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The distribution of the microfilariae of *Onchocerca volvulus* in the different body regions in relation to the attacking behaviour of *Simulium damnosum* s.l. in the Sudan savanna of northern Cameroon.

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Summary

Densities of *Onchocerca volvulus* microfilariae in four volunteers with low to moderate infections were estimated at five body sites by paired skin snips. The landing of *Simulium damnosum* s.l. females on the body of these volunteers was recorded during 12 hours for six days. Most flies fed at the ankles (53% and 51%) and calves (28% and 27% respectively) in both the standing and sitting positions. The density of microfilariae in the skin was highest in the pelvic region (24·1 mf/mg) and relatively low in the calf (14·8 mf/mg) and ankle (1·0 mf/mg) regions. From the biting rate (♀♀/body part) and the microfilarial density (mf/mg) a transmission index was calculated for the different body regions. This was highest for the calves showing that this part of the leg, if unclothed, accounts for the highest rate of contact (50 to 60% of total) between vector and parasite.

Introduction

In human onchocerciasis, highest microfilarial densities are frequently observed in the skin of those parts of the body, which are preferentially attacked by the local *Simulium* vector species. Thus, in Guatemala, the microfilarial density is highest in the upper parts, corresponding to the behaviour of *S. ochraceum* (DE LEON & DUKE, 1966; KAWABATA *et al.*, 1980) whereas in Africa, higher concentrations were found in the lower parts of the body, corresponding to the low-biting habits of the vectors *S. damnosum* s.l. and *S. neavei* s.l. (see KERSHAW *et al.*, 1954; DUKE & BEESLEY, 1958). In Yemen, where the local vector is an apparently new member of the *S. damnosum* complex (see GARMS & KERNER, 1982), the highest concentrations were found at the ankles (ANDERSON *et al.*, 1973).

Protection of those parts of the body preferentially bitten by the vector by clothing or by repellent-treated garments may considerably reduce the risk of infection (RENZ & ENYONG, 1983) and could reduce transmission by infected persons. Thus the quantitative relationship between the distribution of the microfilariae in the exposed parts of the body and the attacking behaviour of the vector should be investigated.

We examined the distribution of microfilariae in the skin of four volunteers, harbouring low to moderate infections of the Sudan savanna strain, and compared it with the sites of blood-feeding of the local *Simulium* vectors on the same persons.

Materials and Methods

The experiments were carried out during the rainy season in North Cameroon at the river Mayo Dokday near Tchollíré, in a Sudan-savanna area with hyperendemic onchocerciasis (FUGLSANG & ANDERSON, 1977). In this region the vectors during the rainy season are, as determined by cytotaxonomic identification of larvae from a nearby breeding site, mainly *S. damnosum* s. str. and *S. sirbanum*, with a small proportion of *S. squamosum*.†

†The identification of larvae was kindly provided by Dr. Vajime, OCP, Ouagadougo, Haute Volta.

Sites of skin biopsies (Fig. 1)

Skin snips were taken from four people with different degrees of infection, between 07 and 08 hours. Two snips were taken from the right and left side of the body: upper body (scapula one and breast one), iliac crest, calf, ankle and fore-arm (medial and lateral). The skin snips were obtained with a scleral punch (Weiss "S", London), weighed on a torsion balance and incubated in saline in the wells of a microtitre plate. After one hour the emerged microfilariae were counted and the density per mg skin was estimated. The geometric mean densities (gM) were calculated according to the log n+1 method of WILLIAMS (1964).

Recording of the attacking behaviour of the flies

A volunteer stood or was seated on a white cloth 1·5×1·5 m to facilitate observation of flies. The volunteer changed position every hour, wearing only a slip as clothing. After six hours the volunteer was changed for the second half of the day. One of us (P.W.) observed the back of the volunteer and recorded each landing of a *Simulium* female and the corresponding body site. Several consecutive landings of an individual fly were recorded as one attack, as long as the same body region was selected. Only when the fly settled for several seconds was an attack on a given anatomical region recorded. Most flies landed at least twice on the same region and started to probe when left undisturbed. The fly was then caught with a sucking tube.

