

## The Density of *Onchocerca volvulus* Microfilariae in the Skin at Different Times of the Day in Guatemala

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Concerning the concentration of *Onchocerca volvulus* microfilariae in the skin of African patients, Lartigue (1967) found the fewest numbers between 10.00 and 12.00 hours in Haute-Volta; Duke *et al.* (1967) reported an elevation in numbers during afternoon hours which correlated with the curves for temperature/saturation deficiency and for the biting density of *Simulium damnosum* in Cameroon. Recently, Picq et Jardel (1973) reported no significant changes in microfilaria density (Mfd) throughout the day in Haute-Volta when assessed quantitatively.

In order to elucidate the possible variations in Mfd in the skin at different times of the day, the authors carried out experiments in Guatemala using the quantitative skin snip method of Tada *et al.* (1973).

### Materials and Methods

During the period of our epidemiological study of onchocerciasis in Guatemala, infected individuals were selected as volunteers for several experiments in Fincas Rosario Chuarramos, Monte de oro, and Nimaya. Among them, 6 persons (# 11-16) in Finca Rosario Chuarramos and 3 (# 22-24) in Finca Monte de oro were examined during the period between Dec. 11 and 18, 1973. Skin snips were taken from the right scapular region of these volunteers 6 times at 4-hour intervals starting at 16.00 hours on the first day until 12.00 hours of the second day.

Each snip was taken approximately 1 cm away from the site of the previous one, finally forming a circle. The snips taken were put in drops of physiological saline on slides.

The actual skin area in mm<sup>2</sup> was calculated using a simple optical apparatus which projected the shape of the biopsy onto section paper. The skin snips were not teased but were incubated in a plastic box which contained a small amount of water in order to keep the slides wet; the snips were then transferred to other slides at 15 minutes, 1, 2, 6 and 12 hours, respectively. The number of microfilariae found using 50 X magnification in all the slides used in single series was totaled and the microfilarial density (Mfd) was expressed as the total number of microfilariae in mm<sup>2</sup> of skin area. Room temperature was recorded in the laboratory where skin snips were taken and incubated. On Dec. 18, the Mfd of the skins from 4 volunteers was examined only during the day between 7.00 and 17.00 hours in Finca Monte de oro. Skin snips were taken 6 times at an interval of every 2 hours from each volunteer. Collections of biting black flies were run in the same area using human bait for 15 minutes at 2-hour intervals from 7.00 to 17.00 hours of Dec. 18 and 19 paralleling the skin-snippings from the 4 microfilarial positives. The great majority of the collected flies were *Simulium ochraceum*.

For the purpose of determining the effect of the sun on the Mfd in the skin, an experiment was carried out on 8 persons (# 40-47) in Finca Nimaya on Jan. 9, 1974. The right scapular region of all the volunteers resting in a guest house was masked with 12×12 cm<sup>2</sup> black cardboard from 9.00 to 10.00 hours. A skin snip was taken from each person at 10.00 hours. Then, 4 volunteers (# 40-43, control group) were asked to stay in the same place with masks on the same skin region, while the other 4 volunteers (# 44-47, experimental group) exposed the nude upper-half of their bodies to the sun for 1 hour. The second snips were taken at 11.00 hours from all the volunteers. Again, all the persons were made to rest with masks for an additional hour in the house until the third snips were taken at 12.00 hours. The Mfd in the skin snips from the control and experimental groups were compared with each other. The temperature of the open air between 10.00 to 11.00 hours was 31°C while that of the

guest house was 21°C at 9.00 hours, 24°C at 10.00 hours and 25°C at 11.00 and 12.00 hours, respectively.

### Results

The microfilarial density (Mfd) in skin snips taken from 9 volunteers at 6 determined times between 16.00 and 12.00 hours of days is summarized in Table 1 and in Fig. 1. Further, the average Mfd per cent of 9 volunteers at each interval in the day was calculated and was plotted in Fig. 2 with the temperature of the laboratory on the days of examination. In this experiment, as is evident from Fig. 1, the Mfd at different times of the day of all those examined showed an undulation to some extent on the logarithmic scale but not a circadian rhythm such as that seen in the periodic forms of *Wuchereria bancrofti* or *Brugia malayi* microfilariae in the blood. As shown in Fig. 2, however, the average Mfd percentage curve depicted in the regular scale seems to

Table 1 Microfilarial density (Mfd) in 9 volunteers infected with *O. volvulus* during the time from 16.00 to 12.00 (Dec. 11-18, 1973, Guatemala)

