Analytical study of the periodicity of blood microfilaria in blood donors consulted at Buta General Reference Hospital in the Democratic Republic of Congo

Abstract:

Filariasis reaches about 150 million people worldwide in 80 countries. 120 million people are already affected by filariasis, 49% of them in Asia, 34% in Africa and 16% in the Western Pacific. *Loa loa*, known as a diurnal microfilaria, is also highly endemic in African regions. The geographical location of the environment favours the conditions for the multiplication of Chrysops, allowing a man to receive about 3500 to 4000 bites of this vector per year with a monthly aggressiveness rate varying between 0.5 to 5.5 infesting bites per individual depending on the month of transmission (June and September). Currently, Non-Governmental Organizations are well engaged in the fight against this disease in the Democratic Republic of Congo. Despite this effort, in Buta, in the province of Bas-Uélé where we conducted our investigation, it turned out that among all the respondents, family donors represented 73.4% and volunteers 26.6%. Of this result, during the night the *Loa loa* had 60% against 28.9% of *Wuchereria bancrofti*. The most affected age group was between 18 and 27 years of age (37.1%) and the least affected between 48 and 57 years of age (11.1%), while the male sex had 63% compared to 37% of the female sex among donors. This study provides information on the nocturnal periodicity of *Loa loa* observed in donors who donate blood at night at the Buta General Reference Hospital, in contrast to its known diurnal periodicity.

Keyword: *Frequency, patients, blood microfilaria, General Reference Hospital, DR Congo.*

INTRODUCTION

Filariasis are parasitic diseases that exist in many countries around the world. According to the World Health Organization (WHO), filariasis is the set of pathological manifestations that determine the parasitism of a human organism by filaria belonging to the order of Nematodes [1].

This organization estimates that more than 150 million people suffer from filariasis in the intertropical zone. India is the most affected country with 45.5 million people infected, followed by the sub-Saharan region with 41 million people infected. [2].

Worldwide, filariasis causes alterations in the lymphatic system, hypertrophy of certain parts of the body, causing pain, severe disability and social stigmatization.

The different filariasis are: lymphatic filariasis, onchocerciasis, Medina filariasis and loase [3].

Indeed, these diseases are currently a real public health problem, as filariasis as a whole represents the third largest parasitic endemic disease in the world, after malaria and schistosomiasis. [2].

In Africa, Southern Sudan, Central African Republic, Angola and the Democratic Republic of Congo are among the countries with high endemicity due to microfilaria. [4].

In Congo Kinshasa, filariasis remains a major public health concern because 26 million Congolese are exposed to the risks of contamination, half of whom are already affected. Of the 13 million people affected by microfilaria, those who are actually disabled (blindness, hypertrophy of certain parts of the body) are estimated at 70 miles. [4].

The prevalence in the surveyed areas during the years 2007 - 2008 in the Democratic Republic of Congo was 40.7%. Recent surveys conducted between 2010 – 2013 indicate that filariasis remains focused in much of the Democratic Republic of Congo. [1].

It should be noted that at present, Non-Governmental Organizations are well engaged in the fight against this disease in the Democratic Republic of Congo. This is the case of the CBM (Mesoamerican Biological Corridor), located in Kasai Province, Uélé, Tshopo, North and South Ubangi, North Tshoapa, Rutshuru, Goma, Ituri and Sankuru.

It should be recalled that the vital activity of most Congolese people in general and that of the population of Bas-Uélé in particular remains focused on the primary sector (agriculture, hunting and fishing).

The activities of the primary sector oblige the practitioners to remain in permanent contact with insects belonging to the order of Diptera, probably a vector of this pathology. In forest areas, a man receives 3500 to 4000 Chrysops bites per year. The monthly rate of aggressiveness varies between 0.5 and 5.5 infective bites per individual depending on the month of transmission (June and September) [3].

Given the geographical location of the microfilaria mentioned above, blood transfusion centres are more interested in blood microfilaria that can be transmitted from donor to recipient through blood.

In Bas-Uélé Province, the city of Buta and its surroundings, the microfilaria *Loa loa* was targeted for our study in the laboratory analysis before blood transfusion due to the presence of chrysopes (its vector) in the forests.

