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Molecular detection of *Wuchereria bancrofti*, *Loa loa* and *Mansonella perstans* from dried blood spots taken from pregnant women in rural Burkina Faso

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Abstract: Introduction: Human filariasis causes high morbidity and severe illness. There is a link between helminth infection and anemia. The objective of this study was to estimate the prevalence of blood-dwelling microfilariae among pregnant women in Burkina Faso using a molecular technique and attempt to find an association between anemia and filarial infection. **Methods:** A total of 1018 dried blood spot samples (DBS) were collected from pregnant women at the Health District of Nanoro. The DNA was isolated from DBS samples using a rapid and simple method. Afterward, the isolated DNA was assayed using the Filaria real-time PCR (F-RT-PCR) method. **Results:** Ten F-RT-PCR-positive samples were obtained as follows: two *W. bancrofti* (0.2%), four *L. loa* (0.39%), and four *M. perstans* (0.39%). No concomitant filarial infections were detected, as well as no coinfections between filarial disease and malaria. There was no link between the presence of *W. bancrofti*, *L. loa*, or *M. perstans* and anemia in pregnant women. **Conclusions:** The prevalence and intensity of human filariasis in this study were low for all of the samples in which microfilariae were detected. The F-RT-PCR can be a confirmatory test for diagnosis in remote areas due to its effectiveness in detecting and differentiating, both sensitively and specifically, a wide range of filarial parasites.

Keywords: *Wuchereria bancrofti*; *Loa loa*; *Mansonella perstans*; dried blood spot; Burkina Faso; real-time PCR

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1. Introduction

Human filarial diseases are caused by parasitic roundworms whose progeny, the microfilariae, can be found in the peripheral blood or skin, depending on the filarial species [1]. To summarize, onchocerciasis or river blindness is caused by *Onchocerca volvulus*, and it is associated with vision impairment or blindness as well as severe dermatitis [2]. Lymphatic filariasis (LF) is caused by *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*. LF patients suffer from lymphedema in their lower extremities, lymphedema in the scrotum (hydrocele), or tropical pulmonary eosinophilia, which is characterized by asthma-like symptoms. Loiasis, the eye-worm, is caused by *Loa loa*; its typical clinical signs are the subconjunctival migration of the adult worm, Calabar swellings, pruritus, oedema, and arthralgia. Mansonellosis is caused by the following three main species: *Mansonella perstans*, *Mansonella ozzardi*, and *Mansonella streptocerca*, normally with no specific symptoms [3–5]. These filarial nematodes have an important social and economic impact on the affected populations, causing high morbidity and serious illnesses resulting in social stigmatization, marginalization, and a loss of work for the afflicted [6].

The life cycles of filariae are similar. All filarial parasites are transmitted by the bite of a female blood-sucking arthropod or vector (mosquitoes, black flies, and deerflies) [1,3,7]. Third-stage larvae (L3) infect a new host when the vector feeds. They penetrate into the skin through the wound inflicted by the insect, L3 larvae, undergo two molts and eventually develop into sexually mature adult worms. Adult females are viviparous; they release thousands of microfilariae into the blood or skin, where they are picked up by vectors during their blood meal after being fertilized by adult male parasites. Ingested microfilariae undergo two molts to become infective L3 larvae that are transmitted to the human host during subsequent bloodmeals [1,3,4].

In 2000, The Global Programme to Eliminate Lymphatic Filariasis (GPELF) was launched with the objective of eliminating this disease as a public health problem by 2020 [8]. The eradication of LF relies on mass drug administration (MDA) using the following three drugs that are currently available for treatment: diethylcarbamazine (DEC), albendazole, and ivermectin [6,9]. GPELF ended in 2020 without achieving the goal of globally eliminating LF by 2020. Since the start of GPELF, the number of infections has been reduced by 74% globally. The latest estimate is that 51.4 million people are infected [10,11]. The goal of the latest Neglected Tropical Diseases Roadmap 2021–2030 is to eliminate LF as a public health problem in 80% of endemic countries by 2030 [11].

Loa loa is endemic in eleven African countries based on the prevalence of eye worm history through a Rapid Assessment Procedure for Loiasis (RAPLOA) [12]. An estimated 14.4 million people live in areas with high infection rates. Another 15.2 million people live in areas where 20–40% of people are reported to have had past eye worm history [13]. In several endemic areas, it co-exists with onchocerciasis and LF, and this represents a public health issue because the ivermectin and DEC treatment administered during MDA programs against LF and ivermectin MDA for onchocerciasis can lead to severe adverse effects (SAEs), including fatal cases of encephalopathy, especially with a high *L. loa* microfilarial load [14,15].

