

Onchocerciasis in the Amazonian focus of southern Venezuela: altitude and blackfly species composition as predictors of endemicity to select communities for ivermectin control programmes

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Abstract

In preparation for an ivermectin distribution programme, the prevalence and intensity of infection due to *Onchocerca volvulus* as well as the species composition and abundance of *Simulium* vectors were investigated in 22 Yanomami communities situated along 2 altitudinal transects in the southern Venezuelan onchocerciasis focus. These transects corresponded to the Ocamo–Putaco and Orinoco–Orinoquito river systems, covering a range of elevation between 50 m and 740 m above sea level (asl). A total of 831 people underwent parasitological examination in this survey and an additional 196 patients from a previous study, at an altitude of 950 m, were included in the analysis. A total of 92 659 man-biting blackflies were collected and identified to morphospecies. *S. oyapockense* s.l. was the predominant simuliid up to 150 m asl, whereas *S. guianense* s.l. and *S. incrustatum* s.l. prevailed above 150 m. Communities located below 150 m were found to range from hypo- to mesoendemic; all villages above 150 m proved to be hyperendemic (>60% microfilarial prevalence) and mass ivermectin treatment should be implemented. Age above 10–14 years, altitude of the village and biting rate of *S. guianense* s.l. up to 200 m asl were found to be statistically significant independent predictors of infection by multivariate logistic regression using a spline model. There were no differences in infection status according to sex. Above 200 m, microfilarial rate and density remained approximately constant, prevalence averaging 79% regardless of blackfly abundance. For the implementation of ivermectin-based onchocerciasis control programmes in the Amazonian focus, altitude and species composition of the blackfly population might be adopted as useful indicators aiding selection of the most affected communities. However, below 200 m additional parasitological indicators may also be necessary. As a direct result of this study, regular mass-ivermectin delivery to meso- and hyperendemic communities is now in progress.

Keywords: onchocerciasis, *Onchocerca volvulus*, endemicity, altitude, Simuliidae, ivermectin, Amazonian focus, Venezuela

Introduction

Previous studies have indicated that, in the southern Venezuelan focus, human onchocerciasis is present in Sierra Parima, in the upper Orinoco river basin, and in some highland parts of the upper Caura area (RASSI *et al.*, 1977; GODOY, 1982; GODOY *et al.*, 1989; YARZÁBAL *et al.*, 1983, 1985b). More recently, a southward extension of this focus has been reported by BOTTO *et al.* (1997). This zone, contiguous with the Brazilian endemic area, comprises the so-called Amazonian onchocerciasis focus, affecting mainly the indigenous Yanomami Amerindian group. In the highlands of this area *Simulium guianense* s.l. has been incriminated as a main and efficient vector, whereas in the lowlands *S. oyapockense* s.l./*S. roraimense** is a poorer insect host (RASSI *et al.*, 1977; TAKAOKA *et al.*, 1984b; SHELLEY *et al.*, 1987; BASÁÑEZ *et al.*, 1988). A variation in endemicity has been reported, the higher levels of prevalence and intensity being observed at higher altitude (RASSI *et al.*, 1977; MORAES *et al.*, 1979). Apart from *Onchocerca volvulus*, the presence of *Mansonella ozzardi* in skin snips has also been detected in the area (MORAES, 1976; YARZÁBAL *et al.*, 1983); both filariae might be transmitted by the same vectors (SHELLEY *et al.*, 1980, 1997; YARZÁBAL *et al.*, 1985a).

In addition to *S. guianense* s.l. (also referred to as *S.*

pintoï) and *S. oyapockense* s.l. (as *S. amazonicum* and *S. cuasisanguineum*), there are other anthropophilic blackfly species such as *S. incrustatum* s.l.† (as *S. limbatum*† and *S. yarzabali*), and *S. bipunctatum* (as *S. antillarum* and *S. pseudoantillarum*) (TAKAOKA *et al.*, 1985; SHELLEY *et al.*, 1997), whose vectorial role has yet to be determined. Apart from the crude division already mentioned between the highlands (high prevalence) and the lowlands (low prevalence), the relationship between elevation, endemicity, and species composition has not previously been systematically studied in this focus.

Altitude has been found to be positively associated with onchocerciasis endemicity in Guatemala and Mexico within the temperature limits required for the completion of the parasite extrinsic incubation period within the fly. *S. ochraceum* s.l. (the main vector in these foci) prevails in the communities located at the highest altitude, *S. metallicum* s.l. at intermediate locations, and *S. haematopotum* (a less efficient host) in the lowlands (TAKAOKA *et al.*, 1984a; OCHOA *et al.*, 1987; ORTEGA *et al.*, 1989). Elevation and proximity to the breeding sites have been found to be important predictors of onchocerciasis prevalence and severity in the forest zone of Cameroon. These associations were used in the context of efforts to stratify communities according to their risk of onchocercal disease with a view to the rational implementation of control programmes based on ivermectin distribution (NGOUMOU *et al.*, 1994; MENDOZA-

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*Because of taxonomic proximity of *S. oyapockense* s.l. and *S. roraimense*, probable sympatry in the lowland localities of the Amazonian focus, and owing to the difficulty in separating the adult females of these 2 species (SHELLEY *et al.*, 1997), when we refer to *S. oyapockense* s.l. this may also include *S. roraimense*.

