

Loiasis: the individual factors associated with the presence of microfilaraemia

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No microfilariae are detectable in a significant percentage of those infected with the filarial worm *Loa loa*. While the probability of an infected individual becoming microfilaraemic is known to increase with age, the mechanisms underlying this trend are not well understood. Epidemiological data from an endemic village in central Cameroon were therefore explored, in an attempt to determine if, after taking into account any history of filaricidal treatment, the presence of *Loa* microfilaraemia in an individual was related to his/her gender, age, and/or exposure to the human-infective larvae of the parasite. An index of exposure, based on the monthly transmission potentials of the *Chrysops* in each of the main types of vegetation in a village and on the activity schedule of each inhabitant of the village, was developed. The results of the data analysis confirm that the acquisition of microfilaraemia is gender-dependent (males generally being more likely to be microfilaraemic than females), and indicate that, in males, a high level of exposure to infective larvae determines the shift from amicrofilaraemic to microfilaraemic status. They also indicate that filaricidal treatments have a long-lasting suppressive effect on *Loa* microfilaraemia, an observation that may have important implications for any strategy to limit the risk of *Loa*-associated encephalopathy following ivermectin treatment.

Following treatment with ivermectin, individuals harbouring high *Loa loa* microfilarial loads may develop serious adverse reactions, including a fatal encephalopathy (Gardon *et al.*, 1997a). There is therefore a particular interest in identifying the factors that are associated with the presence or absence of *Loa* microfilaraemia in individuals living in endemic areas. Curiously, microfilariae (mff) are never detected in samples of peripheral blood from many people who — because they have each suffered from the

sub-conjunctival migration of at least one adult worm — are known to have been infected with *L. loa* (Kershaw, 1950; Dupont *et al.*, 1988; Pinder, 1988). The mechanisms underlying this phenomenon, of so-called 'occult loiasis', are obscure (Wahl and Georges, 1995). In their study of the village of Ngat, in the Central province of Cameroon, Garcia *et al.* (1999) found about 60% of the population to be genetically predisposed to become microfilaraemic when infected with *L. loa*, although this predisposition did not account for all the variation seen in the risk of developing microfilaraemia. Elsewhere in central Cameroon, a detailed analysis of *L. loa* infection in a population of 8467

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people (Pion *et al.*, 2004) confirmed that, as previously observed (Kershaw *et al.*, 1953; Rippert *et al.*, 1977, 1980), the probability of becoming microfilaraemic increases with age, in a gender-dependent fashion. Males were found to show a progressive increase between the ages of 15 and 50 years, followed by a plateau, whereas females showed no significant increase between the ages of 15 and 50 years but did show one in later life (Pion *et al.*, 2004). An increase in the prevalence of microfilaraemia with age is expected because the risk of an individual being infected, via the bite of an infected *Chrysops*, increases with time, and because a person who has become microfilaraemic remains so throughout life (Van Hoegaerden *et al.*, 1987; Noireau *et al.*, 1989; Garcia *et al.*, 1995). It is also possible that, at any given age, the probability of becoming microfilaraemic increases with the level of exposure to the bites of infected *Chrysops* [i.e. to the number of the human-infective, third-stage larvae (L₃) of *L. loa* that are inoculated]. Surprisingly, in their multivariate analysis of data from 667 villagers in Ngat, Garcia *et al.* (1995) observed that, after adjustment for age, neither occupation (categorized as forest-related and not forest-related) nor the time spent outdoors between 06.00 and 10.00 hours (categorized as <50% of the time or at least 50%) affected the prevalence of *Loa* microfilaraemia. At the time of this study, however, the level of endemicity in Ngat was clearly fairly high (37.6% of all adults were microfilaraemic, carrying a mean of 18,148 mff/ml blood). In such a setting, where almost everyone is highly exposed to infection, it is perhaps genetic predisposition that is the main factor governing the presence/absence of a *Loa* microfilaraemia in a given individual. To help clarify this issue, the present study was based in a village in central Cameroon where the level of endemicity for loiasis was lower than that in Ngat. The main aim of this study was to evaluate whether the presence of *Loa* microfilaraemia was related to gender, age

and/or the level of exposure to L₃ over the previous 10 years.

