Microfilarial distribution of *Loa loa* in the human host: population dynamics and epidemiological implications

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**SUMMARY**

Severe adverse events (SAEs) following ivermectin treatment may occur in people harbouring high *Loa loa* microfilarial (mf) densities. In the context of mass ivermectin distribution for onchocerciasis control in Africa, it is crucial to define precisely the geographical distribution of *L. loa* in relation to that of *Onchocerca volvulus* and predict the prevalence of heavy infections. To this end, we analysed the distribution of mf loads in 4183 individuals living in 36 villages of central Cameroon. Mf loads were assessed quantitatively by calibrated blood smears, collected prior to ivermectin distribution. We explored the pattern of *L. loa* mf aggregation by fitting the (zero-truncated) negative binomial distribution and estimating its overdispersion parameter *k* by maximum likelihood. The value of *k* varied around 0.3 independently of mf intensity, host age, village and endemicity level. Based on these results, we developed a semi-empirical model to predict the prevalence of heavy *L. loa* mf loads in a community given its overall mf prevalence. If validated at the continental scale and linked to predictive spatial models of loiasis distribution, this approach would be particularly useful for optimizing the identification of areas at risk of SAEs and providing estimates of populations at risk in localities where *L. loa* and *O. volvulus* are co-endemic.

**Key words:** *Loa loa*, microfilarial aggregation, ivermectin, Cameroon.

**INTRODUCTION**

The African filarial worm *Loa loa* is well known for spectacularly migrating under the eye conjunctiva, and its association with transient oedemas called ‘Calabar swelling’. Even if these signs make it one of the primary causes of consultation in the endemic areas (Boulesteix and Carme, 1986), loiasis is not regarded as a very serious disease. However, individuals harbouring high microfilaraemias may, exceptionally, develop serious spontaneous neurological or renal complications (Cauchie et al. 1965; Zuidema, 1971). More significantly, it is well known that high microfilarial (mf) loads are associated with a risk of developing neurological serious adverse events (SAEs) after treatment with the filarialid drugs diethylcarbamazine (DEC) and ivermectin (Fain, 1978; Carme et al. 1991; Gardon et al. 1997a; Boussinesq et al. 1998). Thus, following ivermectin treatment, it has been demonstrated that individuals presenting with high mf loads (>8000 mf/ml), and those with very high mf loads (>30 000 mf/ml) had, respectively, an increased risk of developing severe adverse reactions without neurological involvement, and SAEs (Gardon et al. 1997a). In the context of the Community Directed Treatment with Ivermectin (CDTI) carried out in Africa, these SAEs are of crucial concern as they can lead to fatal outcomes and jeopardize the success of the African Programme for Onchocerciasis Control (APOC) (Twum-Danso, 2003).

In loiasis, many individuals do not present with microfilariae (mfs) in their peripheral blood, a phenomenon usually described as ‘occult loiasis’, yet they may prove to be infected because of previous history of subconjunctival worm passage. While genetic epidemiology (Garcia et al. 1999) and immunological studies (Winkler et al. 1999; Akue et al. 2002; Walker-Deemin et al. 2004) have brought useful insights into understanding the processes leading to some individuals developing *L. loa* microfilaraemia, the population dynamics of *L. loa* remains poorly documented. In a previous paper, we presented a detailed analysis of the structure of the microfilarial reservoir of *L. loa* in an endemic...
population through the study of host age- and sex-
specific parasitological profiles in terms of preva-
ience and intensity of microfilaraemia (Pion et al.
2004). Our results indicate that the prevalence of 
microfilaraemia increases with age, is higher for 
males than females and, more unexpectedly, that, for 
given level of endemicity, the mean intensity 
among microfilaraemic individuals remains nearly 
unchanged with host age.

The prevalence and intensity of an infection are 
but two characteristics of the distribution of parasites 
among hosts. In addition, parasite distributions are 
typically overdispersed. The degree of parasite over-
dispersion is a key parameter of the stability and dy-
namics of a host-parasite system (May and Anderson, 
1978; Dobson and Hudson, 1992) and, according to 
some authors, it is constant and characteristic for a 
given host-parasite system (Bliss and Fisher, 1953; 
However, it would be expected that the distribution 
of parasites per host is a dynamic property within a 
given host-parasite system depending, for instance, 
on the intensity of transmission, the age-structure of 
the host population, and the operation of age-
dependent and/or density-dependent processes 
(Adler and Kretzschmar, 1992; Pugliese et al. 1998). 
Since, to our knowledge, mf aggregation has never 
been characterized for L. loa, we focus, in the present 
paper, on the distribution of the L. loa mf loads in the 
human population.

