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Analytic Survey of the Periodicity of the Blood Microfilariae among the Donors of Blood Consulted to the General Hospital of References of Buta in Republic Democratic of Congo

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Authors' contributions

This work was done in collaboration among all the authors. Author BME designed the study, performed the statistical analysis, wrote the protocol and produced the first draft of the manuscript. Author HMP managed the analyses of the study. Author AAK managed the bibliographical research. All authors read and approved the final manuscript.

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ABSTRACT

Non-Governmental Organizations in collaboration with the National Program for the Fight against Onchocerciasis ''PNLO'', are well engaged in the fight against filariasis in the Democratic Republic of Congo.

This study, conducted at the Buta General Reference Hospital, aims to search for the *Loa loa* microfilaria in blood donors during the night in order to verify the theory that this microfilaria species was exclusively observed during the day (at daytime periodicity).

The study involved 45 donors (family and volunteer donors) parasitized by microfilariae. The majority of them were *Loa loa*, the predominance of which came from night-time collection between

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18:00 and 22:00. Donors from the primary activity sector (agriculture, hunting and fishing) [81.5%], those in the 18-27 age group (37.1%) and men (63%) were more infested with microfilaria. Life in rural areas (66.7%) was one of the factors favouring microfilaria infestation, taking into account the above-mentioned vital activities of the population living there. The markers detected in donors outside the microfilaria tested were hepatitis B antibodies (HBsAg) (6.5%) and HIV antibodies (8%). We have observed that the periodicity of the parasite (*Loa loa*) which is (known) diurnal can be reversed depending on the activity of the parasitized person; depending on whether he or she works during the day or at night. There is also the hypothesis that the period of activity of the vector selects the periodicity of the parasite.

Keywords: Periodicity; patients; blood microfilaria; general hospital of reference; DR Congo.

1. INTRODUCTION

Filariasis are parasitic diseases that exist in many countries around the world.

WHO estimates that more than 150 million people suffer from filariasis in the intertropical zone India is the most affected country with 45.5 million infected people, followed by the sub-Saharan region with 41 million infected people. Indeed, these diseases are currently a real public health problem, as filariasis as a whole is the third most widespread parasitic endemic in the world, after malaria and schistosomiasis [1]. The history of filariasis is probably the history of human migration [2].

It is in this context that human filariasis is widespread in tropical countries (more than 70 countries in the world). The severity of the disease varies: it can be mild or severely disabling [3].

In the French territories of these regions, it is still present in Polynesia (aperiodic form). In Mayotte (periodic form), which was still considered one of the most important centres in the world about 40 years ago with 35.3% microfilariae in 1976, filariasis has considerably decreased with a prevalence of 1.9% in 2003. Entomological surveys conducted in 2003 and 2004 did not reveal the presence of filarial larvae (communication from Dr. N. Elissa) [4].

However, it should be recalled that Culex pipiens autogenicus captured in the Parisian subway allows Wichereria bancrofti to develop to the infective stage at 25-27ºC [5].

Investigations have been conducted on the effect of various procedures to increase or decrease the number of Pacific type *Wuchereria bancrofti* microfilaria in peripheral blood. An increase in body temperature in the early morning hours was followed by a moderate decrease in the number of microfilariae. An increase in the amount of oxygen breathed, or a reduction in oxygen (hypoxia) or an increase in carbon dioxide, or the ingestion of sodium bicarbonate did not produce a constant and significant change in the number of microfilaria [6].

Lymphatic filariasis is transmitted by different types of mosquitoes, including those of the genera Culex, which are widely distributed in urban and semi-urban areas, Anopheles, which is found mainly in rural areas, and Aedes, which is treated mainly in endemic Pacific islands [7].

In addition, other studies have shown that eliminating Lymphatic Filariasis is an "easy to do" and inexpensive health intervention that offers significant benefits "beyond filariasis", illustrates partnership and is easy to evaluate. A future without Lymphatic Filariasis will reduce poverty and improve the halth of the poor, prevent disability, strengthen health systems and build partnerships [8].

In Oceania, filariasis is aperiodic: the peripheral population of microfilariae is stable during a boreal owl outbreak. This biological form is called *Wichereria bancrofti* var. pacifica. It seems that the phenotypes of the microfilaria are selected by the trophic periodicities of the vectors [9].

Lymphatic filariasis can take asymptomatic, acute or chronic forms. The majority of infections are asymptomatic, with no outward signs of infection. However, these asymptomatic infections cause damage to the lymphatic system, kidney damage and damage to the immune system [10].

In tropical and subtropical regions, mass treatment, if done properly, reduces the incidence of filariasis. Improvements in living and housing conditions, as well as anti-vector

campaigns against malaria, are also important contributors to the overall downward trend in Bancroft's disease [11].