SUBJECTS AND METHODS

Study Area and Population

The study was performed in Kokodo, a village located (at 4°12'N, 11°17'E) in an area of degraded forest, in the Lékié division of the Central province of Cameroon. Two criteria were considered when selecting this village as the study area. Firstly, the level of loiasis endemicity had to be relatively moderate, and secondly the environment (especially the vegetation) had to be sufficiently heterogeneous that individuals living in the area would exhibit a wide range of exposure to *Chrysops*.

According to the latest national census, 1177 people lived in Kokodo in 1987. In 1996, as part of a study of risk factors associated with post-ivermectin, *Loa*-associated encephalopathy (Gardon *et al.*, 1997a), the prevalence of *Loa* mff among 69 of the residents of Kokodo who were aged 15 years was found to be 29%. At that time, the arithmetic mean of the counts of mff in the villagers found to be microfilaraemic was 10,401 mff/ml (i.e. about half that recorded in Ngat). The main occupations of the male and female residents of Kokodo are cocoa farming and the growing of food crops/housework, respectively.

Parasitological Survey

In April 1999, the villagers of Kokodo who were then at least 15 years of age were invited to participate in a parasitological survey, so that the prevalences and intensities of *Loa* microfilaraemia could be evaluated. A 50- μ l sample of fingerprick blood was collected, between 10.00 and 16.00 hours, from each subject who gave his or her informed consent, and used to make a thick smear. The smears were then Giemsa-stained before being fully checked for *Loa*

mff. All *Loa* mff detected were counted. Individual microfilarial status (IMS) was simply coded 1 (when at least one microfilaria was observed in the thick bloodsmear) or 0 (when no mff were observed). Each subject was told the result of the examination of the smear of his or her blood.

Inclusion Criteria

A standardized questionnaire and interview were used, immediately after the collection of the blood sample, to collect relevant information from each participant. The gender and age of each subject, and detailed information on the dates on which he or she had taken a filaricidal drug, on the periods (of at least 1 month) he or she had spent away from Kokodo and neighbouring communities (especially in a town), and on his or her daily and seasonal patterns of activity over the previous year (see below) were recorded.

As filaricidal drugs, including ivermectin, are known to have a long-lasting effect on *Loa* microfilaraemia (Gardon *et al.*, 1997b), only the data on the subjects who said that they had not ingested such drugs in the 3 years prior to the interviews were included in the final analyses. The data on the villagers who reported spending >24 months beyond Kokodo and its neighbouring communities in the previous 10 years were excluded, so that the villagers included in the analysis would reflect the risk of infection in the study area.

Evaluation of the Level of Exposure to New Infections with *Loa loa* during the Last Decade

To estimate the decadal transmission potential (DTP) for each subject (i.e. the number of *L. loa* L₃ potentially inoculated over the previous 10 years), the data collected in the interviews were combined with those collected by Demanou *et al.* (2001) during an entomological survey in the study village. For the entomological survey, *Chrysops* were collected, over three consecutive days every

2 weeks, between May 1999 and April 2000 (Demanou *et al.*, 2001). The flies were caught at 10 sampling stations selected to be representative of four types of vegetation: open areas near households; crop fields; cocoa plantations; and unfarmed forest. During each collection period, two people maintained a woodfire at each sampling station between 07.00 and 18.00 hours, in order to attract the vectors (Duke, 1955). The *Chrysops* attracted were caught, dissected and examined for *Loa* infection. The results of the collections and dissections were then used to estimate two entomological indicators for each month in each vegetation type: the mean monthly biting rate (MBR; the theoretical number of *Chrysops* bites/person-month) and the monthly transmission potential (MTP; the theoretical number of *L. loa* L₃ inoculated/person-month). The results of the entomological study by Demanou *et al.* (2001) showed that: (1) *C. silacea* was probably the main vector in the area (representing 92.1% of the flies caught, the rest being *C. dimidiata*); (2) the intensity of transmission increased progressively from the open areas to the forest (open areas < crop fields < cocoa plantation < forest); and (3) there were three seasonal peaks in the intensity of transmission — one in April (the short rainy season), one in August–September (the beginning of the long rainy season), and one in December (the beginning of the long dry season). The locations of the sampling stations are shown on a map of Kokodo by Demanou *et al.* (2001), together with the MBR and MTP for each month and each vegetation type.