Case		Time of day					
		16.00	20.00	0.00	4.00	8.00	12.00
# 11	Mfd	101.7	52.3	49.5	75.4	69.5	171.6
	%*	19.6	10.1	9.5	14.5	13.4	33.0
# 12	Mfd	5.9	8.1	5.4	5.5	10.0	4.5
	%	15.0	20.6	13.7	14.0	25.4	11.4
# 13	Mfd	91.0	103.6	69.3	105.9	102.1	130.3
	%	15.1	17.2	11.5	17.6	17.0	21.6
# 14	Mfd	123.9	229.3	113.6	147.5	123.6	98.1
	%	14.8	27.4	13.6	17.6	14.8	11.7
# 15	Mfd	55.8	60.4	78.6	52.1	100.8	44.0
	%	14.2	15.4	20.1	13.3	25.7	11.2
# 16	Mfd	23.5	42.8	44.3	57.9	39.8	51.5
	%	9.0	16.5	17.1	22.3	15.3	19.8
# 22	Mfd	2.0	20.8	24.6	15.2	24.8	13.8
	%	2.0	20.6	24.3	15.0	24.5	13.6
# 23	Mfd	21.1	25.1	28.3	49.8	37.9	23.8
	%	11.3	13.5	15.2	26.8	20.4	12.8
# 24	Mfd	9.8	15.7	9.2	11.5	10.9	11.1
	%	14.4	23.0	13.5	16.9	16.0	16.3
Average Mfd %		12.8	18.3	15.4	17.6	19.2	16.8

\* Percentage of the individual Mfd of the total obtained.

Table 2 Microfilarial density (Mfd) in 4 volunteers infected with *O. volvulus* during the day from 7.00 to 17.00 (Dec. 18, 1973, Guatemala)

Case		Time of day					
		7.00	9.00	11.00	13.00	15.00	17.00
# 17	Mfd	3.2	4.8	6.4	1.8	5.4	7.1
	%	11.1	16.7	22.3	6.3	18.8	24.7
# 18	Mfd	16.2	10.6	28.3	28.5	9.5	23.9
	%	13.8	9.1	24.2	24.4	8.1	20.4
# 19	Mfd	15.4	33.8	51.4	37.2	60.2	40.2
	%	6.5	14.2	21.6	15.6	25.3	16.9
# 20	Mfd	3.1	8.3	2.3	4.9	3.0	2.5
	%	12.9	34.4	9.5	20.3	12.4	10.4
Average Mfd %		11.1	18.6	19.4	16.7	16.2	18.1

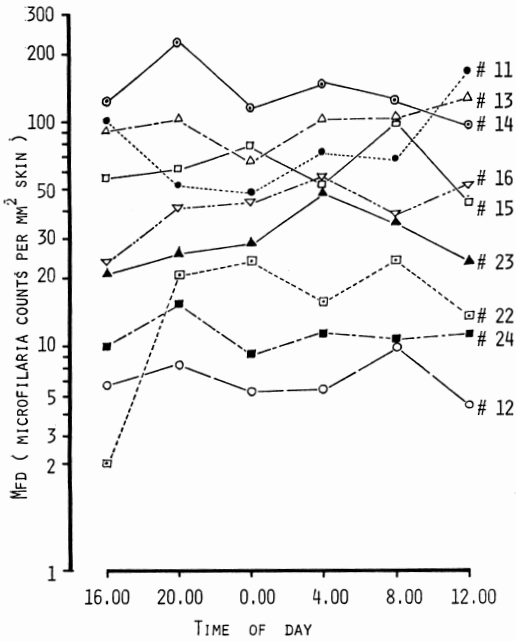


Fig. 1 Microfilarial densities in the skin snips of 9 volunteers at different times of day (Dec., 1973).

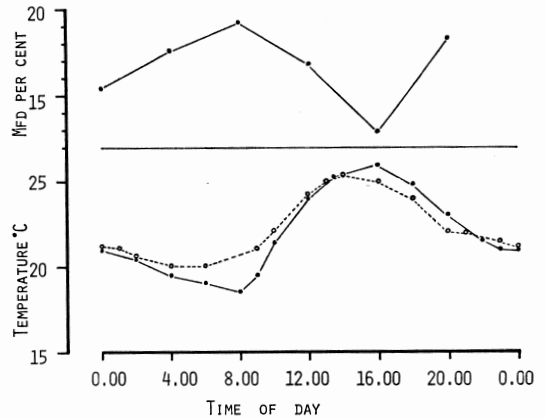


Fig. 2 The temperature of the laboratory and the average Mfd percentages of 9 volunteers at different times of day (Dec., 1973).

give a different result. There, the minimum Mfd percentage at 16.00 hours is coincident to the maximum of the temperature curve. The elevations in the Mfd are seen at 8.00 hours in the morning and 8.00 hours in the evening.

