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Prevalence of blood-borne parasitosis: Case of *Loa loa* filariasis among first-time blood donors attending the Benjamin Ngoubou Regional Hospital of Tchibanga, Nyanga province, Southwestern Gabon

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ABSTRACT

Background: Many blood receivers around the world are exposed to blood-borne diseases such as filariasis. Unfortunately, microfilaria worms are not screened for during blood transfusion in Gabon and other endemic countries. **Objective:** The objective of this study was to determine the prevalence *Loa loa* filariasis among first-time blood donors attending the Benjamin Ngoubou Regional Hospital of Tchibanga, South-western Gabon. **Methods:** This cross-sectional study was conducted from May 22 to August 18, 2023 at the medical analysis laboratory of the Tchibanga Regional Hospital. Blood samples were collected from first-time donors who signed a written informed consent form. *Loa loa* diagnosis was carried out using direct optical microscopy and cytoconcentration techniques. Prospective blood donors who agreed to participate in the study were subjected to a structured questionnaire containing sociodemographic information and *Loa loa* associated risk factors. The collected data were entered into an Excel 2016 spreadsheet, exported to R software (version 4.1.1.) in its R Commander Interface, and analyzed using logistic regression. **Results:** A total of 620 first-time blood donors, with a mean age of 33 ± 7.7 years, were included in this study. The prevalence of *Loa loa* microfilariae among the first-time donors in this study was 5.16% (95% CI: [0.04- 0.80]), (n= 32). Univariate and multivariate analyses using logistic regression revealed that being a student (adjusted Odds Ratio = 0.004; 95% CI: [0.000 - 0.07]) working in the informal sector (adjusted Odds Ratio = 0.006;

95% CI: [0.001 - 0.08]), and belonging to the blood group AB+ (adjusted Odds Ratio = 0.11; 95% CI: [4.93 - 26. 2]) appear to be protected from *Loa loa* infection. In contrast, prospective blood donors residing in rural areas (adjusted Odds Ratio = 5.48; 95% CI: [1.42 -1. 48], belonging to blood groups A+ (adjusted Odds Ratio = 40.7; 95% CI: [5.11 - 33.07]) and B+ (adjusted Odds Ratio = 29.1; 95% CI: [14.1 - 6.73]), having a history of blood transfusions (adjusted Odds Ratio = 56.36; 95% CI: [8.13 - 36.24]), and being anemic (adjusted Odds Ratio = 1.67; 95% CI: [0.29 - 0.96]) were significantly more exposed to *Loa loa* microfilariae. *Conclusion:* The results of this study raises concerns and highlights the need to include the diagnosis of blood - borne parasites during blood donation and blood transfusion in Gabon.

Keywords: Prevalence, *Loa loa* filariasis, First-time donors, Tchibanga, Gabon.

1. INTRODUCTION

Giving blood to a patient in distress in a hospital or health facility emergency room is a life-saving act for the patient. According to the WHO, nearly 118.5 million blood donations are annually collected worldwide, 40% of which are in high-income countries, where 16% of the world's population lives. Nearly 13300 blood centers in 169 countries reported collecting 106 million donations. In Africa, WHO supports countries through the development of national blood transfusion policies, screening and testing strategies, and blood product preparation and quality control. Due to various health problems, such as anemia, medical conditions, or risk of transfusion-transmissible infections, many prospective blood donors have often been refused to donate blood (Mremi et al., 2021). Therefore, safe blood transfusion is necessary to avoid hemoparasites such as human immunodeficiency virus (HIV), hepatitis C virus, hepatitis B virus, syphilis and blood-borne parasites such as, *Plasmodium spp* and microfilariae (Alharazi et al., 2022).

Although the latter infection is not often reported in blood transfusions or blood donations Drews et al., (2021), it is highly endemic in Central African countries, especially Gabon where the tropical forest covers 85% the territory. Loasis is transmitted by an arthropod of the genus *Chrysops* (Pallara et al., 2022). This bloodsucking fly lives in the trees of tropical forests (Tompaloumla et al., 2020). Typically, loasis manifests as a red, pruritic subcutaneous swelling called Calabar edema on the dorsal surface of the hands and the wrists, and it is estimated that more than 10 million people in West and Central Africa are infected with *Loa loa* thread-like nematodes (Metzger and Mordmüller, 2014). Varying from country to country where this public health problem is endemic, a prevalence exceeding 50% has been reported in four localities in Cameroon Kamgno and Boussinesq, (2001) and an overall prevalence of 66% for the history of eyeworms and 22% for microfilaremia in Gabon (Akue et al., 2011).