Results

Distribution of microfilarial densities (Table I)

In all, 80 skin-snips (average weight 2·0 mg, s.d.±0·7 mg) were taken from the ankles, calves, iliac crest, upper body (breast and shoulder) and arms of four volunteers, four snips from each site (Fig. 1). The highest density of microfilariae was calculated in the 16 biopsies from the iliac crest (gM=24·1 mf/mg), followed by the calves (14.8 mf/mg). However, the higher density at the iliac crest was not a constant feature, as among 16 paired snips from the calf and iliac crest, five showed higher densities at the calf and 11 at the iliac crest. The densities on the upper body

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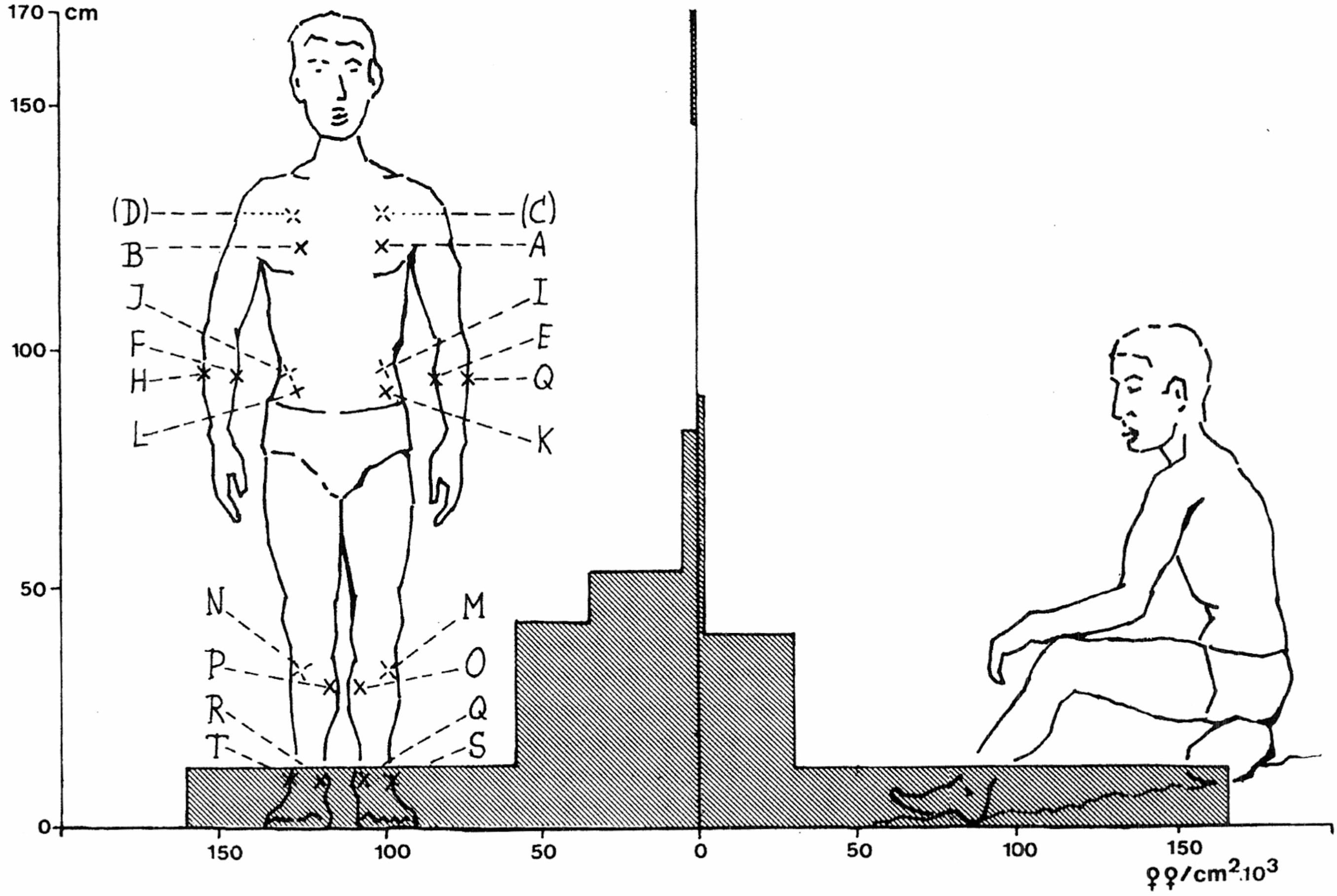

Fig. 1. The *Simulium damnosum* biting density (♀♀/cm² × 10³) in relation to the distance from the ground (cm) in standing (total 324 fly attacks) and in sitting position (total 352 fly attacks). Letters A-T refer to the sites where the skin snips were taken.

Table I—The distribution of microfilarial densities in the skin of four carriers of the Sudan savanna strain of *O. volvulus*. See Fig. I for the sites, where the skin snips were taken (A-T).