In order to determine if there was a similarity between the Mfd-curve and the biting density of the black fly vector, skin snips from 4 volunteers (# 17-20) were

examined only during the day. The results obtained are shown in Table 2 and Fig. 3. In this experiment, the average Mfd in percentage during the observation hours showed a smooth and flat curve from 9.00 to 17.00 hours and a slightly lower value at 7.00 hours as shown in Fig. 3-A. There was no correlation between the Mfd-curve and the biting density of the black fly, *S. ochraceum*, (B) or the temperature curve (C).

The effect of the sun on the Mfd in the skin was examined according to the above mentioned experimental design. The Mfd in the skin of the controls and the experimental groups was plotted on the logarithmic scale in Fig. 4-A and -B, respectively. In

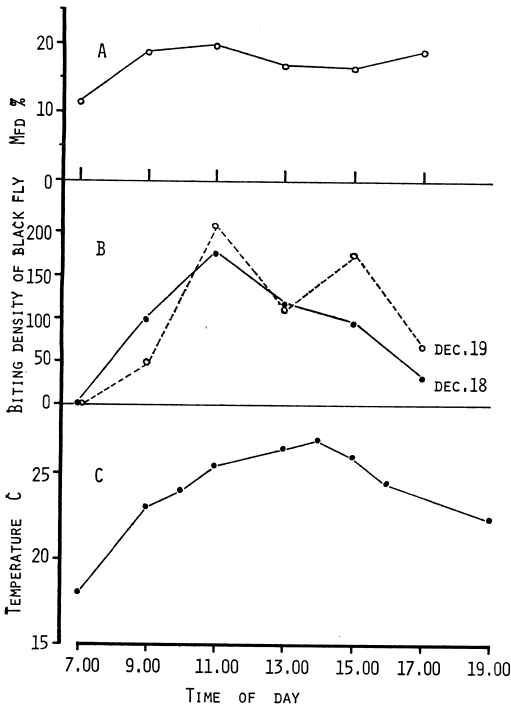


Fig. 3 Showing the average Mfd as a percentage in the skin snips of 4 volunteers (A), biting density of *S. ochraceum* expressed as the number of flies/boy/15 min. at 2-hour interval (B), and the room temperature (C). (Dec., 1973, Finca Monte de oro).

the control individuals, there was a slightly variant Mfd during the 2 hours of observations as shown in Fig. 4-A, while in the experimental group, # 45 and 47 showed only small changes in their Mfd. However, the Mfd of # 46 increased and on the other hand that of # 44 decreased remarkably after exposure. From this brief experiment, it might be concluded that there were no significant changes in the Mfd in the skin after exposure of the skin to the sun.

**Discussion**

The skin snip which was taken at 16.00 hours in case # 22 showed an exceptionally low value, 2.0, in comparison with others ranging from 15.2 to 24.8 during the time

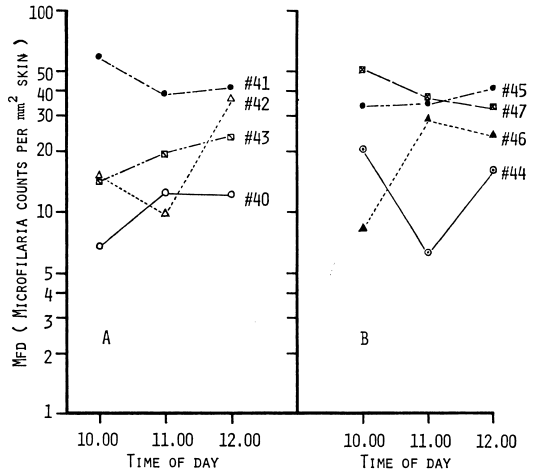


Fig. 4 Variations in microfilaria density in the 4 controls (A) and the 4 experimental individuals (B) whose skins were exposed to the sun between 10.00 and 11.00 hours.

from 20.00 to 12.00 hours (Table 1). It is difficult to explain this phenomenon clearly.

