Amazonian onchocerciasis: parasitological profiles by host-age, sex, and endemicity in southern Venezuela

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SUMMARY

This paper describes, for the human onchocerciasis focus of southern Venezuela, the age profiles of *Onchocerca volvulus* microfilarial (mf) and nodule prevalence, mf intensity, and mf aggregation for the whole examined population (836 Yanomami people) living in 20 villages, and for these communities classified according to endemicity levels (hypoendemic: $\leqslant 20\%$; mesoendemic: 21–59%; hyperendemic: $\geqslant 60\%$ infected). Mf prevalence and intensity increased with age, particularly in the hyperendemic areas, and there were no marked differences between the sexes. The prevalence of nodules followed the same age pattern. Fifty percent mf prevalence was reached in the 15–19 year age-class when the population was taken as a whole; nearly in the 10 to 14-year-olds for the hyperendemic level, in those aged 20–29 years in mesoendemic areas, and not reached at all in hypoendemic villages. The degree of mf aggregation was measured by the k value of the negative binomial distribution and by the variance to mean ratio (VMR). The relationship between the standard deviation (S.D.) of mf counts and the mean mf density was also explored. These 3 indices (k, VMR, and S.D.) showed a tendency to increase with both mean mf load and host age. Since infection intensity and host age were themselves positively related, it was not possible to draw definite conclusions about age-specific changes of parasite aggregation. There was not a significant decrease of mf intensity after an earlier peak neither was there a shift towards younger ages of the maximum no. of mf/mg reached as the endemicity level increased. These results are discussed in relation to detection of density dependence in the human host, selection of an indicator age-group for rapid epidemiological assessment (REA) methods, and strategies of ivermectin distribution in the Amazonian focus. It is recommended that, for the Amazonian onchocerciasis focus, the indicator group for REA consists of all those aged 15 years and over.

Key words: onchocerciasis, infection age-profiles, parasite aggregation, indicator group for REA, southern Venezuela, Yanomami.

INTRODUCTION

Previous studies have shown that in the hyperendemic communities of the onchocerciasis focus of southern Venezuela, the prevalence and intensity of microfilarial infection increase with host age, perhaps reflecting a reduced ability of the population to control infection (Yarzábal *et al*. 1983, Basáñez & Yarzábal, 1989; Botto *et al*. 1997). However, age-profiles of infection have not been systematically studied in areas with different levels of endemicity within this focus. The investigation of such profiles is important because they (a) reflect the balance between the average rates of parasite input (immigration) and parasite loss (death) taking place in communities exposed to different transmission intensities (Anderson & May, 1991); (b) have been used to explore the possible operation of regulatory processes acting to constrain parasite population growth (Woolhouse, 1992); (c) underlie patterns of onchocerciasis morbidity (Renz *et al*. 1987), and (d) allow a decision about which age-bands may constitute indicator groups for rapid epidemiological assessment of infection prevalence for control programmes (Knüttgen & Büttner, 1969; Taylor, Duke & Muñoz, 1992). The design of control strategies should also depend on the possible existence of acquired immunity to infection. High levels of herd immunity warrant high levels of coverage to achieve the goal of reducing transmission without causing undesirable changes in the patterns of morbidity (Anderson & May, 1991).

An approach for the detection, by indirect means, of protective immunity in helminth infections of humans has been the examination of age-profiles of infection prevalence, intensity, and aggregation in

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populations challenged by different intensities of transmission. Given the assumption of a gradually acquired protective immunity, it is expected that the intensity of infection would decline with age after a peak, that there would be a shift of this peak towards younger ages as the intensity of transmission increases, and that the degree of parasite overdispersion would decrease with host age (Woolhouse *et al*. 1991; Fulford *et al*. 1992). However, other density-dependent, as well as non density-dependent, mechanisms may generate some or all of these patterns (Fulford *et al*. 1992, 1996; Woolhouse, Ndamba & Bradley, 1994; Quinnell, Grafen & Woolhouse, 1995).

The inspection of age-profiles of infection can also provide information about the epidemiological status of the community permitting identification of indicator age-groups from which indices of infection prevalence, intensity, and associated morbidity can be estimated. Examples of this are the youngest age-class for which microfilarial prevalence reaches 50 % (AI_{50}) as a measure of onchocerciasis endemicity in a population (Knüttgen & Büttner, 1969; Renz *et al*. 1987), and a cohort of adults who are at least 20 years of age for the purposes of measuring epidemiological changes resulting from *Simulium* control in the Onchocerciasis Control Programme of West Africa (Remme *et al*. 1986).

The work presented in this and the next paper forms part of a more extensive study of onchocerciasis epidemiology in the Amazonian focus of southern Venezuela with the aims of generating indicators of risk, occurrence, and severity of infection for rapid epidemiological mapping (REMO) and assessment (REA) within the context of an ongoing ivermectin-based control programme coordinated regionally by CAICET. This paper describes the parasitological profiles of *Onchocerca volvulus* infection and aggregation in the examined population taken as a whole and classified according to levels of endemicity. Vivas-Martínez *et al*. (2000) provide indicators for rapid assessment of onchocerciasis prevalence in the area.

MATERIALS AND METHODS

Study area and parasitological methods

Twenty Yanomami communities (average size ± S.D. equal to 60 ± 37 people per village) were surveyed for the presence of *O. volvulus* infection. These communities were located along 2 river systems: (A) the Ocamo–Putaco rivers, and (B) the Orinoco–Orinoquito rivers. Details of the geography, climate, and vegetation of the area, as well as the altitude, name and location of each village can be found in Vivas-Martínez *et al*. (1998). A sample of 836 people ($\geqslant$ 5 years of age) out of a total population of 1225 were skin-snipped and 766 individuals were examined for onchocercomata. Consent for taking skin biopsies and practising a clinical examination was obtained from each adult individual and parental permission was sought for all children enrolled in the survey. Two biopsies (one from the left and other from the right side) were taken from both the scapular and iliac body regions of each person with a Holth-type corneoscleral punch. Snips were incubated in microtitration plates with buffered saline solution. After 24 h incubation, emerging microfilariae (mf) were counted, identified as *O. volvulus* based on morphological criteria, the snips weighed and the arithmetic mean no. of mf/mg per person calculated.