Many studies have shown that *Loa loa* is a diurnal blood microfilaria that ends up in the peripheral blood circulation between 10:00 am and 4:00 pm with all the risks of transmission during blood transfusion [1].

At the Buta General References Hospital, the ordered and permanent non-operationality of the blood bank means that blood donors are searched each time there is an urgent case of severe anemia requiring a transfusion; this conditions blood tests outside the four markers prescribed by the National Blood Transfusion Program, in addition to the search for blood microfilaria outside the scheduled hours (10:00 a.m. to 4:00 p.m.) by many scientists.

The main purpose of this study was to search for the *Loa-loa* microfilaria in blood donors at night to verify the theory that this species is exclusively observed during the day.

MATERIALS AND METHODS

Description of the study environment

Our study was conducted in the biomedical laboratory (during transfusion safety activities) of the General References Hospital in Buta, Bas-Uélé province in the Democratic Republic of Congo from January 3 to March 28, 2018.

Methodology and type of study

The population of our study consisted of 410 volunteer and family donors who consulted the blood donation service from 6:00 p. m. to 6:00 a. m.; of these 410 samples, 45 cases were of interest to our research. This cross-sectional - descriptive study allowed us to collect and analyze the samples. While the data processing used the percentage calculation below to interpret the results and present them in the various tables.

Statistical analysis of data

To analyze and interpret our data, we have grouped them into different tables and calculated percentages according to the following formula:

$$\% = \frac{fx100}{F}$$

(f = Observed frequency; F = Expected frequency; 100 = Constant et % = Percentage)

RESULTS

Table 1. Repartition of donors by quality or type

Donor type	Frequency	%
Family	301	73,4
Volunteer	109	26,6
Total	**410**	**100**

It can be seen from this table that the majority of our respondents are family donors with 73.4%; while volunteer donors represent only 26.6%.

Table 2. Distribution of donors by microfilaria positivity

Microfilariaemia test	Frequency	%
Positive	45	11
Négative	365	89
Total	**410**	**100**

The analysis of this table shows that the positive microfilariaemia observed among our respondents reaches 11%. While 89% of donors did not present the microfilariae in their blood on direct (fresh) examination.

Table 3: Distribution of parasitized donors by microfilaria species

Species of microwire	Frequency	%
Loa loa	27	60
Wuchereria bancrofti	13	28.9
Négative	5	11.1
Total	**45**	**100**

The table above shows the results of the examinations after staining. It can be seen from this table that the species *Loa loa* reaches a frequency of 60%. While Wuchereria bancrofti represents 28.9%; and 11.1% of blades are reported negative.

Table 4. Distribution of parasitized *Loa loa* donors by age group.

Age group	Frequency	%
18 – 27 years	10	37,1
28 – 37 years	08	29,6
38 – 47 years	06	22,2
48 – 57 years	03	11,1
Total	**27**	**100**

It can be seen from this table that the most affected age group is between 18 and 27 years old, i.e. 37.1%, and the least affected is between 48 and 57 years old, with 11.1%.

Table 5. Distribution of parasitic donors by the *Loa loa* microfilaria according to sex

Gender	Frequency	%
Male	17	63
Female	10	37
Total	**27**	**100**

It can be seen from this table that more than half of the respondents are male with 63% compared to 37% of female donors.

Table 6. Distribution of parasitic donors by *Loa loa* microfilaria by place of residence

Residence	Frequency	%
Urban environment	09	33,3
Rural area	18	66,7
Total	**27**	**100**

It should be noted in this table that the frequency of donors infected by the *Loa loa* microfilaria in rural areas is twice as high, 66.7%, as that of donors residing in urban areas, 33.3%.

Table 7. Repartition of parasitic donors by the *Loa loa* microfilaria function of sampling time

Sampling time	Frequency	%
6pm - 10pm	17	63
22h - 2h 07	07	25,9
2h - 6h	03	11,1
Total	**27**	**100**

Examination of this table shows that nearly two thirds, or 63% of cases of positivity in Loa loa, relate to samples taken between 6pm and 10pm. While samples taken between 22:00 and 2:00, and those taken between 2:00 and 6:00 represent 25.9 and 11.1% respectively.