Mansonellosis, the most benign human filariasis, remains a neglected human filarial infection despite the fact that 600 million people are at high risk of infection in Africa and parts of Central and South America [16–18]. Studies on *M. perstans* infections in Burkina Faso are scarce. Kyelem et al. [19] conducted a study to determine the impact of long-term ivermectin mass drug administration (MDA) on the *W. bancrofti* and *M. perstans* infections in Burkina Faso. In 2012, *M. perstans* microfilariae was observed in an atypical and exceptional location from the cervicovaginal smear of a patient from Mangodara (Burkina Faso) during cervical cancer screening [20]. To date, the incidence of mansonellosis in Burkina Faso is underestimated, and health authorities have paid little attention to this disease.

There is an association between helminth infection and anemia, and there is also the possibility that this host's immune responses are modulated by the helminths infecting them [21]. Anemia in pregnancy has a very important socio-economic impact since it can adversely affect maternal and fetal well-being, resulting in increased morbidity and fetal death among populations with low socio-economic status [22,23].

Estimating the prevalence of blood-dwelling microfilariae among pregnant women in rural areas is of great interest as it can inform public health protocols. Therefore, this study was conducted to evaluate, through the use of molecular techniques, the prevalence of *W. bancrofti*, *L. loa*, and *M. perstans* microfilariae in pregnant women from some communities in Burkina Faso and to lay a foundation for future epidemiological and distribution studies of human filarial infection. Since the prevalence and intensity of human filariasis in Burkina Faso were expected to be low due to the MDA program, which intervened by implementing ivermectin and albendazole in this country, a technique with high sensitivity and specificity was required. For these reasons, the chosen technique was the Filaria real-time PCR (F-RT-PCR). Additionally, this study was an attempt to find an association between anemia in pregnant women and filarial infection.

2. Materials and Methods

2.1. Study area: brief description

The present study was carried out by the Clinical Research Unit of Nanoro (CRUN) in the Nanoro Health District (Figure 1). The Nanoro Health District is one of the seven (07) districts of the center-west health region. The district headquarters is in the Nanoro department, 90 kilometers from Ouagadougou, the capital city. This district's area is 1302 km^2 or 5.98% of the center-west region's area of 21752 km^2. This district is characterized by a high migration flux among youngsters toward the capital city and the bordering countries. The literacy rate is about 23% of the population. Since February 2009, the CRUN has implemented a health and demographic surveillance system (HDSS) involving 66,409 individuals (up to the year 2022).

2.2. Study design and eligibility criteria

Blood samples were collected from a cross-sectional study aiming to characterize the clinical epidemiology of pregnant women attending antenatal care (ANC) for the first time. For this reason, all pregnant women attending routine ANC visits at the Health District of Nanoro centers (Nanoro, Pella, Soaw, and Kindi) (Figure 1), regardless of their gestational age, were invited to participate in the study.

