Original article

Epidemiology of Loa Loa and Mansonella Perstans Filariasis in the Akonolinga Health District, Centre Region, Cameroon

Épidémiologie des Filarioses à Loa Loa et Mansonella Perstans dans le District Sanitaire d'Akonolinga, Région du Centre, Cameroun

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ABSTRACT

OBJECTIVE

To provide complementary parasitological data on Loa loa and *Mansonella perstans* filariasis in the Centre Region of Cameroon.

METHODS

For each enrolled subject, a 50 µl calibrated thick blood film was performed using capillary blood obtained between 11 a.m. and 3 p.m. Giemsa stained slides where examined under optical microscope in order to identify and count *L. loa* and *M. perstans* microfilariae**.**

RESULTS

A total of 1511 subjects took part in the survey. The prevalence of loiasis was 23%. As regards the microfilarial load, arithmetic and Williams' geometric means were respectively 2666.3 and 4.9 mf/ml. The intensity of infection, as well as the prevalence, were significantly higher in males than in females and were found to increase with age. The prevalence of high microfilarial loads (≥ 8000 mf/ml) was 7.9% and that of very high microfilarial loads (≥ 30 000 mf/ml) was 2.4%. The prevalence of the infestation with *Mansonella perstans* was 12.4 % and that of the co-infection *L. loa – M. perstans* was 5.1 %.

CONCLUSION

1) The Akonolinga health district is hyperendemic for loiasis. 2) Data collected in this area are similar to those published in most of the surveys carried out in other endemic areas. 3) *M. perstans* filariasis has a relatively high prevalence. These data will be helpful for the Lymphatic filariasis elimination program. The implementation of the treatment for lymphatic filariasis in this area should take into consideration these data for the prevention of severe adverse events that may occur in patients with very high *L. loa* microfiloarial loads after treatment with ivermectin.

KEY WORDS: *Loa loa*, *Mansonella perstans*, prevalence, intensity, co-infection

RÉSUMÉ

OBJECTIFS

Reporter des données parasitologiques complémentaires sur les filarioses *Loa loa* et *Mansonella perstans* dans la Région du Centre au Cameroun.

MÉTHODOLOGIE

Chez tous les sujets, une goutte épaisse calibrée de 50µl de sang capillaire a été réalisée entre 11 heures et 15 heures. Ces gouttes épaisses ont été colorées au Giemsa et examinées pour l'identification et le dénombrement des microfilaires de *L. loa* et *M. perstans*.

RESULTATS

1521 personnes ont participé à cette étude. La prévalence de la loase était de 23%. Concernant les charges parasitaires, la moyenne arithmétique était de 2666,3 mf/ml et la moyenne géométrique de Williams de 4,9 mf/ml. La prévalence ainsi que les charges parasitaires étaient significativement plus élevées chez les hommes et augmentaient avec l'âge. La prévalence des fortes microfilarémies (≥ 8000 mf/ml) était de 7,9%, et celle des très fortes microfilarémie (≥ 30 000 mf/ml) était de 2,4%. La prévalence de l'infection par *M. perstans* était de 5,1%.

CONCLUSION

1) Le District d'Akonolinga est hyperendémique pour la loase. 2) Les résultats obtenus dans cette zone sont similaires à ceux relevés dans plusieurs autres zones d'endémie. 3) La prévalence de la filaire *M. perstans* est assez élevée. Ces résultats seront utiles pour le programme d'élimination de la filariose lymphatique. La mise en œuvre du traitement de la filariose lymphatique dans cette zone devra prendre en compte ces données pour la prévention des effets indésirables graves qui pourraient survenir après le traitement par l'ivermectine chez les patients ayant de fortes microfilarémies de *L. loa*.

