

A Comparative Study of Several Diagnostic Measures Applied in Guatemalan Onchocerciasis

ISAO TADA¹⁾, MASATAKA KORENAGA¹⁾, TATSUYUKI MIMORI¹⁾,
MAKOTO SAKAMOTO²⁾, TAKESUMI YOSHIMURA³⁾,
MANUEL M. RECINOS C.⁴⁾, OTTO F. FLORFS⁴⁾,
ARACELY LUJAN T.⁴⁾, J. ONOFRE OCHOA A.⁴⁾,
JULIO C. CASTRO⁴⁾ AND GUILLERMO ZEA F.⁴⁾

(Received for publication; October 30, 1984)

Key words: onchocerciasis, Guatemala, diagnostic measure, comparison, ELISA, IHA

Various diagnostic measures have been developed to follow up the effect of vector control program started in San Vicente Pacaya county in Guatemala since 1975; skin snipping by Tada and Figueroa (1974); Kawabata *et al.* (1980); indirect hemagglutination test by Ikeda *et al.* (1978, 1979); skin test by Hashiguchi *et al.* (1979); Ito *et al.* (1981); double diffusion test by Takaoka *et al.* (1983); enzyme-linked immunosorbent assay by Korenaga *et al.* (1983) and Ito *et al.* (1983). Thus it was needed to compare the nature of individual diagnostic measures ever adopted with each other. In this context, we undertook a multi-disciplinary study in 2 fincas (=plantations) in Suchitepequez Province, Guatemala, with special emphasis on the clarification of relationship among various di-

agnostic measures.

Materials and Methods

During the period between 27 September and 1 October, 1982, a survey was made at Santa Inés and El Regalo, the population of which was 306 and 200, respectively. According to the classification of the Ministry of Public Health, Guatemala, this region belonged to Zone 3 endemic focus (Figueroa, 1974). Santa Inés was located at 900-1,400 m above sea level and El Regalo, 500 m, on the Pacific slope of the Sierra Madre mountains.

Skin snips were taken from 2 body portions of the examinees on; bilateral scapular regions in females and left scapular and iliac regions in males by using Holth type corneal punch (2mm). Because these skin regions were found being provided with higher microfilarial density in this country (Kawabata *et al.*, 1980). Unteased snips were incubated for one hour on a glass slide in saline for enumeration of microfilariae released and later in the well of microtiter plate with saline for further 23-24 hours for counting of all the emerging microfilariae. In order to assess microfilarial density (MFD) quantitatively, snip area was measured using a projection device (Tada *et al.*, 1974). The MFD was expressed in the average number of microfilariae in the above 2 snips per 10 mm².

Skin test (ST) was performed using whole

This investigation received Grant in Aid for Special Research Promotion (No. 57123117) entitled "Fundamental studies on the control of tropical parasitic diseases" and Overseas Scientific Research Program (No. 57041041), the Ministry of Education, Science and Culture, Japan.

¹⁾ Department of Parasitic Diseases, Kumamoto University Medical School, Kumamoto 860, Japan;

²⁾ Department of Parasitology, Institute for Tropical Medicine, Nagasaki University, Nagasaki 852, Japan; ³⁾ Department of Clinical Epidemiology, University of Occupational and Environmental Health Japan, Kitakyushu 807, Japan; ⁴⁾ Departamento de Oncocercosis, Servicio Nacional de Erradicación de la Malaria, Guatemala C. A.

adult *Onchocerca volvulus* antigen extracted by veronal buffered saline (VBS) at the concentration of 20 µg/ml protein at pH 7.8, according to the procedure by Ito *et al.* (1981). It was tentatively considered positive reaction when the wheal size (length plus width in mm) exceeded 21 mm, 15 minutes after injection of 0.02 ml antigen solution.

Using the eluent of dried blood sample taken on filter paper from the ear-lobe of examinees, indirect hemagglutination test (IHA) was performed according to our previously mentioned procedure (Ikeda *et al.*, 1978). As the antigen, for both IHA and ELISA, crude extract of whole adult *O. volvulus* isolated from nodules by using the collagenase technique (Schulz-Key *et al.*, 1977) was used. In order to adjust antigen concentration for the sensitization of erythrocytes, titration was undertaken by using standard positive serum obtained from immunized rabbit. The starting dilution of the blood eluent used was 1 : 30. The blood samples thus examined were considered positive if the reciprocal IHA titer was 60 or more.

In order to measure *O. volvulus*-specific IgG antibody by an enzyme-linked immunosorbent assay (ELISA), the aliquot blood eluent was used. The detailed procedure and

specificity of this method were reported elsewhere (Korenaga *et al.*, 1983). The ELISA value was expressed in the optical density at 500 nm. In the previous study, it was considered as significant increase of specific IgG production, when O. D. was 0.50 or more (Korenaga *et al.*, 1983). This criterion was adopted in this study.

Onchocercal nodules were examined by a systematic palpation of the whole body of the examinee. The nodules thus found were removed surgically by the end of the present survey period.