†SHELLEY *et al.* (op. cit.) consider that *S. limbatum* is absent from the Brazilian part of the Amazonian focus but, since RAMÍREZ-PÉREZ *et al.* (1982) report this species in the Venezuelan part, when we refer to *S. incrustatum* s.l., we do not rule out the possibility of it including *S. limbatum* until further taxonomic work is carried out.

ALDANA *et al.*, 1997). Similarly, the initiation of ivermectin control programmes in Latin America under the auspices of OEPA (Onchocerciasis Elimination Program for the Americas) requires the identification and selection of appropriate indicators in order to prioritize communities for the rational delivery of treatment. This necessity is even stronger in the Amazonian focus, where, although population density is low (there are approximately 13 000 Yanomami in Venezuela inhabiting about 360 villages), the affected communities are scattered in an extensive and remote area of 192 000 km² (OCEI, 1993; COLCHESTER, 1995).

The results presented in this paper form part of a more extensive study coordinated by CAICET in southern Venezuela with the purpose of obtaining entomological, parasitological, and clinical indicators appropriate to the evaluation of onchocerciasis endemicity and severity in the Amazonian focus. In particular, this work tests the hypothesis that in southern Venezuela there is clinical variation of onchocerciasis endemicity according to altitude and related to the species composition and abundance of biting simuliids which, in turn, vary in their vectorial capacity and competence. This paper presents updated epidemiological and entomological information gathered along an altitudinal gradient of Yanomami villages with the objective of obtaining indicators that aid identification of high-risk communities for the purpose of ivermectin control programmes. The parasitological and clinical findings will be discussed elsewhere.

Materials and Methods

Study area and population

Two altitudinal transects were chosen which ranged from the lowlands at 50 m to about 740 m above sea level

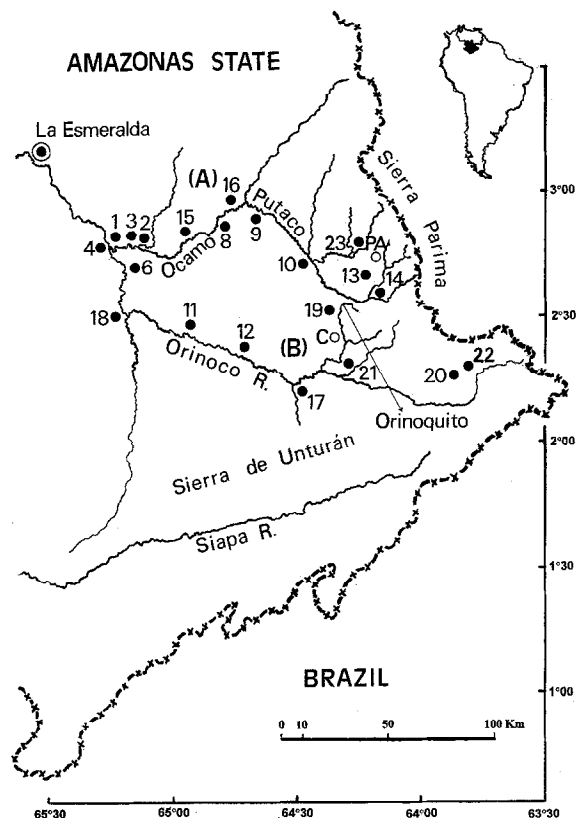


Fig. 1. Study area and location of the Yanomami villages referred to in this work. (A): Ocamo-Putaco rivers (comprises villages no. 1, 2, 3, 8, 9, 10, 13, 14, 15 and 16); (B) Orinoco-Orinoquito rivers (no. 4, 6, 11, 12, 17, 18, 19, 20, 21, 22). Village 23 is Niyayowë-theri (Parima-B), C is Coyowë-theri, and PA is Parima-A. The insert shows the relative situation of the Amazonas State and Venezuela in South America.

el (m asl) towards the highlands of Sierra Parima. These transects corresponded to 2 river systems: (A) the Ocamo and Putaco rivers, and (B) the Orinoco and Orinoquito rivers (see Fig. 1). The area covered approximately 10 000 km². A total of 20 Yanomami communities were studied, 10 along each cline. These villages were reached by boat and on foot, and sometimes by air. Their altitude was measured with an Avocet Vertech Alpine altimeter (Avocet in Silicon Valley, CA, USA). Analyses included the village of Niyayowë-theri (situated in Parima-B), whose raw data had originally been collected in the study reported by YARZÁBAL *et al.* (1983). This extended the range of altitude to 950 m.

The lowlands are characterized by average temperatures of 26–27°C and 3750–5000 mm annual rainfall, lying in very humid tropical forest, whereas the highlands exhibit mean temperatures of 18–24°C and annual rainfall of 1100–1200 mm, frequently comprising secondary savannah areas surrounded by pre-montane and semi-deciduous forest (EWEL *et al.*, 1976; HUBER *et al.*, 1984). Although there is virtually no season without rain in either the lowlands or the highlands, the months with less precipitation in Ocamo are January, February and March (COCCO, 1987). In the Parima mountains, the drier months are usually December to February (SHELLEY *et al.*, 1997). Taking the study area as a whole, the rainiest months are those from April to October, and the driest include November through March.