Data analysis

Cross-sectional age-specific profiles of mf and nodule prevalence, mf intensity, and degree of parasite aggregation were analysed for the whole examined population and for communities grouped according to their level of onchocerciasis endemicity. Since visual inspection of age-related profiles of infection did not show steep changes of intensity and prevalence, broad age-groups were chosen to ensure reasonable sample sizes (Table 1). For each village, mf prevalence was age- and sex-adjusted using the direct method (Kirkwood, 1988). Endemicity levels followed the criteria of the Onchocerciasis Elimination Programme for the Americas, i.e. hypoendemic, mesoendemic, and hyperendemic communities being defined according to mf prevalence of $\leqslant$ 20 %, 21–59 % and $\geqslant$ 60 % respectively (OEPA, 1996). Exact confidence limits were calculated for the proportion of people with mf and nodules in each age-band (Armitage & Berry, 1994). Trends in the proportion infected with age and sex were explored by logistic regression methods with age as a categorical variable, adjusting for non-independence of individuals within communities, and using the youngest age-class and the male sex as the baseline groups. However, because some analyses further subdivided the data into a larger number of categories, the younger age-groups were left with very few infected individuals. In these cases, the baseline was reversed to the oldest age-class.

As in many other macroparasitic infections, the frequency of mf counts among hosts was not normally distributed (see below). In consequence, for purposes of graphical representation, a logarithmic transformation (adding 1 to the mf/mg counts so as to include the zeros) was used. Negative binomial regressions were used to explore the relationship between mf counts (taking into account snip weight), age-group and sex (in the whole study population), and between mf counts, age-group, sex, and endemicity level (in the population classified by prevalence group). A constant value of the parameter k was assumed (this assumption, as a first approximation, was justified from the results presented

Table 1. Age and sex distribution of the source and study populations of 20 Yanomami communities of the Amazonian onchocerciasis focus of southern Venezuela

Age-group (year)	Source population (100%)			Study population (68%)		
	Males (%)	Females (%)	Total (%)	Males (%)	Females (%)	Total (%)
5–9	135 (11·0)	110 (9·0)	245 (20·0)	99 (11·8)	58 (6·9)	157 (18·7)
10–14	100 (8·2)	61 (5·0)	161 (13·2)	75 (9·0)	38 (4·6)	113 (13·6)
15–19	80 (6·5)	64 (5·2)	144 (11·7)	64 (7·7)	42 (5·0)	106 (12·7)
20–29	143 (11·7)	113 (9·2)	256 (20·9)	104 (12·4)	74 (8·9)	178 (21·3)
30–39	108 (8·8)	109 (8·9)	217 (17·7)	69 (8·2)	61 (7·3)	130 (15·5)
40+	112 (9·1)	90 (7·4)	202 (16·5)	85 (10·2)	67 (8·0)	152 (18·2)
Total	678 (55·3)	547 (44·7)	1225 (100·0)	496 (59·3)	340 (40·7)	836 (100·0)

Table 2. Age- and sex-adjusted microfilarial prevalence, its 95% confidence limits, and the corresponding level of endemicity in the communities of the Amazonian onchocerciasis focus, surveyed along the rivers Ocamo–Putaco and Orinoco–Orinoquito, southern Venezuela

Code no.* and village name	Positive/ Examined	Prevalence %	95% CL	Endemicity level
		(A) Ocamo–Putaco		
(3) Kashorawë-theri†	0/39	0·0	0·0–9·0	Hypoendemic
(1) Ocamo	2/117	2·4	0·4–7·3	Hypoendemic
(2) Iyewëi-theri	1/29	5·4	0·4–20·9	Hypoendemic
(15) Yepropë-theri	2/38	5·8	0·7–18·8	Hypoendemic
(8) Maweti-theri	17/46	24·3	12·8–39·3	Mesoendemic
(16) Toothothopiwei-theri	13/46	33·9	20·5–49·4	Mesoendemic
(13) Yoreshiana A	45/78	63·6	51·9–74·2	Hyperendemic
(14) Yoreshiana B	20/32	65·4	46·4–81·3	Hyperendemic
(9) Awei-theri	15/24	66·8	44·5–84·8	Hyperendemic
(23) Niyayowë-theri‡	144/196	77·5	71·0–83·1	Hyperendemic
(10) Pashopeka-theri	32/38	79·8	63·5–91·1	Hyperendemic
		(B) Orinoco–Orinoquito		
(6) Shashanawë-theri	0/12	0·0	0·0–26·5	Hypoendemic
(4) Yohoopë-theri	1/22	2·9	0·0–20·8	Hypoendemic
(18) Purima-theri	6/38	13·9	4·7–29·2	Hypoendemic
(11) Mahekoto-theri	23/54	41·4	28·1–55·7	Mesoendemic
(22) Harau-theri B	12/22	62·9	39·7–82·4	Hyperendemic
(12) Cerrito	20/26	64·6	53·7–89·6	Hyperendemic
(20) Harau-theri A	19/28	66·3	45·8–83·2	Hyperendemic
(21) Hokotopiwei-theri	50/59	74·8	61·5–85·2	Hyperendemic
(17) Hasupiwei-theri	36/45	78·4	63·5–89·3	Hyperendemic
(19) Maiyo-theri	38/43	86·8	72·8–95·2	Hyperendemic

* Location of these villages by code number can be seen in Vivas-Martínez *et al*. (1998).
† Includes 4 people from nearby San Benito, which consisted solely of a family of 6 people. The 2 localities are in close geographical proximity and *S. oyapockense s.l.* is the only man-biting species in both.
‡ Raw data from Yarzábal *et al*. (1983).

by Vivas-Martínez *et al*. 1998). The log-likelihood ratio test (LRT) was used to compare models with and without interaction between the explanatory variables (Clayton & Hills, 1993).