Since 2012, French, American and Congolese researchers have been administering albendazole every six months to the inhabitants of the village of Seke Pembe in the Republic of Congo, in parallel with WHO. For three years, 80% of the 900 inhabitants of this village have received this treatment. While the prevalence of the disease was about 17 per cent at the beginning of the treatment, it fell to 5 per cent by the end of the treatment. An unexpected and satisfactory result for the research team [12].

Numerous studies have shown that *Loa loa* is a diurnal blood microfilaria that enters the peripheral blood between 10:00 am and 4:00 pm with all risks of transmission during blood transfusion [13,14].

Currently, non-governmental organizations are well engaged in the fight against this disease. It should be recalled that the vital activity of most Congolese in general and of the population of Bas-Uélé in particular remains concentrated on the primary sector (agriculture, hunting and fishing). These activities oblige practitioners to remain in permanent contact with insects belonging to the Diptera order, probably the vector of this pathology [15,16].

Given the geographical location of the microfilariae mentioned above, blood transfusion centers are more interested in blood microfilariae that can be transmitted from donor to recipient through blood. In the province of Bas-Uélé, the city of Buta and its surroundings, the microfilaria *Loa loa* was targeted for our study in laboratory analyses prior to blood transfusion because of the presence of laces (its vector) in forests.

At the General Referral Hospital of Buta, the orderly and permanent non-operation of the blood bank means that blood donors are sought whenever there is an urgent case of severe anemia requiring transfusion; this condition among blood tests outside the four markers decreed by the National Blood Transfusion Program (NBTP), in addition to the search for blood microfilariae outside the scheduled hours (10:00-16:00) by many scientists.

The objective of this study is to search for the Loa-loa microfilaria in blood donors during the night in order to verify the theory that this species is exclusively observed during the day (at diurnal periodicity).

2. MATERIALS AND METHODS

2.1 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.

2.2 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.

2.3 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:

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

Legend

f = Observed frequency;
F = Expected frequency;
100 = Constant;
% = Percentage

3. RESULTS

It emerges from Table 1 that the majority of our respondents are family donors with 73.4%, while volunteer donors represent only 26.6%.

Table 1. Repartition of donors by quality or type

Donor type	Frequency	%
Family	301	73.4
Volunteer	109	26.6
Total	**410**	**100**

The analysis of Table 2 shows that the positive microfilaremia observed among our respondents reaches 11%. While 89% of donors did not present the microfilariae in their blood for direct examination (at cost).

Table 2. Distribution of donors by microfilaremia positivity

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

The Table 3 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 40%.

Table 3. Distribution of parasitized donors by microfilaria species

Species of microfilaria	Frequency	%
Loa loa	27	60
Wuchereria bancrofti	18	40
Total	**45**	**100**

The result of Table 4 shows that apart from microfilaria, other donors were positive for the marker HBsAg (6.5%) and HIV (8%).

Table 4. Distribution of donors by positivity with other markers

Markers	Frequency	%
HBsAg	4	8 .5
HIV	3	6.0
Négative	38	85.5
Total	**45**	**100**

Table 5 shows that the most affected age group is 18 to 27, followed by 28 to 37 (29.6%), 38 to 47 (22.2%) and 48 to 57 (11.1%).

Table 5. Distribution of *Loa loa* parasitized donors by age group

Age range	Frequency	%
18 - 27 years old	10	37.1
28 - 37 years old	08	29.6
38 - 47 years old	06	22.2
48 - 57 years old	03	11.1
Total	**27**	**100**

It emerges from Table 6 that more than half of the respondents are male with 63% compared to 37% of female donors.

We note in Table 7 that the frequency of donors parasitized by the *Loa loa* microfilaria in rural areas is double (66.7%) that of donors residing in urban areas (33.3%).

Table 6. Distribution of *Loa loa* microfilariate parasitized donors by gender

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

Table 7. Distribution of *Loa loa* microfilaria infected donors by location of residence

Places of residence	Frequency	%
Urban environment	09	33.3
Area environment	18	66.7
Total	**27**	**100**

Table 8. Distribution of parasitic donors by microfilaria *Loa loa* according to collection time

Sampling time	Frequency	%
6:00 pm - 10:00 pm	17	63
10:00 pm - 2:00 am	07	25.9
2:00 am - 6:00 am	03	11.1
Total	**27**	**100**

The result of Table 8 shows that nearly two thirds or 63% of positive cases at *Loa loa* refer to samples taken between 6:00 pm and 10:00 pm. While the samples taken between 10:00 pm and 2:00 am and those taken between 2:00 am and 6:00 am represent 25.9 and 11.1% respectively.