MOTS CLÉS**:** *Loa loa*, *Mansonella perstans*, prévalence, charges parasitaires, co-infection

INTRODUCTION

Loiasis is a helminthiasis known to be endemic in the Central African rain forest [1]. It is estimated that some 14.4 million people live in high risk areas for loiasis [2]. In some regions where loiasis and onchocerciasis and/or lymphatic filariasis (LF) are co-endemic, people harbouring high *L. Loa* microfilarial loads are at risk of developing severe adverse reactions and even encephalopathies after ivermectin treatment [3, 4]. A precise delineation of areas where there is a risk of severe adverse events (SAEs) after treatment with ivermectin is therefore a priority for the African Programme for Onchocerciasis Control (APOC) and the Global Alliance to Eliminate Lymphatic Filariasis (GAELF). Such delineation could help to plan safer strategies for mass drug distribution for the control of onchocerciasis and/or the elimination of LF. Some data on the prevalence of *L. loa* filariasis in Centre Cameroon [5, 6] where there is a high risk of developing SAEs have already been collected, but they are still incomplete. In the Akonolinga health district for example, such data concerning the prevalence and the intensity of *L. loa* are lacking. Since this area is endemic for LF - a disease targeted for elimination -, it is necessary to clarify the level of endemicity of loiasis and estimate the proportion of patients at risk of developing post-ivermectin SAEs before any intervention.

Mansonella perstans infection is another human helminthiasis that is widely distributed in Africa and parts of Central and South America and the Caribbean. Because it has been difficult to associate this filarial infection with a clear and specific clinical picture, it received very little interest. In Cameroon, data on *M. perstans* filariasis are scanty and also need to be provided.

METHODS

A. Study area

The study was carried out from January to December 2009 in the Akonolinga health district located in the Centre Region of Cameroon, at approximately 100 kilometres away from Yaounde, the capital city. It covers a 4300 km^2 area with a bit more than 100,000 inhabitants. The vegetation here is the equatorial rain forest. The main activities of the populations are agriculture, hunting and fishing.

B. Sampling and ethical considerations

Subjects were recruited according to a convenience sampling technique. All individuals aged 15 years old and above were invited to take part in the survey. Individual who agreed to take part in the study signed an informed consent form before undergoing parasitological examinations. At the same time, their identity, age and gender were also recorded.

C. Parasitological examination

For each subject included in the survey, a 50 µl calibrated thick blood film (CTBF) was performed using capillary blood obtained between 11 a.m. and 3 p.m. Giemsa stained slides where analysed under optical microscope (magnification x400) in order to identify and count *L. Loa* and *M. perstans* microfilariae (mf). Microfilarial loads were then expressed in microfilariae per millilitre (mf/ml) by multiplying the crude mf count of on the preparations by 20. Calibrated thick blood films were examined at the Centre for Research on Filariasis and other Tropical Diseases (CRFilMT) by two experienced lab technicians and when any discrepancy was found, a second examination was performed by each.

D. Data analysis

Microfilarial loads were described using the arithmetic mean [8], the geometric mean [9, 10] and the Williams' Geometric Mean (WGM) [6] of microfilarial loads. For *M. perstans*, only arithmetic and Williams' geometric means of microfilarial loads were calculated.

The WGM was computed as follow:

$$WGM = e^{\sum \frac{Ln(x+1)}{n}} - 1$$

In this formula**,** x is the subject's microfilarial load and n the total number of subjects examined.

In this survey, we also used two other indicators to better evaluate the intensity of *L. loa* infection and its association with the risk for developing severe adverse events: the prevalence of high microfilarial loads (≥ 8000 mf/ml – level of significant risk of SAEs after ivermectin treatment) and the prevalence of very high microfilarial loads (≥ 30 000 mf/ml – level of significant risk of SAEs with neurological signs after ivermectin treatment).

Chi-square test was used to compare proportions of individuals presenting with the parasite according to their gender and age group. The non-parametric Mann-Whitney test was used to compare means between genders and age groups since a normal distribution was not expected from counting data.

Data collected were analysed using STATA 12 software and the threshold for statistical significance was set at 0.05.

RESULTS

A. Sample description

A total of 1511 volunteers from five health areas (Abem, Edjom, Ekoudou, Endom, Yeme Yeme) of the Akonolinga health district took part in the survey. Their mean age was 47.7 (95% confidence interval

(CI): 46.8- 48.6) years old, with a minimum of 15 years old and a maximum of 91 years old.

B. Characteristics of L. loa filariasis infection

Prevalence

The prevalence of *L. loa* infection based on CTBF results was 23% (95% CI: 21-25%). The infection rate was 32.3% in males and 17.2% in females ($p < 0.001$). The prevalence of loiasis was found to increase progressively with age from 13.2% in subjects aged less than 30 years to 33.8% in those aged 76 years and above. Details of the prevalence according in the different health areas surveyed are given in **Table I**. The highest prevalence of *L. loa* infection (25.8 %) was found in Yeme Yeme whereas Ekoudou had the lowest prevalence (17.2 %).

