamburgo    | 33        | 36            | 22,363     |
| Queb. El Amate    | 8         | 5             | 3,397      |
| Queb. El Guineo   | 6         | 3,143         |
| Queb. La Campana  | 4         | 4             | 1,810      |
| Rio Guachipilin   | 23        | 11            | 20,766     |
| Queb. La Culebra  | 11        | 6             | 11,015     |
| Rio El Carmen     | 11        |               | 8,015      |
| Queb. San Nicolás | 6         | 2             | 3,390      |
| Queb. Aldana      | 6         |               | 1,471      |
| Queb. El Balsamo  | 15        | 2             | 10,403     |
| Rio El Camaron    | 13        | 8             | 12,260     |
| Rio Silencio      | 21        | 2             | 17,714     |
| Queb. Ixcanalar   | 5         | 1             | 7,330      |

In total there were 23 streams, 254 tributaries and 109 sub-tributaries with the total of 263,864 linear meters in very rugged terrain where 816 waterfalls and cascades were also located.

The investigations of larvae brought progress of various activities and the systematic surveys in 1982 totaled 635 in Rio Guachipilin, 65 in Rio Lavaderos; 172 in Rio Jazmines, 39 in El Zapote and 62 in Rio Barretal.

As the result of measurements following results were obtained:

Water currents are modified in line with the seasons and entomological studies require adjustments and pinned down convenient sites especially conducive for the development of *S. ochraceum* species which was confirmed as the main species in the transmission of onchocerciasis.

The duration of ovarian gonotrophic cycle in *S. ochraceum* was 3 to 5 days depending on the temperature. The higher the altitude above sea level, the lower the natural mortality of *S. ochraceum*. However the incubation period of *Onchocerca volvulus* in the body increases. On the contrary, the lower the altitude, the higher mortality goes up but the time for parasite incubation is shortened. This evidence explains the difference of endemicity of the disease at different altitudes.

Dichotomous keys for easy recognition of the blackflies in the pilot area were prepared, highlighting the visible features of larvae and pupae for the practical use.

A brigade of five technicians came over exclusively for the evaluation of the results by larval counts inquiries and blackflies. Nine stations were set biweekly for the capture in February at El Injerto, in March in the Sierra Morena plantation plus 3 tentative stations. In each session, the capture of blackflies was performed from human bait for 150 minutes. The aim of the capture was to give alarm to modify operations if abnormal increases of the vector bites are recorded.

By the larval and adult blackflies capture inquiries, the following ten species were found: *Simulium ochraceum, S. metallicum, S. callidum, S. mexicanum, S. gonzalesi, S. rubicundulum, S. acatenangoensis, S. jacumba, S. pulvurulentum* and *S. corronsi*. In this country there have been 46 blackfly species recorded and only eight of which are anthropophilic, human biting.

Out of ten species found in the pilot area, only the first three are incriminated for the transmission of onchocerciasis. Microfilariae can attain to the 3rd stage in *S. metallicum* and *S. callidum* but in *S. ochraceum* this was in much lower proportion, so that its transmission activity is uncertain. As in the cattle and equine, infections with *Onchocerca guturosa* and *O. cervicalis* are widely distributed, it is very important to identify *O. volvulus* in the blackflies studied.

The reproduction of *S. ochraceum* starts in May with
the onset of rains and is the most abundant from Oct. to Feb. showing its peak in Dec. and Jan. The lowest season is in Jun. The minimum current flow faster than 0.1 to 10 liters per second are their ideal habitat, unlike *S. metallicum* which is the most abundant and their larvae are always in larger streams.

A new anthropophilic species was registered in 1980 as *Simulium horacioi* by entomologists Okazawa and Onishi during the first stage of the project. The name is a tribute to Dr. Horacio Figueroa, a prominent national researcher and the promoter of the Guatemala and Japan collaboration project.

*S. ochraceum* is anthropophilic and despises the blood of other animals. Their bites are manifested preferentially in the upper part of the human body, unlike *S. metallicum* which prefers biting other animals and the lower human body parts when you do a search.