Degree of mf aggregation. Frequency distributions of mf counts per person departed significantly from Poisson and conformed to a negative binomial ($\chi^2 = 47{\cdot}7$, 38 D.F., $P = 0{\cdot}134$). Therefore, negative binomial regression was used to estimate, by maximum likelihood, the parameters of this distribution (mean and k) from raw mf counts taking into account snip weight. Two measures of the degree of parasite clumping in the host population were calculated; the negative binomial aggregation parameter, k, and the variance to mean ratio, VMR. The former is an inverse measure of the degree of dispersion whereas the latter is proportional to the dispersion. In addition, the relationship between the standard deviation (S.D.) and the mean of the mf counts was also explored. Spearman correlation analyses were carried out between the indices of dispersion and mean mf load or host age.

All statistical analyses were performed using Stata ver. 5.0 (Stata® Corporation, College Station, TX).

Table 3. Number of people positive for skin microfilariae out of the total examined by skin-snip in each sex and age-class in 20 Yanomami villages of the Amazonian onchocerciasis focus of southern Venezuela

Age-group (years)	Males	Females
5–9	15/99	11/58
10–14	18/75	10/38
15–19	32/64	20/42
20–29	56/104	33/74
30–39	39/69	29/61
⩾ 40	51/85	38/67
Total	211/496	141/340
Percentage infected	42·5	41·5

RESULTS

Populations of 18 out of the 20 villages investigated were found to be positive for *O. volvulus* mf. Overall prevalence was 42 % (352/836) and varied between 2·4 % and 86·8 % in the villages with non-zero prevalence. Taking into account all 20 villages, 7 of them were identified as hypoendemic, 3 as mesoendemic, and 10 as hyperendemic (see Table 2). The number of people examined and found positive for mf and nodules in each age-class, and endemicity level is presented in Tables 3–5.

Age-profiles

Figure 1 shows the age-profiles of mf prevalence and mf intensity for males and females in the entire examined population. There was no difference between the sexes regarding the proportion infected in the population as a whole (Odds Ratio = 0·96, 95 % CI = 0·72–1·27, $P = 0{\cdot}760$). The prevalence of infection, using the 5 to 9-year-olds as baseline, increased significantly with age from the 10–14 year age-group onwards (all P values $< 0{\cdot}001$, Appendix A). There was no significant interaction between age-class and sex regarding mf status (LRT = 2·17, 5 D.F., $P = 0{\cdot}825$). The intensity of infection increased with age (all P values $< 0{\cdot}05$, Appendix B) but this time sex was borderline significant ($P = 0{\cdot}049$), and the interaction between age-group and sex improved the model (LRT = 18·08, 5 D.F., $P = 0{\cdot}003$).

Figure 2A presents the age-profiles of mf prevalence by endemicity level. At the hyperendemic level, mf prevalence increased significantly with age from the 10 to 14-year-olds onwards (all P values $< 0{\cdot}001$ taking the 5–9 year band as the baseline group, Appendix C). For the mesoendemic villages there was no significant trend in infection status with age-class (Appendix D). In the hypoendemic communities, P values were 0·004 for the 15 to 19-year-olds and 0·049 for the 20–29 year class when those aged ⩾ 40 years were used as the baseline (Appendix E).

Regarding the intensity of mf infection as a function of age, sex, and endemicity level, the negative binomial regression showed no significant effect of sex or of its interaction with either age- or prevalence group. In consequence, males and females were pooled together and the analysis repeated. There was a positive significant effect of both endemicity level and age-class on mf load in the model without interaction (all P values $< 0{\cdot}001$, Appendix F). Consideration of interaction between age-group and endemicity level improved the model significantly (LRT = 60·3, $P < 0{\cdot}001$, 10 D.F.), with this effect being attributable to the interaction between the meso- and hyperendemic prevalence groups and those age-classes ⩾ 15 years (Fig. 2B). The value of the constant parameter k was equal to 0·2454 for the model with interaction.

Figure 2C shows the age profiles of the prevalence of palpable nodules by endemicity level. Values for the hyperendemic village of Niyayowë-theri (original data from Yarzábal *et al.* 1983) are also shown for comparison. Palpable nodules tend to be detected at

Table 4. Number of people positive for skin microfilariae out of the total examined by skin-snip in each age- and endemicity category in 20 Yanomami villages of the Amazonian onchocerciasis focus of southern Venezuela

Age-group (years)	Endemicity level		
	Hypoendemic	Mesoendemic	Hyperendemic
5–9	0/56	0/23	26/78
10–14	0/39	4/19	24/55
15–19	1/33	7/18	44/55
20–29	2/64	19/37	68/77
30–39	2/47	15/28	51/55
⩾ 40	7/56	8/21	74/75
Total	12/295	53/146	287/395
Percentage infected	4·1	36·3	72·7

Table 5. Number of people positive for palpable nodules out of the total examined for onchocercomata in each age- and endemicity category in 20 Yanomami villages of the Amazonian onchocerciasis focus of southern Venezuela

(Data for the hyperendemic village of Niyayowë-theri are included for comparison (see text).)