The traditional Yanomami organize themselves into small and scattered villages, inhabiting a communal, open and round house called 'shabono'. This, together with their scanty clothing, leads to an almost continuous exposure to biting flies. Yanomami are semi-nomadic with migrations following 2 main patterns: micro- and macro-movements. The former, taking place about every 2–3 years, are likely to happen in the close vicinity of the area they occupy and are possibly due to overgrowth of their vegetable garden and general decay of the shabono. The latter, due to complex patterns of alliances, kinship, and hostilities, may involve population growth, fission, and spread into new areas, leading to major changes in the location of the shabono (COLCHESTER, 1995; CHAGNON, 1997). Although there is evidence suggesting that about 250 such large-scale relocations have taken place in the past 150 years, there are also many instances of the same village remaining in the same area for 60–80 years (COCCO, 1987; CHAGNON, 1997). In this work it will be assumed that, although onchocerciasis may not be at endemic equilibrium within each village, the infection is acquired through local exposure to simuliid bites taking place in the shabono and surrounding plantation. People included in the study comprised those older than 5 years of age who were living in a particular community for at least 1 year. Ethical clearance was sought from the Ethics Committee of the London School of Hygiene and Tropical Medicine, the Regional Health Direction of the Amazonas State (Venezuelan Ministry of Health), the Indigenous Affairs Section of the Amazonas State, and the Upper Orinoco Health District. Approval for the survey was also requested from community leaders. Consent for taking skin biopsies was obtained from each adult individual, and parental permission was sought for all children enrolled in the study.

Parasitological methods

Four skin snips were taken; 2 from the scapular region and 2 from the iliac crest, using a Holth-type corneal punch disinfected with 2% glutaraldehyde and isopropyl alcohol as recommended by the Inter-American Conference on Onchocerciasis (IACO, 1992). Skin biopsies were incubated for 24 h in micro-titration plates containing buffered saline solution. Emerging microfilariae (mf) were fixed with 2% formal-

dehyde, stained with Mayer's haemalum, counted and identified to species (*O. volvulus* and *M. ozzardi*). The snips were weighed to allow calculation of mean number of mf/mg of skin (YARZÁBAL *et al.*, 1983).

Entomological methods

In each community, and during 3 to 5 consecutive days, all the blackflies that landed to bite on Yanomami volunteers in the shabono were caught for the first half hour of each hour from 07:00 to 18:30. Two subjects participated each day, working for alternate hours. The anthropophilic simuliids were counted, identified to species according to the keys of RAMÍREZ-PÉREZ *et al.* (1982) and SHELLEY *et al.* (1997), and preserved in 80% ethanol for further staining and dissection (NELSON, 1958). Some villages, chosen for more detailed entomological studies, were visited again on various occasions and data collected during both dry and rainy seasons. Results for these communities regarding infection and parity status in the fly population will be presented elsewhere.

Data analysis

Endemicity was measured as age- and sex-adjusted onchocercal mf prevalence and intensity. Individual microfilarial load was calculated as the arithmetic mean number of mf/mg in the 4 skin snips taken. Overall village intensity was estimated as the arithmetic mean and the geometric mean of Williams (WILLIAMS, 1937) of all the people examined, both positives and negatives. Criteria for classification of communities as hypo-, meso-, and hyperendemic followed OEPA (1996) recommendations, i.e., mf prevalence <20%, between 20 and 59%, and >60%, respectively. Microfilarial prevalence and intensity were standardized by the direct method using the census data of the whole population under study (KIRKWOOD, 1988). The relationship between the proportion of people infected, *p*, and mean worm load, *m*, was explored by assuming an underlying negative binomial distribution of microfilarial counts

with aggregation parameter, *k*. Previous analyses (data not shown) had indicated that this assumption was justified. The function $p = 1 - \left(1 + \frac{m}{k}\right)^{-k}$ was fitted to the data and the parameter *k* estimated by maximum likelihood (GUYATT *et al.*, 1990). As an alternative to constant *k*, the assumption of a linear *k*, i.e., $k(m) = k_0 + k_1 m$ was also considered. The likelihoods of both models were compared using the log-likelihood ratio test, which, under the null hypothesis, is approximately distributed as a chi-square with d.f. equal to the difference in the number of parameters estimated by each model (COX & OAKES, 1984).

Simuliid species composition in each village is reported as the proportion of each species in the total collected during the sampling days. Daily biting rates (DBRs) were calculated by multiplying the number of flies caught in each half-hour by 2, adding the hourly totals and averaging the daily totals. Arithmetic means were used for this purpose because there was little variation between days regarding such totals. Between-season variation was greater. Consequently, when species composition and DBRs were available for dry and rainy seasons at a particular locality, both results were used separately or combined into a single measure calculated as the geometric mean (PORTER *et al.*, 1988) weighted by the proportion of dry and wet months in a year.