Several species of blackflies were studied at different sites on the seasonal variation, degree of anthropophily, activity by time, the type of outbreaks and seasonal larval densities. At low temperature the life of vector is longer and microfilarial load is lower. At higher temperature more parasite develop and a marked reduction of the life is seen in the blackfly.

The capture rounds of adult blackfly with human bait performed in 88 seasons over seven points for each round in average by a couple of technical staff maneuvering for 15 minutes. In 1982 this activity sessions with 1,621 reported catching 1,764 were *S. ochraceum* blackflies (20%) and 6,845 of other species (80%) at stations within the treatment area. At stations which were installed outside the protected area, the collections were 3,099 (34%) *S. ochraceum* and 5,961 other species (66%). These indicators are repeated in other years.

The entomological task was directed to: a) natural infection in the field and laboratory, b) the study of larvae and pupae, c) an ecological study of water courses, d) the flight range of blackflies and e) the various evidence for the use of insecticides. Chlorofoxin phiripos-methyl was more lethal to the larvae of blackflies, while Temephos (Abate) was chosen as the larvicide to be used for the control as judged by the low toxicity to humans and other organisms in the collateral ecosystems.

The conclusion was that the use of insecticide Temephos (Abate), applied in the streams is possible to reduce blackfly larvae without the risk of affecting other species which were not involved in the transmission. *Bacillus thuringiensis* was tested among other possible insecticides to see the damage to the ecosystem, but the result was not satisfactory.

Under the background of rigorous knowledge and the results of the preliminary studies, the preferable area was selected to fight against *S. ochraceum* in 14 basins 91.5 square kilometers and the surveillance extended to seven basins which were not achieved watershed protection.

On Mar. 1979 blackfly control began using Abate in the Barretal river and El Zapote river with fortnightly applications in the tributaries of the 201 existing sites then spread to Lavaderos (Corresponding studies indicate that applications can be made up at intervals of 30 days). The initial dose of 3 ppm was poured into the current flow in 10 minutes.

In the beginning solid briquettes of Temephos placed in wire cages were applied for ten minutes or more within the pre-measured current. But the practice showed that the wettable powder formulation was more efficient and practicable. Consistent manual applicators are used in plastic bags containing soluble dose of Temephos, which is dissolved in the bag filled with water and the contents are discharged into the stream.

The designated area for treatment at baseline was 7.6 km² calculated as reduced area for two workers but usually in 10 km² due to subsequent expansion to compensate possible migrations of *S. ochraceum*. Doubts fell in the Guachipilin and Chilar rivers. By Jun. 1981 the current capacity assessment was made before applying Abate. From Jul. the previous capacity assessment was dismissed and applications were made every 100 meters in selected streams.

In 1981 the antilarval treatment was continued in Lavaderos, Barretal and Zapote rivers. In Jul. 1982, the Jasmines river and in Dec. of that year Conacaste and Chilcas rivers were added. Also In Mar. 1981 the application was extended to the bed of the Guachipilin river. On Dec. 30, 22 biweekly applications were completed in 15,851 sites, equivalent to 19,468 pounds of 50% wettable powder.

This operation has lowered the blackfly density from the risk limit to the minimum limit. The catch rates of adult flies indicating the interruption of the transmission, too. However it was found that the seasonal migration of the blackfly from the neighboring areas was not under the treatment and this was a worrying situation.

In seven watersheds, 434 tributary streams were treated, only Guachipilin River were completed with 26 scheduled cycles by 1982.

By the last year of the Project in 1983, vector control was done in 57.4 square kilometers encompassing parts of the municipalities of San Vicente Pacaya and Palin. In the mid of the year, treatment was extended to the west basin and stream of Calamote and the part of the east side of Marinalá river after larval inquiries prior mapping in El
Pajal, La Verde and Rodeo of the Municipality of Palin. Marinalá that is in the Municipality of Escuintla and the stream of Hamburg in the Municipality of San Vicente Pacaya.

Larviciding was done in 13,224 segments of waterways, employing 1,748 wages. The population of *S. ochraceum* was reduced to the transmission limit, seven in 13 blackflies per month.