Information on each subject's main activities, and on the type of field (either cocoa plantation or crop field) that they cultivated, was gathered during the interviews. This was used, retrospectively, to establish a time-table of each person's field activities during the 12 months preceding the parasitological examinations, and the number of years this time-table had been followed over the preceding decade. A DTP, expressed as

the number of L_3 inoculated/decade, was then calculated for each subject, using the formula:

$$DTP = \prod_a \sum_v \sum_{j=1}^{12} MTP_{v,j} T_{v,j}$$

where a is the number of years of the last decade during which the individual's field-activity time-table was followed, j represents the month ($j=1$ for January), v the type of field cultivated (cocoa plantation or crop field), and $T_{v,j}$ the time spent in field v during month j ($T=0$ if the individual did not work in field of type v during month j , $T=1$ if he/she worked full-time, and $T=0.5$ if he/she worked part-time).

Statistical Analysis

Version 10.0 of the SPSS software package (SPSS Inc, Chicago, IL) was used to analyse the data. The association between presence of *Loa* microfilaraemia (IMS) and each of four potentially explanatory variables — gender, age, having received a filaricidal treatment before mid-1996, and DTP — was explored. The participants were divided into three age-groups, of villagers aged 15–29, 30–49 and 50 years. Those who had received at least one filaricidal treatment before mid-1996 were coded 1, and those who had never received any filaricidal drug were coded 0. Lastly, the subjects were divided into two groups according to their DTP: a 'high-exposure' group (DTP >14,000 L_3 /decade) and a 'low-exposure' group (DTP ≤14,000 L_3 /decade). The threshold value of 14,000 L_3 /decade was chosen to split the study population into two groups of similar size.

The analysis was done in two steps. Univariate analyses [χ^2 tests and calculation of odds ratios (OR) with 95% confidence intervals (CI)] were run first, to verify whether there were associations between each variable and the IMS. A multivariate analysis was then performed, using logistic

regression models. These models were assessed using a backward stepwise procedure, starting from a full model, including two- and three-way interaction terms between gender, age and exposure (see below). At each step, the significance of the variables was tested using the Wald statistic at a level of 5%, and likelihood-ratio tests were used to evaluate improvement in the model. For the final models, goodness of fit was assessed using the test described by Hosmer and Lemeshow (1989).

RESULTS

Description of the Population

Of the 441 subjects who were examined, 258 were excluded from the data analysis only because they had received filaricidal treatment (mainly with ivermectin) in the 3 years prior to interview (91), only because they had spent <8 years in or close to Kokodo during the last decade (117), or for both of these reasons (50). Of the remaining 183 subjects (the 86 males and 97 females) who were included in the analysis, all but one (a women who was seriously physically handicapped and claimed never to leave her household) had a DTP >0, and 124 (68%) had never been treated with a filaricidal drug. Table 1 presents the distributions of the individuals included in the data analysis, split by age, DTP, and filaricidal treatment before mid-1996.

Prevalence and Univariate Analysis

The prevalence of *Loa* microfilaraemia and the mean, standard deviation and median of the DTP in the different age-groups and two gender classes are given in Table 2.

The prevalence of *Loa* microfilaraemia was found to be significantly higher in the male subjects than in the female (37.2% *v.* 21.6%; $P=0.022$; OR=2.15; CI=1.12–4.16), and in those who had never received any filaricidal treatment than in those who had been treated (prior to mid-1996) (33.9% *v.* 18.6%; $P=0.034$; OR=2.24;

TABLE 1. The numbers of individuals included in the data analysis, split by age, gender, history of filaricidal treatment, and exposure [measured as the number of third-stage *Loa loa* larvae (L_3) theoretically inoculated in the previous decade]

Gender	Age (years)	No previous treatment, and exposed to:*		Previous treatment, and exposed to:*	
		≤ 14,000 L_3 /decade	>14,000 L_3 /decade	≤ 14,000 L_3 /decade	>14,000 L_3 /decade
Male	15-29	9	11	1	1
	30-49	2	20	2	5
	≥50	1	18	2	14
Female	15-29	13	6	5	0
	30-49	19	3	5	10
	≥50	16	6	13	1

*'Previous treatment' refers to treatment with diethylcarbamazine or ivermectin received before mid-1996 (i.e. >3 years before the parasitological examinations in April 1999).