Age-group (years)	Endemicity level			
	Hypoendemic	Mesoendemic	Hyperendemic	Niyayowë
5–9	0/52	0/23	17/69	7/34
10–14	0/38	0/19	4/48	7/30
15–19	0/31	2/18	8/45	1/29
20–29	7/60	7/37	17/66	8/32
30–39	5/42	1/28	15/49	9/35
⩾ 40	6/53	5/21	40/67	17/35
Total	18/276	15/146	101/344	49/195
Percentage infected	6·5	10·3	29·4	25·1

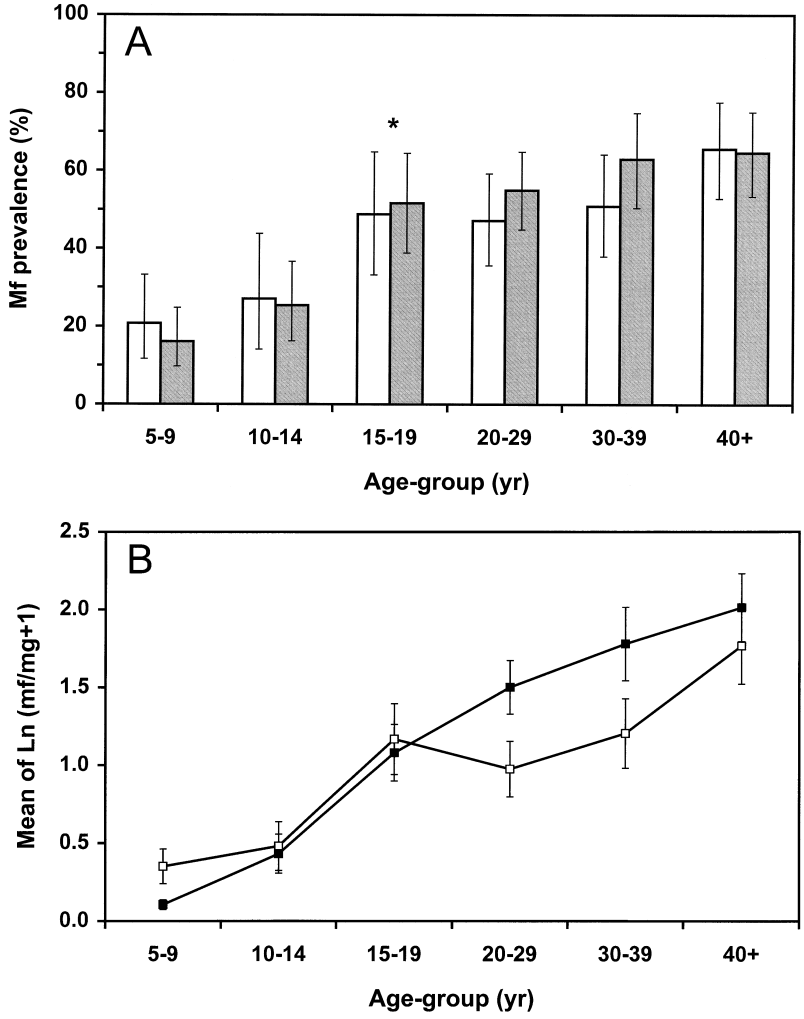

Fig. 1. Age-profiles of microfilarial (mf) prevalence (A) and mf intensity (B) in the whole examined population (n = 836) from the Amazonian onchocerciasis focus of southern Venezuela. White bars and markers: males; grey bars and black markers: females. Error bars for (A) and (B) are, respectively, the 95% confidence limits for prevalence values and the S.E. of the mean mf load; the asterisk indicates the AI_{50} i.e. the youngest age-group for which the proportion infected is ⩾ 50%.

