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GUATEMALAN ONCHOCERCIASIS: SKIN SNIPPING METHODS AND MICROFILARIAL DENSITIES IN A GIVEN MINUTE AREA OF THE SKIN[1]

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Abstract: To establish a convenient and reliable method for diagnosis and epidemiological surveys on Guatemalan onchocerciasis, three skin biopsy instruments were evaluated in 108 volunteers. The German-made Holth type, the Japan-made Walser type corneoscleral punches and a lancet and disposable scalpels were used for the purposes of taking skin snips. Better results were obtained with the lancet and scalpel, indicating a higher detection rate or a lower false negative rate for the patients. This method, however, showed some disadvantages in the present study. The volunteers had various complaints and/or rejected our examinations because of the painfulness of the scalpel procedure. On the other hand, the Holth punch was better than the Walser punch which indicated a relatively high false negative case and was rather troublesome in handling. From the results obtained, we recommend the Holth punch as the skin snip apparatus in Guatemala. To examine the microfilarial densities in a given minute area of the skin, six skin snips each (12 snips in total), 0.5 cm apart, were taken by the Holth punch from 33 subjects. In this study, there were considerable numbers of negative skin snips, especially in those patients who had a relatively low density of microfilariae. This would be of importance for diagnosis and epidemiological surveys for Guatemalan onchocerciasis. The results seem to indicate that if one skin snip was taken from the patients of a low endemic area in Guatemala, the prevalence of onchocerciasis in the area might be underestimated with false negative skin snips. Moreover, there was a great variation in the microfilarial densities in the 12 skin snips from each subject. The maximum to minimum rate of microfilarial densities in average was 1: 9.7 in the left scapula and 1: 7.1 in the left iliac crest of the 33 subjects. These facts offer some useful suggestions for us, when we examine the intensity of infection in an endemic area or investigate a diurnal or seasonal variation of microfilarial densities of *Onchocerca volvulus* in Guatemala.

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Introduction

In the diagnosis of onchocerciasis, the use of skin snips is the most convenient and reliable method to detect patients with the disease. For this purpose, hitherto, biopsy instruments, such as various types of corneoscleral punches, sharp scissors and blades have been investigated and used for taking skin snips. According to a recent study, the Holth type punch is the best one in epidemiological surveys of African onchocerciasis (Rougemont *et al.*, 1975). This kind of information on Latin American onchocerciasis has not been generally available up to the present time. We, therefore, investigated the usefulness of several biopsy instruments, with regard to their handling in surveys and detecting rates of onchocerciasis in Guatemalan patients. On the other hand, microfilarial densities derived from skin biopsies have been recognized as an important indicator of the intensity of infection in endemic areas of the disease. Microfilarial densities, however, differ in various areas of the skin of infected persons (DeLeon and Duke, 1966) and also varies with geographical differences of the disease as demonstrated by the World Health Organization Expert Committee (WHO, 1976). A better understanding of variation in microfilarial densities of *O. volvulus* in the skin would contribute to our epidemiological knowledge including diurnal or seasonal variations in densities. From these view points, we also examined microfilarial densities in a given minute area of the skin of patients in an onchocerciasis zone in Guatemala.

Material and Methods

In the comparison of skin snip methods for Guatemalan onchocerciasis patients, the following three apparatuses, *viz.*, the German-made Holth type and Japan-made Walser type corneoscleral punches and the lancet and disposable scalpel, were employed. Three skin snips each, 1 cm apart from each other, were taken from the left scapular region of each subject by the three instruments. Skin snips taken were put onto a microscopical slide glass and incubated at room temperature in 0.9 per cent physiological saline for one hour. Unstained microfilariae emerging from skin snips were counted immediately at $\times 40$ magnification under a compound microscope. The number of microfilariae obtained per 1 mm^2 was used as the expression of microfilarial density in the present study. Examinations were carried out in August, 1977 at Finca Valle de Oro, Municipio de Chicacao, Departamento de Suchitepequez, Guatemala. One hundred and eight male and female subjects, aged from 15 to 45, were examined by each instrument.

