



Microfilaremic Loiasis and *Mansonella perstans* Infection Prevalence and Sociodemographic Risk Factors in Urban and Rural Provinces of Gabon, Central Africa

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: <https://doi.org/10.9734/ijtdh/2025/v46i11620>

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Cite as: Akomezoghe, Luccheri Ndong, Noé Patrick M'bondoukwé, Reinne Moutongo Mouandza, Jacques Mari Ndong Ngomo, Bridy Chesly Moutombi Ditombi, Coella Joyce Mihindou, Dimitri Ardin Moussavou Mabicka, Mérédith Flore Ada Mengome, Bédrieh Pongui Ngondza, Maryvonne Kombila, Marielle Karine Bouyou Akotet, and Denise Patricia Mawili Mboumba. 2025. "Microfilaremic Loiasis and *Mansonella Perstans* Infection Prevalence and Sociodemographic Risk Factors in Urban and Rural Provinces of Gabon, Central Africa". *International Journal of TROPICAL DISEASE & Health* 46 (1):43-53. <https://doi.org/10.9734/ijtdh/2025/v46i11620>.

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/129062>

Original Research Article

Received: 01/12/2024

Accepted: 01/01/2025

Published: 10/01/2025

ABSTRACT

Introduction: Loiasis and blood mansonellosis are parasitic vector borne diseases, the most widespread in Gabon after malaria. Loiasis presents all characteristics of a Neglected Tropical Diseases with recent implication on excess mortality among hypermicrofilaremia patients and *Mansonella perstans* seems to interact with other pathogens. The present study aimed to determine the prevalence and sociodemographic risk factors associated with loiasis and blood mansonellosis in three provinces of Gabon with different levels of urbanization.

Methods: Participants were recruited in the province of Woleu-Ntem (rural area), Ngounié (rural area) and Estuaire (urban area). This cross-sectional study was conducted from December 2020 to April 2022. Sociodemographic data were collected and venous blood was collected in an EDTA tube for detection of *Loa loa* and *Mansonella perstans* microfilariae by direct microscopic examination and leukoconcentration techniques.

Results: A total of 2,132 participants were included: 1,342 in the Woleu-Ntem, 492 in the Estuaire and 298 in the Ngounié. Loiasis prevalence was 23.6% (n=503/2,132), 8.7% (n= 185/2,132) for *Mansonella perstans* infection and 2.4% (n=51/2,131) for co-infection in the study population. *Loa loa* was found more frequently in Woleu-Ntem compared to Ngounié (OR: 1.9 [CI 95%: 1.4-2.8]; p< 0.01) and Estuaire (OR: 2.9 [CI 95%: 2.1-4.1]; p< 0.01). Regarding *Mansonella perstans*, the risk of having microfilaremia was 14 and 7 times higher in Ngounié compared to Estuaire (OR: 14.7 [CI 95%: 7.9-29.8]) (p< 0.01) and Woleu-Ntem (OR: 7.3 [CI 95%: 5.2-10.3]) (p< 0.01). The microfilaremia geometric mean was significantly higher in the group of infected inhabitants from Ngounié sites (p< 0.01). No risk factors were found in Estuaire province for both filariasis. In Ngounié, participants older than 29 years old were less frequently infected by *Loa loa* and *Mansonella perstans* (p< 0.01). In Woleu-Ntem, men and the older people were more infected than their counterparts (p< 0.01).

Conclusions: Loiasis and blood mansonellosis burden are higher in rural settings, although also present in the Estuaire. Age and gender were differently associated with these blood filariasis carriage in the three provinces.

Keywords: Gabon; *Loa loa*; *Mansonella perstans*; urbanization, risk factors.

ABBREVIATIONS

CNER : National Ethical Committee for the Scientific Research
DPMTM : Department of Parasitology-Mycology-Tropical Medicine
EDTA : Ethyldiaminetetraacetyl
IVM : Ivermectin
L. loa : *Loa loa*
M. perstans : *Mansonella perstans*
MDA : Mass drug administration
NTDs : Neglected Tropical Diseases

1. INTRODUCTION

Loiasis and blood mansonellosis are parasitic vector borne diseases, the most widespread in

