Age-dependent prevalence of *Loa loa* amicrofilaremia and microfilaremia status as defined by two markers: microfilaria and specific IgG4

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Loiasis infection is characterised by long term stability in infection status. The bases of such stability are not well known. As preliminary step toward verification of possible genetic involvement in this stability, a survey in a homogeneous population (n = 106) of a village from an endemic zone of Gabon was undertaken. The distribution of *Loa loa* microfilaremia according to age revealed a significant relationship between age and the presence of microfilariae in the blood ($p = 0.0059$). The proportion of microfilaricmic individuals increased with age until 45 years old, and did not exceed 34% as its maximum. The other marker (specific IgG4) increased also significantly with age ($p = 0.0038$), but in contrast to microfilaremia, the prevalence of specific IgG4 in the group from 45 years onward reached 100%. These observations show the importance of age for the definition of the amicrofilaricmic or microfilaricmic individual status in an endemic area and are in agreement with the hypothesis suggesting the existence of genetic factors controlling the outcome of the parasitological status in *L. loa* infection.

**Key words**: *Loa loa*, prevalence, age, IgG4, genetic.

INTRODUCTION

Loiasis is caused by the human filarial *Loa loa* which is endemic in the west and Central African forest block. An estimated 10 millions individuals are thought to harbour the parasite (Sasa, 1976; Akue et al., 2011; Zouré et al., 2011) and in some regions, the loiasis is the second cause of hospital consultation after malaria (Boulestiex and Carme, 1986). Several clinical symptoms varying from mild to severe have been documented in *L. loa* infection (Nutman et al., 1988; Klion et al., 1991; Akue, 2011). The treatment available (diethylcarbamazine citrate and Ivermectine) are mostly active on circulating microfilariae (Richard-Lenoble et al., 1988). Vector control is an unrealistic approach.

Therefore, a better understanding of this infection in human population from endemic area is required in order to elaborate new strategy for its control. Epidemiological studies have shown the diversity of infection status and the microfilaricmic status was used for long time to define loiasis (Richard-Lenoble, 1980). However, it is admitted today that some are infected without any
microfilariae in their blood by microscopic technique even after blood concentration exist (Dupont et al., 1988). These individuals are designated as amicrofilaremic or occult infected. A good characterisation of these occult infected is principal for the understanding of mechanisms which control the infection. Besides the microscopic technique and the ocular passage of adult worms, new methods such as detection of Loa loa specific IgG4 (Akue et al., 1994) are now available. Furthermore, by using some of these methods, it appears clearly that occult infected individuals are more prevalent than microfilaraemic ones (Akue et al., 1996). Interestingly, different studies have shown the stability within the space concerning the prevalence of microfilariae carriers which never exceed one third of the Bantu population in any endemic country (Pinder et al., 1988). In contrast, Pampiglione et al. (1979) have shown up to 88% of microfilariae carriers in Bambutis Pygmy of Noth Zaire. There is not only stability in time for infection status but also for the density of parasite in the blood (Garcia et al., 1995; Akue et al., 1996). The existence of different parasitological status temporally stable suggests that some individuals once infected are capable of controlling durably their microfilaraemia (occult infected); in contrast to the others (microfilaraemic), remain susceptible for life span. The phenomenon can be under control of some genetic factors as it was noticed in other parasitic infections (Wakelin and Blackwell, 1988; Bouchery et al., 2012). This study was carried out in the light of new tools for diagnosis to understand whether the absence of microfilaria is just time dependent or a genetic character for some people.

MATERIALS AND METHODS

Study population

The survey was carried out in the village of Dienga. A field station of Centre International de Recherches Medicales de Franceville (CIRMF) to study infectious diseases. Dienga is situated in the south-east of Gabon, Ogooue Lolo province, near the Congolese border. The climate is of equatorial type with the vegetation of savannah. Habitations are grouped along the main road. The population was recently evaluated at 1050 individuals. A proportional random sample was obtained within the population aged over five years which was divided into “stratums” according to age and sex. This random sampling method ensures the representativity of the results, and the structure of the sample to reflect the one in the general population from which the sample was taken. It also allows having sample from individuals of all age, and all level of exposure to infective bites of chrysops vector. Stratums were designed as follow: 1) Less than 6 years, very protected in general. 2) From 6 to 15 years old, spending most of their time in the villages and most often are school children. 3) From 16 to 45 years old, active population, farmers in general. 4) More than 45 years, less active, but sometimes participating during harvesting time.

Each of those individuals had 5 ml of blood taken once, after their agreement, and treated if found positive. Blood was taken in tube containing EDTA and kept at 4°C for a maximum of 72 h at Dienga before it was taken to CIRMF laboratory where 1.1 ml was used for microscopic examination for the presence of microfilariae. The remaining blood samples were centrifuged and the plasma kept at -20°C.