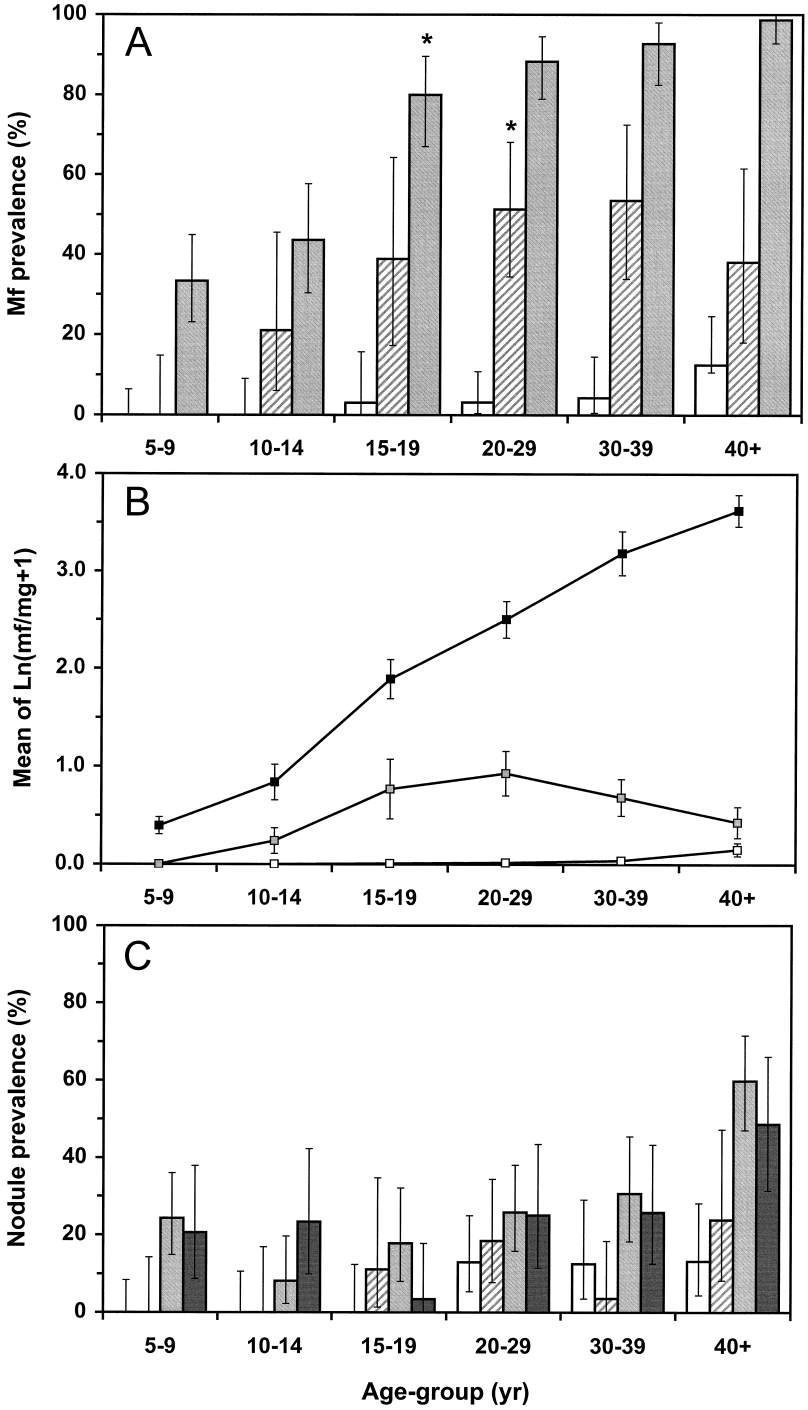

Fig. 2. Age-profiles of mf prevalence (A), mf intensity (B), and nodule prevalence (C) by endemicity level. White bars and markers: hypoendemic villages ($\leqslant 20\%$ prevalence); hatched bars and light-grey markers: mesoendemic localities (21–59%); grey bars and black markers: hyperendemic communities ($\geqslant 60\%$); black bars in (C): hyperendemic village of Niyayowë-theri (see text). Error bars for (A) and (C) are the 95% confidence limits of prevalence values; for (B) they are the S.E. of the mean mf load. Asterisks as in Fig. 1: no age-band reaches 50% prevalence in the hypoendemic level; AI_{50} corresponds to the 20–29 year age-class in the mesoendemic category but the 95% CL of the prevalence for the 15–19 year age-group include 50%; similarly, although the AI_{50} for the hyperendemic villages would be the 15–19 year age-band, the 95% CL of the prevalence for the 10 to 14-year-olds include 50%.

a later age than skin mf at hypo- and mesoendemic levels. In general, the age profiles of nodule prevalence for these two levels were similar to those of mf prevalence, but the trend became significant only when age was considered as a linear term (OR = 1·05, 95% CI = 1·03–1·07, $P < 0·001$ for hypo- and OR = 1·04 (1·00–1·08), $P = 0·030$ for mesoendemic areas). For the hyperendemic category, and taking the 5 to 9-year-olds as baseline, there was a significant increase only for the oldest age-class due

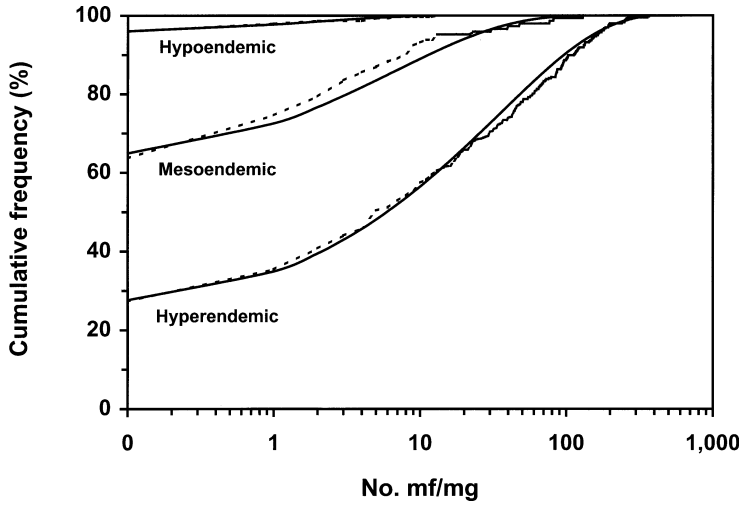

Fig. 3. Cumulative frequency distributions of the number of mf/mg among the examined population grouped by endemicity levels. (- - - - -) Observed values; (———) fitted model (negative binomial) with mean and k values as in the text. Maximum mf load = 12·1 mf/mg for the hypoendemic level; 130·0 mf/mg for the mesoendemic level; 366·0 mf/mg for the hyperendemic level.