Preliminary data exploration showed that both microfilarial prevalence and intensity increased with age and elevation. A multivariate logistic regression model was fitted to estimate the individual risk of being infected with *O. volvulus* according to age (as a categorical variable), sex, and altitude (as a linear spline, GOULD, 1993), with the 5-9-years age-group as the baseline. Since there were no differences in infection status attributable to sex, this variable was dropped in all subsequent analyses. These included investigation of the relationship between risk of infection and vector abundance by incorporating DBRs per blackfly species and season in addition to age and altitude. All regression

Table 1. Age- and sex-standardized prevalence and intensity of *O. volvulus* microfilariae in villages in the Amazonian onchocerciasis focus, southern Venezuela (see Fig. 1)

Code no.	Village ^a	Altitude (m asl)	No. mf +ves/ no. examined	Adjusted prevalence (%)	Adjusted intensity (mf/mg) AM ^b	SE ^c	WM ^d
6	Shashanawë-theri	50	0/12	0.00	0.00	0.00	0.00
4	Yohoopë-theri	50	1/22	2.86	0.01	0.02	0.01
3	Kashorawë-theri	60	0/35	0.00	0.00	0.00	0.00
2	Iyewëi-theri	60	1/29	5.43	0.01	0.01	0.01
1	Ocamo	90	2/117	2.41	0.06	0.15	0.03
18	Purima-theri	110	6/38	13.87	0.34	0.55	0.19
16	Toothothopiwei-theri (Mo)	125	13/46	33.87	1.32	1.68	0.47
8	Maweti-theri(Mo)	140	17/46	24.32	0.60	0.80	0.31
11	Mahekoto-theri	140	23/54	41.38	6.97	12.05	1.23
15	Yepropë-theri	150	2/38	5.79	0.58	0.90	0.14
9	Awei-theri(Mo)	162	15/24	66.75	64.70	27.01	14.61
12	Cerrito	165	20/26	64.63	13.75	10.86	2.97
17	Hasupiwei-theri	200	36/45	78.39	40.66	39.33	12.35
10	Pashopeka-theri(Mo)	240	32/38	79.76	25.41	24.53	6.72
21	Hokotopiwei-theri	400	50/59	74.77	60.92	45.35	18.92
14	Yoreshiana B	475	20/32	65.38	37.91	44.95	5.49
20	Harau-theri A	642	19/28	66.25	29.95	21.33	6.19
13	Yoreshiana A	690	45/78	63.59	23.40	22.06	4.57
19	Maiyo-theri(Mo)	720	38/43	86.75	33.37	34.06	8.44
22	Harau-theri B	740	12/22	62.88	17.77	8.32	5.13
23	Niyayowë-theri ^e	950	145/196	77.53	66.45	78.66	12.15

^aThe naming of Yanomami villages follows the guidelines proposed by LIZOT (1975).

^bArithmetic mean considering both positives and negatives.

^cStandard error of the arithmetic mean.

^dGeometric mean of Williams.

^eFigures recalculated from raw data gathered by YARZÁBAL *et al.* (1983).

(Mo)Indicates presence of *Mansonella ozzardi* in skin snips.

models assumed observations to be independent between clusters (communities), but not necessarily within communities, thus obtaining robust variance estimates. The statistical analyses were conducted using STATA ver. 5.0 (Stata Corporation, TX, USA).

Results

A total of 831 people out of 1220 (68%) were examined in this survey. Overall onchocercal prevalence was 42% (352 mf-positives). The prevalence of *M. ozzardi* in the studied population was 2.5% (21/831); this species was present in 5 of the 20 villages investigated and in all cases it was found in individuals who were also positive for *O. volvulus* (see Table 1).

Table 1 summarizes the elevation, prevalence, and intensity data for each village. These data show a sharp increase of onchocercal infection up to about 200 m and a levelling off thereafter. The Spearman correlation coefficients between prevalence and altitude, and intensity and altitude were, respectively, 0.829 ($P < 0.001$) and 0.827 ($P < 0.001$). A multivariate spline logistic regression model was therefore applied to explore the relationship between individual infection status, age, and elevation. This model allowed altitude below and above 200 m to be used as a linear term exploring the contribution to the change in prevalence due to the differential of altitude above this threshold. Results are presented in Figure 2A and Table 2. In addition to age