Table 8. Distribution of parasitic donors by sector of activity

Activity sector	Frequency	%
Primary	22	81,5
Secondary	5	18,5
Tertiary	0	0,0
Total	**27**	**100**

The analysis of this table shows that more than three quarters, or 81.5% of donors infected by the *Loa loa* microfilaria, are involved in primary sector activities. However, no parasitized donors were found among tertiary sector practitioners.

DISCUSSIONS

Our study shows that the majority of donors are family donors (73.4%) compared to 26.6% of volunteer donors. The explanation for these results can be summarized as that the population of the City of Buta is not sufficiently aware of the practice of voluntary blood donation. The results of this survey do not differ significantly from a study in Kisangani that examined the barriers to voluntary blood donation. Several factors hinder voluntary blood donation, according to the survey. The results of the latter show that 81.5% of the subjects surveyed had knowledge of this practice; however, 57.9% of them have never donated blood [1].

In addition, this study revealed that the positive microfilaremia observed among our respondents reached 11%. This microfilar positivity refers to the two species found in Table 5 where *Loa loa* represents 60%, *Wichereria bancrofti* represents 28.9% and 11.1% are slides declared negative. These negative results may be justified by the storage conditions of the samples because insects can gnaw at the microfilaria spreads of some slides before fixing and reading.

It should be noted that the positivity to microfilaremia observed is not surprising given that filariasis has been found to be endemic in 51% of the health zones of the Democratic Republic of Congo and precisely in the dismembered Orientale Province where the City of Buta is located, as well as the Kasai and Equateur provinces. Generally speaking, filariasis occurs in 10 of the 11 former provinces of the Democratic Republic of Congo [2].

Positivities to other markers

The positivity to other markers of donors not parasitized by microfilaria that this work relates to the analytical study of the periodicity of blood microfilariae, we considered useful to report the frequencies observed at the General Hospital of Buta References to the different markers in our respondents. For this purpose, the highest frequency (8.5%) refers to Hepatitis B followed by HIV with 6%.

Frequency of parasitaemia in *Loa loa* by gender and sector of activity

During our study, we found that the most affected or parasitic sex is the male sex with 63% versus 37% of female subjects. In addition, 81.5% of parasitized donors are involved in activities in the primary sector, which includes agriculture, hunting and fishing.

The superiority of male parasites (63%) can be justified by the fact that hunting and fishing activities practiced in forests where *Loa loa* vectors (Chrysops) are found are more common among men.

Frequency of parasitaemia in *Loa loa* by place of residence

The results obtained indicate that 66.7% of donors live in rural areas compared to 33.3% in urban areas.

The result of our research is combined with that of a survey carried out in the Democratic Republic of Congo which showed that in forest areas, a man receives 3500 to 4000 Chrysops infestation bites per year [1].

Frequency of parasitaemia in *Loa loa* as a function of age group

It has been obtained from Table 4 that the 18-27 and 28-37 age groups are more parasitized in decreasing order of 37.1% and 29.6% than the other age groups.

This situation is explained by the fact that these age groups are favourable to primary sector activities.

Frequency of parasitaemia at *Loa loa* as a function of sampling time

Our research has shown that 63% of parasitaemia in *Loa loa* refers to samples taken between 6pm and 10pm.

Samples taken between 22:00 and 2:00 and from 2:00 to 6:00 in the morning represent 25.9% and 11.1% respectively. This situation is linked to the decrease in the number of patients attending the Hospital as a function of the night's advancement time.

Our scientific investigation has shown that the species *Loa loa* is found in the collection of samples taken at night.

However, Marc GENTILINI's theory says that the microfilaria *Loa loa* is only active during the day (10:00 am to 4:00 pm) [1].

To this end, it was noted that the periodicity of the parasite (*Loa loa*), which is diurnal, may be reversed depending on the activity of the parasitized person. Depending on whether she works day or night. In addition, there is also the hypothesis that the period of vector activity selects the periodicity of the parasite [2].

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In relation to the result of our research which indicates the positivity of Loa loa microfilaremia in nocturnal sampling of peripheral blood samples, our justifications at this stage can only be based on the hypotheses as mentioned above by adding that of the nature of the parasite, so to speak, that there would probably be other subspecies of *Loa loa* not yet identified.