In the examinations for microfilarial densities obtained from various skin snips taken from each subject, only the Holth type corneoscleral punch was used. The incubation of the skin snips and counting of microfilariae were conducted as mentioned above, unless otherwise noted. Six skin snips each were taken from the left scapula and the left iliac crest of the subject respectively. The distance between each snip was 0.5 cm as shown in Figure 1. Only male volunteers, aged 15 or over, were examined in this study. The examination was done in April 1978 and in November

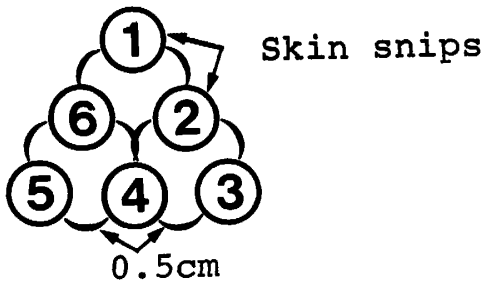

Figure 1 Schema for six skin snips, 0.5 cm apart, taken from the left scapula and the left iliac crest of 33 subjects.

1978 at Finca San Rafael Sumatan, Municipio de Yepocapa, Departamento de Chimaltenango, Guatemala.

Results

Comparison of the skin snipping methods

Thickness and areas of the skin snips taken by each instrument are shown in Table 1. The thickest skin snip was obtained by scalpel, while the thickness was nearly equal in skin snips taken by the other instruments. In the examinees, the positive rate for microfilariae by the scalpel (61.1%) was higher than the rates by the

Table 1 Thickness and area of the skin snips taken by each instrument

Instrument	Thickness in mm*	Area in mm^2
Scalpel	1.01±0.21**(n=31)	5.46±2.99 (n=108)
Holth punch	0.63±0.27 (n=31)	5.54±2.53 (n=108)
Walser punch	0.60±0.21 (n=33)	4.75±1.95 (n=108)

* Fixed specimens with 10% formalin.
** Standard deviation.

Table 2 Results of skin snips in the residents of Finca Valle de Oro by three different instruments

Instrument	Microfilarial density* in positives		Positive rate for MF (%)	False negative rate (%)
	Arithmetic mean	Geometric mean		
Scalpel	5.79±12.67**	2.37	66/108 (61.1)	8/76 (10.5)
Holth punch	2.86± 4.60	1.55	60/108 (55.6)	11/76 (14.5)
Walser punch	2.51± 3.94	1.39	56/108 (51.9)	18/76 (23.7)

* Microfilarial density per 1 mm^2 skin snip.
** Standard deviation.

Holth punch (55.6%) and Walser punch (51.9%) as shown in Table 2. The false negative rate in all of the positives for microfilariae was 10.5 per cent by scalpel and 14.5 per cent by Holth punch but it was considerably higher with the Walser punch (23.7). The rate of agreement of the results obtained by each instrument was examined among the combinations of the two methods, respectively. When we form a hypothesis in which the positives or the negatives may be similarly detected by two of these three instruments, the rate of agreement was 83.3 per cent between scalpel and Holth punch ($36+54/108\times100$) and also between Holth punch and Walser punch ($41+49/108\times100$). It was 84.3 per cent between the scalpel and Walser punch ($53+38/108\times100$). The detection rate for microfilariae from the positive subjects by each instrument was 85.5 per cent by Holth punch, 76.3 per cent by Walser punch and 89.5 per cent by scalpel. In both the arithmetic and geometric means, the microfilarial density by scalpel was significantly higher ($p<0.001$) than that by the two punches. Frequency distributions of microfilarial densities in the skin snips taken are shown in Figure 2. Comparing the densities obtained by the three instruments, they demonstrated nearly an equal tendency in the density per 1 mm^2 skin snip.

