LONGITUDINAL SURVEY OF LOA LOA FILARIASIS IN SOUTHERN CAMEROON: LONG-TERM STABILITY AND FACTORS INFLUENCING INDIVIDUAL MICROFILARIAL STATUS

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Abstract. A longitudinal, one-year survey of Loa loa infection was carried out in an endemic area of southern Cameroon. Parasitologic samplings (calibrated thick blood smears) were performed every two months to study the evolution of loiasis infection at both the population and the individual level. The mean number of measurements by subject was 3.8 (range 1–6). At the population level, prevalence of infection and microfilaremic load were found to be very stable over time. This observation is consistent with the existence of an important reserve of parasitic material available for vectors and the maintenance of high levels of transmission. At the individual level, both the microfilarial status (microfilaremic/nonmicrofilaremic) and the level of parasitemia showed a remarkable stability over time. Age was the relevant factor that influenced the individual microfilaremic status in the whole population. When only microfilaremic individuals were taken into account, age did not influence the level of microfilaremia, suggesting that loiasis could be considered as a noncumulative disease. The stability of individual microfilaremic status and the pattern of infection variations observed with age support the view that genetic factors might be involved in host defense mechanisms against loiasis infection.

Loa loa filariasis is found only in the tropical rain forest of western and central Africa. More than 30 million people live in endemic areas.1 Whereas the seriousness of the two characteristic clinical features of this disease are not very serious (Calabar swelling or subconjunctival migration of an adult worm),2 rare severe complications, including eardrum fibrosis and renal diseases, have been reported.3–5 Within hyperendemic regions, exposure to L. loa may approach 100%6,7 with approximately 30% of the population being microfilaremic.8 This characteristic profile was reported by Kershaw in a hyperendemic area of central Africa.9 To explain this relatively low rate of microfilaremic subjects, various hypotheses, including the existence of a genetic influence on the outcome of filarial infections, have been put forward.10,11

Epidemiologic studies on loiasis are most often cross-sectional, and the infection status of an individual is determined from a single parasitologic examination of microfilariae in the peripheral blood. The present study is a longitudinal survey of a population living in an endemic area. Several measurements of individual microfilarial density have been performed to describe the loiasis situation, and its evolution in the village, and to study the long-term stability of individual microfilarial status. Both the quantitative (parasite density) and the qualitative (microfilaremic/nonmicrofilaremic) aspects of individual microfilarial status have been studied. Furthermore, environmental, behavioral, and individual factors have been investigated to isolate those that could influence the individual microfilarial status.

SUBJECTS AND METHODS

Subjects. The survey was conducted between April 1992 and April 1993 in a southern Cameroon village (region of Mbalmayo) located 70 km from Yaounde. Data on the population (738 subjects from the Ewondo ethnic group) were recorded between February and March 1992. Habitations consist of small dwellings located on both sides of a 10-km-long trail across the forest. The village has been divided into four areas. All subjects of the village performed their usual activities during the study. A physician with access to full treatment facilities was present every week to visit sick people. Since no severe manifestation of loiasis was observed, no treatment against filariasis was distributed during the survey.

Laboratory methods. Parasitologic measurements were made every two months during the follow-up (one year) from calibrated thick blood smears (30 mm²) obtained by fingerprick between 10:00 AM and 3:00 PM. At each measurement, a blood smear was taken from all persons more than one year of age present in the village. These calibrated thick blood smears were stained with Giemsa, and the microfilariae were identified and counted. Parasitemia was expressed as number of microfilariae/30 mm². Additional analyses on parasite density (PD) were conducted using a natural logarithmic transformation based on log(PD + 1) to allow for zero counts. This log-transformed parasite density will be denoted as the LPD.

Variables of interest and strategy of analysis. At the population level, loiasis infection was quantified at each visit by both the mean of the LPDs (quantitative) and the proportion of microfilaremic individuals (denoted as prevalence of infection) computed in present subjects. The evolution of these two indicators was studied to describe the status of loiasis in the village.

In this work, we were also interested in studying the individual microfilarial status (IMS). Each individual was submitted to several measurements, and to obtain a unique variable accounting for the degree of loiasis infection, a mean parasite density (MPD) was defined. Before calculating the MPD, we checked by one-way analysis of variance that the period of measurement had no significant effect on LPDs (see details in the results). The MPD was then computed for each individual as the mean of their LPD values and thus, represents the quantitative aspect of the IMS. The MPD was equal to 0 for more than 60% of the subjects (see details in the re-
Results. i.e., the PD was 0 in all measurements for these individuals. This observation had two consequences on the strategy for the analysis: 1) studies on MPD were performed in the whole population and in the population with positive MPD values (positive population consisting in 195 individuals sampled at least twice) and 2) a binary variable was also considered as accounting for the qualitative aspect of IMS: each individual was classified as either noninfected if their MPD was 0 or infected if their MPD was positive. Both the quantitative and the qualitative aspects of IMS were used to study 1) the long-term stability of the IMS and 2) the factors that can influence the IMS.