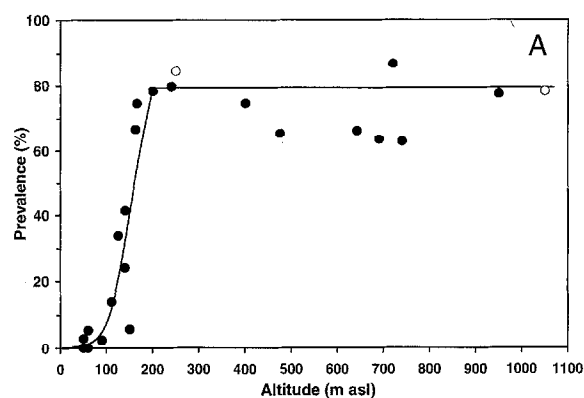


Fig 2A. Microfilarial prevalence (%) as a function of altitude (m asl). Closed circles are the age and sex-adjusted prevalence values for each community. Open circles are the values for Coyowë-theri (250 m) and Parima-A (1050 m) according to YARZÁBAL *et al.* (1983, 1985). Solid line is the predicted prevalence, p , according to the results of the logistic regression model presented in Table 2, as follows:

$$p = \left[\sum_i \left(\frac{e^{\alpha + \beta_1 x_1 + \beta_2 x_2}}{1 + e^{\alpha + \beta_1 x_1 + \beta_2 x_2}} \right) \omega_i \right] 100,$$

where α is the constant, β_1 is the logarithm of the odds ratio for the age-group i , x_1 is the variable age-group, β_2 is the logarithm of the odds ratio for altitude < 200 m, x_2 is altitude in metres, and ω_i is the proportion of people in each age-group i . Above 200 m prevalence is constant.

Table 2. Results of the spline logistic regression model of infection status with age and altitude as independent variables

Variable	Odds ratio ^a	z value	P	95% lower CL	95% upper CL
Age-group					
10-14 years	1.579	1.370	0.171	0.821	3.035
15-19 years	6.999	4.711	<0.001	3.115	15.727
20-29 years	16.023	10.527	<0.001	9.559	26.854
30-39 years	25.535	11.802	<0.001	14.908	43.734
≥40 years	28.858	8.977	<0.001	13.850	60.128
Altitude					
<200 m asl	1.051	9.573	<0.001	1.040	1.061
≥200 m asl	0.999	-1.537	0.124	0.997	1.000

^aOdds ratios measure the increasing risk of infection per age-group or per metre of altitude above sea level (asl).

above 10-14 years as an independent risk factor of infection, there was a highly significant positive linear trend in the odds of microfilarial infection up to 200 m ($P < 0.0001$), whereas beyond this altitude the trend was found to be non-significant ($P = 0.124$). Consequently, the odds of being infected above 200 m were assumed to be constant and the prevalence to be equal to 79.4%. For the sake of completeness, Figure 2A includes 2 data points from YARZÁBAL *et al.* (1983, 1985), namely Coyowë-theri (250 m) and Parima-A (1050 m). These villages were not considered in the logistic regression because their raw data were not available at the time of the analysis. The relationship between microfilarial prevalence and intensity is depicted in Figure 2B. The estimated value of the aggregation parameter, k , was 0.2493. The assumption that k varies linearly with mean mf load did not significantly improve the fit (log-likelihood ratio statistic = 3.240, $P = 0.072$, 1 d.f.).

The total number of flies collected and the DBR for dry and/or rainy season are presented in Table 3 for all the anthropophilic blackfly species found in the Yanomami communities. A total of 92659 flies were collected (91.6% *S. oyapockense s.l.*, 6.1% *S. incrustatum s.l.*, 1.3% *S. guianense s.l.*, and 1.0% *S. bipunctatum*). In addition to the species included in Table 3, a total of 26 *S. exiguum s.l.* were collected biting man in the communities of Maweti and Pashopeka. Figure 3 shows the species composition as the proportion of the total col-

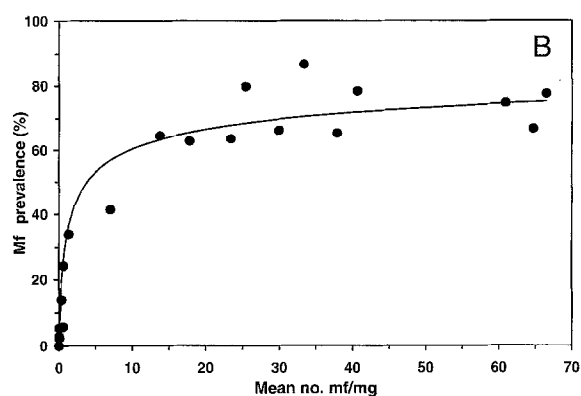


Fig 2B. Microfilarial prevalence (%) as a function of arithmetic mean microfilarial load (mf/mg). Closed circles are the age and sex-adjusted prevalence values for each village. Solid line is the predicted prevalence according to the equation

$p = \left[1 - \left(1 + \frac{m}{k} \right)^{-k} \right] 100$, where k is the aggregation parameter of the negative binomial ($k = 0.2493$).

lected by altitude. It can be seen that *S. oyapockense s.l.* was predominant below 150 m whereas *S. guianense s.l.* and *S. incrustatum s.l.* began to prevail above this elevation. *S. bipunctatum* appeared in smaller numbers at the highest altitudes.