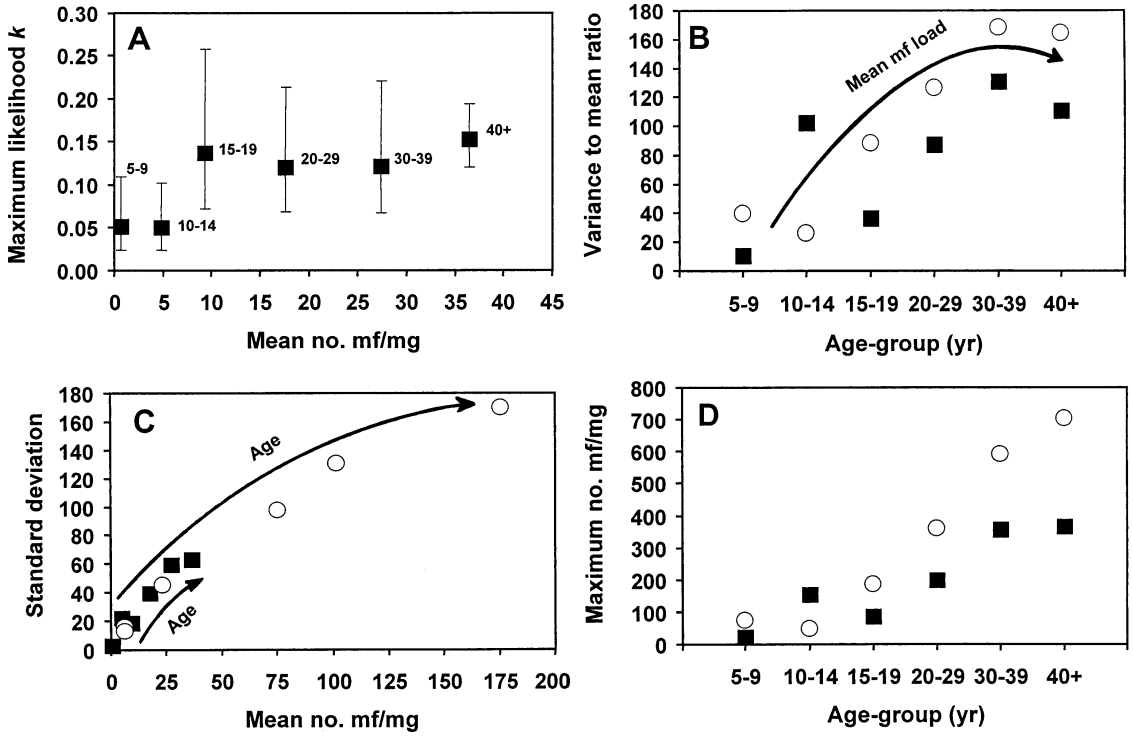

Fig. 4. Indices of parasite dispersion plotted against age-specific mean mf load and age-group for the whole examined population (■) and for Niyayowë-theri (○). (A) Estimate of k and its 95% confidence limits *versus* mean intensity of infection; age-groups identified. (B) *VMR versus* age-band (the arrow indicates the direction of increase in mean mf load). (C) S.D. *versus* mean no. mf/mg (the arrow indicates the direction of increase in host age). (D) Maximum mf burden observed in each age-class.

to the high prevalence of nodules in those ⩾ 40 years (OR = 4·53, 95% CI = 1·81–11·36, $P = 0·001$). The same pattern was observed for Niyayowë-theri.

Aggregation patterns

The distribution of mf counts in the examined population as a whole ($n = 836$) was strongly aggregated ($VMR = 113·28$, $P \ll 0·001$ when compared to Poisson), with a mean of 16·57 mf/mg and a k estimate of 0·0911 (95% CL(k) = 0·0811–0·1025). Figure 3 shows the observed and expected cumulative frequency distributions by endemicity level. As the prevalence of onchocerciasis increases, the tail of the distribution is extended, indicating a higher proportion of individuals with heavier mf loads. The

mean mf burden per person, the k value (95% CL), and the VMR increased with endemicity: 0·11 mf/mg, $k = 0{\cdot}0134$ (0·0058–0·0306), $VMR = 7{\cdot}02$ for the hypoendemic level; 3·96 mf/mg, $k = 0{\cdot}0920$ (0·0574–0·1474), $VMR = 58{\cdot}11$ for the mesoendemic category, and 33·53 mf/mg, $k = 0{\cdot}2318$ (0·2026–0·2651), $VMR = 99{\cdot}72$ for the hyperendemic class). Figure 4 shows, for all communities analysed together, the changes with mean mf burden and age-class in parasite dispersion when this is measured by the negative binomial parameter k (Fig. 4A), the VMR (Fig. 4B), and the S.D. of the mf counts (Fig. 4C). The maximum mf density recorded for each age-band is also shown (Fig. 4D). For the VMR and S.D. *versus* mean, but not for k, data from the hyperendemic village of Niyayowë-theri are included for comparison. The age-specific sample sizes in Niyayowë-theri did not allow for a reliable estimation of the k values; for the same reason, age-specific k values were not calculated separately for each endemicity level. All 3 indices showed a tendency to increase with age-group and intensity of infection, but correlations between k and mean mf load or mean age, and between VMR and mean mf density or mean age were not highly significant for the communities studied in this work ($r_S = 0{\cdot}771$, $P = 0{\cdot}072$, 4 D.F., in all cases). For the hyperendemic village of Niyayowë-theri, correlations were strongly significant between mean intensity and mean age ($r_S = 0{\cdot}943$, $P = 0{\cdot}005$), between VMR and mean intensity (0·943, $P = 0{\cdot}005$), and between VMR and mean age (0·886, $P = 0{\cdot}019$). Therefore, there is strong confounding between host age and the intensity and dispersion of microfilarial infection. However, for the 40+ year-olds, the dispersion would seem to decrease or show a tendency to level off.

Age-group with 50% mf prevalence

Fifty percent prevalence was already reached in the 15–19 year age-group in the population taken as a whole (Fig. 1A) and in the hyperendemic villages (Fig. 2A). However, the 95% CL for the prevalence of the previous age-band (10–14 years) included 50%. By contrast, this level of infection was reached in the 20–29 year age-class in mesoendemic localities (confidence limits overlapped widely with those of the 15–19 year age-group). No such prevalence was reached in hypoendemic areas.

DISCUSSION

By contrast with other helminth infections of humans such as schistosomiasis and those caused by intestinal worms, in which mean worm load peaks in the younger age-groups, the intensity of onchocerciasis in the Amazonian focus of southern Venezuela increases progressively with age, particularly in the hyperendemic areas. This is in agreement with previous studies in areas of high transmission within this focus (Yarzábal *et al*. 1983, Basáñez & Yarzábal, 1989; Botto *et al*. 1997) which, as in the present work, did not find marked differences between the sexes. In the hypo- and mesoendemic villages, infection tended to increase slightly or to level off. Microfilarial and nodule burdens which either reach a plateau or increase nearly monotonically with age have been reported in savanna and forest areas of West and Central Africa when transmission ranges from low and moderate to very intense (Duke, Moore & Anderson, 1972; Anderson *et al*. 1974; Duke, Anderson & Fuglsang, 1975; Thylefors, Philippon & Prost, 1978; Renz *et al*. 1987). However, these authors report a marked difference between the sexes, with males more heavily infected than females. The relative contribution of physiological and behavioural factors to this difference has long been debated (Bundy, 1988). The results presented here suggest that exposure may be important in explaining the observed patterns. The Yanomami males and females are equally engaged in activities exposing them more evenly to blackfly bites from an early age (see also Renz, Fuglsang & Anderson, 1987). However, there was a borderline significant effect of sex and a significant interaction between sex and age-class when the age profile of mf burden was analysed for the population as a whole. Such effects were not detected when the profiles were explored for the population further subdivided into prevalence levels.