CI=1.05-4.75). It was also higher in the subjects in the high-exposure group than in those in the low-exposure group but the difference was not statistically significant (33.7% *v.* 23.9%; $P=0.145$; OR=1.62; CI=0.85-3.10). The prevalences also differed with age-group, with values of 17.4%, 31.8% and 33.8% recorded for the subjects aged 15-29, 30-49, and ≥50 years, respectively ($P=0.13$). When the youngest age-group was taken as the reference, the OR for the presence of *Loa loa* microfilaraemia in the subjects aged 30-49 (OR=2.22; CI=0.88-5.57; $P=0.091$) and ≥50 years (OR=2.43; CI=0.98-6.00; $P=0.056$) were of borderline, statistical significance.

Multivariate Analysis

Most (80.2%) of the male subjects but only 26.8% of females were exposed to DTP

exceeding 14,000 L_3 /decade (Table 1), and the DTP for the males were generally much higher than those for the females (a Wilcoxon's non-parametric test giving a P -value of <0.001). Surprisingly, the prevalence of *Loa loa* microfilaraemia amongst the 71 female subjects assigned to the low-exposure group was far higher than that amongst the 26 females assigned to the high-exposure group (28.2% *v.* 3.8%). The corresponding values for the males were 5.9% in the low-exposure group ($N=17$) and 44.9% in the high-exposure ($N=69$). In the light of these observations, which (although the number of females in the high-exposure group was fairly low) indicate that males and females do not have the same pattern of response to exposure, three models — of the total population, of the male population, and of the female population — were developed.

TABLE 2. Prevalences of *Loa loa* microfilaraemia and decadal transmission potentials (DTP) for the subjects included in the data analysis, split by age and gender

		Males			Females					
Age (years)	No.	Prevalence (%)	Mean DTP (S.D.) and [median] (L_3 /decade)			No.	Prevalence (%)	Mean DTP (S.D.) and [median] (L_3 /decade)		
			15-29	22	22.7			13,110	(8540)	[14,351]
30-49	29	41.4	22,888	(10,906)	[23,829]	37	24.3	13,921	(7579)	[12,454]
≥50	35	42.9	29,572	(11,191)	[28,622]	36	25.0	13,718	(6631)	[11,563]
≥15	86	37.2	23,107	(12,263)	[23,363]	97	21.6	13,041	(7658)	[11,893]

L_3 , Third-stage larvae of *L. loa*.

As indicated above, four variables, namely gender (only in the total-population model), age, previous filaricidal treatment and DTP, were included in the models, as main-effect factors. Interaction terms between gender, age and exposure (i.e. gender \times exposure and gender \times age \times exposure for the total-population model and age \times exposure for all three models) were also considered. With DTP, the cut-off value was kept at 14,000 L_3 /decade for the total-population model but — as this variable differed markedly between males and females — changed to 23,000 L_3 /decade for the male-population model, and to 12,000 L_3 /decade for the female-population model. As previously, these threshold values were chosen to give similar numbers of individuals of each gender in the high- and low-exposure groups: 47 males and 42 females in the high-exposure, and 42 males and 55 females in the low-exposure. The reference categories in each of the models were females, age of 15–29 years, an history of filaricidal-drug treatment before mid-1996, and low exposure. The results presented in Table 3 correspond to the last iteration of the selection process. The factors found to be

not significantly associated with the presence of a *Loa* microfilaraemia are therefore not included in this table.

The total-population model ($N=183$) revealed that the presence of a *Loa* microfilaraemia was significantly associated with the male gender and high exposure and that there was a confounding effect between gender and exposure. This model also indicated that, among those who had not received any filaricidal treatment before mid-1996, the probability of having a *Loa* microfilaraemia was approximately double that of the other subjects (OR=2.18; CI=0.99–4.80), although this trend was only of borderline statistical significance. Age, either as a main-effect factor or included in an interaction term with exposure, was not found to be associated with IMS.