Because there may be individuals harboring adult worms without detectable microfilariae in the blood at any time Pinder, (1988), and because these occult carriers are difficult to identify by microscopy or by sensitive and specific molecular diagnosis Haselow et al., (2003), Choudhury et al., (2003), *Loa loa* microfilariae may be transmissible by blood transfusion, a route considered as a dead-end transmission for the parasite because it cannot reproduce or grow. Despite numerous efforts by the health authorities of this country, the disease remains highly endemic and is not always systematically screened during blood donations or blood transfusions. This situation, which endangers naive blood recipients, prompted this study to determine the prevalence of blood-borne parasitosis: the case of *Loa loa* microfilariae among first-time blood donors at the Tchibanga Regional Hospital, Nyanga Province, in South-western Gabon.

2. DONORS AND METHODS

Setting and period of the study

This cross-sectional study was conducted at the medical analysis laboratory of the Benjamin Ngoubou Regional (BNR) Hospital of Tchibanga (Southwestern Gabon) from May 22 to August 18 of year 2023. Although there is another health structure located 150 km away (Bongolo Hospital), the BNR hospital is the largest public health structure in the province of Nyanga. It receives thousands of patients each year, including blood donors, to manage all aspects of transfusion, from the recruitment of blood donors to the distribution of safe blood.

Inclusion and exclusion criteria

The study population consisted only first-time blood donors, regardless of gender, who came to the BNR Hospital in Tchibanga to donate blood during the study period and had given their verbal and written consent. In addition, those who did not have a reliable telephone contact or who did not fill out the questionnaire properly were excluded, as well as those whose names were on a laboratory deny list, exhibited risky behavior, were under 18 years of age, weighed less than 50 kg, pregnant, menstruating or breastfeeding women.

Data collection

Based on mixed methods including qualitative and quantitative tools before providing a blood sample, all first-time blood donors in the study were subjected to a standard questionnaire to collect information such as sociodemographic characteristics (gender, age, marital status, occupation, place of residence, type of donation). This survey generally lasted between 4 and 5 minutes depending on the type of donation which was either family or voluntary donation.

Blood collection and analysis

Ten (10) ml of fresh venous blood was collected on an EDTA tube from each study participant. Parasitological diagnosis of microfilariae, consisted of direct examination with ten (10 μ l) of blood deposited with a calibrated micropipette on a wet slide and examined under a light microscope with a 10 X eye objective (Touré et al., 1998). As described previously, the parasitemia was expressed as microfilariae per milliliter (mf/ml) of blood (Heddle et al., 2009). To refine the diagnosis of *Loa loa* microfilariae, a cytoconcentration technique was applied to each blood sample as described by (Petithory et al., 1986). One (1) ml of blood was diluted in 9 ml of PBS and lysed with 200 μ l of saponin (2%). Next, the tubes were centrifuged for 10 minutes at 500 rpm and the supernatant discarded. The entire pellet was then resuspended in 3 ml of 0.9% physiological water and centrifuged at 2,000 rpm for an additional 10 minutes. Then, a drop of the suspension was placed in the center of a glass slide using a Pasteur pipette covered with a coverslip and read under a light microscope using the 10 X objective.

Statistical analysis of the data

The data collected through the questionnaire forms were entered and processed in the Excel 2016 spreadsheet. The R software (version 4.1.1.) with the R command interface was used for statistical analysis with Chi-square tests, one-factor ANOVA, and multinomial logistic regression. A p-value < 0.05 was considered statistically significant.

3. RESULTS

Prevalence of *Loa loa* microfilaria in the study population (N = 620)

A total of 620 first-time blood donors, with a mean age of 33 ± 7.7 years, participated in this study. With a sex ratio (M/F) of 8.25, men predominated over women. The prevalence of *Loa loa* microfilariae in primary donors in this study was 5.16% (95% CI: [0.04- 0.80]) (n= 32).