A decline in mean worm burden with age following an earlier peak, particularly in areas of intense transmission, has been interpreted as evidence of the operation of processes constraining parasite population growth such as acquired immunity and parasite-induced host mortality, and/or host age-related changes in exposure or parasite survival and fecundity (Anderson & May, 1991). In addition, a peak shift towards younger ages with increasing infection intensity in the community has been documented in schistosome (Woolhouse *et al*. 1991; Fulford *et al*. 1992) and lymphatic filarial infections (Michael & Bundy, 1998). The results presented here show an opposite trend, with higher mean mf loads being reached at older ages as the endemicity level increases (see also Basáñez & Boussinesq (1999) for Cameroonian savanna villages). For the mesoendemic level, mf loads did not show an increase with host age, and the interaction between age-class and endemicity level was significant. This effect could be due to a small number of mesoendemic communities or to a genuine biological effect. A similar pattern between mf intensity, age-class, and prevalence group has been reported in forest areas of Cameroon (Bradley *et al*. 1998). Not only does the mean mf burden increase with age in highly endemic areas but also the tails of the frequency distributions

become longer, indicating a higher proportion of individuals with heavier worm loads. These distributions are strongly aggregated, with a low value of negative binomial k (0·0911) for the mf counts in the examined population as a whole. This value is lower than a previous estimate (0·2493) based on the relationship between age- and sex-adjusted mf prevalence and intensity at the community level (Vivas-Martínez *et al*. 1998), possibly due to the larger amount of variation present in the data before stratifying by age-class and/or endemicity level. For the model with interaction between age-class and endemicity level, the value of k (0·2454) was very similar to the previous estimate.

The inspection of age-related changes in the degree of parasite aggregation has also been used to detect the possible operation of density-dependent effects. The argument is that a decrease of overdispersion with host age (a surrogate of cumulative exposure) may reflect processes shortening the tails of the distributions by acting on the most heavily parasitized hosts (Gordon & Rau, 1982). Alternatively, changes in the heterogeneity of host exposure with age, and aggregation of infection events may produce similar patterns (Fulford *et al*. 1992; Woolhouse, Ndamba & Bradley, 1994; Quinnell, Grafen & Woolhouse, 1995). The results presented here would suggest a decreasing degree of parasite clumping with host age when aggregation is measured by k but an increasing degree of overdispersion according to the variance to mean ratio (with the possible exception of the oldest age-group). However, a problem when detecting overdispersion patterns in helminth studies using the indices applied here (the most commonly used in the parasitological literature), is that mean infection intensity and its variance are often confounded with host age, and it is difficult to break this confounding. In fact, both the S.D. of mf counts and the mean mf density increased with age in this study, in contrast with the results obtained for schistosome infections (Fulford *et al*. 1992). Other techniques, which fit models to data exploring explicitly the effect of age on variation of parasite counts have been applied successfully to larger data sets (Fulford *et al*. 1992, 1996).

Although the existence of apparently uninfected individuals exposed for long periods in areas of high transmission has suggested the operation of protective immunity in onchocerciasis (the so-called putative immune, Elson *et al*. 1994), acquired resistance may be very slow to develop. Resistance to infection in onchocerciasis seems to be more related to the cellular than to the humoral arm of the immune response (Bradley *et al*. 1995), with stronger Th1-type responses in the putative immune (Lüder *et al*. 1996) as opposed to antibody-dominated responses in the heavily infected (biased towards Th2, Elson *et al*. 1995). Parasite antigens have been found to immunosuppress cellular responses both *in vivo* (Arango *et al*. 1983) and *in vitro* (Elkhalifa *et al*. 1991), a phenomenon reversed by ivermectin treatment (Soboslay *et al*. 1994). In hyperendemic areas, where transmission is already intense at a young age, the process of early infection may predispose (tolerize) most individuals to acquiring increasingly higher worm burdens as age progresses (Maizels & Lawrence, 1991), explaining the increase in intensity of infection observed in Figs 1 and 2. The maximum mf density also increased with host age both in the communities studied here and in Niyayowë-theri. In those situations where hosts are immunocompromised, or a significant proportion of the population develops immunological tolerance, parasite loads may carry on increasing and peak shifts might not apply (Woolhouse, 1998).

The lack of a marked difference in mf prevalence and intensity between Yanomami males and females; the observation that prevalence may reach at least 50% from the 15–19 year age-group onwards; the fact that this age-group is more amenable to examination than Yanomami children, and the possibility that a sample comprising only adult males aged $\geqslant 20$ years as recommended for Africa (Taylor, Duke & Muñoz, 1992) could be too scanty in already small Yanomami villages (a mean of 18 adult males per village), all suggest that the indicator group for rapid epidemiological assessment in the Amazonian focus might consist of all individuals aged $\geqslant 15$ years (both sexes included). This section of the population would comprise on average 41 people, and provide a sample size of approximately 28–30 individuals per community. Yanomami communities do not usually exceed 200 inhabitants, so the sampling issues raised for larger villages (WHO, 1992) would not apply here.

The indicator groups, proposed here and by WHO (1992), both differ from that recommended by Knüttgen & Büttner (1969), which was either the AI_{50} (the youngest age-band with a mf prevalence $\geqslant 50\%$), or the group comprising those aged $\leqslant 14$ years (from whom mf prevalence would be estimated). These authors were more interested in classifying communities into broad categories of endemicity than in estimating their point prevalences, an objective of REA methods. When mf prevalence varied between 0 and 17% in the whole village (their study was conducted in Guinea), no age-group reached 50% prevalence, in agreement with the results obtained for hypoendemic communities of the Amazonian focus ($\leqslant 20\%$ mf prevalence); for prevalences ranging from 17 to 50%, the AI_{50} comprised those in the 15–29 year age-band (similar to the mesoendemic setting analysed here); for values of 50 to 67% mf prevalence in the general population, AI_{50} corresponded to the 10 to 14-year-olds, close to the results for the hyperendemic level as defined in this work ($\geqslant 60\%$ infected with mf).