In the model restricted to the male population, DTP and absence of previous filaricidal treatment were still associated with *Loa* microfilaraemia. In the model restricted to the female population, however, no variables were found to be significantly associated with the presence of *Loa* microfilaraemia.

TABLE 3. Coefficients and odds-ratios [with their 95% confidence intervals (CI)] estimated, by logistic regression, from the model of the total population included in the analysis and from the model of the male subpopulation*

Variable	Regression coefficient and (S.D.)	P	Odds-ratio and (CI)
TOTAL-POPULATION MODEL			
Gender (male)	2.97 (1.05)	0.005	19.43 (2.48–152.50)
Exposure [$>14,000$ third-stage larvae (L_3)/decade]	2.24 (1.06)	0.034	9.40 (1.19–74.51)
Exposure ($>14,000$ L_3 /decade) \times gender (male)	-4.85 (1.50)	0.008	0.69 (0.00–0.15)
Absence of previous treatment	0.78 (0.40)	0.055	2.18 (0.99–4.80)
Constant	-3.73 (1.07)	<0.001	–
MALE-POPULATION MODEL			
Exposure ($>23,000$ L_3 /decade)	1.21 (0.50)	0.017	3.34 (1.25–8.97)
Absence of previous treatment	1.75 (0.63)	0.006	5.73 (1.66–19.57)
Constant	-2.50 (0.69)	<0.001	–

*The total- and male-population models have non-significant Hosmer and Lemeshow goodness-of-fit statistics ($P=0.19$ with seven degrees of freedom, and $P=0.99$ with three degrees of freedom, respectively). The reference categories were females, age 15–29 years, an history of filaricidal drug treatment before mid-1996 (i.e. 'previous treatment'), and a decadal transmission potential (DTP) of $<14,000$ L_3 /decade for the total-population model and of $<23,000$ L_3 /decade for the male-population model.

DISCUSSION

Few studies have addressed the issue of the relationships, at the level of the individual, between the intensity of exposure to filarial parasites and the outcome of infection. The main reason for this is the difficulty in measuring the degree of exposure at an individual level. Several such studies have been conducted on onchocerciasis (Renz *et al.*, 1987; Bockarie and Davies, 1990; Cadot *et al.*, 1998) but little information is available regarding loiasis. The only relevant study on loiasis appears to be the one performed by Garcia *et al.* (1995) in Ngat, Cameroon.

In the total-population model developed in the present study, gender and exposure were found to be associated with the presence of *L. loa* microfilaraemia. In this model, an history of filaricidal treatment taken >3 years previously was found to be not quite significantly associated with microfilaraemia.

Males were more likely than females to present with a *Loa* microfilaraemia. This bias has been reported in most surveys on loiasis (Kershaw *et al.*, 1953; Ripert *et al.*, 1977, 1980), and was recently confirmed by Pion *et al.* (2004), when they performed a detailed multivariate analysis on data from a large number of subjects living in villages representing a wide range of endemicity levels. The mechanisms underlying this phenomenon are still unknown but experimental infections in animal models have demonstrated gender differences in susceptibility to, or the development of, filarial infections (Ash, 1971; Denham, 1974) and the possible role of hormonal factors in these differences (Reynouard *et al.*, 1984; Nakanishi *et al.*, 1989; Rajan *et al.*, 1994). No significant association was found between gender and microfilaraemic status in Ngat (Garcia *et al.*, 1995) but this may have been because the intensity of transmission of *L. loa* was particularly high in the village. Whereas the annual transmission potentials (ATP) varied, in Kokodo, from

568 L₃/person-year near the households to 2883 L₃/person-year in the cocoa plantations (Demanou *et al.*, 2001), the ATP measured in 1992–1993 in Ngat was much higher, at 9289 L₃/person-year (J.-P. Chippaux, unpubl. obs.). In Ngat, therefore, any intrinsic factors associated with the lower prevalence of *Loa* microfilaraemia in females could have been obscured by the very high level of exposure. The present analysis showed an interaction between gender and exposure, indicating differences between males and females in the relationship between exposure and the presence of microfilaraemia.