Besides the population dynamics aspects, char-
acterizing the distribution of L. loa mfs among 
humans may be particularly useful for assessing the 
proportion of the host population at risk of post-
treatment SAEs. In particular, the negative binomial 
distribution (NBD) provides a simple relationship 
which depends on the magnitude and functional form of the overdispersion parameter 
(Anderson, 1982). This relationship has been used to 
investigate the distribution of helminths parasites in 
humans (Anderson and May, 1985; Guyatt et al.
1990; Basañez and Boussinesq, 1999), wildlife hosts 
(Shaw and Dobson, 1995; Shaw et al. 1998), and 
vectors (Cheke et al. 1982; Renz, 1987; Basañez et al.
1995). If the overdispersion parameter can be de-
termined for L. loa, it would be possible, in principle, 
to estimate the mean mf load in a community given its 
prevalence. This approach has been used success-
fully for Onchocerca volvulus in humans (Basañez 
et al. 2002) and vectors (Basañez et al. 1998).

Since a predictive spatial model for prevalence of 
L. loa microfilaraemia from environmental data ob-
tained by remote sensing has been developed and 
validated (Thomson et al. 2004), prevalence estimates 
can easily be obtained for the whole distribution area 
of the parasite. If the NBD model were also vali-
dated, merging the results obtained by the Thomson 
et al. model with a well-defined relationship between 
community prevalence of microfilaraemia and preva-
ience of heavy infections, would provide a useful tool 
to aid SAEs surveillance in CDTI campaigns.

In the present study, we explore the patterns of 
L. loa mf aggregation in endemic populations, and 
develop and test a model to predict the prevalence of 
heavy L. loa mf loads in a community given its mf 
prevalence.

PATIENTS AND METHODS

Study area and parasitological surveys

The study areas and the methods used for selecting 
and examining subjects have been previously de-
scribed (Gardon et al. 1997a; Boussinesq et al. 2001; 
Pion et al. 2004). Briefly, the data analysed in the 
present paper were collected as part of a trial con-
ducted in 1995–1996 in the Lékie Division (Central 
Province, Cameroon) to evaluate the incidence of 
L. loa related post-ivermectin SAEs and to identify 
risk factors associated with the latter. During this 
trial, 4183 subjects aged ≥15 years were examined in 
36 communities. This age group was chosen because, 
at the time of this trial, all the SAEs reported so far 
had occurred in individuals ≥15 years.

From each consenting individual, a blood sample 
was collected by finger-prick, between 10.00 and 
16.00 h, in a non-heparinized capillary tube, and 
calibrated thick blood films were immediately pre-
pared, using 50 µl of blood. Each Giemsa-stained 
smear was then examined under a low-power 
microscope and all the L. loa mfs present on the slide 
were identified and counted. All the persons ex-
amined had been questioned as to whether they had 
received any antifilarial treatment previously, and 
the data from those few who had been treated during 
the last 5 years were discarded from analysis.

Statistical analysis

Method to assess overdispersion. Various methods to 
assess the degree of parasite contagion or aggregation 
have been advocated in the literature, among which 
the variance to mean ratio (VMR) investigates dis-
crepancy from the Poisson or random distribution 
(VMR = 1), and the index of discrepancy measures 
departures from the uniform distribution (all hosts 
harbour the same number of parasites) (Poulin, 
1993; Poulin and Morand, 2000). We chose to assess 
aggregation through the parameter k of the NBD 
fitting observed mf distributions in population 
strata as defined in the following section. However, 
during preliminary analyses, the NBD model, when 
fitting to the complete observed distributions of mf 
densities (including zero densities), did not provide 
satisfactory fits, whereas the zero-truncated NBD 
model provided adequate fits. Thus, assuming that 
the zero count class may not be reliable because only
~60% of the infected population would be genetically predisposed to present with microfilaraemia (Garcia et al. 1999), and that some individuals may be false-negatives (due to the lack of sensitivity of the blood film method when microfilaraemia is low), we chose the zero-truncated NBD (tNBD) model (Pichon et al. 1980; Grenfell et al. 1990).