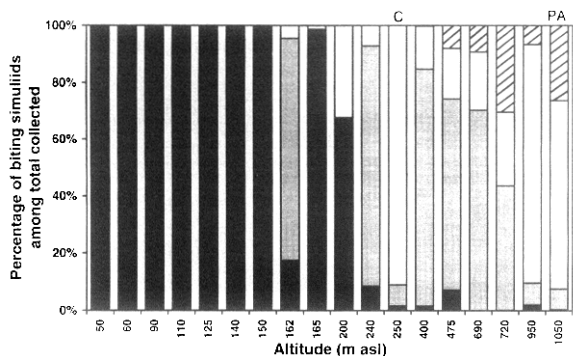


Fig. 3. Species composition of anthropophilic simuliids against altitude. *S. oyapockense*: black bars; *S. incrustatum*: grey bars; *S. guianense*: white bars; *S. bipunctatum*: hatched bars. Graph includes data for Coyowé-theri (C, 250 m) and Parima-A (PA, 1050 m) from BASÁÑEZ (1992).

In order to investigate the contribution to the risk of infection due to biting rate, this variable was incorporated into the previous spline model. Neither *S. exiguum s.l.* nor *S. bipunctatum* were considered in this analysis because available data had indicated that none of these species was naturally infected with larvae indistinguishable from *O. volvulus* (BASÁÑEZ, 1992). The combined geometric mean biting rate was preferred to separate seasonal data because it was considered that this average represented better the vector contact to which people are exposed through time. The model was fitted with and without altitude ≥ 200 m asl (a non-significant variable as shown in Table 2). The log-likelihood ratio test indicated no difference between these options ($P=0.492$) and, therefore, the most parsimonious model was chosen. Of the 3 simuliid species included, only *S. guianense s.l.* was found to be positively associated with infection status below 200 m ($P=0.006$). Results are summarized in Table 4. Figure 4 depicts the relationship between biting rate and altitude for each simuliid species and season.

Discussion

In the southern Venezuelan onchocerciasis focus, prevalence of microfilarial infection increased with age and altitude; there were no significant differences between the sexes. Microfilarial prevalence increased rapidly with elevation up to 200 m asl, rising from 0 to 78% and remaining relatively constant above this altitude. Hypo- and mesoendemic communities were found to be situated at elevations ≤ 150 m, whereas all villages located above 150 m proved to be hyperendemic. Although not considered in the spline logistic regression model, Figure 2 includes the altitude and mf rate of Coyowé-theri and Parima-A reported by YARZÁBAL *et al.* (1983, 1985) in order to stress the consistency of our results with those from previous surveys. A similar trend is followed by infection intensity, with low parasite loads below 150 m in contrast to mf densities ranging from 14 to 66 mf/mg (arithmetic means), or from 3 to 19 mf/mg (Williams' means) above 150 m (Table 1). The relationship between microfilarial prevalence and intensity was markedly non-linear as is the case in many other macroparasitic infections, the small value of the clumping *k* parameter reflecting strong aggregation of parasite abundance within host populations (ANDERSON & MAY, 1985, 1991). This corresponds to a wide variability of infection intensity (and possibly onchocerciasis morbidity) among the communities considered to be hyperendemic.

The trends exhibited by infection prevalence and intensity according to altitude seem to correspond well with accompanying changes in blackfly species composition. *S. oyapockense s.l.* was the predominant species below 150 m with 1256 to 6097 bites/person daily; *S. incrustatum s.l.* and *S. guianense s.l.* prevailed above this

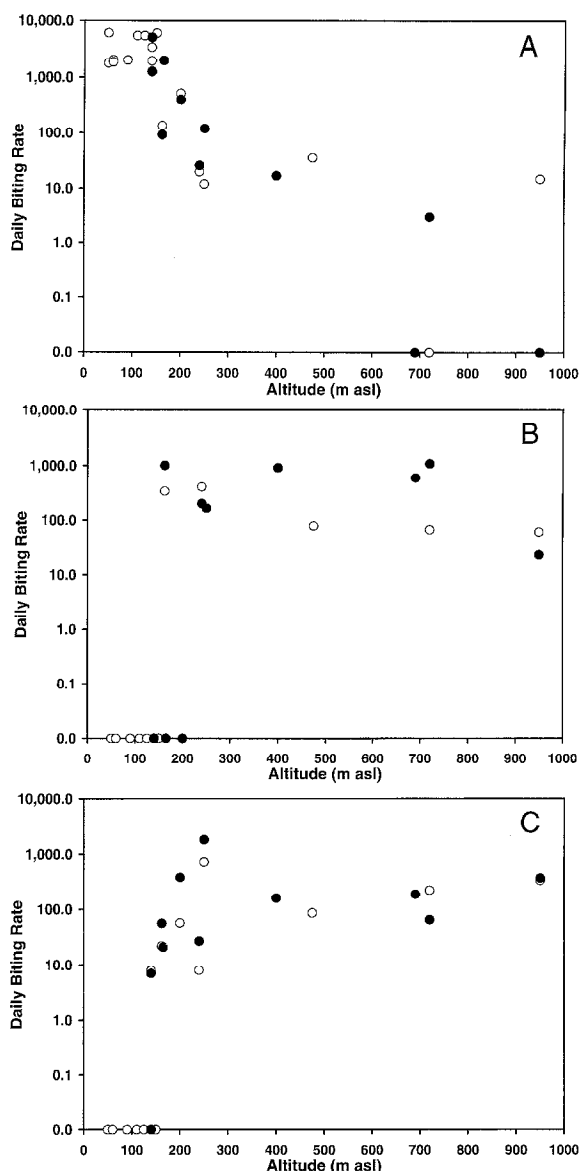


Fig. 4. Daily biting rate against altitude for *S. oyapockense* (A), *S. incrustatum* (B), and *S. guianense* (C). Closed and open circles represent, respectively, rainy and dry seasons. Data for Coyowé-theri (250 m) from BASÁÑEZ (1992) are included as follows: *S. oyapockense* (r: 120, d:12); *S. incrustatum* (r: 165); *S. guianense* (r: 1819, d: 728).