Gabon after malaria (M'bondoukwé et al. 2018, Akue et al. 2011). These filariasis are mainly found in rural areas due to favorable forest environment for *Chrysops sp. vector for Loa (L.)*

loa and *Culicoides* sp. for *Mansonella* (*M.*) *perstans* in Central Africa (M'bondoukwé et al. 2018, Akue et al. 2011). The specific symptoms of loiasis are Calabar swelling, crawling sensation and adult worm in eye; other symptoms such as pain, arthralgia, pruritus, as well as some rare complications include loss of vision, nephropathy, pulmonary inflammation due to its interference with cardiac complications are also described (Veletzky et al. 2020, Ramharter et al. 2023, Buell et al. 2019). Loiasis has shown a significant interest in public health in the control of river blindness. During mass drug administration (MDA) with ivermectin (IVM) for the elimination of onchocerciasis in coendemic areas, patients with *L. loa* hypermicrofilaremia developed fatal severe adverse events (Klion et al. 1993, Pion et al. 2006, Chippaux et al. 1996). On the other side, in endemic settings, clinical symptoms of blood filariasis are frequent reasons for outpatient consultations. Moreover, it was reported that hypermicrofilaremia was associated with a reduction of lifespan in Cameroon and Democratic Republic of Congo, neighbors countries of Gabon (Hemilembolo et al. 2023, Chesnais et al. 2017). Loiasis also impacts quality of life exposed populations (Veletzky et al. 2020).

Loiasis is considered as a common disease in Gabon; this endemic filariasis shares similar characteristic with Neglected Tropical Diseases (NTDs) such as soil-transmitted helminths. It is more frequent in rural areas where more than 60% of the community members can be infected, chronic carriage is also frequent and clinical symptoms are responsible for non negligible disability-adjusted life-years (DALYs), although not associated with death. Furthermore, there is no standardization of patient case management and no specific actions for loiasis control in endemic settings such as the Central Africa region (Veletzky et al. 2020, Metzger and Mordmüller 2014).

Although considered as non-pathogenic, *M. perstans* can interfere with other filariasis, causing cross-reactivity of rapid diagnostic tests and, consequently, false-positive results (Wanji et al. 2015). In addition, *M. perstans* has been associated with high immunoglobulin E (IgE) levels, which correlate with exacerbated allergic symptoms in some infected individuals (Bouyou-Akotet et al. 2014).

Recent data on the distribution of these two filariasis within the country are scarce (M'bondoukwé et al. 2018, Akue et al. 2011,

Bouyou et al. 2016, M'Bondoukwé et al. 2015, Moutongo et al. 2023). Previous local surveys were performed either in urban or rural settlements, either in onchocerciasis foci, either in a small population size (Ella et al. 2022, Veletzky et al. 2022). Moreover, socioeconomic disparities in urban and rural regions are known to influence the distribution of helminthic diseases that appear in the World Health Organization (WHO) NTDs list. As loiasis and blood mansonellosis share some common risk factors, including socioeconomic ones with these NTDs, their prevalence could also be influenced by these risk factors or by community member care seeking behaviors (Moutongo et al. 2023, Silver et al. 2011, Riaz et al. 2020). Thus, the present study aimed to determine the prevalence and sociodemographic risk factors for loiasis and blood mansonellosis in three provinces of Gabon with different levels of urbanization.

2. METHODS

2.1 Study Sites

This study was carried out in three of the nine provinces of Gabon (Woleu-Ntem, Ngounié and Estuaire), with varying degrees of urbanization. In the Woleu-Ntem (northern province), the survey was performed in very remote rural communities, specifically in villages of the Ntem and Haut-Ntem departments, in the Ngounié (southern province), the survey was carried out in rural communities, more specifically in the Louétsi-Wano department. Finally, in the Estuaire province, the study was carried out in the capital city, Libreville, more precisely in the Department of Parasitology-Mycology-Tropical Medicine (DPMTM) of the Faculty of Medicine at the Université des Sciences de la Santé. The DPMTM is the reference center for the diagnosis and management of filarial infections.

2.2 Study Design

This cross-sectional study was conducted from December, 2020 to April, 2022. In Woleu-Ntem and Ngounié sites, a preliminary visit was organized to inform the local authorities and communities about the purpose, importance and procedures of the study. Awareness-raising campaigns were set up to facilitate the voluntary recruitment, ensuring that volunteers were fully informed about the study objectives and procedures. In both provinces, the surveys were part of two projects (PHYLECOG and OCEAC) in which the burden of endemic parasite carriage

was assessed. In Estuaire, outpatients and those who came for a biological testing for the diagnosis of blood filariasis at the DPMTM, were offered to participate in the study. Their oral consent to the subsequent use of their data for scientific purposes, was previously obtained.