The long-term stability of the quantitative IMS was assessed in the positive population by testing, using analysis of variance, that the variability of the LPDs was less important within individuals than between individuals. For the binary IMS, the following method was used to assess the individual stability of a negative PD across the measurements. The computations are clearly detailed in Table 1. Let $p_0$ be the observed probability of having a negative PD in the total number of measurements ($p_0 = \frac{\text{number of negative PDs}}{\text{total number of PDs}}$). According to the hypothesis that a negative PD occurs at random within individuals (i.e., all measurements are independent), the probability for an individual who had $j$ measurements that all their PDs are equal to 0 is given by $p_0^j$. The expected number of such individuals (sampled $j$ times and being always negative) is $n_j p_0^j$, where $n_j$ is the number of subjects sampled $j$ times. These expected numbers were compared with the observed ones by a chi-square test for individuals having more than two measurements. Rejection of the null hypothesis of independence of measurements means that individuals maintain their negative binary IMS over time.

The explanatory variables tested in this analysis as potentially influencing the IMS were 1) sex; 2) habitat areas (four areas); 3) occupation and activity divided into two categories: field workers walking several hours per day across the forest to go to the fields that are some distance from the village, and others denoted as nonfield workers (teachers, schoolchildren, older people, etc.); 4) age, which was used both as a quantitative variable and as a binary one (the motivations of the cutoff point will be detailed in the results); and 5) percentage of time spent inside houses between 6:00 AM and 10:00 AM, denoted as indoor time, which was used both as a quantitative variable and as a binary one (less or more than 50%). Indoor time was assessed by two Cameroon sociologist investigators from the Cameroon University of Science, who spent two months in the village living with the population. Within the families that accepted their presence, indoor time was assessed by both interviews with the family members and their own observations.

Statistical methods. In spite of log-transformation, the distribution of MPD was far from the assumptions required to use parametric analysis methods. Consequently, univariate nonparametric methods were performed to study the relationship between the MPD and the explanatory variables. For the binary IMS, multivariate analysis was also performed by means of multiple logistic regression. The goodness of fit of a given logistic model was tested by the Hosmer-Lemeshow test.

All computations were carried out using BMDP statistical software (University of California, Los Angeles, CA).

### RESULTS

#### Description of the population under study

Of the 738 subjects, 667 were sampled at least once and represented the population under study. The 71 nonparticipants were not resident in the village at the time of the study and were either children in junior high school or adults working in Yaounde. The mean number of measurements by individual in this population was 3.8 (range 1–6) (Table 2). The mean age of the studied population was 28.5 years (range 1–90) with a sex ratio (male/female) of 0.9. Whereas the number of individuals living in the four areas was significantly different ($P < 0.01$), mean age and sex ratio did not vary significantly in the four areas ($P > 0.2$ and $P > 0.9$, respectively). Thirty-three percent of the subjects were field workers. Among them, the proportion of men (59%) was significantly higher than among nonfield workers ($P < 0.001$). Mean age was not different according to sex ($P > 0.35$). However, the mean age (SEM) of field workers, 46.9 years (1.32), was significantly higher ($P < 0.001$) than that of nonfield workers, 19.3 years (0.9).

The percentage of indoor time was available only for a
subsample of the population (n = 430). The subsample consisted in families that accepted the presence of an investigator for several hours per day several days per week. There was no significant difference between the subsample and the whole population according to sex, activity, habitation area, and age. Both the mean MPD and the proportion of infected subjects observed in the subsample were not significantly different from the ones observed in the whole population. There was a significant (P < 0.001) negative correlation (r = -0.16) between age and time spent indoors, indicating that the percentage of time spent inside houses decreased with age.

Population results. Table 3 shows the variation by date of visit of the mean LPD and the prevalence in the whole population. The period of measurements had no significant effect either on the LPDs (P > 0.6) or on the prevalence of infection (P > 0.6). Annual prevalence of loiasis in this village was 28.3%. The number of blood smears carried out at each visit increased significantly (P < 0.0001) and stabilized around 450 (65%) at the third passage. Mean age was not significantly different by visit.

Long-term stability of the IMS. For the quantitative aspect of the IMS, the variance of the LPDs, computed for the subsample of the population (n = 195) and in the positive population (n = 667) and in the whole population (n = 224). The mean MPD (SEM) was 3.325 (0.13) for men and 1.85 (0.12) for women in the whole population: among positive subjects, the mean MPD was 3.916 (0.24) for men and 3.875 (0.23) for women.