In order to analyze the relationship among various diagnostic measures, the data from combined populations of 2 fincas, about 375, were processed by computers, VAX 11-720 and Heulett-Packard model 87. The population structure of 375 examinees is that: 41 males and 35 females in ages 0-9; 50 and 50 in ages 10-19; 82 and 75 in ages 20-49; and 29 and 13 in ages 50+, respectively. The age composition of both sexes was considered almost even.

Results

1. Skin snipping

In Santa Inés, 86 (40.8%) out of 211 inhabitants examined were positive for microfilariae.

Table 1 The results of skin snipping in Santa Inés and El Regalo, Suchitepequez, arranged by sex and age

Finca	Age (years)	Male			Female		
		No. examined	No. positive (%)	MFD*	No. examined	No. positive (%)	MFD
Santa Inés	0-9	22	7(31.8)	2.2	9	1(11.1)	0.3
	10-19	33	15(45.5)	15.6	26	2(7.7)	0.4
	20-49	54	27(50.0)	18.5	46	21(45.7)	14.9
	50-	15	9(60.0)	24.8	7	4(57.1)	6.8
	Total	124	58(46.8)	15.6	88	28(31.8)	8.5
El Regalo	0-9	19	2(10.5)	0.2	26	0(0.0)	0.0
	10-19	17	2(11.8)	5.1	24	2(8.3)	4.2
	20-49	28	10(35.7)	15.8	29	7(24.1)	10.4
	50-	14	1(7.1)	3.5	6	2(33.3)	10.0
	Total	78	15(19.2)	7.5	85	11(12.9)	5.4

* Mean number of microfilariae per all the examinees

ariae by the two-clip biopsy method. In El Regalo, 26 (16.0%) were positive out of 163. Microfilarial positives by age and sex in these fincas were shown in Table 1. In Santa Inés, the positive rate for microfilariae increased with age in both sexes. In this finca, it was noted that the infection rate was considerably high even among male children under 10 years of age. On the other hand, in El Regalo, the positive rate did not show consistent increase with age, even the maximum rate was 35.7% at 20-49 year old group, which was almost equal to that (31.8%) of male 0-9 year-old children in the previous finca. The average MFD for microfilarial positives among males in Santa Inés was 33.4 and that of females, 26.7, while those were 38.8 and 41.9, respectively, in El Regalo. Apparently so far as the average MFD in microfilarial positives is concerned, the MFD was rather high in the

latter finca, in spite of its lower microfilarial rate. However, the average MFD per examinee were 15.6 in males and 8.5 in females in Santa Inés. Those were reasonably low, 7.5 and 5.4, respectively, in El Regalo. The MFD per examinee would thus be considered to be an adequate indicator for the endemicity than MFD per positive. The distribution patterns of average MFD by age group in 2 fincas run roughly parallel with those of microfilarial rate in fincas, too.

2. Nodule palpation

Systematic palpation revealed that in Santa Inés, 16 (13.0%) out of 123 males and 2 (2.3%) out of 88 females were with onchocercal nodules, whereas those were 4 (5.2%) out of 77 males and 4 (4.7%) out of 86 females in El Regalo. Among all the 26 nodule carriers, 19 (73.1%) were less than 29 years old. The number of male nodule carriers in the former

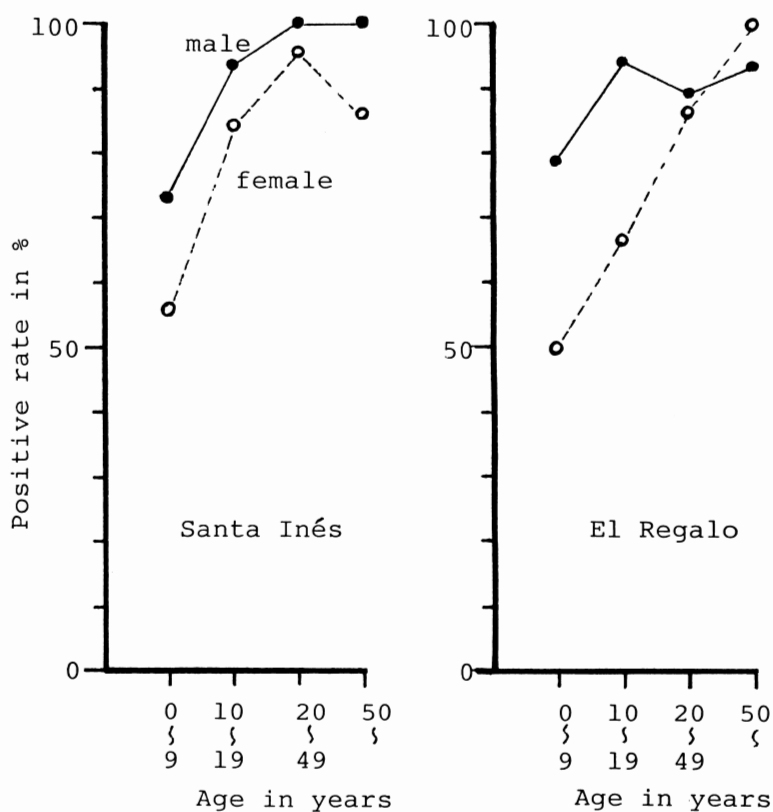


Fig. 1 The result of skin test on the inhabitants from two fincas, Santa Inés and El Regalo arranged by age and sex.

fincas reflected a high prevalence of this disease in this region.