2.3 Study Population

2.3.1 Inclusion criteria and data collection

All participants who accepted to be tested for a filarial diagnosis in all the three sites, and who provided their informed consent were included. If the person was a minor, consent was sought from their parents or legal guardians. For each participant, age, gender, location and the results of blood tests were reported on a dedicated case report from.

2.4 Sample Size Calculation

The required sample size for each site was calculated according to previously reported loiasis prevalence in the different sites (39.5%, 20.2% and 18.6% for Estuaire, Woleu-Ntem and Ngounié respectively) (Akuje et al. 2011, Bouyou et al. 2016). The precision was fixed at 5% marginal error, and standard score at 95%. The minimum sample size determined were 280, 245 and 233 for Estuaire, Woleu-Ntem and Ngounié respectively.

2.5 Blood Testing

A volume of 5 ml venous blood sample was taken in an EDTA tube, between 10 am and 3 pm. In Libreville, the participants were directly sampled at the DPMTM. In Woleu-Ntem and Ngounié, samples were collected in the villages, stored in a refrigerated cooler, and transported to the field laboratory. The direct examination of 10 μ L of blood and the leukoconcentration of 5 mL using the Ho Thi Sang and Petithory method allowed the detection and quantification of *L. loa* and *M. perstans* microfilaremia, as previously described (M'Bondoukwé et al. 2024, Ho and Petithory 1963). The Microfilarial density was expressed as the number of microfilariae per milliliter of blood (mf/mL). Hypermicrofilaremia was defined for a parasite density higher or equal than 8,000 mf/mL. *M. perstans* and *L. loa* were identified according to the microfilaria size, presence of sheath and nucleus size.

2.6 Ethical Considerations

The study protocol was approved by the National Ethical Committee for the Scientific Research

(CNER) under the reference number PROT No 0053/2022/CNER/P/SG for the Woleu-Ntem sites, and under the reference number 0081/2019/SG/PR/CNR for Ngounié and Estuaire sites. An authorization was also obtained from the General Direction of Health as well as the village chiefs.

2.7 Statistical Analysis

The data was double entered in Microsoft Excel® version 2016, with the involvement of an operator and a verifier to guarantee the integrity and accuracy of the information in each site. Then, the three data sets were merged and backups were preserved. Data analysis was then carried out using R software version 4.3.0. The quantitative variable "age" was transformed in qualitative variable "age groups" as follows: less than 30 years old, between 30 and 60 years old and superior or equal to 60 years old. The qualitative data were presented in absolute values and percentages (95% confidence intervals). To determine the association between age groups, gender, provinces and *L. loa* and *M. perstans* infections, the chi-square test was used. In the case of small numbers, the Fisher exact test was used. Microfilaremia and age were tested for normality using Karposi test. The *L. loa* and *M. perstans* microfilaremia was expressed in geometric mean and compared between the different provinces according to, age and gender using the Kruskal-Wallis test. A *p*-value below 0.05 was considered significant.

3. RESULTS

3.1 Characteristics of the Study Population

The participant distribution according to age and gender is summarized in the Table 1. A total of 2,132 participants were recruited in the three study sites, 1,342 in the Woleu-Ntem, 492 in the Estuaire and 298 in the Ngounié. In all three sites, women predominated, the sex ratio was 0.52 in the Estuaire, 0.76 in the Woleu-Ntem and 0.96 in the Ngounié provinces. The median age was 51 [2;97] years old; more than 90.0% of participants from Estuaire and Woleu-Ntem were over 30 years old (Table 1).

3.2 Prevalence of Filariasis and Microfilaremia in the Study Sites

The overall, prevalence of *L. loa* microfilaremia was 23.6% (n=503/2,132) in the whole study

population. This prevalence showed a significant variability between the three provinces (Table 2). It was found to be almost twice as high in Woleu-Ntem compared to Ngounié (OR: 1.9 [CI 95%: 1.4-2.8]) (p< 0.01). Participants from Woleu-Ntem were at 2.9 times at higher risk of being infected by *L. loa* compared to participants from Estuaire (OR: 2.9 [CI 95%: 2.1-4.1]) (p< 0.01) (Table 2). Regarding *M. perstans*, its overall prevalence was 8.7% (n= 185/2,132). The risk of having *M. perstans* microfilaremia was 14 and 7 times higher in Ngounié compared to Estuaire (OR: 14.7 [CI 95%: 7.9-29.8]) (p< 0.01) and Woleu-Ntem (OR: 7.3 [CI 95%: 5.2-10.3]) (p< 0.01) (Table 2). Only 51 of 637 infected persons had dual *L. loa* + *M. perstans* infection, giving a prevalence of 2.4% (n=51/2,131). Likewise, coinfection was also more frequent among the inhabitants of Ngounié (p< 0.01) (Table 2).