Estimates of $k$ (and corresponding variance) were obtained using the maximum likelihood method (MLM) proposed by Sampford (1955) and confidence intervals were obtained by bootstrapping (1000 simulations for each stratum). The fits to tNBD and calculations of confidence intervals were performed using Stata 9.0. Goodness of fit was tested using $\chi^2$ tests with the number of degrees of freedom equal to the number of frequency classes – 3 (Elliott, 1977).

Patterns of microfilarial aggregation with host age and sex by level of endemicity. Investigation of parasite overdispersion with age and transmission intensity has been used to obtain insights into the possible operation of age- or parasite density-related processes regulating population dynamics (Anderson and Gordon, 1982; Pacala and Dobson, 1988; Fulford et al. 1992; Woolhouse et al. 1994; Das et al. 1995; Filipe et al. 2005). We classified the villages according to 3 endemicity levels based on the prevalence of microfilaraemia in the population aged \( \geq 15 \) years, as a proxy for transmission intensity. These levels were: low endemicity (<25% mf prevalence), moderate endemicity (25–34.9%) and high endemicity (\( \geq 35 \%) \). In each of these categories, the populations were subsequently sorted by sex and age according to the following age classes: 15–19, 20–29, 30–39, 40–49, 50–59 and \( \geq 60 \) years. The total population was thus divided in 36 different strata (3 prevalence classes x 2 sexes x 6 age classes). We estimated $k$ for each separate stratum. We then tested whether $k$ varied by group using a linear regression of $k$ on age-sex-endemicity group; such a method has been used to investigate aggregation patterns of *Schistosoma haematobium* in human populations (Woolhouse et al. 1994).

Predicting prevalence of heavy infection given mf prevalence. We aimed at developing a model to predict the prevalence of heavy *Loa loa* mf loads in a community given the prevalence of microfilaraemia in those aged \( \geq 15 \) years in such a community. To this end, we considered the village as the epidemiological unit, so this part of the analysis was conducted at community level.

Let \( X_i \) denote the random variable equal to the mf count in an individual aged \( \geq 15 \) years old and living in community \( i \); \( x_i \) the actual value of \( X_i \); \( \pi_i \) the overall prevalence of microfilaraemia in those aged \( \geq 15 \) years in community \( i \) (i.e. \( \pi_i = \text{Prob}(X_i > 0) \)), and \( T \) the microfilaraemia threshold above which an individual is considered to have heavy infection.

Then, following the results obtained in the first part of the analysis, we assume that, in a given village \((i)\), the frequency distribution of mf counts in those microfilaraemic follows a truncated NBD with parameter \( M_i \), the mean mf intensity, and \( k_i \), the overdispersion index, i.e. \( X_i \sim \text{tNBD}(M_i, k_i) \). Thus, the proportion of people in community \( i \) presenting with more than \( T \) mf/ml is:

\[
P_i(X_i > T) = 1 - \sum_{x=0}^{T} p_i(x_i) \pi_i \quad \text{Equation (1)}
\]

Explicitly, the tNBD of mf counts is given by:

\[
p_i(x) = \frac{\Gamma(x+k_i)}{\Gamma(k_i)x!} \frac{q_i^x(1-q_i)^{k-i}}{[1-(1-q_i)^{k}]}
\]

where \( q_i = \frac{M_i}{M_i+k_i} \) and \( \Gamma \) is the gamma function.

Our aim was to render expression (1) exclusively in terms of \( \pi_i \). For this purpose, we modelled, on the one hand, \( M_i \) as a function of \( \pi_i \), and, on the other hand, \( k_i \) as a function of \( \pi_i \).

(i) Relationship between \( M_i \) and \( \pi_i \)

As the simplest possible approximation and motivated by inspection of the data, we assumed a linear relationship, across communities, between the mean microfilarial load and the microfilarial prevalence in those aged \( \geq 15 \) years,

\[
M_i = A \pi_i \quad \text{Equation (2)}
\]

(ii) Relationship between \( k_i \) and \( M_i \)

Parameter \( k_i \) was estimated for each separate village \( i \) using the maximum likelihood method described in the first part of the analysis. Then, parameter \( k_i \) was included in equation (1) using 2 alternative functional forms: a constant value \( k_i = 0.3 \) (the mean \( k \) value obtained either in the ‘per age and sex stratum’ analysis or in the ‘per village’ analysis of the current data, see Results section), and a log-linear relationship with mean mf intensity, \( k_i = \alpha + \beta \log(M_i) \). This model has been chosen because \( M_i > 0 \). In this latter model, we also used the relationship between \( M_i \) and \( \pi_i \) derived from equation (2). The empirical relationships between \( M_i \) and \( \pi_i \), and between \( k_i \) and \( M_i \) were fitted to the current study data using the least squares method, analogous to the linear regression approach used above.