In the whole population, the Spearman rank correlation coefficient between age and MPD (r = 0.31) was highly significant (P < 0.0001), indicating that the older individuals were the most infected (Figure 1a). In the positive population (Figure 1b), the correlation between age and MPD (r = 0.13) was not significant (P > 0.05) suggesting that among infected people, age did not influence the MPD levels. As mentioned previously, age was also correlated with activity and indoor time, and may be a confounding factor in evaluating the relationships between 1) MPD and activity and 2) MPD and indoor time described in the following paragraphs. Consequently, age was also considered as a binary variable to study both these relationships for two levels of the age factor. Two cutoff points could be used in view of the results in Figure 1a: 30 or 40 years. The results were similar using either one, and will be presented using the 30-year cutoff point. The mean MPD (SEM) of people younger than 30, 0.724 (0.10), was significantly lower (P < 0.0001) than the mean MPD (SEM) of the older people, 1.971 (0.16).

In the whole population, the mean MPD (SEM) was significantly higher (P < 0.0001) for field workers, 1.893 (0.18), than for others, 1.016 (0.10). In the positive population, the activity factor was borderline nonsignificant (P > 0.05) with a mean MPD (SEM) of 4.346 (0.24) for the field workers and 3.616 (0.23) for the others. However, age may be considered as a confounding variable since field workers are older than the others. In the whole population, the effect of activity on the MPD was no longer significant among subjects younger than 30 (P > 0.6) and among subjects older than 30 (P > 0.57). The same pattern was observed when the positive population was studied.
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The effect of indoor time was studied in the subsample of 430 subjects. There was a negative ($r = -0.17$) significant correlation between the percentage of indoor time and the MPD ($P < 0.001$), indicating that the intensity of individual infections decreased with the percentage of time spent inside houses. Considering the positive population (120 individuals) of this subsample, the same correlation was borderline nonsignificant ($P > 0.05$). Age may be again considered as a confounding variable since age and indoor time were significantly correlated. In both the whole and the positive population, the correlation between MPD and indoor time was clearly not significant when computed separately among people younger and older than 30.

Factors influencing the binary IMS. Using univariate methods, neither sex nor habitation area had a significant effect on the binary IMS. The effect of age on the probability of being infected is presented in Figure 2. Infected subjects were significantly older than noninfected ones, with a mean age (SEM) of 37.9 years (1.54) and 23.9 years (1.01), respectively ($P < 0.0001$). The proportion of infected subject was 21% among people younger than 30 and 49% among people older than 30 ($P < 0.0001$). Field workers were significantly ($P < 0.0001$) more infected (44%) than the others (28%). In the subsample, the proportion of infected people was significantly higher ($P < 0.02$) in the population spending less than 50% of the time inside houses.

Multivariate analysis was then performed by means of logistic regression using the three variables found significant by the univariate analysis, i.e., age, activity, and indoor time (indoor time was considered only in the subsample). When age (either as a quantitative or a binary variable), was entered in the logistic model, activity and indoor time were no longer significant variables, predicting the probability of infection ($P > 0.50$, and $P > 0.20$, respectively). The variations of the probability of infection with age predicted by the logistic model including age as a quantitative variable (model Q) is shown in Figure 2 (bold line). When age was considered as a binary variable (model B), the regression coefficient was easily interpretable in terms of odds ratio. The odds of infection between the subjects older than 30 and those younger than 30 was 3.87, 95% confidence interval (CI) = 2.71, 5.53. Model Q and model B were not nested and could not be directly tested by standard procedures (likelihood ratio test). However, we noted that the goodness of fit provided by the model B was more satisfactory than the one provided by the model Q according to the Hosmer-Lemeshow test ($P = 0.67$ and $P = 0.14$, respectively).

DISCUSSION

This work represents, to our knowledge, the first longitudinal epidemiologic survey of loiasis including several measurements for each individual and studying the long-term stability of loiasis infection at both the population and the individual level. At the population level, both the qualitative (prevalence of microfilaremia subjects) and the quantitative (mean LFDs) aspects of loiasis infection were found stable over time. These results confirm those of cross-sectional studies, showing a remarkable stability of the microfilarial density in different age groups of an adult population. A practical implication of this result is that the epidemiologic situation in a region may be assessed by a one-time survey, without important loss of information. A direct consequence of this long-term stability is the existence of an important reserve of parasitic material available for the vectors, and thus, the persistence over time of high transmission levels in populations living in endemic areas, as it had been shown in the Congo and Gabon on the basis of both entomologic observations and immunologic or clinical data. However, this high transmission does not explain our observations that only 28% of the population have circulating microfilariae, and that microfilariae carriers are generally the same individuals over time. Among the 128 subject sampled six times, 61% were never microfilaremic whereas the expected figure would have been 13% according to the hypothesis of random occurrence of negative PDs within individuals (Table 1). Furthermore, microfilariae carriers presented a very low variability of their microfilaremia over time, confirming the long-term stability of both the qualitative and quantitative aspects of individual microfilarial status. Similar results concerning the stability of the individual microfilarial status have been found in subjects sampled twice, both in Gabon and the Congo, with a one-year and a three-year interval between the two measurements, respectively.