**Epidemiology and Parasitology**

The parasite *Onchocerca volvulus* in Africa lives in the human host for ten years if there is no reinfection. In America this data has not been verified.

Being chosen as the Pilot Area centered in San Vicente Pacaya, priority review of infection of the inhabitants revealed the disproportions of infestation due to the latitudes of the different sites of human dwellings. The drastic height differences are due to that the whole Pilot Area is located on the slopes of Pacaya volcano and it showed great features like the intense migration of workers in coffee plantations which constitute the region.

One of the preliminary objectives was to obtain the infection status. And the first result was as follows:

- **Village**
  - Aldea Las Chilcas: 88% infection.
  - Finca Guachipilín: 86%
  - Finca El Camarón: 16.5%
  - Aldea El Cedar: 13%

Tentative data showed that in the people living in areas between 400 and 500 meters above the sea level, the reported infection rates was 21%; those living between 1,220 to 1,370 m the rate was 71% and above 1,720 m the infection rate was reduced to 13.7%.

The systematic palpation of subcutaneous nodules, onchocercoma, was the most immediate diagnostic method and the brigades investigated the area revealing the following results in the initial survey:

| Village                  | No. of examined people | No. of nodules |
|-------------------------|------------------------|---------------|
| Aldea de Villa Canales  | 5                      | 118           |
| Aldea de Escuintla      | 3                      | 91            |
| Palin (Pueblo)          | 1                      | 818           |
| Aldea de Santa Lucia Ctz.| 1                      | 23            |
| Aldea de Guanagazapa    | 8                      | 344           |
| Mun. San Vicente Pacaya | 25                     | 2,690         |

The confirmation of diagnostic measures was performed and the result was as follows:

By the palpation, 93 were positive out of 1,136 people with 124 nodules in 11 or more locations. It means that 8.3% were the patients. On the other hand, by the examination with biopsies, difference was evident showing 145 persons (27.9%) out of the examined were positive.

Six localities, villages and farms, were chosen for systematic epidemiological investigations since 1978 and they were visited to compare the results of skin biopsies, palpation and removal of nodules, visual acuity and other ophthalmic observations. These operations were aimed at the assessment of the results of vector control.

In 1980 the results obtained by the general by examinations. The result was as follows:

| Municipality        | Locality | No. examined person | No. positive |
|---------------------|----------|---------------------|-------------|
| San Vicente Pacaya  | 9        | 1,236               | 56          |
| Palin               | 3        | 138                 | 30          |

In 338 nodules removed, the size was between 5 and 12 mm, although some exceptional nodule measured 20 mm, in 82.5% measured. The average size of head nodule was 7 mm and the chest nodule, 8 mm. In 873 nodules examined, over 80, about 10%, were without adult filarias inside or not onchocercoma.

Anatomical study of 77 excised nodules revealed that adult male worms were from 1.0 to 5.2 mm in length and females, 16.8 to 69.7 mm by the measurement. Approximately 40 to 67% of the nodules examined harbored only female worms. Inoculations of microfilaria into white mice allowed us to see the survival of immature stage of onchocerciasis. This finding will provide us necessary measures for parasitological and immunological studies.

The microfilariae (mf) in mice appeared in the eyes, ears, viscera, skin, skeleton and finally accumulated in the tail. These mf remained alive up to 12 weeks after inoculation, which was an encouraging achievement for the different type of studies. Because the previous reports shows that the ideal animal for these purposes was chimpanzee, but its use in our environment would be expensive and complicated.

Skin biopsies showed higher efficiency in the diagnosis of onchocerciasis than body palpation method. However it was decided to combine both methods, because it was found that if only one is applied, positivity will be lost 20% to 30% of the examined cases, especially in the low grade of infections.

In the comparison of searching methods of mf, among those positive by skin biopsies, 23.5% were with mf positive in the urine in the inhabitants of Berlin plantation and 20.6% in Sumatán plantation. Three patients who were
negative by biopsy were positive in their urine.