We applied expression (1) to 2 different threshold values: \( T = 8000 \text{ mf/ml} \) and \( 30000 \text{ mf/ml} \). The first value corresponds to the threshold above which there is a significant increase in the relative risk of occurrence of functional impairment following ivermectin treatment, and the second threshold, to the value above which the risk of occurrence of serious neurological reactions is increased (Gardon et al. 1997a). Deviation of these predictions from the observed data was assessed using $\chi^2$ tests.
Patterns of microfilarial aggregation amongst the different strata of the host population

Table 1 shows the number of microfilaraemic individuals out of the total number of subjects examined and the arithmetic mean of the positive mf loads in the 36 different strata of the population. The 36 values of $k$ estimated by MLM ranged between 0.07 and 0.66 (Fig. 1). The average, common $k$, calculated as the mean of the age-, sex- and endemicity-specific $k$ values, weighted by the reciprocal of each value’s estimated variance, was 0.30. According to $\chi^2$ tests, the tNBD provided satisfactory fits to the observed data in all but 1 stratum (males aged 15–19 years in high endemicity villages, $k=0.29$). The use of $k_c$ to represent $k_i$ for each village is supported by the linear regression model, in which parameter $k$ was independent of sex and age of the host, or level of endemicity in the village, either as main effect or included in 2-way interactions (Table 2). It should be noted that the test is approximate, and that a non-significant regression coefficient for any of the factors considered only indicates such a factor is likely to be unrelated to $k$.

Predicting the prevalence of high microfilarial loads from the prevalence of microfilaraemia

Relationships between mean intensity $M_i$ and microfilarial prevalence $\pi_i$ at community level. As a first approximation, a linear relationship between the prevalence and mean intensity of $L. loa$ microfilaraemia for every village did not provide a very satisfactory fit (coefficient of determination, $R^2=0.27$). To obtain a more robust relationship to be used in the subsequent modelling, we aggregated the villages according to their $\pi_i$ values, in the 6 following groups [12–22], [22–27], [27–32], [32–35], [35–38] and $\geq38\%$, with 6 villages in each group. The linear
relationship obtained using this grouping ($M_i = 316.4 \pi$) was very similar to that obtained when considering separate communities ($M_i = 314.4 \pi$) but considerably improved the fit ($R^2 = 0.78$); while the improvement was largely due to a reduction in the number of data points and therefore in the variability of the data, the similarity of the fits suggests that the regression estimate is essentially independent of data grouping (Fig. 2).

### Table 2. Estimates of the linear regression coefficients of the NBD overdispersion parameter $k_i$ on individual-level variables (host age and sex) and village-level variable (endemicity level) for the distribution of *Loa loa* microfilarial loads in the Lékié Division, Central Province, Cameroon

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>-0.111</td>
<td>-0.411-0.190</td>
<td>0.457</td>
</tr>
<tr>
<td>Age group</td>
<td>-0.064</td>
<td>-0.131-0.003</td>
<td>0.059</td>
</tr>
<tr>
<td>Endemicity</td>
<td>-0.125</td>
<td>-0.273-0.022</td>
<td>0.092</td>
</tr>
<tr>
<td>Sex x Age group</td>
<td>0.004</td>
<td>-0.043-0.051</td>
<td>0.780</td>
</tr>
<tr>
<td>Sex x Endemicity</td>
<td>0.073</td>
<td>-0.026-0.171</td>
<td>0.143</td>
</tr>
<tr>
<td>Age group x</td>
<td>0.024</td>
<td>-0.005-0.053</td>
<td>0.019</td>
</tr>
<tr>
<td>Endemicity</td>
<td>0.005</td>
<td>0.102</td>
<td>0.001</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.609</td>
<td>0.273-0.944</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**Prediction of heavy microfilarial loads.** The predicted prevalence of heavy infections (eqn 1), for $T = 8000$ and $T = 30 000$ mf/ml, respectively, were in good agreement with the observed distribution of heavy mf loads (Fig. 4A and B). The model assuming a constant value for the degree distribution of heavy mf loads was in good agreement with the observed distribution of heavy mf loads.

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**Discussion**

**Population dynamics insights**

At the time of the surveys, mass ivermectin distribution had not been initiated, and to our knowledge, no significant environmental or ecological changes had taken place in the area. Therefore, our assumption is that the *L. loa* population was at endemic equilibrium with its human and vector hosts. Our work thus contributes to the characterization of the distribution of *L. loa* among humans and to highlight its epidemiological implications in natural, non-intervened settings.