Examination for the presence of microfilariae in the blood

A wet preparation of 10 µl of uncoagulated blood plus a drop of saponine was systematically examined under microscope. In addition, a concentration technique was performed according to Akue et al. (1996). Briefly, in a conical 15 ml tube, the following mix was made: 1 ml of blood, 200 µl of saponine and 9 ml of phosphate buffered saline (PBS). This mixture was incubated at room temperature for 5 min, then centrifuge for 10 min at 500 g. The whole pellet was examined under microscope. Microfilariae (mf) were counted and the total obtained was expressed as mf/ml. The identification of microfilariae species was based on their size, mobility, the presence or the absence of sheath.

Diagnosis of L. loa infection by specific IgG4 detection

This was based on an enzyme linked immunosorbertent assay (ELISA) using adult worm L. loa antigen as describe elsewhere (Akue et al., 1994). Briefly, adult worms removed from eye of patient during their oculur migration were washed and homogenised in Tris-HCl 10 mM buffer plus protease inhibitors and antigen extracted with 1% sodium deoxycholate. The ELISA was performed with 10 µg/ml of antigen and ant-human IgG4 (HP 6011) diluted at 1/30000 as described. Individuals with a mean optical density (OD) higher than the mean OD of African outside endemic zone of L. loa (Gambie, Mali) plus one standard deviation of this mean were considered positive.

Statistical analysis

The analysis were performed using Epi info 6 program, Chi square test was used for the comparison between group and Yates correction was performed when necessary. Fischer exact test was used for comparison of prevalence of microfilariae between age group, and for seroprevalence result in men and women. P ≤ 0.05 was considered significant.

RESULTS

Characteristic of the study population

The individuals are Bantu from Nzebi ethnic group, farmers in general with women mostly involved in agriculture. Most of the children attended a school which is situated in the centre of the village. Only people who agreed were enrolled. Finally, the study population was formed by 106 persons from both sexes with age varying from 6 to more than 45 years old. Sex ratio (women/men = 1.25) with mean age of 26 is shown in Table 1.

Prevalence of microfilaraemia

Among the 106 individuals examined, 13 had microfilariae of L. loa (12.26%), 95% CI (confidence interval): 6.18-18.36. The microfilaraemia vary from 3 to 7475 mf/ml. Mansonella perstans was also present but less
Table 1. Study population.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Male (n)</th>
<th>Female (n)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-15</td>
<td>24</td>
<td>19</td>
<td>43</td>
</tr>
<tr>
<td>16-45</td>
<td>13</td>
<td>21</td>
<td>34</td>
</tr>
<tr>
<td>&gt;45</td>
<td>9</td>
<td>20</td>
<td>29</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>60</td>
<td>106</td>
</tr>
</tbody>
</table>

Table 2. Prevalence of microfilaraemia in different age groups.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Microfilaraemic individuals (n)</th>
<th>Number (N)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-15</td>
<td>1</td>
<td>43</td>
<td>2.35</td>
</tr>
<tr>
<td>16-45</td>
<td>4</td>
<td>34</td>
<td>11.43</td>
</tr>
<tr>
<td>&gt;45</td>
<td>8</td>
<td>29</td>
<td>26.66</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>66</td>
<td>12.26</td>
</tr>
</tbody>
</table>

Table 3. Prevalence of specific IgG4 positive/negative individuals in different age group.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Specific IgG4+ n (%)</th>
<th>Specific IgG4- n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-15</td>
<td>24 (55.81)</td>
<td>19 (44.19)</td>
</tr>
<tr>
<td>16-45</td>
<td>27 (79.41)</td>
<td>7 (20.59)</td>
</tr>
<tr>
<td>&gt;45</td>
<td>26 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>77 (74)</td>
<td>29 (26)</td>
</tr>
</tbody>
</table>

Prevalence of infection as detected by specific IgG4 test

Samples (n = 103) were analysed for the prevalence of specific IgG4 by ELISA. As shown in Table 3, it appears that 74.75% (95% CI: 65.55 - 82.47) of individuals were positive using this test. Again, there was a significant relationship between age and seroprevalence (p = 0.0038). The risk of being IgG4 positive increase with increasing age (p = 0.00118). When males and females were analysed separately, a significant difference was seen among female (p = 0.0052) and male (p= 0.042). The sex was not a risk factor for loiasis infection according to the serological test (p= 0.54). Following the dynamic of specific IgG4 (Figure 1, sero prevalence curve), the seroprevalence was already high between 6 and 15 years age (55.26%), but continued to increase to reach 100% of individuals at 45 years and above. Specific IgG4 remained in plateau at that level until 85 years olds.

DISCUSSION

Although, previous studies did not agreement with this (Garcia et al., 1995; Noireau et al., 1989; Pampiglione et al., 1979), it is shown that age affects the prevalence of microfilaraemic in such a way that the percentage of microfilariae carriers increases and reaches a plateau at about 45 years with a maximum of 33.33% in this study. It seems likely that individuals potentially "susceptible microfilariae carriers" are recruited gradually until they all become infected.