Association of *Loa loa* Infection and Sociodemographic Characteristics of Blood Donors Attending the BNR Hospital of Tchibanga (Southwestern Gabon)

After exploring the possible associations between the sociodemographic characteristics of the study population (n=620) and the prevalence of *Loa loa* microfilariae, using univariate analysis, the variables were fitted in a multivariate logistic regression model. The results indicate that first-time blood donors with student status (adjusted Odds Ratio = 0.004; 95% CI: [0.000 - 0.07]) or working in the informal sector (adjusted Odds Ratio = 0.006; 95% CI: [0.001 - 0.08]), and belonging to the blood group AB+ (adjusted Odds Ratio = 0.11; 95% CI: [4.93 - 26.2]) seemed to be protected from *Loa loa* infection; Whereas, those residing in rural areas (adjusted Odds Ratio = 5.48; 95% CI: [1.42 - 1. 48], belonging to blood groups A+ (adjusted Odds Ratio = 40.7; 95% CI: [5.11 - 33.07]) and B+ (adjusted Odds Ratio = 29.1; 95% CI: [14.1 - 6.73]) were significantly more exposed to *Loa loa* microfilaria (Table 1).

Table 1 Association of *Loa loa* and Sociodemographic Characteristics of BNR Hospital Blood Donors.

Variables	Number of first-time study donors screened N (%)	Prevalence of <i>Loa loa</i> microfilariae		Univariate analysis		Multivariate analysis	
		Positive N (%)	Négative N (%)	COR CI 95%	p	AOR CI 95%	p
Gender							
Male	553 (89.19)	29 (5.24)	524 (94.76)	Reference	-	-	-
Female	67 (10.81)	3 (4,48)	64 (95.52)	1.2 [0.35 - 4.09]	0.76	8.75 [0.25 -1.95]	0.18
Age groups (years)							
18 - 25	139 (22.42)	10 (7.19)	129 (92.81)	1.62. [0.75 - 3.51]	0.22	0.52 [0.11- 2.4]	0.403
26 - 33	116 (18.71)	8 (6.90)	108 (93.1)	1.45 [0.63 -3.32].	0.18	0.50 [0.1- 2.52]	0.4
34 - 41	80 (12.9)	10 (12.5)	70 (87.5)	2.27. [1.13 - 4.55]	0.001	0.25 [0.04. - 1.48]	0.13
42 - 50	285 (45.97)	4 (1.40)	281 (98.6)	Reference	-	-	-
Marital status							
Married	231 (37.26)	7 (3.03)	224(96.97)	Reference	-	-	-
Single	255 (41.13)	15 (5.88)	240 (94.12)	1.28 [0.63-2.61]	0.50	1.43 [0.19 - 10.68]	0.73
Cohabiting	134 (21.61)	10 (7.46)	124 (92,54)	1.7 [0.78 - 3.68]	0.17	0.26 [0.04 -1.7]	0.16
Professional status							
Pupil/ Student	158 (25.48)	11(6.96)	147 (93.04)	1.57 [0.74 - 3.33]	0.24	0.004 [0.00 -0.7]	0.004*
Formal sector	295 (47.58)	14 (4.75)	281 (95.25)	Reference	-	-	-
Informal sector	167 (26.94)	7 (4.19))	160 (95.81)	0.75 [0.32- 1.77]	0.51	0.006 [0.01 -0.08]	0.006*
Residence							
Urban	353 (56.94)	24(6.80)	329 (93.2)	Reference	-	-	-
Rural	267 (43.06)	8 (3)	259 (97)	0.42b [0.19 - 0.95]	0.034	5.48 [1.42 - 21.28]	0.014*
Donor type							
Family	496 (80)	20 (4.03)	476 (95.97)	Reference	-	-	-
Volunteer/ Replacement	124 (20)	12 (9.68)	112 (90.32)	2.55 [2.21 - 5.37]	0.011	0.4 [0.09 - 1.87]	0.3
Blood types							
O+	76 (12,25)	8 (10.53)	68 (89.47)	Reference	-	-	-
AB+	241 (38.87)	13 (5.39)	228 (94.61)	1.08 [0.52 - 2.23]	0.8	11.1 [4.93 - 26.2]	0.003*
A+	167 (26.94)	7 (4.19)	160 (95.81)	0.75 [0.32 - 1.77]	0.51	40.7 [5.11 - 33.07]	0.000
B+	136 (21.94)	4 (2.94)	132 (97.06)	0.49 [0.17 - 1.42]	0.15	29.1 [14.1 - 6.73]	0.000*

COR= Crude Odd ratio; AOR= Ajusted Odd ratio; CI= Confidence Interval; * = Significant p-values in bold

Risk Factors and Clinical Manifestations Associated with *Loa loa* microfilariae among First-time Blood Donors.