3. Skin test (ST)

The age-prevalence of skin test positives arranged by sex was illustrated in Fig. 1. In Santa Inés, the positive rate increased with age from 72.7% in 0-9 year old group to 100% in 20+ year old group in males. The distribution pattern of positive rate by age in females tended to run parallel with that of males. The patterns of ST positives in both sexes were quite similar between two populations. As was seen in the microfilarial positive rate, positive rate in ST among females was again lower than that of males, particularly in the younger generations.

The correlation between the results of ST and MFD was analyzed on 375 examinees from the above mentioned two fincas and the result was shown in a cross-tabulation (Table 2). Out of 263 examinees free of microfilarodermia, 215 (81.7%) reacted positively in ST, although the rate was rather high among those with microfilarodermia. On the contrary, in 53 examinees whose wheal size were 20 mm or less (negative reaction), 48 (90.6%) were without microfilariae. However, even in those whose wheal were 27 mm or more, 120 out of 192 examinees (62.5%) were apparently without microfilariae. Although the sensitivity of ST is high, the rate of false positive reaction was considered too high from the diagnostic and also from epidemiological view.

4. Indirect hemagglutination test (IHA)

The distribution patterns of positive rate by age and sex in IHA in two fincas were shown in Fig. 2. Positive rate in the IHA increased with age. In Santa Inés, even male youngsters less than 19 years of age showed a high rate, more than 40%, while it attained approximately to 80% in those more than 20 years of age. In both fincas, at any age groups except 50+ year old in El Regalo, the positive rates in males were markedly higher than those of females. This tendency was analogous to the pattern seen in microfilarial rate.

Table 3 shows the relationship between IHA and MFD among 374 examinees. Among 231 IHA negatives, 91% were microfilarial negatives. On the contrary, this rate was reduced to 36.4% (52/143) among the positives in IHA. It is noteworthy that out of 112 microfilarial positives, 91 (81.3%) were positives by IHA, too, whereas out of 262 without microfilariae, only 52 (19.8%) were with positive titers.

Out of 374 examinees, 44 were negatives for both IHA and ST, while 135 were positive for both tests. Thus the coincidence rate was as low as 47.9% (179/374) between these tests. On the other hand, ST positive rate out of 143 IHA positives and 231 negatives was quite similar to each other, 94.4% and 81.0%, respectively. Further, in comparison with the high correlation between microfilarodermia and IHA, ST would be considered as rather unrelevant due to its extremely high sensitivity (Table 2). However, there was a

Table 2 The relationship between the result of skin test and microfilarial density (MFD) of 375 inhabitants from Santa Inés and El Regalo

MFD*	No. of examinee	Wheal size in skin test (mm)				Positive rate† in skin test (%)
		-14	15-20	21-26	27+	
0	263	6	42	95	120	81.7
1-9	44	0	4	10	30	90.9
10-49	44	0	0	15	29	100.0
50-	24	0	1	10	13	95.8
Total	375	6	47	130	192	85.9

* Average number of microfilariae in 10 mm² skin area of 2 snips from scapular and iliac regions.