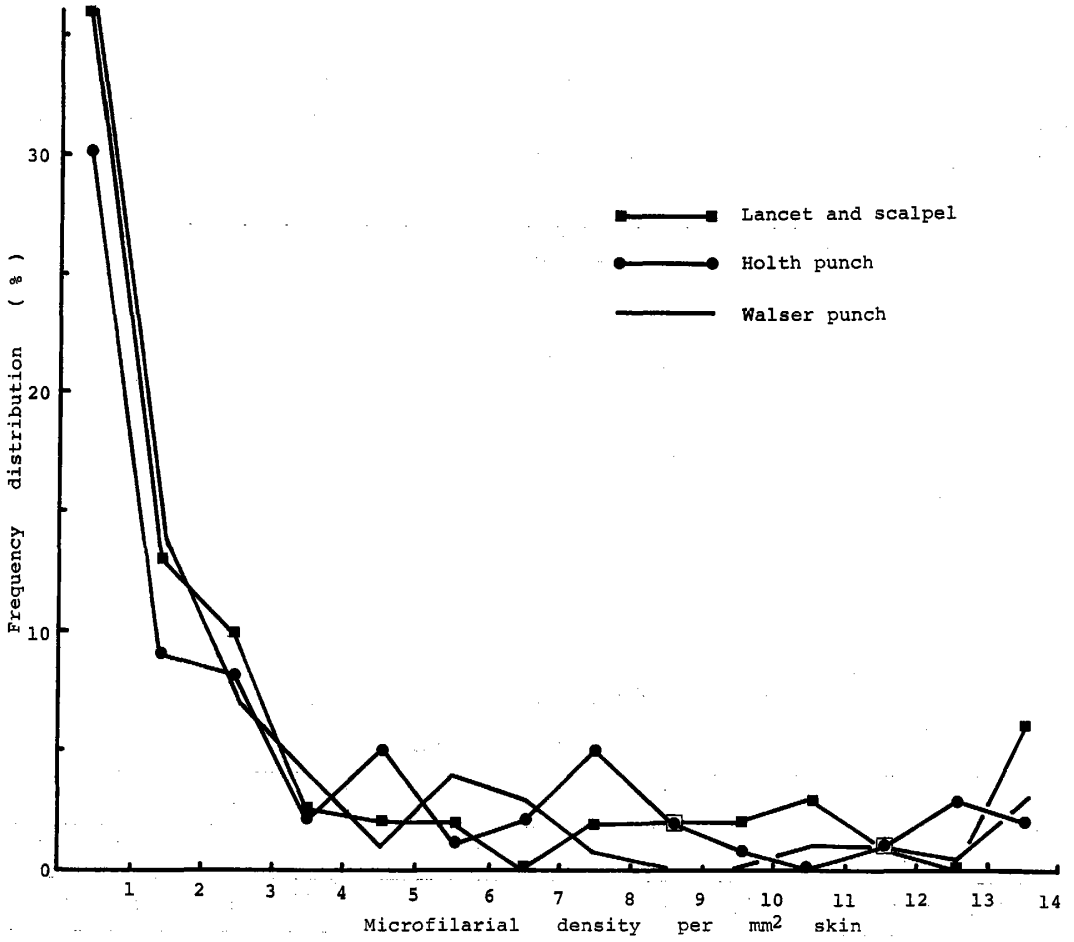

Figure 2 Frequency distributions of microfilarial densities per 1 mm^2 skin snip taken by three different biopsy instruments.

Microfilarial densities in given minute areas of the patient's skin

The results from 33 volunteers are shown in Table 3. In this examination,

Table 3 Summary of the microfilarial densities in six skin snips each, 0.5 cm apart from each other, taken from both the left scapula and left iliac crest of male patients