Volunteers	Site	O. mff.	O. mff./mg	J.-P. mff.	J.-P. mff./mg	S. mff.	S. mff./mg	N. mff.	N. mff./mg	Geometric Mean mff./mg	n^+/n^t
Upper body	A	0	0	3	1·1	21	6·5	7	3·6	3·3	13/16
	B	1	0·7	3	1·0	26	11·4	12	7·4		
	C	2	1·7	0	0	12	8·3	33	26·8		
	D	0	0	1	0·6	21	9·7	73	37·3		
Fore-arm	E	0	0	0	0	32	13·1	6	3·8	1.1	8/16
	F	0	0	10	3·8	26	23·6	1	0·4		
	G	0	0	1	1·3	4	1·4	0	0		
	H	0	0	0	0	1	0·7	0	0		
Iliac crest	I	12	6·7	50	13·7	140	58·1	248	79·2	24·1	16/16
	J	11	4·4	42	19·9	72	48·0	166	53·2		
	K	3	1·5	61	22·3	132	56·9	107	53·5		
	L	26	12·6	28	15·6	134	63·5	139	55·2		
Calf	M	1	1·1	10	9·2	56	60·9	62	24·3	14·8	15/16
	N	18	14·2	16	9·0	13	13·5	212	111·6		
	O	0	0	14	9·6	70	46·1	28	11·1		
	P	17	8·5	25	16·7	26	24·1	158	67·2		
Ankle	Q	0	0	1	0·4	1	0·6	5	1·7	1·0	10/16
	R	0	0	3	1·0	92	24·7	11	5·4		
	S	0	0	10	6·7	1	0·5	0	0		
	T	0	0	3	1·6	0	0	1	0·6		

n^+/n^t number of positive skin snips/total snips.

Table II—*Simulium damnosum* s.l. biting rates, *Onchocerca volvulus* microfilarial densities and calculation of the transmission index for various body-parts in the *standing* position: The transmission index is the product of the *S. damnosum* s.l. biting rate per body part with the corresponding microfilarial density

Parameter	Dimension	Ankles	Calves	Knees	Thighs	Hands	Abdomen	Arms	Upper Body	Head
Distance from ground	cm height	0-12	12-43	43-53	53-83	67-83	83-115	83-137	115-146	146-170
S. damnosum s.l. biting rate per body-part	♀♀ attacks	177	94	28	13	4	1	4	1	2
Total no. of flies below waist: 316 flies = 97·5%							above waist: 8 flies = 2·5%			
Surface area* of body part	cm^2	1,100**	1,644	830	3,034	890	3,100	2,450	3,100	1,200
Microfilarial density	mf/mg	1.0	14.8	(16)†	(20)	(1)	24·1	1·1	3·3	(3)
S. damnosum s.l. biting density	♀♀/$cm^2 10^3$	161	57	34	4·3	4·5	0·3	1·6	0·3	1·7
Transmission index per body part	♀♀ mf/mg	177	1391	(448)	(260)	(4)	24	4	3	(6)
% of total transmission index:		8	60	19	11	0	1	0	0	0

*calculated according to DU BOIS & DU BOIS (1915); **without sole of the foot, †values in parentheses indicate estimated nos. of microfilariae or estimated transmission indices, see text.

Table III—Sitting position. (See Table II for legend.)

Parameter	Dimension	Ankles	Calves	Knees	Thighs	Hands	Abdomen	Arms	Upper Body	Head
Distance from ground	cm height	0-12	12-36	30-40	23-40	40-45	23-49	45-75	49-78	78-102
S. damnosum s.l. biting rate per body-part	♀♀ attacks	181	94	14	49	3	4	4	3	0
Total no. of flies below waist: 341 flies = 96·9%							above waist: 11 flies ≙ 3·1%			
Surface area* of body part	cm^2	1,100**	1,644	830	3,034	890	3,100	2,450	3,100	1,200
Microfilarial density	mf/mg	1.0	14.8	(16)†	(20)	(1)	24·1	1·1	3·3	(3)
S. damnosum s.l. biting density	♀♀/$cm^2 10^3$	165	57	17	16	3·4	1·3	1·6	1·0	0·0
Transmission index per body part	♀♀ mf/mg	181	1391	(224)	(980)	(3)	96	4	10	(0)
% of total transmission index:		6%	48%	8%	34%	0%	3%	0%	0%	0%

(gM=3·3 mf/mg) were significantly lower than at the calves, and the ankles and fore-arms had very low microfilarial densities (1·0 and 1·1) mf/mg respectively). None of the 16 biopsies from the iliac crest was negative, but no microfilariae were seen in one of 16 biopsies from the calf, three of 16 from the upper body, six of 16 from the ankle and eight of 16 from the forearm. Only one of the volunteers (S) showed one small palpable nodule at the lowest right rib.

Considering the patience of our volunteers, no skin snips were taken at the knees, thighs, hands and head. An estimate for the approximate microfilarial density at these sites, which is given in Tables II and III, was therefore obtained from the adjacent values.