Factors contributing to the stability of parasitemia may be inherent both to the parasite (dynamics of production of microfilariae by adult worms) and to the host (immune defense mechanisms). In hyperendemic regions, individuals may be exposed to an infective bite every five days and thus be...
peatedly inoculated with *L. loa* infective larvae (*L*$_3$). As described for other filarial infections, the host may, by an immune defense reaction, control the level of infective *L*$_3$ larvae and thus the number of adult worms able to produce microfilariae. The dynamics of production of microfilariae by adult worms in a host is not well-documented, and no clear correlation between the number of gravid females and the intensity of microfilaraemia have been shown in loiasis infection.$^{15-17}$ To account for the heterogeneity between individuals (60% never infected versus 40% always infected), several arguments suggest the role of genetic factors involved in host defense mechanisms. Experimental studies have shown that an important component involved in the control of microfilaraemia after experimental infection with *Dipetalonema vitae* is a genetically determined capacity to produce cuticle-specific IgM.$^{18}$ In human pathology, various possible explanations for familial clustering in Bancroftian filariasis have been reported. In utero exposure to filarial antigens might lead to a lower filarial-specific cellular response and thus, influence the individual microfilarial status.$^{19,20}$ In a study of a large Polynesian population, a segregation analysis was found to be consistent with a genetic involvement in the predisposition to filarial susceptibility, whereas no linkage was observed with the HLA-A or B loci.$^{21}$ An association between HLA-B15 and predisposition to elephantiasis was reported in Senegal and west Malaysia by Chan and others.$^{22}$ Furthermore, the role of a major gene controlling resistance/susceptibility to other parasitic infections, such as schistosomiasis or malaria, has been already found by means of specific statistical methods.$^{23,24}$ Further familial studies using segregation and linkage analyses are now needed to determine the nature of the genetic component potentially involved in filariasis infection.

In the whole population, the results of univariate analysis showed that forest-related activities, time spent outside, and age increased the risk of infection and the level of microfilaraemia. The effects of activity and indoor time were no longer significant after adjustment for age. Logistic regression results confirm that activity and indoor time have no significant effect on the probability of infection when age is entered in the model. Transmission of loiasis caused by *Chrysops trituberculatus* (Chrysops dimidiata or *Chrysops silacea) occurs during the day. Entomologic studies have shown a seasonal pattern of host-seeking activity for both *C. dimidiata* and *C. silacea*. However, Noireau and others$^{25}$ have shown in the Congo that landing densities of *C. dimidiata* were greatest in the fields than in the village, whereas these densities were similar in all sites for *C. silacea*. The pattern of our results observed in an endemic area suggests that even if forest-related activities and time spent outside represent an increase of exposure, these factors do not increase the probability of being infected and the level of microfilaraemia. However, in areas of low transmission, these factors may increase the risk of infection.

In the whole population, age was found to be the relevant factor influencing both the qualitative (microfilaremic/non-microfilaremic) and the quantitative (MPD) aspect of individual microfilarial status, indicating that the probability of infection and the level of microfilaraemia increase with age. In view of Figures 1a and 2, this increase seems to present a plateau around 30 years, consistent with the pattern observed by Noireau and others$^{23}$ on the basis of one measurement with a plateau around 20 years. This result supports the view that for an individual who was not parasite-positive until the age of 30 years, the probability of microfilaraemia is very low. In the positive population (MPD > 0, subjects with positive MPD), the effect of age on the microfilaraemia is borderline nonsignificant. This pattern of results suggests that among infected subjects, the quantitative aspect of the individual infection status has no significant variation with age (Figure 1b) and is consistent with a very long-term stability of this infection status. Furthermore, it indicates that loiasis could be considered as a noncumulative disease, and that children who are infected may reveal a high level of microfilaraemia even if the proportion of infected individuals is lower among children (Figure 2). Consequently, adverse reactions observed in high-microfilaricmic individuals treated with ivermectin, as reported by Chippaux and others,$^{27}$ could occur in children as well as in adults.

Finally, the stability of individual microfilarial status and the varied pattern of infection variations observed with age suggest that genetic factors could be involved in the susceptibility to loiasis infection, as it has been shown in animal models and in other parasitic diseases. The knowledge of such genetic factors could help improve understanding the dynamics and physiopathology of this filariasis and may have practical implications for control strategies for this disease.

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REFERENCES