Patient No.	Age	Left scapula			Left iliac crest		
		Mean* density	S. D.**	Maximum/ minimum	Mean density	S. D.	Maximum/ minimum
1	35	16.0	10.4	7.8	4.9	2.4	6.4
2	29	19.5	18.0	9.6	25.2	20.9	6.3
3	60	10.4	6.5	5.9	28.7	18.9	9.6
4	78	3.9	2.6	5.7	9.6	9.2	11.4
5	47	2.4	2.4	9.6	10.3	8.0	7.5
6	21	1.0	2.4	—	9.7	5.3	4.8
7	21	19.3	13.4	9.7	18.8	15.8	11.0
8	50	37.9	19.9	7.6	33.9	18.3	4.9
9	55	11.3	6.7	8.7	33.2	23.9	5.5
10	72	9.8	5.5	5.3	42.1	14.2	2.8
11	58	6.7	11.0	94.3	12.7	10.3	7.8
12	59	4.2	5.0	19.9	17.7	13.7	8.4
13	59	0.3	0.2	1.7	25.4	25.3	8.9
14	33	9.5	10.9	25.2	65.4	18.5	2.4
15	37	0.9	1.3	4.1	50.7	34.7	8.5
16	45	7.2	6.7	17.7	1.3	1.4	11.7
17	33	0.3	0.4	1.7	14.5	6.2	4.7
18	41	1.6	1.0	3.0	0.03	0.08	—
19	38	4.5	3.6	11.1	13.2	5.7	4.6
20	46	0	—	—	0.1	0.2	—
21	49	7.5	3.6	3.1	4.6	6.3	21.5
22	52	0.6	0.4	5.0	9.3	5.4	4.1
23	62	0.3	0.4	9.0	0.7	0.7	10.0
24	19	6.0	4.8	2.0	1.2	0.3	2.5
25	58	11.0	2.9	2.0	26.2	7.9	2.3
26	54	9.8	4.2	3.0	10.1	6.1	7.0
27	43	5.8	2.4	3.4	0.2	0.2	5.0
28	26	6.5	5.2	5.5	10.0	4.7	3.6
29	27	2.7	1.5	4.7	0.9	0.9	11.0
30	23	25.2	8.1	2.2	10.7	8.6	6.8
31	51	10.5	2.9	2.1	0.4	0.8	19.0
32	37	5.5	2.9	5.3	18.4	12.2	5.4
33	32	3.9	2.6	5.0	1.0	0.5	3.2

* Mean microfilarial density per 1 mm^3 skin snip.

** Standard deviation.

Table 4 Microfilarial densities in fifteen cases of the 33 subjects with one or more negative skin snips; 12 snips were taken from the left scapula and the left iliac crest (density per 1 mm^2 skin snip)

Patient No.	Left scapula						Left iliac crest					
	1	2	3	4	5	6	1	2	3	4	5	6
5	2.1	0.7	6.7	0	1.7	3.1	3.8	5.2	3.2	24.1	13.3	11.9
6	0	0	0	5.8	0	0	6.6	3.8	8.0	18.1	7.8	13.8
11	0	4.5	28.3	0.3	0	6.8	28.1	22.4	11.7	3.6	6.7	3.7
13	0.5	0.4	0.3	0	0.5	0	7.7	63.4	50.6	10.5	7.2	11.8
14	7.0	1.1	3.6	0	17.5	27.7	67.0	65.5	77.8	38.1	52.9	90.9
15	3.3	0	0.8	0	0	1.3	89.2	73.3	26.4	22.3	10.5	82.6
16	1.0	17.7	3.6	1.5	12.5	6.7	0.3	0	0	2.4	3.5	1.3
17	0	0	0	1.0	0.6	0	12.8	20.4	4.3	19.8	18.2	11.2
18	2.2	2.2	0.9	1.4	2.7	0	0.2	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0.6	0
22	0.9	0	0.5	0.2	0.7	1.0	11.4	9.4	4.6	5.9	18.9	5.4
23	0	0.9	0	0.1	0.6	0.3	0.7	0.5	0.2	0.2	2.0	0.4
27	9.5	2.8	5.0	5.5	7.7	4.3	0.2	0	0.1	0.3	0.5	0.3
29	0.9	1.2	2.2	4.2	4.0	3.8	0.3	1.0	1.4	0	0.2	2.2
31	14.3	11.3	9.1	6.9	13.1	8.3	0	0	0	0.1	1.9	0.1

Table 5 Microfilarial densities in six snips, 0.5 cm apart, taken from the left scapula or the left iliac crest of each male subject (snip incubation at 32 C)