Vivas-Martínez *et al*. (2000) use the results presented here to propose, for the selected indicator age-group, rapid methods for prevalence assessment in the Amazonian focus based on parasitological and other indicators already described (Vivas-Martínez *et al*. 1998), and evaluate the ability of such indicators, used alone or in combination, to correctly allocate communities into categories of high and low priority for mass treatment with ivermectin.

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Appendix A. Odds ratios (OR) and significance levels for logistic regression of *O. volvulus* microfilarial prevalence on sex and age-class for the whole data set ($n = 836$) examined in southern Venezuela. The baselines are the male sex and the 5–9 year age-group

Variable	OR	95% CI	*P*
Sex (Female)	0·86	0·64–1·15	0·302
Age-group 2 (10–14 years)	1·65	0·78–3·51	0·191
Age-group 3 (15–19 years)	4·88	2·67–8·91	< 0·001
Age-group 4 (20–29 years)	5·08	2·86–9·04	< 0·001
Age-group 5 (30–39 years)	5·62	3·30–9·57	< 0·001
Age-group 6 (⩾ 40 years)	7·21	3·92–13·27	< 0·001

Appendix B. Coefficients and significance levels for negative binomial regression of *O. volvulus* microfilarial load on host sex, age-class, and interaction between sex and age-groups for the whole data set ($n = 836$) examined in southern Venezuela. The baselines are the male sex and the 5–9 year age-group

Variable	Coefficient*	95% CI	*P*
Sex (Female)	1·01	0·00–2·01	0·049
Age-group 2 (10–14 years)	2·92	0·69–5·16	0·010
Age-group 3 (15–19 years)	2·93	0·65–5·21	0·012
Age-group 4 (20–29 years)	3·52	1·52–5·52	0·001
Age-group 5 (30–39 years)	3·80	1·62–5·99	0·001
Age-group 6 (⩾ 40 years)	3·69	1·60–5·78	0·001
Age-group 2 × Sex	−2·55	−4·10–−1·01	0·001
Age-group 3 × Sex	−2·04	−3·58–−0·51	0·009
Age-group 4 × Sex	−2·44	−3·78–−1·09	< 0·001
Age-group 5 × Sex	−2·51	−3·95–−1·08	0·001
Age-group 6 × Sex	−2·14	−3·53–−0·75	0·003

* Ln (mean mf load).
The log-likelihoods were −2359·21 and −2368·25 for the models with and without interaction, respectively.

Appendix C. Odds ratios (OR) and significance levels for logistic regression of microfilarial prevalence on age-class for hyperendemic onchocerciasis in southern Venezuela ($n = 395$). The baseline is the 5–9 year age-group

Variable	OR	95% CI	*P*
Age-group 2 (10–14 years)	1·55	0·65–3·69	0·323
Age-group 3 (15–19 years)	8·00	3·83–16·69	< 0·001
Age-group 4 (20–29 years)	15·11	7·69–29·70	< 0·001
Age-group 5 (30–39 years)	25·50	10·73–60·60	< 0·001
Age-group 6 (⩾ 40 years)	148·00	30·53–717·51	< 0·001

Appendix D. Odds ratios (OR) and significance levels for logistic regression of microfilarial prevalence on age-class for the onchocerciasis mesoendemic level in southern Venezuela ($n = 123$). The 5–9 year age-group was dropped (23 negative observations). The baseline is the ⩾ 40 year age-group

Variable	OR	95% CI	*P*
Age-group 2 (10–14 years)	0·43	0·04–4·50	0·484
Age-group 3 (15–19 years)	1·03	0·19–5·71	0·969
Age-group 4 (20–29 years)	1·72	0·73–4·04	0·217
Age-group 5 (30–39 years)	1·88	0·58–6·02	0·291

Appendix E. Odds ratios (OR) and significance levels for logistic regression of microfilarial prevalence on age-class for the onchocerciasis hypoendemic level in southern Venezuela ($n = 200$). Age groups 5–9 years (56 negative observations) and 10–14 years (39 negatives) were dropped. The baseline is the ⩾ 40 year age-group

Variable	OR	95% CI	*P*
Age-group 3 (15–19 years)	0·22	0·08–0·62	0·004
Age-group 4 (20–29 years)	0·23	0·05–1·00	0·049
Age-group 5 (30–39 years)	0·31	0·04–2·44	0·267

Appendix F. Coefficients and significance levels for negative binomial regression of *O. volvulus* microfilarial load on host age-class and endemicity level for the whole data set ($n = 836$) examined in southern Venezuela. Baselines are the 5–9 year age-group and the hypoendemic level

Variable	Coefficient*	95% CI	*P*
Age-group 2 (10–14 years)	1·88 ± 0·32	1·26–2·50	< 0·001
Age-group 3 (15–19 years)	2·86 ± 0·33	2·22–3·50	< 0·001
Age-group 4 (20–29 years)	3·57 ± 0·29	3·00–4·15	< 0·001
Age-group 5 (30–39 years)	3·71 ± 0·30	3·11–4·30	< 0·001
Age-group 6 (⩾ 40 years)	4·56 ± 0·32	3·94–5·19	< 0·001
Endemicity level 2 (mesoendemic)	4·06 ± 0·30	3·48–4·65	< 0·001
Endemicity level 3 (hyperendemic)	6·16 ± 0·24	5·69–6·64	< 0·001

* Ln (mean mf load).
The log-likelihoods were −2137·51 and −2167·66 for the models with and without interaction, respectively.

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