To test the association between *Loa loa* microfilaria exposure, risk factors, and clinical manifestations among first-time blood donors in the study, a crude analysis of the variables was performed. Using multivariate logistic regression, it was found that having a history of blood transfusion (adjusted Odds Ratio = 56.36; 95% CI: [8.13 - 36.24]), and being 'anemic' (adjusted Odds Ratio = 1.67; 95% CI: [0.29 - 0.96]), were significantly associated with *Loa loa* microfilaria infection (Table 2).

Table 2 Association of *Loa loa* Infection with Risk Factors and Clinical Manifestations of Blood Donors Attending the BNR Hospital of Tchibanga (Southwestern Gabon).

Variables	Number of first-time study donors screened N (%)	Prevalence of <i>Loa loa</i> microfilariae		Univariate analysis		Multivariate analysis	
		Positive N (%)	Négative N (%)	COR CI 95%	p	AOR CI 95%	p
Clinical Symptoms							
Absent	573 (92.42)	23 (4.02)	550 (95.98)	Reference	-	-	-
Dermatosis	8 (1.29)	3 (37.5)	5 (62.5)	12.06 [2.79-52.93]	<0.001	-	-
Pruritus	26 (4.19)	2(7.69)	24 (92.31)	1.57 [0.35 - 6.96]	0.253	-	-
Calabar Swelling	13 (2.10)	4 (30.77)	9 (69.23)	9.19 [2.67-31.67]	<0.001	-	-
Tattooed							
No	417 (67.26)	20 (4.80)	397 (95.2)	Reference	-	-	-
Yes	203 (32.74)	12 (5.91)	191 (94.09)	1.25 [0.6 - 2.61]	0.56	2.37 [0.66- 8.50]	0.19
History of blood transfusion							
No	579 (93.39)	21 (3.63)	558 (96.37)	Reference			
Yes	41 (6.61)	11(26.83)	30 (73.17)	9.74 [4.3- 22.04]	< 0.001	56.36 [8.9 - 36.24]	0.000*
Anemia							
No	466 (75.16)	24 (5.15))	442 (94.85)	Reference		-	
Yes	154 (24.84)	8 (5.19)	146 (94.81)	1.1 [0.48 –2.52]	0.98	1.67 [0.29 –0.96]	0.045*

COR= Crude Odd ratio; AOR= Adjusted Odd ratio; CI= Confidence Interval; * = Significant p-values in bold

4. DISCUSSION

The present study aimed to determine the prevalence of *Loa loa* microfilariae among first-time blood donors attending the BNR Hospital of Tchibanga (Nyanga Province, southwestern Gabon). The present study indicated that men were in the majority compared to women. This finding is consistent with previous studies that reported a low female representation among blood donors Jun et al., (2012), Hé et al., (2017), (Armél et al., 2021), and in line with the global trend. This result could be due to the various contraindications associated with blood donation in women such as hypotension, anemia, pregnancy, breastfeeding or menstruation, lactation and low body weight (Danic and Bigey, 2009). The prevalence of 5.16% was reported for *Loa loa* microfilariae among first-time blood donors in the present study. This result is higher than prevalences of 1.56% and 3.5% reported respectively in Franceville (Southern Gabon) and Nigeria (Ünlü et al., 2018; Kenguele et al., 2022).

But, lower than the prevalence of 40.9 % reported by Bola et al., (2014) in Nigeria. The variability of the prevalences of these parasites in these studies could be explained by many parameters such as the difference in the timing of the studies, the seasons and

years, the sampling methods, the differences in the levels of education or awareness of the transmission and prevention of *Loa loa* in the study area and the geographical diversities of the study sites (Campillo et al., 2023). In addition, bioclimatic conditions could also influence the differences in prevalence reported here (Badia-Rius et al., 2021). Exploration of potential associations between sociodemographic characteristics of the study population and the prevalence of *Loa loa* microfilariae, using univariate analysis and adjustment of variables in a multivariate logistic regression model, revealed seven determinants such as student status, working in the informal sector, rural residence, and blood group AB, A+, and B+, were significantly associated with microfilaria in *Loa loa*.