† Positives; 21 mm or more in wheal size

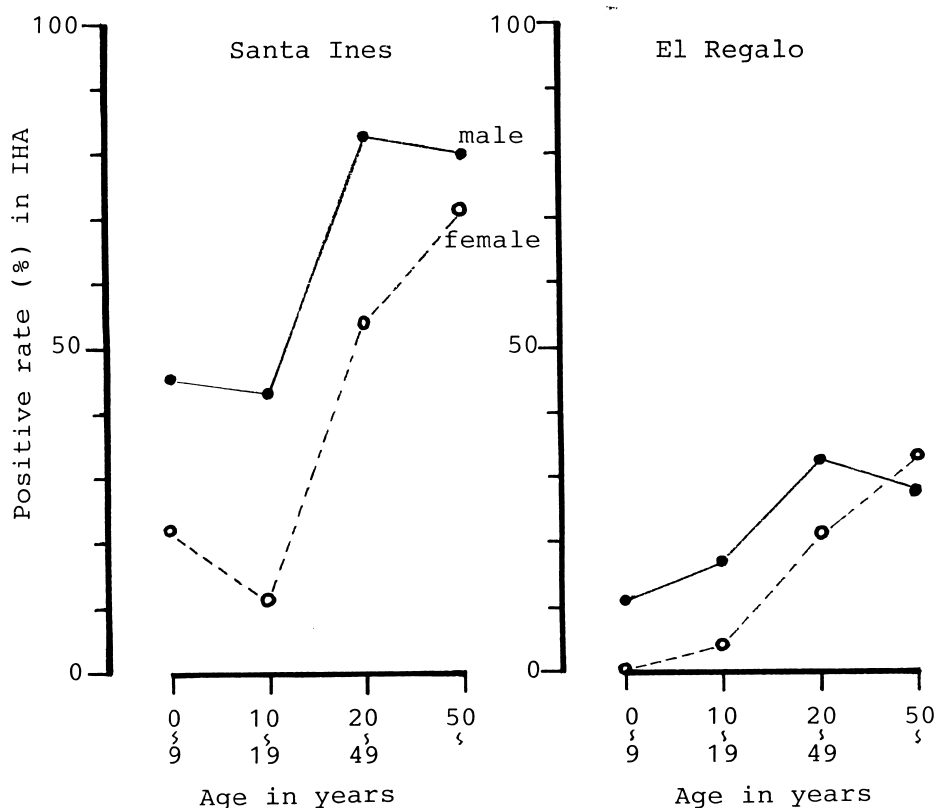


Fig. 2 Age distribution of the positive rate in IHA among the inhabitants from Santa Inés and El Regalo arranged by sex.

Table 3 The relationship between the result of indirect hemagglutination (IHA) and microfilarial density (MFD) of 374 examinees from Santa Inés and El Regalo

MFD*	No. of examinees	Reciprocal IHA titer				Positive rate† in IHA (%)
		≤30X	60X	120-240X	480X ≤	
0	262	210	9	24	19	19.8
1-9	44	9	10	17	8	79.5
10-49	44	7	7	16	14	84.1
50-	24	5	1	12	6	79.2
Total	374	231	27	69	47	38.2

* MFD; definition shown in Table 2

† 60 X or more in reciprocal IHA titer

‡ difference is statistically significant ($p < 0.005$)

rough correlation between the positivity in IHA and wheal size by ST: Positive rate in IHA was 0.0% (0/6) in those with wheal size less than 14 mm, 17.4% (8/46) in those with

15-20 mm; 32.3% (42/130) in those with 21-26 mm; and 48.4% in those more than 27 mm. In other words, the positive rate in IHA tended to increase in proportion to wheal size

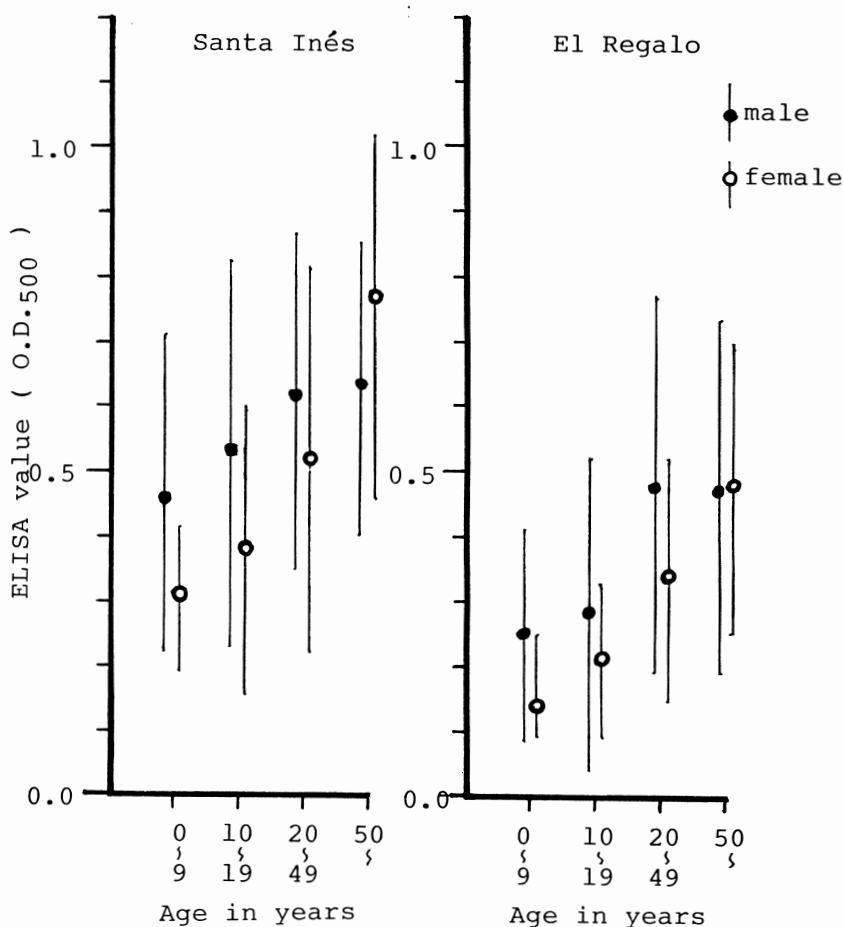


Fig. 3 Age-sex distribution of average ELISA values among the inhabitants from Santa Inés and El Regalo (average O.D.±S.D.)

in ST.