Considering the 593 *L. loa*-infected participants, *L. loa* geometric mean microfilaremia was 321.7 mf/mL. The microfilaremia ranged from 1 to 12,000 mf/mL in infected individuals from Estuaire, from 100 to 30,200 mf/ml in Ngounié and from 1 to 105,900 mf/mL in Woleu-Ntem. The geometric mean microfilaremia tended to be higher in the group of infected inhabitants from Ngounié sites (p= 0.056) (Table 2).

The *M. perstans* geometric mean parasitemia of the 185 parasite carriers, was 59.7 mf/mL. It was the highest at Ngounié province where the microfilaremia ranged from 100 to 105,900 mf/ml; this range was of 3 to 500 mf/ml in Woleu-Ntem and 1 to 5 mf/mL in Estuaire. *L. loa* hypermicrofilaremia was detected in 3.2% (n= 69/2,132) participants. Those living in Ngounié and Woleu-Ntem were 5 and 4 times at higher risk of having hypermicrofilaremia compared to those living in the Estuaire (OR: 5.2 [CI 95%: 1.2-

35.5]; p< 0.01 for Ngounié and OR :4.2 [CI 95%: 1.2-25.9]; p<0.01 for Woleu-Ntem).

3.3 Factors Associated with *Loa loa* and *Mansonella perstans* Single or Co-Infection

In the Estuaire province, neither age nor gender were found associated with the presence of both filariasis. Participants older than 29 years old and living at Ngounié province were more frequently infected (p< 0.01) (Table 3). In Woleu-Ntem, men had more often a filariasis than women (p< 0.01) (Table 3). Regarding the parasite species, participants aged below 30 years had the lowest frequencies of *L. loa* and *M. perstans* infections (Table 3). Older than 60 years old participants had the highest rates of *L. loa* or *M. perstans* in the Woleu-Ntem provinces (Table 3). Patients aged between 30 and 59 years old from Ngounié had the highest prevalence of both filariasis infection (Table 3). Indeed, an additional analysis showed that *L. Loa*-infected participants from Ngounié were significantly younger [mean age: 47.0 (±20.7) years] than those from Estuaire [mean age: 57.8 (±19.1) years] and from Woleu-Ntem [mean age: 54.9 (±16.1) years] (p< 0.01).

The *L. loa* and *M. perstans* parasite densities, were higher in older inhabitants from Ngounié (Table 3). Moreover, their geometric mean parasite density was twice higher than that of their younger counterparts (Table 3).

4. DISCUSSION

This study is the first in describing the mapping and comparing *L. loa* and *M. perstans* microfilaremia in large populations of urban and rural settlements of Gabon. Not surprisingly, mansonellosis and loiasis were more prevalent in rural sites. This is in accordance with previous reports in Gabon (M'bondoukwé et al. 2018, M'Bondoukwé et al. 2015).

Table 1. Characteristics of the study population in different localities

Variables	Provinces					
	Estuaire		Ngounié		Woleu-Ntem	
	N	%	N	%	N	%
Gender	405		298		1341	
Female	266	65.7	152	51.0	762	56.8
Male	139	34.3	146	49.0	579	43.2
Age (years)	396		294		1330	
<30	35	8.8	153	52.0	131	9.8
[30; 60[182	46.0	85	28.9	719	54.1
≥60	179	45.2	56	19.1	480	36.1

Table 2. Prevalence of *Loa loa* and *Mansonella perstans* infections and microfilaremia in different localities

Prevalence	Estuaire (N=492)		Ngounié (N=298)		Woleu-Ntem (N=1342)		p-value
	n	% (95% CI)*	n	% (95% CI)	n	% (95% CI)	
<i>Loa loa</i>							
Positive	51	10.4 (0.7-11.5)	46	15.4 (11.6-20.1)	406	30.2 (27.8-32.8)	<0.0001
Hypermicrofilaremia**	2	3.9 (7.9-13.5)	8	17.4 (8.3-31.9)	59	14.5 (11.3-18.4)	0.044
<i>Mansonella perstans</i>	12	2.4 (1.3-4.3)	107	35.9 (30.5-41.7)	66	4.9 (3.8-6.2)	<0.0001
<i>Loa loa</i> + <i>Mansonella perstans</i> coinfection	3	0.6 (0.2-1.9)	23	7.7 (5.1-11.5)	25	1.9 (1.2 -2.8)	<0.0001
Microfilaremia		Gmean (mf/mL)		Gmean (mf/mL)		Gmean (mf/mL)	
<i>Loa loa</i>	51	204.4	46	969.7	406	300.5	0.056
<i>Mansonella perstans</i>	12	46.1	107	389.2	66	1.7	<0.0001