Students, workers of the informal sector, and belonging to the blood type AB+ were significantly less prone to *Loa loa* infection. Students and workers of the informal sector could be practicing preventive measures and/or not living in a *Loa loa* endemic area. Regarding donors of the blood type AB+, the result is consistent with the study in Nigeria which reported no microfilaria infection among donors of this group (Bola et al., 2014). Also, our study indicated that rural residents were 5.48 times more exposed to *Loa loa* infection compared to those living in an urban setting (adjusted Odds Ratio = 5.48; 95% CI: [1.42 - 1.48]). This finding could be explained by the fact that in rural areas, the daily activities of these populations (farming, fishing, hunting) expose them to chrysops and mosquito bites. In addition, it should be noted that in rural areas, most villages are surrounded by equatorial forest and vegetation, which may constitute potential reservoirs of Chrysops (Kenguele et al., 2022).

Blood types A+ (adjusted Odds Ratio = 40.7; 95% CI: [5.11 - 33.07]) and B+ (adjusted Odds Ratio = 29.1; 95% CI: [14.1 - 6.73]) were significantly more vulnerable to *Loa loa* microfilariae infection. This result supports the hypothesis that individuals with blood group O are less vulnerable to infection than other blood groups. To test the association between clinical manifestations of first-time blood donors and exposure to *Loa loa* microfilariae, univariate and multivariate analyses were done using logistic regression. The results revealed that donors with a history of blood transfusion (adjusted Odds Ratio = 56.36; 95% CI: [8.13 - 36.24]), and those suffering from anemia (adjusted Odds Ratio = 1.67; 95% CI: [0.29 - 0.96]) had a very high probability of being infected with *Loa loa* microfilaria.

Indeed, first-time donors with a history of blood transfusion (adjusted Odds Ratio = 56.36; 95% CI: [8.13 - 36.24]) were 56.36 times more likely to be infected with *Loa loa* microfilaria than those without a history of blood transfusion. This result agrees with a previous study conducted in Gabon Parkouda et al., (2024) and the fact that *Loa loa* microfilariae is transmissible through blood transfusion (Choudhury et al., 2003). In the present study, first-time donors with anemia (adjusted Odds Ratio = 1.67; 95% CI: [0.29 - 0.96]) had a 1.67-fold probability of being infected with *Loa loa* microfilariae. This result is consistent with a fairly recent study conducted in Lambaréné (Central Western Gabon) which reported that 36.5% of first-time blood donors suffered from anemia (Parkouda et al., 2024).

Limitations of the study

Despite the contributions made, there are some limitations to this study that should be considered in future studies. Within the conceptual framework of the study, we identified some risk factors associated with *Loa loa* microfilaria infection. The laboratory examinations performed in this study were limited to microscopy. In the presence of healthy donor with occult disease, a negative serological screening poses a threat to blood transfusion. The application of nucleic acid-based detection techniques such as polymerase chain reaction (PCR) or immunological methods such as detection of IgG4 directed against antigens would have been appropriate to detect submicroscopic parasites.

5. CONCLUSION

Student status, working in the informal sector, living in a rural area, having blood groups AB, A+, and B+, having a history of blood transfusion, and being anemic were significantly associated with *Loa loa* microfilaria among first-time donors in this study. This study showed that *Loa loa* microfilaria infection was associated with sociodemographic characteristics and risk factors. These results could guide health authorities in Gabon in the control and prevention of *Loa loa* microfilaria infections. To avoid infectious transmissions to blood recipients, they should introduce molecular and immunological diagnostic methods for blood donations or blood transfusions in areas where these parasites are endemic. The authorities could for instance improve sanitation to reduce the prevalence of *Loa loa* microfilaria infections and alleviate the burden of parasitic diseases among the population living in the city of Tchibanga and surrounding villages.

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Abbreviations

OR: Odds ratio

AOR: Adjusted Odds ratio

CI: Confidence interval

N: Number of participants

(%): Percentage

Author Contributions

TNM, and HMK were responsible for the study design. TNM, and LCOE were responsible for the conduct of the study. HMK, ULOS and AJEM were involved in data analysis and interpretation. Statistical analyses were performed by TNM, HMK, MA, and LCOE. Finally, the manuscript was written and edited by TNM, JMA, and LCOE. All authors read and approved the final version of the manuscript for publication.

Ethical Considerations

The identity of the participants was kept secret and authorization was obtained from the general management of the BNR Hospital of Tchibanga. The study protocol followed the ethical rules contained in the Declaration of Helsinki.

Informed consent

Written & Oral informed consent was obtained from individual participants included in the study.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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Data and materials availability

All data associated with this study are present in the paper.

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