5. Enzyme-linked immunosorbent assay (ELISA)

The eluent of dried blood samples from the inhabitants of 2 fincas was assayed by micro ELISA technique. The results, expressed as the O. D. value, were shown in Fig. 3 by sex and age in each finca. It was noted that the average O. D. value was considerably high in Santa Inés even in 0-9 year old males. Generally, it is likely that ELISA value was proportional to the intensity of onchocercal infection in the age groups. The distribution curves of ELISA value were similar to those of microfilarial rate.

A clear relationship was found between

MFD, an index of the intensity of infection, and the amount of *O. volvulus*-specific IgG antibodies detected by ELISA (Table 4). In 263 cases without microfilarodermia living in endemic area, 215 (81.8%) revealed 0.49 or less in ELISA value. The number of those with higher ELISA value increased with MFD. Among those whose MFD were 50 or more, only 20.9% showed less than 0.49 in ELISA value, the upper limit in normal control (Korenaga *et al.*, 1983). In other words, 79.1% of those with higher MFD had higher value, too, much more enhanced than those from lightly infected areas.

As seen in Fig. 4, there was a clear relationship between ELISA value and positive

Table 4 The relationship between microfilarial density (MFD) and ELISA value among 375 examinees from Santa Inés and El Regalo

MFD*	No. of examinee	ELISA value				Positive rate† in ELISA
		≤0.24	0.25-0.49	0.50-0.99	1.00≤	
0	263	115	100	40	8	18.2
1-9	44	3	13	26	2	63.6
10-49	44	1	7	29	7	81.8
50-	24	1	4	17	2	79.1
Total	375	120	124	112	19	—

* MFD; definition shown in Table 2

† Positives; 0.50 or more in ELISA value

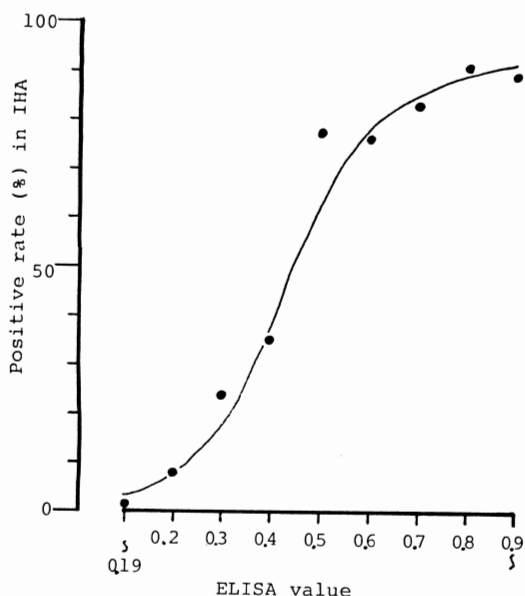


Fig. 4 Relationship between ELISA value and the positive rate in IHA tested in 374 examinees of two fincas.

rate in IHA in 374 examinees. The positive rate in IHA, 4.7% in those with less than 0.29 in ELISA value, increased in a sigmoid curve with the latter value and attained to 90.9% in those with 0.80-0.89. The correlation coefficient between classified IHA titers and ELISA values was $r=0.741$ (plotted values, 373). Fig. 5 shows the sexual difference in the composition of examinees of two fincas by categorized IHA titer and ELISA value. These 2 immunodiagnostic methods revealed essentially the same tendency in the

reactions: Males were composed of individuals with higher IHA titer or ELISA value than females. This finding suggests that males were more strongly infected than females. As the negative criterion for ELISA lies at 0.49 or less, the negative rate in males is 55.5% and that in females is 76.3%, respectively. These figures are almost equal to the negative rates in IHA, 51.0% in males and 74.4% in females, respectively, indicating a high correlation of results between IHA and ELISA.

A possible relationship was assessed between ELISA value and positive rate in ST in this population. The positive rate in skin test was 75.8% in those whose ELISA values were 0.24 or less and it increased slightly with the rise in ELISA value up to 95.5% of those with 0.50-0.99. A blunt correlation may also be noted between skin reactivity and specific IgG antibody by ELISA, although the antibody classes concerned in each measure are different.