* CI confidence interval

** Hypermicrofilaremia corresponding to ≥ 8000 mf/ml

Table 3. *Loa loa* and *Mansonella perstans* microfilaremia prevalence and parasite density in the three study sites by gender and age

Variables	% (95% CI*)	Provinces										
		Estuaire			Ngounié			Woleu-Ntem				
		p-value	Gmean	p-value	% (95% CI)	p-value	Gmean	p-value	% (95% CI)	p-value	Gmean	p-value
<i>Loa loa</i>												
Gender												
Male	12.2 (7.5-19.1)	0.90	166.8	0.5	17.8 (12.2-25.2)	0.34	1201.3	0.3	38.0 (34.0-42.1)	<0.0001	372.3	0.14
Female	11.3 (7.9-15.9)		217.2		13.2 (8.4-19.8)		734.0		24.4 (21.4-27.6)		233.3	
Age (years)												
< 30	8.6 (2.2-24.1)	0.20	303.8	0.4	5.2 (2.4-10.4)	<0.0001	1168.9	0.6	17.6 (11.7-25.4)	0.0009	286.3	0.8
[30; 60[9.3 (5.7-14.8)		636.6		27.1 (18.3-38.0)		570.5		29.6 (26.3-33.1)		196.6	
≥ 60	15.1 (10.3-21.4)		158.0		23.2 (13.4-36.7)		1017.0		34.4 (30.2-38.8)		346.1	
<i>Mansonella perstans</i>												
Gender												
Male	2.1 (0.6-6.7)	1	100.0	1	39.0 (31.2-47.5)	0.32	384.5	0.6	7.2 (5.3-9.8)	0.0009	1.7	0.13
Female	2.6 (1.1-5.6)		49.3		32.9 (25.6-41.0)		394.6		3.1 (2.1-4.7)		1.49	
Age (years)												
< 30	0.0 (0.0-12.3)	0.35	217.2	0.8	24.2 (17.8-31.9)	0.0001	342.3	0.036	3.0 (1.0-8.1)	0.037	1.83	0.4
[30;60[1.6 (0.4-5.1)		N/A		50.6 (39.6-61.5)		314.2		3.9 (2.6-5.6)		1.0	
≥ 60	3.3 (1.4-7.5)		78.6		42.9 (30.0-56.7)		740.3		6.9 (4.8-9.6)		1.6	

* CI confidence interval, ** Gmean: geometric mean

In Estuaire, the rates of infection were the lowest, 10.4% and 2.4% respectively for loiasis and mansonellosis, similarly to prevalences recorded 8 years ago in the Estuaire (4.7% for loiasis and 1.1% for mansonellosis) and in the south of Gabon (4.8% for loiasis and 0.8% for mansonellosis) (M'Bondoukwé et al. 2015, Dieki et al. 2023). Study design, sample size and techniques used for the parasitological diagnosis can explain this difference. On contrary, Bouyou and colleagues reported a prevalence four fold higher (39.5%) in the DPMTM (Bouyou et al. 2016). Thus, loiasis prevalence decreased between 2016 and 2021 in the capital city. Two major explanation can be raised. First, before 2020, the DPMTM was the single center historically recognized as the reference for filariasis diagnosis and case management. Outpatients who underwent a biological testing came from the nine provinces of the country. Secondly, in 2016, occult loiasis detected with serology was the most frequent form of loiasis, while indirect immunofluorescence was not performed in the present study (Bouyou et al. 2016). Thus, the burden of loiasis could be underestimated here. In another way, infected participants diagnosed at the DPMTM in 2021-2022 lived in the Estuaire. It is important to notice that the majority of them performed food crops or had weekly activities in their own plantations that are located in the suburban areas of Estuaire. These plantations are in savannah areas and burning is the main tool used to clean the land, fertilize the soil and prepare it for new planting, creating a favourable environment for *Chrysops* development (Badia-Rius et al. 2019, Sevidzem et al. 2021). The frequent visits in rural provinces, from where some inhabitants of the Estuaire province come, would also explain the presence of *L. loa* and *M. perstans* infected people in the capital city. Thus, although entomological data are lacking, there is a possible local transmission of loiasis in the Estuaire province.