One of the main motivations for using the NBD model is that once the degree of overdispersion has been characterized, the theoretical frequency distribution is entirely defined by the arithmetic mean. As the latter is related to the prevalence, it is possible to estimate, from prevalence values, the proportion of hosts harbouring mf densities above an arbitrary threshold (Guyatt and Bundy, 1991). However, the standard NBD model did not fit well the distribution of the *L. loa* mf loads probably because in southern Cameroon only ~60% of the population is genetically predisposed to present with microfilaraemia (Garcia et al. 1999). Instead, the zero-truncated negative binomial distribution, used to describe *Wuchereria bancrofti* (Pichon et al. 1980; Das et al. 1990; Grenfell et al. 1990) and *Brugia malayi* (Srividy et al. 1991) mf densities in some foci of lymphatic filariasis, was found to fit particularly well with the data.
well the distributions of *L. loa* mf loads among the positives.

Some of the models proposed to understand the mechanisms generating overdispersion in host-parasite systems, predict a decrease in the level of aggregation (increase of $k$) with host age in the presence of down regulatory density dependence. Such a trend has been taken to indicate operation of parasite-induced mortality of individuals harbouring high parasite densities (Anderson and Gordon, 1982; Pacala *et al.* 1988) or the development of acquired immunity with age and exposure to infection (Woolhouse *et al.* 1991; Fulford *et al.* 1992). We observed a very stable degree of aggregation in the different strata of the population. The fact that we did not observe any trend in the overdispersion pattern with host age does not, however, necessarily imply the absence of processes regulating abundance of *L. loa* mfs within an individual. Different complex processes, acting simultaneously, may lead to this apparently simple pattern (Duerr *et al.* 2003). In the case of loiasis, since spontaneous lethal complications are quite uncommon, it seems reasonable to discard a process of parasite-induced mortality of heavily infected hosts in the absence of antifilarial treatment.

**Comparison with previous studies on aggregation in filarial infections**

Comparing values of $k$ between different species for which the mean infection intensities are different has some limitations (Taylor *et al.* 1979; Gregory and Woolhouse, 1993). Nonetheless, the range of overdispersion values observed for *L. loa* was very similar to those observed for other filarial species such as *W. bancrofti*. For this species the value of $k$ has been estimated, using the tNBD model and from independent population samples, as $\sim 0.3$ (Pichon *et al.* 1980; Grenfell *et al.* 1990). For *O. volvulus*, an age-structured model using a zero-inflated NBD yielded $k$ around 0.5 for hosts aged $\geq 15$ years (Filipe *et al.* 2005).

**Predictions of the proportion of the population at risk of SAEs**

We developed a semi-empirical model, aiming at predicting the proportion of the population at risk of post-filaricidal treatment SAEs given the prevalence of microfilaraemia among those aged $\geq 15$ years in the community. We developed 2 alternative models, incorporating different assumptions about the relationship $k_i(M_i)$ between overdispersion and mean mf load among the positives in a community: one
with $k_i$ varying with $M_i$ and another with a common $k_i$. The predictions were not very sensitive to the assumption about $k_i$, so adopting a constant value for $k_i$ ($k_i=0.3$) would be the most parsimonious and practical approach.

However, if $k_i \sim 0.3$ corresponds to endemic equilibrium, the patterns of aggregation are likely to change in communities where large-scale filaricidal treatment is organized. This might constitute a limitation of our present modelling approach, which stands on the analysis of undisturbed populations. Yet, the SAEs are most likely to occur among individuals receiving their first treatment with ivermectin, the post-treatment mf loads usually remaining below the risk threshold until the next treatment round (Gardon et al. 1997b). A model including a $k_i(M_i)$ function would, in principle, be better suited for tracking changes with mean mf densities following treatments.

The maps provided by the model developed by Thomson et al. (2004) are now used to support APOC’s activities, when setting up the CDTI in ivermectin-naïve areas. Such maps give a particularly useful indication of locations where the SAEs surveillance procedures have to be strengthened. As a useful addition to the information generated by the spatial maps, our model would provide information on the proportion of the population at risk of SAEs. The Thomson et al. model gives an indication of location and overall prevalence but not of likely challenge. APOC is now facing a breakthrough regarding the ‘Loa challenge’ APOC is now facing.

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