Discussion

As was mentioned by Wada (1982), the intensity of infection with onchocerciasis was closely associated with the distribution and abundance of vector blackfly, *Simulium ochraceum*. Thus the location of residential area is primarily important from the viewpoint of disease transmission. In El Regalo, the microfilarial positives were quite few among those below 19 years of age, particularly in females. This fact indicates

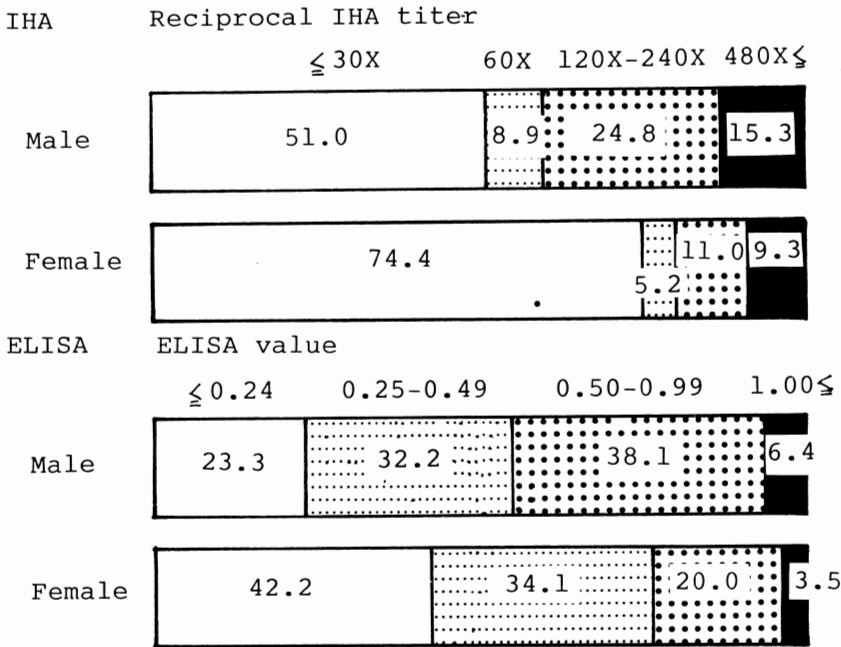


Fig. 5 Composition of categorized IHA titer and ELISA value in 375 examinees from two fincas. Each figure in the box shows the frequency percent in the total.

that adult people are mainly exposed to vector-biting while visiting and working in mountainous focus of transmission which is generally corresponding to coffee fields. Wada (1982) explained sexual difference in microfilarial rates based on the difference of "r", the force of infection in Muench's simple catalytic model. Yoshimura *et al.* (1982) calculated annual incidence rates of males and females to be 0.080 and 0.038, respectively, in San Vicente Pacaya, Guatemala, based on the results of skin snipping. In the present study, not only the microfilarial and nodule rates, but also MFD, IHA titer and ELISA value revealed the differences in the infection intensity between two sexes and among age-groups.

As for onchocercal nodules, we stated that in early stage of infection, especially in low endemic area, the palpation would be a sensitive diagnostic measure (Tada *et al.*, 1979). Further, Aoki *et al.* (1983) stressed that appearance of onchocercal nodule after nodulectomy in a population concerned would be useful in assessing the incidence of the disease. In spite of periodic denodulization

campaign, many newly developed nodules were found especially in males in Santa Inés in this study. Thus it may be considered that nodule detection would reveal the intensity of onchocercal transmission.

A high correlation was shown in a previous paper between IHA and MFD in a population, in which the increase of MFD accompanied with the increase of the people with high IHA titers (Ikeda *et al.*, 1979). An identical close relationship seen in the present study representing marked difference ($p < 0.005$) in the result of IHA between microfilarial positives and negatives (Table 3) indicates the favorable feature of IHA as an immunological index. Because the IHA revealed immune response corresponding to the microfilarodermia.

In the present study, a high correlation was seen between categorized MFD and ELISA values (Table 4), suggesting the presence of a close relationship between the intensity of infection and antibody (IgG) production. In a previous paper, we showed graphically the relations among various populations with different microfilarial rates and the correspond-

ing ELISA value (Korenaga *et al.*, 1983). Ito *et al.* (1983) also observed a high correlation between ELISA-positive rate and/or ELISA value and the mode of infection intensities (microfilarodermia and onchocercal nodule). Recently, Campbell *et al.* (1983) reported that ELISA rather correlated with the Mazzotti reaction, even when the microfilarial density was extremely low. They concluded that ELISA was available in the diagnosis of early stage of infection among children in endemic areas. These reports suggest that the detection of specific IgG with ELISA is a sensitive measure for immunodiagnosis of onchocerciasis.

Our results on the positive relationship between the positive rate in IHA and categorized ELISA values (Fig. 4) support similar ones shown by us (Korenaga *et al.*, 1983) and Ito *et al.* (1983). Thus it may be considered that these two immunodiagnostic measures reveal quite similar results, as both of them rest on the closely relating immunological basis. In this context, it will be worth-while to introduce ELISA technique in lieu of IHA, because (1) this method is of space and time saving, (2) it does not require erythrocytes and complicated sensitization, and (3) it does not need standardization of antigen concentration, when once determined.