elevation with, respectively, 0–1078 and 0–383 bites/person daily (Table 3). However, only *S. guianense s.l.* showed a significantly positive relationship between biting rate and microfilarial infection up to the 200 m endemicity threshold. Above this altitude, prevalence remained approximately constant despite changes in blackfly biting rate. Nevertheless, blackfly abundance is only one of other important components of vectorial capacity, namely vector competence and survival. The role of between-species and between-season variations in parity rates (a possible indicator of vector survival or of differential dispersion of nullipars and parous flies from breeding sites), as well as infective biting rates and transmission potentials, will be discussed elsewhere. Even without this information, available knowledge of intrinsic susceptibility and mechanisms of parasite regulation within simuliid vectors may aid interpretation of the results obtained thus far.

S. oyapockense s.l. possesses a well-developed cibarial armature, destroying a large proportion of ingested mf and exhibiting an overall low vector competence (SHEL-

Table 3. Blackfly species abundance measured as the total collected in each village and as daily biting rate (DBR) during the total number of catching days in dry (d) and/or rainy (r) seasons, and weighted geometric averaged value (a) as described in text

Village no. ^a	Season	<i>S. oyapockense</i> s.l.		<i>S. guianense</i> s.l.		<i>S. incrustatum</i> s.l.		<i>S. bipunctatum</i>	
		n	DBR	n	DBR	n	DBR	n	DBR
4	d	8563	6097	0	0	0	0	0	0
3	d	3054	1861	0	0	0	0	0	0
2	d	5264	2019	0	0	0	0	0	0
1	d	4014	2007	0	0	0	0	0	0
18	d	8125	5448	0	0	0	0	0	0
16	d	6917	5430	0	0	0	0	0	0
8	d	3297	3368	3	–	0	0	0	0
	r	25510	5073	0	0	1	–	0	0
	a		4277		0		0		0
11	d	4982	1942	19	8	0	0	0	0
	r	2392	1256	32	7	0	0	0	0
	a		1506		7		0		0
15	d	9428	6013	0	0	0	0	0	0
9	d	74	135	12	22	175	339	0	0
	r	58	93	29	56	514	1012	0	0
	a		109		38		642		0
12	r	2392	1979	32	21	0	0	0	0
17	d	268	522	29	57	0	0	0	0
	r	354	404	330	383	0	0	0	0
	a		450		174		0		0
10	d	46	20	18	8	957	406	0	0
	r	66	26	64	27	451	201	0	0
	a		23		16		269		0
21	r	24	17	222	160	1224	918	3	–
14	d	9	36	22	86	84	79	10	29
13	r	0	0	185	187	642	597	85	78
19	d	0	0	83	221	25	67	36	96
	r	4	–	91	65	1575	1078	865	577
	a		0		108		340		274
23 ^b	d	98	15	1715	335	281	61	128	20
	r	0	0	1899	369	149	23	197	28
	a		3		354		35		24

^aVillages are ordered according to increasing altitude as in Table 1.

^bData from BASÁNEZ (1992).

Table 4. Results of the spline logistic regression model of infection status with age, altitude, and vector biting rate as independent variables

Variable	Odds ratio ^a	z value	P	95% lower CL	95% upper CL
Age-group					
10–14 years	1.597	1.335	0.182	0.802	3.177
15–19 years	6.008	4.251	<0.001	2.628	13.735
20–29 years	15.461	10.532	<0.001	9.288	25.738
30–39 years	26.324	10.848	<0.001	14.579	47.532
≥40 years	27.422	8.831	<0.001	13.149	57.186
Altitude <200 m asl					
	1.042	4.615	<0.001	1.024	1.060
Daily biting rate					
<i>S. oyapockense</i> <200 m	0.999	–0.607	0.544	0.999	1.000
<i>S. oyapockense</i> ≥200 m	1.001	1.668	0.095	0.999	1.003
<i>S. guianense</i> <200 m	1.076	2.739	0.006	1.021	1.135
<i>S. guianense</i> ≥200 m	0.999	–0.164	0.870	0.996	1.003
<i>S. incrustatum</i> <200 m	0.997	–2.924	0.003	0.995	0.999
<i>S. incrustatum</i> ≥200 m	1.000	0.405	0.685	0.998	1.002

^aOdds ratios measure the increasing risk of infection per age-group, per metre of altitude, or per simuliid bite.