CONCLUSION

At the end of our work focused on the search for blood microfilaria overnight, using the above methodological approach, on 410 donors who constituted the population of our study, apart from the other results among the 45 positive cases, the study showed that *Loa Loa* was present at the interval of 18h00 - 22h00 (63%) followed by sampling between 22h00 - 2h00 (25.9%) and finally between 2h00 - 6h00 (11.1%).

In the light of all these results, only the positivity of the microfilaria *Loa loa* can be observed also in the nocturnal sampling of peripheral blood samples.

In doing so, we suggest the following:

- *To medical bio-technologists***:** To take into account that in transfusion safety and other hematological analyses, nocturnal sampling of peripheral blood samples deserves Loa loa research, as its periodicity is not strictly diurnal.
- *To future researchers:* To deepen this study on the period of Loa loa's activity, which raises hypotheses whose causes are not yet clearly elucidated with the notion of searching, it seems a species related to *Loa loa* if it exists.

REFERENCES

1. Bernard and Geneviève Pierre, Medical Dictionary, 2002.
2. B. Carme, "Filarioses", La Revue du Praticien, no 2, 31 January 2007, p.157-164
3. J. Courtejoie, Laboratory and Health, 1992; p.16.
4. J. Courtejoie, Basic manual for the biomedical laboratory, 2001. p.204-206.
5. M. Gentilini, Tropical Medicine, Flammarion, 1993, p.196-220
6. L. Paris, "Epidémiologie mondiale des maladies parasitaires", La Revue du Praticien, no 2, 31 janvier 2007, p.135
7. Bain O., Passage of microfilaria through the stomach wall of the vector: study techniques and epidemiological importance. Bulletin of the World Health Organization, 1976, p54.
8. Brengues J., Bain O., passage from microfilaria to the hemocele of the vector, in the pairs *Wuchereria bancrofti*-anophèles gambiae, *W. Bancrofti*-aèdes aegypti and *Setaria labiatopapillosa*. Cahiers orstom, series entomologie médicale et parasitologie, 1972, p10.
9. Brumpt E., Précis de parasitologie, Ed. Masson et Cie, Paris, collections de précis médicaux, 1936, p 10.
10. Callot J., Ratignier a. Et al., 1967 - infection of Culex pipiens autogenicus roubaud de france by an American strain of *Wuchereria bancrofti*. Bulletin of the Society of Exotic Pathology 60, p184-187.
11. Giraldo-Calderón G. I., Pérez M. et al., 2008 - evaluation of the triflumuron and the mixture of *Bacillus thuringiensis* plus *Bacillus sphaericus* for control of the immature stages of *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: culicidae) in catch basins. Biomedica. p224- 33.
12. Hawking F., Jennings T. et al, the mechanisms which affect the periodic cycle of Pacific *Wuchereria bancrofti* microfilariae. Journal of helminthology, 1981, p 55: 95-100.
13. Hawking F., the distribution of human filariasis throughout the world. Part IV. America. World Health Organization document, 1975, p75:136. 1-20.
14. Molyneux D., lymphatic filariasis (elephantiasis) elimination: a public health success and development oppportunity. Filaria journal, 2003, p 9:13.
15. Monkey, Presbytis cristatus eschscholtz, 1921. Journal of parasitology, 1980, p66: 645-651.
16. Sissoko D. Infectious and parasitic diseases in Mayotte. Proposal for an epidemiological alert and surveillance system. Mayotte, report. With the collaboration of Renault P., Solet J. L., Quatresous I., Paquet C., Desenclos J.-C.

APPENDICES

Fig1, 2 and 3. *Loa loa* in thin smears after Giemsa staining.

Fig.1

Fig.2

Fig.3

RESEARCH PROTOCOL USED

No.	Age	Gender	Adress	Occupation	Donor type		Sampling time	Examinations		Results and species	
01					FD	VD		FD	TD	*Loa loa*	Other
02											
03											
04											
05											
06											
07											
08											

Legend:

FD = Family Donor

VD = Volunteer Donor

FD = Fresh drip

TD = Thick drip

No.= Number of orders