Site of snipping	Patient No.	Age	Snip No.						Mean* density	S. D.**	Max./ min.
			1	2	3	4	5	6			
Left scapula	1	68	0	0.2	0	0	0	0	0.03	0.1	—
	2	17	7.4	2.3	6.8	12.4	3.9	14.2	7.8	4.7	6.2
	3	48	1.8	1.9	1.8	3.0	1.5	0.7	1.8	0.7	4.3
	4	36	7.6	8.6	2.2	10.8	21.8	21.3	12.1	7.9	9.9
	5	38	0	0	0	0	0	0	—	—	—
	6	42	0	0	0	0	0	0	—	—	—
	7	55	0	0	0	0	0	0	—	—	—
Left iliac crest	8	63	0.5	1.5	1.3	0.1	2.1	4.7	1.7	1.6	47.0
	9	17	0	0.3	0	0.2	1.2	0.1	0.3	0.5	4.0
	10	60	0	0	0	0	0.1	0.2	0.1	0.1	2.0
	11	46	10.1	10.0	3.3	22.7	22.4	22.6	15.2	8.5	6.8
	12	38	12.2	8.3	7.3	7.4	18.9	5.8	10.0	4.9	3.3
	13	35	33.5	30.6	10.6	15.2	23.5	14.9	21.4	9.3	3.2
	14	23	0	0	0	0	0	0	—	—	—
	15	78	0	0	0	0	0	0	—	—	—

* Mean microfilarial density per 1 mm^2 skin snip.

** Standard deviation.

negative skin snips taken from the onchocerciasis patients were found in 10 (30.3%) out of 33 cases on the left scapula and in 5 (15.2%) of the total cases on the left iliac crest (Table 4). Thus, a greater number of the negative skin snips were recognized in the cases taken from the left scapula as compared with those from the left iliac crest. When six skin snips each, 12 snips in total, were taken from each subject, patient No. 20 showed the most noticeable result; he revealed only one positive skin snip. Microfilarial densities showed great differences especially in the left scapular skin snips. Maximum to minimum microfilarial densities in Table 3 shows the grades of variations of the density in each patient.

To examine the influence of the incubation temperature on microfilarial emergence from skin snips, 15 subjects aged 15 or over were selected as examinees (Table 5). No previous examinations for microfilariae or nodules were done in these cases. Six skin snips were taken from the left scapula or the left iliac crest of the subjects. The skin snips were incubated for two hours at 32 C. Five (33.3%) of the 15 subjects were negative with six skin snips. Moreover, three of the positives showed two or more negative snips; only one of the six skin snips was positive for the microfilariae in patient No. 1. In patient No. 8 the greatest ratio of maximum to minimum microfilarial density was recognized. As mentioned above, the microfilarial densities at a constant temperature also had considerable variation as well as those with incubation at room temperature.

Discussion

Of the three instruments used in the present study, the lancet and scalpel method was the best, giving a high detection rate or low false negative rate for onchocerciasis patients. On the other hand, the Holth type corneoscleral punch showed a 55.6 per cent detection rate and a false negative rate of 14.5 per cent. No significant difference was recognized statistically between both rates of the scalpel and the Holth punch but when the Walser punch was used, the comparative rates were 51.9 per cent and 23.7 per cent for the onchocerciasis patients. Between the rates of the Walser punch and the scalpel, there is a significant difference ($p<0.001$ by student's "t" test). From these results, it is suggested that skin snips with the lancet and scalpel method would be better to detect the onchocerciasis patients in Guatemala. However, when we used this method in an epidemiological survey for Guatemalan onchocerciasis, the residents in the endemic area had various complaints and/or rejected our routine examinations because of the painfulness. Based on this disadvantage, it would be better to employ the Holth type corneoscleral punch in an epidemiological survey of onchocerciasis as recommended by several investigators.

With the corneoscleral punch, it is possible to take skin snips of a uniform size and depth (Picq *et al.*, 1971). This method is fast and painless, and leaves a small lesion that heals rapidly (Buck, 1974). According to Rougemont *et al.* (1975), who worked with the German-made Holth punch, the German-made and the French-made Walser punches in African onchocerciasis, there are no substantial differences in sensitivity and precision of the different types of punches. Therefore, they recommended the Holth model which has a simpler design principle and is more pleasant to

handle and costs less than the other two models tested, from the view point of purely practical considerations. While, in this study the Japan-made Walser punch showed a relatively high false negative rate and also this punch was rather troublesome in handling.