The frequency of *L. loa* and/or *M. perstans* carriage was the highest in rural study sites. Indeed, the forest environment, as well as agriculture which is the main activity of adults in the villages, are recognized risk factors of these filariasis. Furthermore, rural communities does not have easy access to health structures. Even if they recognize the symptoms of loiasis, they usually consult traditional practitioners or treat themselves using plants or other traditional portions for symptomatic treatment (Veletzky et al. 2024). They remain chronically infected and maintain the infection.

Differences in species prevalence were observed between Woleu-Ntem and Ngounié. *M. perstans* was highly prevalent in Ngounié while *L. loa* predominated in Woleu-Ntem. The geographical localizations and ecosystems would explain this observation. Indeed, in Ngounié, the alternate of tropical forest areas with swampy and open ground ones constitute the most favorable ecosystem for *Culicoides* sp. vector development (Kershaw et al. 1953). Whereas, the dense forest which predominates in Woleu-Ntem province, forces farmers to often burn the vegetation to allow a better cleaning, creating the most favourable condition for *Chrysops* sp. development and biting.

Age was differently associated with the rate of filariasis carriage according to the provinces. The mean age of *L. loa* carriers was significantly lower in Ngounié (47,0 years old) compared to Woleu-Ntem (54.9 years old) and Estuaire (57.8 years old) ($p < 0.01$). Akue and colleagues reported a non-significant negative correlation between microfilaremia and age, an association with hunting activities which are preferably performed by adults than the young population (Akue et al. 2011). Nevertheless, retired or older people represent the majority of the active populations in villages of Gabon, while the youngest ones work and live in the main cities. Thus, older adults of Woleu-Ntem are those who are responsible for the rest of the family livelihood. They practice hunting, fishing or agriculture. On contrary, in Ngounié province, several local and international agricultural companies that employed younger adults were established for these last 10 years. Therefore, they are more likely exposed to fly bites and filariasis infection compared to the older community members.

The difference in filariasis infection rates between men and women, in Woleu-Ntem, may be due to the specific behavior of the local population. Agriculture, hunting and fishing are the main activities for daily subsistence in rural areas. Agriculture is practiced by both sexes, but mostly by women. Hunting and fishing, however, are predominantly male activities, especially hunting, which is more practiced than fishing and which is culturally considered an exclusively male activity in this region (Moutongo et al. 2023, Mengome et al. 2024). Hunters can spend 10 to 15 days in the forest, that often means building wood fires for warmth or cooking in the night. This increases their exposition to insect bites, particularly by *Chrysop* sp. This vector is attracted by the smoke from wood fires (Akue et

al. 2011, Wanji et al. 2002). This gender heterogeneity was not observed in the Estuaire, the urban site, where hunting is only occasionally practiced and not by a large number of inhabitants, thus reducing the risk of infection.

This study has some limitations. First it was not performed in all the nine provinces of the country, thus the present data cannot be generalized to the whole population. Secondly, specific geographical characteristic of the study sites as well as participants behaviours and daily activities that influence the exposure to the vectors were not assessed. Nevertheless, this is the first study that assessed human risk factors in different communities with a larger population sample.

5. CONCLUSION

Loiasis and blood mansonellosis burden are higher in rural settings, although present in the Estuaire. Age and gender were differently associated with these blood filariasis carriage. Ecosystem, livelihood activities and the economical environment would also contribute to these differences and to the varying burden of these blood filariasis according to the settlements. Further investigations, including all these factors are needed to provide additional accurate data for a better knowledge of blood filariasis epidemiology.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

CONSENT AND ETHICS APPROVAL

The study protocol was approved by the National Ethical Committee for the Scientific Research (CNER) under the reference number PROT No 0053/2022/CNER/P/SG for the Woleu-Nten sites, and under the reference number 0081/2019/SG/PR/CNR for Ngounié and Estuaire sites. An authorization was also obtained from the General Direction of Health as well as the village chiefs.

ACKNOWLEDGEMENTS

The authors extend their sincere gratitude to all the staff at the Centre de Recherche biomédicale En pathogènes Infectieux et Pathologies

Associées (CREIPA) and the Unité Mixte de Recherche sur les Agents Infectieux et leur Pathologie (UMRAIP) for their invaluable support in participant recruitment in the different sites and their assistance with sample analysis. We also thank all the community members who cooperated and participated in this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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