The infection rate is much higher in adults and especially in the elderly, but mf were found even in the children aged 0–2 years. In all age groups the infection rate was higher in man than women.

For the epidemiological study in the endemic Pilot Area, 4,773 people were examined and the data obtained were recorded in punched cards.

By the closing of the project in 1982, a total of 11,282 skin biopsies were performed and revealed the overall positivity of 28%. The most affected age groups were 15 to 44 and those over 65 years.

**IMMUNOLOGY**

After the surveys by skin biopsies and visual acuity tests, the nodulectomy followed.

With the recommendation, the removed nodules were frozen to use in the parasitological and immunological studies. This was to get the possibility of vaccine research and to establish sensitive and specific immunologic tests. In vitro cultivation of *Onchocerca* parasites was also planned.

Filariae were also separated from cattle and horses in the operating slaughter houses in the region for the study. Canine filarias and the adult *Onchocerca* obtained from the nodules of the patients were used for immunological study.

Several antigens isolated were tested in search of the recommendable procedures for the immunological diagnosis as follows:

a) DD-test, agar double diffusion test with the antigens extracted from *Onchocerca volvulus* mf.

b) VBS Test, veronal buffered solution was used to dissolve antigens made from *Dirofilaria immitis* (dog heartworm) in the intradermal reaction.

c) VBS Test, veronal buffered solution was used to dissolve antigens from adult worms of *Onchocerca volvulus* for the intradermal reaction.

The cutaneous application of these three antigens proved effective in detecting patients of onchocerciasis, even though they were without presenting palpable nodules. The antigen from adult worms of *Onchocerca volvulus* showed higher positivity and fewer false positivity in the indirect hemagglutination (Ikeda et al, 1978).

The antigens accepted and submitted in the diagnostic studies and further eight studies could refine and improve the methods. The major drawback to promote immunological studies was the lack of removed onchocerical nodules from the Pilot Area which afford adult worms required in the studies. In the end of 1982, 2,782 samples were taken without immunological tests for the lack of antigens. To solve this problem, the nodules should have been removed by crews of Onchocerciasis Section in other endemic areas outside the pilot area.

In the skin biopsies Holth type corneoscleral punch was used and snips were taken especially from the area of the iliac crest or the scapular region in men. In women, biopsies were taken from both sides of the shoulder region.

The mf of *Onchocerca volvulus* were mostly in the upper torso. Those were found relatively in smaller amount in the head and in the lower areas of the body. It is noteworthy that there are very few mf near the nodules and mf were sometimes found in the urine.

A total of 4,634 samples of 35 core endemic localities of six departments were examined to compare the skin biopsies with the IHA application.

|               | biopsy positive | biopsy negative | sum  |
|---------------|-----------------|-----------------|------|
| IHA positive  | 558             | 346             | 904  |
| IHA negative  | 253             | 3,477           | 3,730|
| Total         | 811             | 3,823           | 4,634|

The conclusion is that the IHA test was a sensitive and reproducible method for the diagnosis of onchocerciasis.

In 1983, regular brigades of Onchocerciasis Section applied the IHA procedure, skin biopsies and palpation. They removed nodules examining 12,272 people in 96 locations, and the results were: skin biopsies of 15,009 revealed 3,809 positive and after the palpation, 1,782 nodules were excised.

A total of 188 patients of onchocerciasis were admitted to the Amatitlán Hospital and were recommended to do a controlled treatment with Diethylcarbamazine, a recognized microfilaricide. 91 of them were extirpated subcutaneous nodules. The biopsies found 180 positives (95.75% of all graduates). After the Diethyl-carbamazine treatment of 180 positives, 102 of them were found negative.

Diethylcarbamazine with the dose of 3 to 10 mg per kilogram of body weight was applied to 252 patients for ten days. Treatment was suspended for seven of them who showed severe side effects. Of those who completed treatment, 139 revealed negative skin biopsies and 103 continued positive, although they showed fewer mf. It was not possible to examine 10 patients.