The relationship between the results of ST and ELISA was rather blunt, because the sensitivity of the former test was too high at any age-groups even in children. It is quite evident, when we compared the results of ST between 2 fincas (Fig. 1), the distribution curves of ST positivity in 2 populations were quite similar to each other, in contrast to those of IHA (Fig. 2). ST revealed similar age-prevalence of positivity regardless to the microfilarial rate by age. Ito *et al.* (1981) stated that ST revealed positive reaction in 98.8% of microfilarial carriers, while it revealed false positive reactions in 40% of the inhabitants from non-endemic areas. The lack of specificity in ST strongly suggest the presence of cross reactions with other helminths. Mueller *et al.* (1973) reported the result of ST using *O. gutturosa*, which re-

vealed no difference in the positive rates among those with nodules, those with positive skin biopsies but no nodules, those with negative biopsies and negative histories of nodulectomy. This antigen caused positive reaction in 60% of 48 bancroftian filariasis. In the present study, as seen in Table 2, the positive rate in ST was between 91 and 100% among microfilarial carriers, whereas the rate was as high as 82% even among microfilarial negatives. Using microfilarial antigen of *O. volvulus*, Hashiguchi *et al.* (1979) compared the positivity rates between microfilarial positives and negatives. In the former group, the rate was 89.1%, while in the latter, 50.0%. Apparently ST could not distinguish microfilarial positives and negatives in endemic areas. Therefore, ST would be adopted only under some limited conditions, such as in the diagnosis for those who stayed temporarily in the endemic area or for an epidemiological study to assess background infection.

Summary

In 375 inhabitants of two fincas (=plantations) located at high- and low-lands in Guatemalan endemic focus of onchocerciasis, we compared the diagnostic natures among several diagnostic measures ever developed in Guatemala. In the skin snippings, the microfilarial rate of male was higher than that of female at any age-groups. The microfilarial density per examinee was considered to be more reliable index than MFD per microfilarial positive from epidemiological aspect. Skin test (ST) using *O. volvulus* antigen revealed higher false positive reactions in comparison with other tests. The positive rate in ST was 82% in microfilarial negatives. Thus the result of ST was considered as rather equivocal. Both the indirect hemagglutination test (IHA) and enzyme-linked immunosorbent assay (ELISA) using *O. volvulus* antigen and eluent of blood taken on filter paper were considered sensitive measures of immunodiagnosis which were adequately reflecting the intensity of infection (microfilarodermia). The result of IHA and ELISA showed high cor-

relation to each other. In contrast to the qualitative indices such as prevalence and incidence, the microfilarial density, IHA and ELISA were considered to be quantitative ones which reflected the intensity of infection in individuals. The merit of ELISA was stressed briefly.

Acknowledgements

During this study, we owed Dr. Hector A. Godoy B., the director of SNEM, and Dr. Takeshi Suzuki, Chief of Japanese Mission, JICA, for innumerable advices and encouragements. We are highly grateful to Miss Dora Mendez, Mrs. Mirian Cardona and all the members of honorable brigadas belonging to Departamento de Enfermedad de Robles (Onchocercosis), SNEM, for their sincere collaboration with our team. In the data processing, we are favored to have great assistance of Misses Hisako Tanaka and Yoko Nakajima of the University of Occupational and Environmental Health Japan.