LEY *et al.*, 1987). The observation that infective larval output is positively related to mf density suggests that there may be initial facilitation, with very little larval development when mf intakes are low (BASÁNEZ *et al.* 1995). This is likely to be the case in the low altitude hypoendemic villages. These villages are generally less remote, closer to missionary and health posts, and more susceptible to influences alien to traditional Yanomami

culture. Here, the use of clothing, school attendance, and adoption of separate enclosed huts (although still arranged around a central open space), are more frequent. These, among other behavioural changes, may play a part in reducing effective human–fly contact. The measurement of biting rates on humans during 12 diurnal hours is most likely to overestimate true exposure in such settings. In addition, the relative accessibility of

these communities has made them targets of regular anthelmintic chemotherapy campaigns and of previous attempts to control onchocerciasis based on the use of suramin and diethylcarbamazine (with, respectively, macro- and microfilaricidal activity). Finally, malaria endemicity in the region exhibits precisely the opposite pattern with elevation, with highest malaria incidence and extensive chloroquine usage in the lowlands. In other endemic areas chloroquine has been shown to reduce significantly mf loads (GUDERIAN *et al.*, 1991).

These considerations raise questions as to whether *S. oyapockense s.l.* may maintain autochthonous onchocerciasis transmission below 150 m. The communities of Maweti and Toothothopiwei-theri are examples of villages where microfilarial rates are, respectively, 24.3 and 33.9% with no recorded biting by *S. guianense s.l.* DBRs of *S. oyapockense s.l.* were 4277 and 5430, and the proportion of parous flies was very high (69–76%) in Maweti. In this locality, *S. oyapockense s.l.* was found naturally infected with L3 larvae indistinguishable from *O. volvulus* (GRILLET *et al.*, 1997). These findings suggest that *S. oyapockense s.l.* might indeed be responsible for mesoendemic transmission in this area.

In contrast, *S. guianense s.l.* is an efficient vector lacking cibarial teeth, exhibiting density-dependent limitation of infective larval output, and being susceptible to parasite-induced mortality at heavy mf intakes (BASÁÑEZ *et al.*, 1995, 1996). It is more likely to be infected/infective and to survive the extrinsic incubation period when mf densities are low to moderate. Mathematical modelling of onchocerciasis population dynamics suggests that in the case of flies with unarmed cibarium, lower threshold biting rates may suffice for endemic maintenance of onchocerciasis transmission (BASÁÑEZ, 1996). *S. guianense s.l.* may be responsible for meso- to hyperendemic transmission in the Amazonian focus, its biting rate showing a positive association with both elevation and endemicity up to 200 m asl (Table 4 and Fig. 4). The role of *S. incrustatum s.l.* remains to be elucidated. Although sympatric with *S. guianense s.l.* in most localities above 150 m, it also possesses an armed fore-gut, relatively low vector competence in experimental infection studies, and has infrequently been found naturally infected with L3 larvae indistinguishable from *O. volvulus*.

It is unlikely that the slight (non-significant) negative trend between mf rate and altitude above 200 m may be due to decreasing ambient temperatures. Most probably, the range of altitudes explored in this work falls well within the temperature limits for the completion of the extrinsic incubation period of *O. volvulus* within *Simulium*. In Guatemala, TADA *et al.* (1979) report a decrease of mf rate above 1300 m asl, the vector being *S. ochraceum s.l.* The fact that in the Amazonian focus there is no further increase in microfilarial prevalence and intensity above 200 m, regardless of local variations in the biting rate of *S. guianense s.l.*, suggests that in addition to limitation of parasite load within the blackfly, there may also be some regulation of parasite abundance within the human host. Although not included in the logistic regression analyses presented here, the biting rate of *S. guianense s.l.* in Coyowë-theri, situated at 250 m asl (1242 bites/person daily), is more than 3 times as high as in Parima-B (950 m), or Parima-A (1050 m), yet before ivermectin treatment mf prevalence and intensity were very similar in these 3 localities (YARZÁBAL *et al.*, 1983, 1985; BASÁÑEZ, 1992).

In conclusion, age above 10–14 years, as well as altitude and abundance of *S. guianense* below 200 m asl, were found to be independent predictors of individual risk of onchocerciasis infection in the southern Venezuelan focus. However, below this threshold altitude, parasitological indicators such as infection prevalence and community microfilarial load are likely to complement entomological information for the purposes of designing and implementing ivermectin control

programmes (VIVAS-MARTÍNEZ *et al.*, 1998, and in preparation). Above this threshold, all Yanomami villages examined were hyperendemic, establishing that altitude >200 m is an indicator for priority treatment with ivermectin. In fact, as a direct result of this survey mass ivermectin treatment is now being regularly delivered by CAICET to most of the hyper- and mesoendemic communities studied here.

Presence and abundance of *S. oyapockense s.l.* and *S. incrustatum s.l.* were not positively correlated with risk of onchocercal infection in this study. The former was associated with low onchocerciasis endemicity, although there are some doubts as to whether transmission is truly autochthonous in hypoendemic communities. In mesoendemic localities such as Maweti and Mahekoto-theri, heavily infected people could act as reservoirs of infection for these flies, as naturally infective *S. oyapockense s.l.* have been detected. The vectorial role of *S. incrustatum s.l.* will be discussed in subsequent publications.

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