Registered side reactions in this patient group was as follows:
### OphthalmoLOGY

Ophthalmological and dermatological symptoms found in Guatemalan onchocerciasis in the pilot area and its surroundings, were not as severe as those reported in Africa. The rate of blindness was 0.4% even in the center of high endemicity. Major eye diseases were: changes in the cornea with a high incidence of iritis and typical cases of fibrous exudative iridocyclitis were rare, but the incidence of inactive iritis was markedly high.

There was a significant correlation between the nodes of the head and ocular symptoms, especially in the anterior segment of the eye. Half of the people with 500 mf or more in a single skin snip had mf in the anterior chamber of the eye. In San Vicente Pacaya, 30% were the patients with mf positive, 6% had mf in the anterior chamber of the eyes.

In Palin, adjacent to the Pilot Area, where many of the villagers work in the communal lands of El Chilar and migrate to the coffee areas throughout the county, 64 people with advanced blindness were found. Considering the importance of this disease in this population The National Committee on Blindness has established a rural school for the blind.

The 600 registered patients were surveyed for the diagnosis of the evolution of eye injuries:

| Symptom                              | Number   |
|--------------------------------------|----------|
| Itching                              | 176 (69.84%) |
| Fever                                | 112 (44.4%)  |
| Erythema                             | 41 (16.27%)  |
| Edema                                | 31 (12.3%)  |

### SCIENTIFIC AFFAIRS OF INTEREST

Development of a research project of Onchocerciasis in Guatemala, through the cooperation agreement of Guatemala and Japan, sponsored by Japan International Cooperation Agency (JICA) aroused extraordinary interest in national and international scientific communities.

During the eight years of the project, there were several events and recognition of scientific orders and more national, boundary and international meetings. Among these events we can cite as follows:

* Conference on Robles’s disease in National School of Nursing in Jun. 21, 1976.
* Conference in Medical Sciences USAC. Jul. 2, 1976.
* International Meeting from 17 to 20 Oct. 1977. America, Europe and Africa.
* Border Meeting in Tapachula, Chiapas, Mexico (1978).
* International Symposium “Onchocerciasis in Mexico” held in San Cristobal de las Casas (1978).
* A Conference, from 12 to 16 Jan. 1981.
* A Joint Guatemala – Japan Conference. 62 Guatemalan researchers, 25 researchers from Japan and 31 from various countries and international organizations in the field of onchocerciasis attended.

In September 1983 edition and print of the “Manual of Control of Robles Disease (onchocerciasis) in Guatemala” were completed. It was widely circulated to nationally and internationally in interested entities.

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- “Guide of Guatemalan onchocerciasis” was prepared, printed and distributed.

During this project, 74 researchers from various universities and institutions from Japan came and they published more than 100 scientific papers in various media related and contributed to all over the world.

JICA provided the equipment and materials worth Q. 961, 885.44.

The development of the various activities demanded sociological contributions of various kinds of which there may be mentioned: Meetings with community leaders, authorities, school teachers, etc. in these eight years. There were so many home visits, outreaching, awareness and consultation that were not recorded in the statistics.

The social workers did promoting school and community groups. They tasks to others, advising the staff responsible for censuses.

Efforts to CARE, CARITAS, Catholics Services, etc. for foods which were distributed to families in the pilot area.
Formation of groups of ladies in the villages of Patrocinio and Los Rios in partnership with Home Educators, Digesa and FEDECOGUA. Promotion of patients who were admitted to the Amatitlán Hospital.

From Jul. 1983 monthly meetings were scheduled looking for group integration for epidemiological purposes.

**Note**

* The Government of Guatemala awarded Dr. Isao Tada with the Medal “Dr. Rodolfo Robles” in 1992 which was imposed by the Vice-president, Gustavo Espina Salguero.

The eminent Professor Emeritus of University of Tokyo, Dr. Manabu Sasa said in his speech at the 12th Joint Meeting in January 1981:

“...The cost of insecticide that is currently consumed in the pilot area is so small that reaches monthly five dollar, although the dose is ten times higher than that recommended by the Onchocerciasis Control Program in Africa” ... “in this area under the control, people were usually bitten by numerous vector flies before starting the control operations, but now almost no vector bites throughout the year”

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