The vertical distribution of the vector's bites

During six days of observation, 676 attacks of probing flies were counted. Typically, most flies came to feed on the feet around the ankles and, to a lesser extent, on the calves (Table II and III). Less than 5% came to bite above the waist and only two flies (0·3%) attacked the head. This same pattern of distribution was observed, regardless of whether the person was standing or sitting, but the thighs were bitten more frequently in the sitting position.

Comparison of fly behaviour, microfilarial distribution and transmission

The biting density of the flies on the different body regions (♀♀/cm^2, Tables II and III) as a function of the distance from the ground decreases rapidly with increasing height. More than 50% of all bites were below 12 cm from the ground and very few bites were observed higher than 1·0 m. No significant difference was detectable between biting behaviour on a sitting and a standing volunteer (Fig. 1). It may be supposed that the chance of an individual microfilaria being ingested is proportional to the biting density, i.e., to the number of flies per cm^2 of a given part of the body. Accordingly, in a standing position, the highest chance is calculated for the ankles (161 flies/1000 cm^2≙100%), followed by the calves (35%), knees (21%), thighs and hands (2·7%), body trunk and arms (0·7%) and head (1·0%).

The relative importance of the different regions of the body with respect to the availability of the parasite to the vector depends on the respective biting rate and on the corresponding density of microfilariae. The respective "index of transmission" (=biting rate × mf-density) is, in the standing position, at the calves (1391 ♀♀ mf/mg) about eight times higher than at the ankles (177 ♀♀ mf/mg) and five times higher than at the thighs (260 ♀♀ mf/mg). This would mean that the microfilariae in the calves account for 60% of the total uptake of microfilariae in a standing volunteer and 48% in a sitting volunteer (Tables II and III, last line). Only very few microfilariae from above the waist appear to be involved in transmission—1% in the standing and 3% in the sitting position.

The average number of microfilariae available for uptake by the biting flies may be estimated by weighting the distribution of microfilariae with the respective biting rate. In the standing position, the average microfilarial supply is calculated to be 6·9 microfilariae per biting fly, compared with 8·2 microfilariae in the sitting position.

Discussion

In human onchocerciasis of the West African Sudan savanna, there is obviously no good correlation between the microfilarial distribution in the body and the attacking behaviour of *S. damnosum* s. str. and *S. sirbanum*. Particularly in the case of early or light infections, the distribution of microfilariae in the different body regions does not favour transmission. This is in contrast to many examples of animal onchocerciasis where there is a rather close association of highest microfilarial and vector biting densities (WENK, 1976; MULLER, 1979). The distribution of fly bites on the human body in the Sudan savanna did not differ significantly from the distribution in the Cameroonian rain forest (DUKE & BEESLEY, 1958) where the main vector, presumably, is *S. squamosum*. The relative percentages of flies coming to feed at the ankles of a standing person were 53·4% in the forest compared with 54·6% in the savanna, 44·2% at the knees and lower legs (savanna: 37·6%) and 2·0% above the waist (savanna: 3·7%).

The distribution of microfilarial densities in the bodies of our volunteers was comparable to the values given by ANDERSON *et al.* (1975) who examined 250 patients in a neighbouring area in North Cameroon. Their patients were selected on the basis of ocular involvement and this explains the much higher average microfilarial density at the buttock, compared with our results from the iliac crest. As they pointed out, the distribution of microfilariae tends to become more uniform in heavy infections, with increasing proportions of microfilariae at the shoulders and calves. From their results (ANDERSON *et al.*, 1975, Table II) it may be calculated that, in the 92 patients with heavy infections (mf-density at the buttock 100 mf/mg and higher) the concentration of microfilariae at the calf (gM 129 mf/mg) reached levels similar to or even higher than those at the buttock (gM 120 mf/mg).

The great majority of microfilariae which might be available for transmission are situated in the calves, knees and thighs. Protecting these parts of the body by clothes should significantly reduce the number of microfilariae available for uptake by biting flies. Even if the total number of biting flies would not be reduced by covering the leg to the ankles, i.e., if all flies would come to feed on the ankles, the average microfilarial supply should change, according to our experiment, from 6·9 mf/mg in the standing position and 8·2 mf/mg in the sitting position to 1·0 mf/mg at the ankles only. It is not clear if this causes a corresponding reduction of the load of infective larvae per fly feeding there.

However, while the contribution of an infected individual to transmission might be reduced dramatically by wearing clothing which protected the knees, calves and thighs, the risk of receiving an infective bite would, in a similarly protected individual, still be of the order of 50 to 60% of that of an unprotected individual (assuming that the distribution of bites by infective flies was similar to that of the flies recorded in this study).

Almost complete protection would be afforded by covering the feet and ankles also, since the flies apparently do not try to change to the upper, unprotected parts of the body, e.g., head and arms (RENZ & ENYONG, 1983).

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