The low proportion of microfilaraemics in the age group of 6-15 years is in agreement with this hypothesis. The latter is supported by the fact that: Firstly, exposition time of this young people has not been sufficient to facilitate biting by infected chrysops. Another explanation may be related to the prepatent period during which larvae are prevalent. Only three individuals had microfilariae of *M. perstans*. The level of *M. perstans* microfilariae vary from 1 to 56 mf/ml. Only one individual had a double infection: *L. loa* /*M. perstans*. The prevalence of *L. loa* microfilariae carriers according to age group (Table 2) revealed a significant difference among the three groups (p = 0.0037). However, when males and females were taken separately the age did not affect the risk of being microfilaraemic in females (p = 0.235) in contrast to males where age affect this risk (p = 0.0015). No significant difference was seen between male and female for the risk of being microfilaria carrier (p= 0.134). When study population was split into ten years group, it was shown that the proportion of microfilariae carriers increase until 45 years (Figure 1, micro-prevalence curve); at this age, the prevalence reached a maximum of 33.33%, then plateau and decreased at 65 years.
Figure 1. Definition of infection status according to age/microfilaria/serology. Both microfilaria and specific IgG4 prevalence were plotted against age. The grey band shows the probable age for acquisition of a definitive infection status in Dienga.

still immature to produce microfilariae. An alternative is the possible existence of single sex infection (Eveland et al., 1975). Interestingly, evolution of microfilariae carriers prevalence every ten years showed a clear increase, then plateau followed by decrease at age 65. This plateau will not be surprising if we accepted the possible involvement of concomitant immunity which acts against establishment of new infection while the first infection remains (Rajakumar et al., 2006; Specht et al., 2011).

At 65 years, the decline of microfilariae carriers may be concomitant to the natural disappearance of infection acquired in younger age and to the low level of exposure as majority of the individuals over 45 years old age group stay indoor. This is substantiated by the evolution of the mean level of microfilariae density which is low in the younger age class (400 mf/ml) then increases for individuals aged between 16 and 45 (3006 mf/ml) and decreases for individuals over 45 years (627 mf/ml). Although no statistical significant difference was found when comparing means level of microfilariae density between age groups, similar trend was found in south-Cameroon (Garcia et al., 1995). When the prevalence of microfilaraemics is examined according to sex, it appears that relationship between sex and existence of microfilaraemia was significant only in males and not in females. Ripert et al. (1977) also mentioned this fact. Although certain studies have found a link between prevalence of microfilariae carriers and sex (Van Hoegarden et al., 1987; Noireau et al., 1989), our survey did not completely support these findings, but the small size of the sample in the group with defined status (45 years and over) as well as the level of exposure to infective larvae and a previous chemotherapy may affect the interpretation of this result.

Age is therefore a non-negligible factor in the definition of infection status. Thus, only individuals who have been in contact with infective larvae and without circulating microfilariae will be considered as amicrofilaremic. However, evidence to show that an individual has been in contact with L3 larvae is not clearly defined in natural conditions. It becomes necessary to define a "cut-off age" beyond which the infection status of an individual will be finally determined.

When loiasis infection was defined using specific IgG4 serology, sex did not affect the parasitological status. The curve of distribution of specific IgG4 test increases gradually and reaches a plateau at about 45 years. This plateau remains stable. Interestingly, the age of the beginning of this plateau is the same as the one determined by microfilaraemia (Figure 1, grey vertical area). Thus individuals in the group of "good responders" that is, individuals capable of controlling their microfilaraemia will not be identified in another way other than taking into account the notion of "cut-off age".

These results suggest the fact that in a given popula-
tion, the minority have a predisposition of being microfilaremic. These individuals will become micro-filariae positive in continual conditions of transmission before a certain age. This age is probably under influence of transmission intensity and the degree of exposure to infective bite. In the region of Chaullu mountains of Congo, for example, the beginning of pla-teau in the curve showing the prevalence of microfilariae according to age, has been found at 20 years old (Noireau et al., 1989).

In South-Cameroon, it is about 40-50 years old (Garcia et al., 1995). The majority of individuals seem to behave as "good responders". Meaning that their defence system, probably immunological, has capability of controlling microfilariae level. These individuals may also be infected before the "cut of age" in the case of Dienga, it is likely that 33% of the population may be predisposed to the microfilaraemic status but this will be apparent only after a long experience of 45 years. Therefore, during the follow up of a group, about 33% of individuals will probably be microfilariae carriers at 45 years old and the whole population infected before this age if there is transmission remains identical throughout the follow-up time.

It has been shown that transmission intensity does not affect the prevalence of microfilaria and the density of microfilariae (Akue et al., 2002). Similarly, the prevalence of occult infection or amicrofilaremic does not depend on transmission intensity. This suggests that it is only after a sufficient length of exposure that the real status (microfilaric or amicrofilaric) of an individual will be revealed. It is likely that the transmission will only either accelerate (High transmission) or decelerate (Low transmission) the outcome. This need to be taken into account while the objective of the study is to search for genetic or protective mechanisms involved in the control of L. loa microfilaria in a given population. However, the fact that microfilariaemia did not exceed 34% while the serological test shows that 100% of individuals have been infected, suggests the existence of genetic factor which limit microfilaria appearance in only some individuals (Bouchery et al., 2012).

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REFERENCES