TABLE I: MICROFILARIAL LOADS AND PREVALENCE OF *LOA LOA* WITH RESPECT TO HEALTH AREAS OF THE AKONOLINGA HEALTH DISTRICT

Health area	N	(%)	AM* (range)	WGM**
Abem	527	24.5	3275,2 (0 - 122240)	6.2
Edjom	332	21.4	2089.9 (0-62600)	4.3
Ekoudou	58	17.2	1384.8 (0-32460)	3
Endom	373	21.7	2200 (0 - 200000)	3.9
YemeYeme	221	25.8	3202.5 (0-118500)	5.7
Total	1511	23	2666.3 (0-200000)	4.9

* Arithmetic mean (mf/ml) ** William geometric mean (mf/ml)

Intensity of L. loa infection

Fluctuations of the microfilarial load

Microfilarial loads ranged from 0 to 200,000 mf/ml. The arithmetic mean was 2666.3 mf/ml (95% CI: 2088.2 – 3244.4). This mean was significantly higher in males (3574.8) than in female (2092.4) ($p < 0.001$). The distribution of *L. Loa* microfilarial loads with respect to health areas is shown in **Table I**.

Prevalence of high microfilarial loads

The prevalence of high microfilarial loads (≥ 8000 mf/ml) was 7.9% (95% CI: 6.6 – 9.3) and that of very high microfilarial loads (≥ 30 000 mf/ml) was 2.4% (95% CI: 1.6 –3.2). These prevalence of high microfilarial loads and very high microfilarial loads were significantly higher in males than in females ($p < 0.001$).

C. Characteristics of Mansonella perstans infection

The prevalence of *M. perstans* was 12.4% (95% CI: 10.8-14.1%). *M. perstans* microfilarial loads ranged from 0 to 10,300 mf/ml. The arithmetic mean of *M. perstans* microfialarial loads was 47 mf/ml. This arithmetic mean was significantly higher in males (70.6 mf/ml) than in females (32.1 mf/ml) ($p < 0.001$). The Williams' geometric mean of *M. perstans* microfilarial loads was 0.8mf/ml. The prevalence of co-infection with *L. loa* and *M. perstans* was 5.1%. The distribution of *M. perstans* microfilarial loads according to health areas is presented in **Table II**.

TABLE II: MICROFILARIAL LOADS AND PREVALENCE OF *M. PERSTANS* WITH RESPECT TO HEALTH AREAS OF THE AKONOLINGA HEALTH DISTRICT

Health area	N	(%)	AM* (range)	WGM**
Abem	527	19.9	103.8 (0 - 10300)	1,6
Edjom	332	2.1	6.6 (0 - 1600)	0.1
Ekoudou	58	5.2	7.2 (0 - 320)	0.3
Endom	373	13.7	27.1 (0 - 1380)	0.8
YemeYeme	221	10	16.2 (0 - 2400)	0.5
Total	1511	12.4	47 (0 - 10300)	0.8

* Arithmetic mean ** William geometric mean

DISCUSSION

Thomson and collaborators [11] have developed a model to estimate loiasis prevalence from satellite based collection of environmental data (vegetation density, altitude, topography and hydrography). From their predictions, it appears that the prevalence of loiasis in the study area (Akonolinga health district) should vary between 20 and 30%. Our findings then fit the Thomson et al. (2004) model since 23% of the subjects examined were infected with *Loa loa*. This prevalence is also in accordance with the results obtained after the rapid assessment of loiasis (RAPLOA) in Central Africa [2], as well as the results obtained in some communities of the East Region in Cameroon with prevalence greater than 20% [12]. This could be explained by environmental similarities between Centre and Eastern Cameroon. In these two forest regions, the vegetation has been somewhat degraded because of urbanization and forest exploitation.

The prevalence of loiasis is lower in the Akonolinga health district than in Southern Cameroon where it reaches 30.9% [9]. This difference may be due to the fact that the dense equatorial forest found in Southern Cameroon has been less influenced by deforestation and is therefore more favourable to the development of *Chrysops*.