In the present examination, the microfilarial densities of the six skin snips had a great variation in both the left scapula and left iliac crest of the patients. A similar tendency was recognized in the case of incubation of the skin snips at a constant temperature (32 C). Furthermore, several patients from whom were taken a total of 12 skin snips had one or more negative biopsies for microfilariae. This fact is of interest and of importance in light of diagnosis and/or epidemiological surveys for onchocerciasis in Guatemala. Buck (1974) suggested that in prevalence surveys it is sufficient to take one snip from each person at the site most likely to be heavily infected. However, this one snip may produce a considerable false negative rate, especially in the case of onchocerciasis in Guatemala where low microfilarial densities are common in endemic areas. In the present study, the patients who had relatively low microfilarial densities had a tendency to have negative skin snips. This fact appears to indicate that especial attention in epidemiological surveys, must be paid to low endemic areas of the disease. Buck (1974) also recommended that if many snips are taken from the same site it is necessary to leave a distance of 1 cm between each snip, because of the minute irregularities of microfilarial distribution in a given area of the skin. In addition, it was shown by Duke (1962), that the microfilarial density in adjacent snips is usually approximately constant, but in some cases the assessed densities may vary by as much as 1 : 3. Similar facts were also ascertained by Tada *et al.* (1973) who worked with three skin snips each taken in a triangular shape, 1 cm apart from each other, from the left buttock of Ethiopian onchocerciasis patients. While the distance between each snip taken from the present patients was 0.5 cm at both the scapula and iliac crest, the maximum to minimum rates of the density obtained from the 33 subjects varied from 1 : 1.7 to 1 : 94.3 (1 : 9.7 on the average) at the left scapula and from 1 : 2.3 to 1 : 21.5 (1 : 1.7) at the left iliac crest. Thus, the results obtained in the present study on Guatemalan onchocerciasis are quite different from those seen in African onchocerciasis. This discrepancy might be caused by the geographical and/or ecological differences of *O. volvulus* in a minute area of the skin. In Guatemalan onchocerciasis, therefore, repeated skin snips should be taken from a given area of the skin in an individual, in order to have reasonably constant figures for the numbers of microfilariae.

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グアテマラのオンコセルカ症，特に3種の検皮法ならびに皮膚内仔虫密度[1]

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グアテマラ共和国において，オンコセルカ症の診断ならびに疫学調査を実施するにあたり，簡便で信頼度の高い検皮法を見出すべく108名の患者について詳細な調査を行った。検皮にあたっては，ドイツ製 Holth type punch，日本製 Walser type punch およびランセットとメスによる3種の方法によって，患者の皮膚片（skin snip）を採取し，仔虫検出率や簡便性などの面から比較検討した。その結果，ランセットとメスによる検皮法は，高い検出率および低い疑陰性率を示し，最も良い成績であった。ところが，本法は検皮時に患者に対して，かなりの苦痛を与えるため，検診を拒否する例が続出したことから，疫学調査には不向きであると判断された。一方，Holth punch は Walser punch

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に比べ疑陰性率が低く，検出率ではランセット・メス法と大差を示さなかった。また Holth punch は検皮時の取り扱いが最も容易であった。これらのことから，グアテマラ共和国におけるオンコセルカ症の疫学調査においては，Holth punch を採用することを推奨する。次に，33名の患者の皮膚における小面積内での仔虫密度の変動を知る目的で，皮膚片間の間隔を 0.5 cm として，左肩および左腰からそれぞれ6個ずつ，計12個（1人あたり）の皮膚片を Holth punch によって採取し，仔虫密度を調べた。一般に，仔虫密度の低い患者では，小面積内での検皮にもかかわらず，陰性を示す皮膚片が認められ，診断および疫学調査上，注目された。したがって，低浸淫地での本症の疫学調査において，1回の検皮法による診断では，その浸淫地の感染の強度は低く見積られる可能性が高い。さらに，同一患者の肩および腰からの皮膚片であっても，その仔虫密度には，かなりの変動がみられた。患者33名の仔虫密度の平均値による最高密度と最低密度の比は左肩で1：9.7，左腰で1：7.1を示した。以上の成績から，グアテマラ共和国でのオンコセルカ症の疫学調査や，その他の仔虫密度に関する研究では，仔虫密度の変動を考慮した検討が必要である。