Table 9. Distribution of *Loa loa* microfilaria infected donors by industry sector

Sector of activity	Frequency	%
Primary*	22	81.5
Secondary**	5	18.5
Total	**27**	**100**

*Legend: *Primary sector: agriculture, fishing, hunting; **secondary sector: motorcycle taximen, business, education*

Analysis of Table 9 shows that more than three quarters (81.5%) of donors infected with *Loa loa* microfilaria are in the primary sector. While 18.5% of infected donors are those working in the secondary sector, and finally, no infected donors were found among those practicing in the tertiary sector.

4. DISCUSSION

Our study shows that most donors are family donors (73.4%) compared to 26.6% of volunteer donors. The explanation for these results can be summed up by the fact 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 too much from those of a study carried out in Kisangani, which looked at the barriers to voluntary blood donation. The survey found that there are a number of factors that impede voluntary blood donation. The results of the survey show that 81.5% of the respondents had knowledge of voluntary blood donation; however, 57.9% of the respondents had never donated blood.

Furthermore, this study revealed that the positive microfilaremia observed among the respondents reached 11%. This positive microfilaria positivity relates to the two species encountered in Table 5 where *Loa loa* represents 60%, *Wichereria bancrofti* represents 40%. It should be noted that the observed positive microfilariae positivity is not surprising, given that filariasis is endemic in 51% of the health zones of the Democratic Republic of Congo, specifically in the dismembered Eastern Province where the City of Buta is located, as well as in the Province of Kasai and Equateur. Generally speaking, filariasis is present in 10 of the 11 former Provinces of the Democratic Republic of Congo.

4.1 Positivities to Other Markers

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

4.2 Frequency of Parasitaemia in *Loa loa* According to Sex and Sector of Activity

In this study, we found that the sex most affected or parasitized is the male sex with 63% against 37% of female subjects. Subsequently, 81.5% of parasitized donors practice 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 in forests where *Loa loa* (Chrysops) vectors are found are more often carried out by men.

4.3 Frequency of Parasitemia in *Loa loa* According to 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 in line with the results 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.

4.4 Frequency of Parasitemia in *Loa loa* by Age Group

Table 4 shows that the 18-27 and 28-37 age groups are more infected in descending order of 37.1% and 29.6% than the other age groups. This situation can be explained by the fact that these age groups are favouring activities in the primary sector.

4.5 Frequency of *Loa loa* Parasitemia as a Function of Sampling Time

Our research has shown that 63% of parasitemia in *Loa loa* relates to samples taken between 6:00 pm and 10:00 pm.

Samples taken between 10:00 p.m. and 2:00 a.m. and between 2:00 a.m. and 6:00 a.m. represent 25.9% and 11.1% respectively. This situation is linked to the decrease in the number of patients visiting the hospital as the night progresses.

Our scientific investigation has shown that the species *Loa loa* is encountered in the collection of samples during the night.

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

To this effect, it has been noted that the periodicity of the parasite (*Loa loa*) which is diurnal can be reversed depending on the activity of the infected person. Depending on whether they work during the day or at night. According to B. Carma, there is also the hypothesis that the period of activity of the vector selects the periodicity of the parasite.

Compared to the result of our research which indicates the positivity of *Loa loa* microfilaremia in nocturnal samples of peripheral blood, 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 exist other subspecies of *Loa loa* as yet unidentified. We believe that the species could evolve with time and the environment by adapting to it.

5. CONCLUSION

At the end of our work focused on the search for blood microfilaria during the night, 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 6:00 pm and 10:00 pm (63%) followed by sample collection between 22h00 - 2h00 (25.9%) and finally between 2:00 am and 6:00 am (11.1%).

In the light of all these results, only the positivity of the *Loa loa* Microfilaria was found to be positive in the nocturnal collection of peripheral blood samples.

In doing so, we suggest the following:

- **To medical bio-technologists:** Please note that for transfusion safety and other hematological analyses, night-time collection of peripheral blood samples is worthy of *Loa loa* testing, as it is not strictly a daytime procedure.
- **To future researchers:** to deepen this study on the period of activity of *Loa loa* which raises hypotheses whose causes are not yet clearly elucidated with the notion of looking for a related species or a sub-species in *Loa loa* if it would exist.

CONSENT

As per international standard or university standard written patient consent has been collected and preserved by the author(s).

ETHICAL APPRROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX 1

Microfilaria *Loa loa*

Fig. 1. *Loa loa* found in the thickened trade drop

Fig. 2. *Loa loa* found in the blood smear

Fig. 3. *Loa loa* found in the thickened trade drop

APPENDIX 2

Data logging form

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

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