References

- 1) Aoki, Y., Sakamoto, M., Yoshimura, T., Tada, I., Recinos, M. M. and Figueroa M., H. (1983): Onchocerciasis in Guatemala, with special reference to appearance of new nodules and parasite content. *Am. J. Trop. Med. Hyg.*, 32, 741-746.
- 2) Campbell, C. C., Figueroa M., H., Collins, R. C., Lujan L., R. and Collins, W. E. (1983): Diagnosis of *Onchocerca volvulus* infection in Guatemalan children. *Am. J. Trop. Med. Hyg.*, 32, 760-763.
- 3) Figueroa M., H. (1974): Robles' disease (American onchocerciasis) in Guatemala. *Onchocerciasis. Sci. Publ. No. 298, PAHO.*
- 4) Hashiguchi, Y., Kawabata, M., Zea F., G., Recinos C., M. M. and Flores C., O. (1979): The use of an *Onchocerca volvulus* microfilarial antigen skin test in an epidemiological survey of onchocerciasis in Guatemala. *Tr. Roy. Soc. Trop. Med. Hyg.*, 73, 543-548.
- 5) Ikeda, T., Tada, I. and Aoki, Y. (1978): The indirect hemagglutination test for onchocerciasis performed with blood collected on filter paper. *J. Parasitol.*, 64, 786-789.
- 6) Ikeda, T., Aoki, Y., Tada, I., Recinos, M. M., Ochoa A., J. O. and Molina, P. A. (1979): A sero-epidemiological study of onchocerciasis with the indirect hemagglutination test. *J. Parasitol.*, 65, 855-861.
- 7) Ito, M., Lujan T., A., Fukumoto, S. and Kamiya, M. (1983): Enzyme-linked immunosorbent assay (ELISA) as a diagnostic tool for Guatemalan onchocerciasis using a bovine filaria (*Onchocerca gutturosa*) antigen and blood samples collected filter paper. *Jpn. J. Vet. Res.*, 31, 141-150.
- 8) Ito, Y., Sakamoto, M. and Yoshimura, T. (1981): The skin test using *Onchocerca volvulus* adult antigen in the diagnosis of onchocerciasis in Guatemala. *Jpn. J. Parasitol.*, 30(1, Suppl.), 14.
- 9) Kawabata, M., Hashiguchi, Y., Zea F., G., Yamada, H., Aoki, Y., Tada, I., Recinos, C., M. M. and Flores, C. O. (1980): The distribution of microfilariae in the skin of Guatemalan onchocerciasis patients: an evaluation of diagnostic potentials. *J. Helminthol.*, 54, 183-190.
- 10) Korenaga, M., Tada, I., Mimori, T., Sakamoto, M., Lujan T., A., Zea F., G., Castro, J. C. and Yarzabal, L. (1983): Enzyme-linked immunosorbent assay (ELISA) in the detection of IgG antibodies in onchocerciasis using blood collected on filter paper. *Jpn. J. Parasitol.*, 32, 347-355.
- 11) Mueller, J. C., Mitchell, D. W., Garcia-Manzo, G. A., Aguilar, F. J. and Scholtens, R. G. (1973): Evaluation of a skin test for onchocerciasis in Guatemala. *Am. J. Trop. Med. Hyg.*, 22, 337-342.
- 12) Schulz-Key, H., Albiez, E. J. and Buettner, D. W. (1977): Isolation of living adult *Onchocerca volvulus* from nodules. *Z. Tropenmed. Parasitol.*, 28, 428-430.
- 13) Tada, I. and Figueroa, M., H. (1974): The density of *Onchocerca volvulus* microfilariae in the skin at the different time of day in Guatemala. *Jpn. J. Parasitol.*, 23, 220-225.
- 14) Tada, I., Aoki, Y., Rimola, C. E., Ikeda, T., Matsuo, K., Ochoa A., J. O., Recinos C., M., Sato, S., Godoy B., H. A., Castillo O., J. J. and Takahashi, H. (1979): Onchocerciasis in San Vicente Pacaya, Guatemala. *Am. J. Trop. Med. Hyg.*, 28, 67-71.
- 15) Takaoka, M., Lujan T., A., Hashiguchi, Y., Kawabata, M., Ito, Y. and Hayashi, S. (1983): Evaluation of the double diffusion test for the serodiagnosis of onchocerciasis in Guatemala. *Jpn. J. Parasitol.*, 32, 451-457.
- 16) Wada, Y. (1982): Theoretical approach to the epidemiology of onchocerciasis in Guatemala. *Jpn. J. Med. Sci. Biol.*, 35, 183-196.
- 17) Yoshimura, T., Hashiguchi, Y., Kawabata,

M., Flores C., O. F., Guidel, O. O. and Mazariegos L., E. C. (1982): Prevalence and incidence of onchocerciasis as baseline data

for evaluation of vector control in San Vicente Pacaya, Guatemala. Tr. Roy. Soc. Trop. Med. Hyg., 76, 48-53.

グアテマラのオンコセルカ症における各種診断法の比較

多田 功¹⁾ 是永正敬¹⁾ 三森龍之¹⁾ 坂本 信²⁾ 吉村健清³⁾
 M.M. RECINOS C.⁴⁾ O.F. FLORES⁴⁾ A. LUJAN T⁴⁾
 J.O. OCHOA A.⁴⁾ J.C. CASTRO⁴⁾ G. ZEA F⁴⁾

¹⁾ 熊本大学医学部寄生虫病学教室 ²⁾ 長崎大学熱帯医学研究所寄生虫部

³⁾ 産業医科大学臨床疫学教室 ⁴⁾ グアテマラ国厚生省マラリア
 防圧局オンコセルカ部)

グアテマラ国スチテペケス県（オンコセルカ症第3流行地区）に属する2農園，サンタ・イネス（海拔高度900~1,400 m）とエル・レガロ（同500 m）の住民，合計375名について各種のオンコセルカ診断法を実施し，各法による成績相互の比較を行なった。得られた結果は次のとおりである。

1) ホルス型パンチによる2箇所検皮法でサンタ・イネスの住民の40.8%に，エル・レガロでは16.0%に仔虫陽性者を検出した。仔虫率は殆ど，どの年齢でも，男性に高い値が認められた。被検住民全員に対する仔虫密度が浸淫を反映すると考えられた。

2) *Onchocerca volvulus* 粗抗原による皮内反応は他の免疫診断法に比べ偽陽性反応率が高く，仔虫0の対

象において82%が陽性で，診断的実用性が低いと考えられた。

3) 濾紙に採った血液を被検試料として用いる間接赤血球凝集反応（IHA）と酵素抗体法（ELISA）は感染強度をよく反映していた。特に仔虫0の群に対する陽性反応率が低い点で共通している。同一集団におけるIHA 価と ELISA 値との間には強い相関が認められた（ $r=0.741$ ）。

4) 検皮法にもとづく incidence および prevalence のような定性的な疫学指標に対し，仔虫密度，IHA，EILSA は定量的な感染指標として重要と考えられた。技術的には ELISA が使用しやすいと考えられる。