There are, as shown in Fig. 2, two peaks in Mfd at 8.00 and 20.00 hours in the curve of the average Mfd percentage. The lowest Mfd seems to occur coincident to the maximum room temperature in the afternoon. A statistical test for the difference in the values of Mfd between the examinations at 8.00 hours and 16.00 hours was made. The t-test was carried out using the square root values of Mfd instead of the original figures in order to minimize the deviation from the normal distribution. The result indicated that there was no statistically significant difference between the two examination times [ $t=1.167 < t(0.05)=2.306$ , when the degree of freedom is 8]. Furthermore, the peak of this curve at 8.00 hours is only 1.5 times the height of the lowest Mfd at 16.00 hours. In addition, as shown in Fig. 3, the average Mfd percentage of the 4 cases examined in the daytime showed a flat curve from 9.00 to 17.00 hours. Although the lowest average Mfd, 11.1%, was shown at 7.00 hours, the others were approximately the same, ranging between 16.2 and 19.4%. These facts may indicate that the differences in Mfd obtained

are not a reflection of periodicity in the usual sense of the term. The similar conclusion was previously drawn by Duke *et al.* (1967) in their studies in Africa. The results obtained may support the finding by Picq and Jardel (1973) in Haute-Volta but may differ from the conclusions by Wegesa (1966) and Lartigue (1967). Wegesa has shown that the Mfd was highest between 8.00 to 10.00 hours and 18.00 hours which coincided with the biting activity of *S. woodi* in East Africa. Lartigue concluded that the number of *O. volvulus* microfilariae in the skin showed a periodic cycle, a remarkable reduction from 8.00 to 10.00 hours and a rise till 18.00 hours.

Duke *et al.* (1967) recognized an increase in microfilarial concentrations during the afternoon hours and found similarities between the 24-hour curves and the curves for temperature/saturation deficiency and for the biting density of *S. damnosum*. Furthermore, these authors reported that the elevation of the Mfd in the Sudan-savanna strain occurred 2 hours earlier than that of the forest strain. In the present study, an elevation of the Mfd was seen at 8.00 hours and the other one at 20.00 hours. There is a possibility that differences in *Onchocerca* strains gives a different pattern in the Mfd-curve. However, the flat curve of the Mfd obtained from another experiment (Fig. 3) is contradictory to this assumption. For this reason, the present authors can not give a final, definitive conclusion on the relation between the variation of the Mfd and the temperature or biting density of *S. ochraceum* in Guatemala.

In an endemic focus of onchocerciasis in Ethiopia, one of the authors (Tada *et al.*, 1973) examined the effect of warming on the Mfd in the skin and failed to find any correlation between the warming of the skin and the resulting Mfd. In the present study, the authors examined the effect of the sun on the Mfd in the exposed skin. The exposure of the skin to the sun for 1 hour does not seem to have effect on the concentration of microfilariae in the skin. In other

words, the findings mentioned in this article suggest that for the black flies the patients would have almost equal numbers of microfilariae in the skin at any time of the day and during any weather in Guatemala.

### Summary

1) The density of *Onchocerca volvulus* microfilariae in the skin of infected individuals in Guatemala was studied for 20 hour periods at 4-hour intervals and for 10 hours during the day at 2-hour intervals. The effect of the sun on the microfilarial density in exposed skin was also studied.

2) To some extent there were variations in the Mfd in the skins of the volunteers examined at different times of the day. However, the authors did not find any circadian rhythm or periodicity of the Mfd in its generally accepted sense.

3) Exposure of the skin to the sun for 1 hour resulted in no significant changes in the Mfd.

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### グアテマラにおける皮膚内 *Onchocerca volvulus* 仔虫の時間別密度

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グアテマラのオンコセルカ流行地において仔虫陽性者の皮膚内仔虫密度の日内変動の有無を検討した。またこれらと平行して伝搬ブユの時間別採取、気温測定を実施した。実験は1973年12月から1974年1月の間にいくつかのフィンカ（農場）の労働者を対象に実施した。その成績は次のとおりである。

1) 9例の仔虫陽性者について4時間おきに4時 p.m. から翌日正午12時まで20時間について、定量的スキン・スニップ法を用いて仔虫密度を測定した。その結果、個々のヴァリエーションは見られるものの仔虫密度の日内変動は無いものと考えられた。9例の平均仔虫密度%で最高/最低比は1.5であつたが、ある種フィラリ

ア仔虫について見られる定期出現性のような型の日内変動は見られなかつた。

2) 次に4例の仔虫陽性者について昼間のみ、7時 a.m. から5時 p.m. まで2時間おきに仔虫密度を測定した結果も同様に有意の変動は無く、いずれの場合も温度や伝搬ブユの biting activity などと無関係と考えられた。なお伝搬ブユ *S. ochraceum* は9時 a.m. から3時 p.m. の間の吸血活動が最もさかんであつた。

3) 8例の仔虫陽性者につきその半数を実験群として背中を1時間直射日光に暴露し、その前後の仔虫密度を測定し、対照群と比較した。その結果、日光の影響と考えられる仔虫密度の変動は見られなかつた。