The prevalence of loiasis was significantly higher in males (32.3%) than in females (17.2%). These results are coherent with those obtained in the Chaillu mountains in Congo in 1989 [13], in the South Region of Cameroon in 1995 [9], and in many other studies conducted in Africa [10, 14]. As suggested by Pion and collaborators, this situation could be due to uneven exposure to *Chrysops* bites during daily activity [10]. In fact, men used to work outdoors, away from houses and are therefore more exposed to *Chrysops* bites than females who stay at home for household activities. For Reynouard and collaborators, such pattern might be explained by a possible influence of

hormonal factors on individual susceptibility to *L. loa* infection and on tolerance to microfilaraemia [15]. This hypothesis is difficult to apply to our findings since no difference in the prevalence and intensity of *L. loa* infection between males and females was observed in individuals aged less than 30 years. Considering that hormonal impregnation is maximal at about 15 years in both gender, if there was any hormonal influence on the microfilaraemia, the difference at this age would already be significant.

The prevalence of *L. loa* infection was found to increase with age in both genders ($p < 0.001$). This pattern was already observed in Southern Cameroon [16] [9] and in Centre Cameroon [10, 14]. This increase of the prevalence with age seems to be a logical consequence of the increase in the probability to be infected with duration of exposure to the *Chrysops'* bite. In addition, the long term stability of the microfilarial status demonstrated in the South of Cameroun [16] could also be a clue for the understanding of this finding.

The arithmetic mean of microfilarial loads in the Akonolinga health district is lower than that obtained in villages of the Bankim health district, Tikar plain, in the Adamawa Region of Cameroon (5301.7mf/ml) [7]. The difference could be due to genetic factors or to the efficiency of the vector in the Bankim health district.

The WGM *of L. loa* microfilarial loads in our study was lower than that obtained in villages of Lekie division in the Centre Region of Cameroon (13.0 mf/ml) [6]. This can be explained by the fact that the prevalence of loiasis in those villages (31.04%) was also higher than the one in our survey (23%).

The arithmetic mean of *L. Loa* microfilarial loads was higher in males than in females. The same observation was made with the WGM (12.09 for males and 3.72 for females, $p = 0.015$). These results could be a consequence of the fact that the prevalence of loiasis was also higher in males than in females. The increase of the WGM with age could also be explained by the age-related increase in prevalence.

The prevalence of high *L. loa* microfilarial loads (≥8000) was 7.9% and that of very high *L. loa* microfilarial loads (≥30000) was 2.4%. These results are coherent with predictions made by Boussinesq and collaborators after a survey in villages of the Lekie division in Centre Cameroon [6]. They studied the relationship between prevalence and indicators of intensity of *L. loa* infection and concluded that in areas where the prevalence of loiasis ranges between 20 and 30%, the prevalence of high microfilarial loads should be expected to range between 5 and 9% and that of very high microfilarial loads between 1 and 3%. These individuals are at risk of SAEs if they are treated with ivermectin or diethylcarbamazine. The surveillance and proper management of eventual post-treatment SAEs should be taken into consideration while planning the lymphatic filariasis elimination in this area. The prevalence of high microfilarial loads and very high microfilarial loads were higher in males. This result was predictable since there is a correlation between the prevalence of loiasis and indicators of intensity [6].

The prevalence of *M. perstans* filariasis in the Akonolinga health district is lower than those obtained in most of the studies already published. In 1989, Noireau and collaborators obtained a prevalence of 26.2% in Bantus of Southern Congo and a prevalence of 80.6% in Pygmies of the same region [13]. In 1995, a prevalence of 26.6% was found during a survey conducted in Southern Cameroon [9]. Two other surveys have shown a prevalence of 24% in adults of Western Uganda in 1996 [17] and 80% in two endemic communities of Uganda in 2009 [18]. In 1997, in a community of the Bafang health District in Cameroon, another survey showed a prevalence of 54%[19]. The difference between results of these studies and our findings could be due to a difference in environmental factors that influence Culicoïds' survival and activity. Hopkins has demonstrated that their larvae easily breed in organic rich compost [20]. This type of environment is likely more common in areas where higher prevalence was obtained. The prevalence of the co-infestation with *L. loa and M. perstans* was higher than that obtained by Agbolade and collaborators in Nigeria (1%) [21].

In our survey, the arithmetic mean of *M. perstans* microfilarial loads was close to that obtained in 2001 in some communities of the Centre Region of Cameroon (64.9mf/ml) [22]. The small difference observed could be explained by the fact that the prevalence of *M. perstans* infection was a bit higher in that study (16.3%).

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