The results of exploration of the models in the present study indicate that, at least for the total population and for the males, individual microfilarial status was associated with the number of *L. loa* L₃ potentially received. This result seems logical, although little is known about the relationships between the number of L₃ transferred at infection, the number of adult worms that will develop in an infected host, and the number of microfilariae that will be produced by the adult worms. Together, experimental models and mathematical modelling may help to clarify the possible role of the number of adult worms on the appearance of microfilaraemia. The results obtained in Kokodo (present study) contrast with those reported from Ngat (Garcia *et al.*, 1995), where the exposure factor was not found to be associated with microfilaraemic status. The ATP, and thus the force of infection, was much higher in Ngat than in Kokodo, however, making the possible effects of low exposure difficult to evaluate in Ngat. The indicators used in Ngat to quantify the levels of exposure were also very simple, whereas the past history of each individual over the 10 years prior to the interviews was considered in the present study, in Kokodo. Even in Kokodo, however, the methods used to quantify the degree of exposure to L₃ had their limitations. The entomological indices employed were measured over just 1 year, for example. Although there is no indication that that year

differed significantly from any other year in the decade of interest, it is clearly risky, in terms of sampling bias, to extrapolate 1-year's results across a decade. Another, similar limitation of the present study is that the time-table for field work was based on just the year prior to the interviews, recorded in a retrospective manner, and simply assumed to be representative of the 10 years prior to the interviews. Such assumptions may obviously lead to some inaccuracy in the estimates of exposure.

A relationship between exposure to *Chrysops* bites and the presence of a *Loa* microfilaraemia was assumed by Thomson *et al.* (2004), when they developed a spatial model to predict the prevalence of human infection with *L. loa* from environmental factors that were relevant to the biology of *Chrysops*. The present results demonstrate that this assumption was correct.

Surprisingly, given that the prevalences of microfilaraemia observed in the subjects aged 15–29 years were, in comparison with those in the two older age-groups, fairly low, there was no evidence in the present study to indicate that age was significantly associated with IMS. In their study of a very large population, Pion *et al.* (2004) found that prevalence did increase significantly with age. The apparently conflicting result from Kokodo may be explained by the fact that, in the present study, account was made for a possible role of exposure and for possible confounding between age and exposure on the presence of *Loa* microfilaraemia at the individual level. In Kokodo at least, the main deciding factor related to *Loa* microfilaraemia appears to be the level of exposure to the vectors within the last few years, whereas age as such, and thus long-term exposure to *Loa* L₃, plays a relatively minor role. It must be acknowledged, however, that the numbers of individuals in some subgroups of the present study, particularly those of females, were fairly small, and that in such cases the power of multivariate models to detect the associations investigated here may be low.

In Kokodo, it was observed that an history of filaricidal treatment prior to mid-1996 (i.e. treatment received >3 years prior to the interviews and parasitological investigation) tended to decrease the probability of the presence of microfilaraemia. This indicates, for the first time, that a filaricide (in most of the present cases, ivermectin) can have a long-term, suppressive effect on *L. loa* microfilaraemia, that lasts for >3 years post-treatment. This effect seems to be marked because the OR corresponding to the variable 'absence of previous treatment' were fairly high in both the total-population (2.18) and male-population (5.73) models. The mechanisms of action of ivermectin on *L. loa* are poorly documented. Observations on *Onchocerca volvulus* and clinical findings in patients with high *Loa* microfilaraemias (Fobi *et al.*, 2000) indicate, however, that one of the effects of ivermectin may be to paralyse the *Loa* mff. This may facilitate the action of inflammatory cells against the mff, which could then be destroyed in the blood vessels or in the lymphatic system. The long-term effect reported in the present study indicates that ivermectin may also reduce the production of new mff. As suggested by Martin-Prével *et al.* (1993), ivermectin could have a long-term effect on the reproductive activity, or longevity, of the adult worms. Further studies are needed to resolve this issue, which may have important implications for those attempting to limit the risk of *Loa*-associated encephalopathy following ivermectin treatment.

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