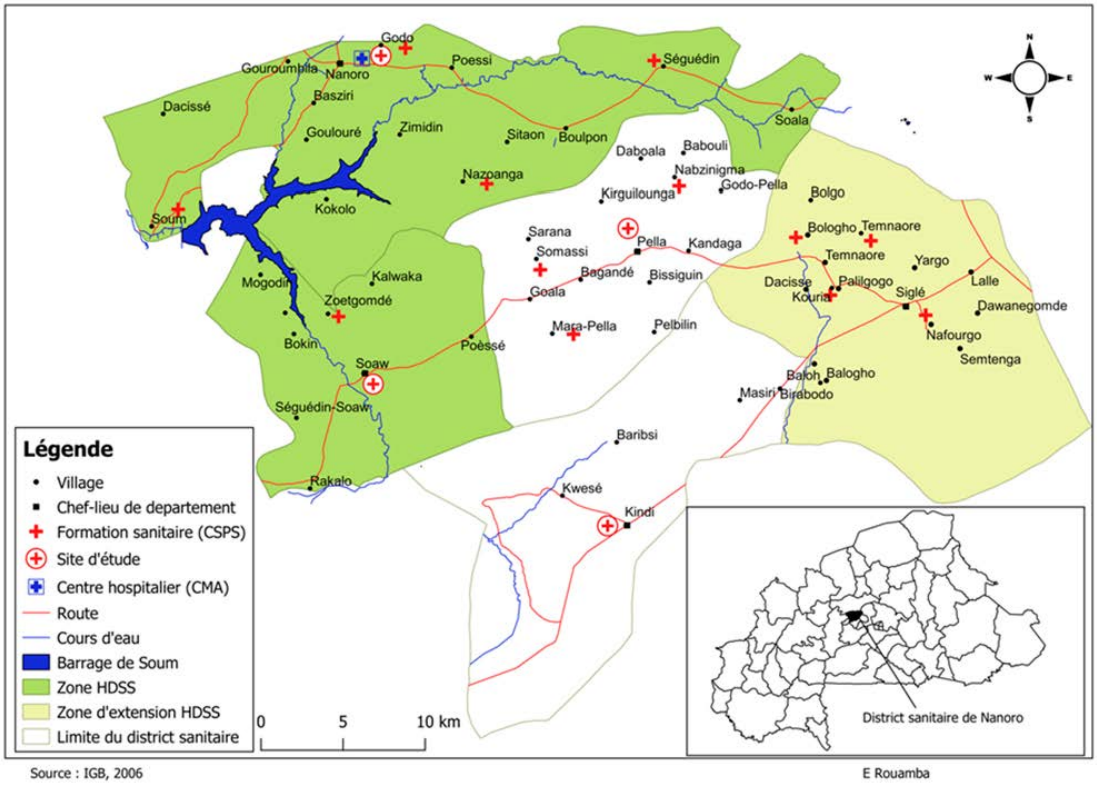

Figure 1: Map of the study area Burkina Faso (in the right down square) showing the Health District of Nanoro and the location of the sampling health centers: Kindi, Nanoro, Pella, and Soaw.

2.3. Study population and sampling

The targeted study population was pregnant women aged between 15 and 45 years old attending ANC at one of the recruiting sites during the study period. During the visit and after obtaining a written informed consent form, a standardized questionnaire was administered to assess socio-demographic data, followed by a physical examination conducted by the study nurses. From each patient, blood samples were collected for malaria rapid diagnostic tests (m-RDT) for prompt management of the study participant, including malaria microscopy and a dried blood spot (DBS). The collected DBS samples were stored in individual plastic bags at adequate temperatures and then transferred to the laboratory of the National Centre of Tropical Medicine (Madrid, Spain).

2.4. Sample size

A total of 1018 DBSs collected from pregnant women living in the recruitment area at the Health District of Nanoro were included in this study. They were collected in the framework of malaria projects from April 2010 to September 2010 and 10 years later, from December 2020 to March 2021.

2.5. Filarial DNA extraction

The DNA from DBS was extracted using a fast and simple non-enzymatic lysis method named Investigator STR GO! Lysis Buffer (QIAGEN GMBH, Germany). A punch 3 mm in diameter was collected from the center of the DBS using a handheld hole puncher. This 3 mm punch was immediately placed into a 1.5 mL tube before adding 100 µL of the lysis buffer, which was vortexed at high speed for 10 s and placed in the heat block at 95 °C for 2.5 min. Then, the tube was put in the centrifuge at full speed (13,000 rpm) for 2 min. The isolated DNA was stored at 4 °C or −20 °C for future analysis. Filter paper samples were handled according to safety procedures to prevent the contamination of samples by disinfecting the handle puncher in NaOH 5 M solution and rinsing the residual NaOH with distilled water.

2.6. Molecular analysis: Filaria Real-Time PCR (F-RT-PCR)

The presence of *W. bancrofti*, *L. loa*, and *M. perstans* in the DNA isolated from DBS samples was assayed using the Filaria real-time PCR (F-RT-PCR) at a final volume of 20 µL, as described by Ta-Tang et al. [24,25]. Briefly, F-RT-PCR was performed using Luna®Universal qPCR Master Mix 2x (New England Biolabs), and the amplification conditions were 1 min at 95 °C followed by 40 cycles of 15 s at 95 °C, 15 s at 48 °C, 30 s at 60 °C and 2 s at 75 °C. All F-RT-PCR reactions were performed using a Rotor-Gene Q 5plex (QIAGEN GMBH, Germany). Clinical samples were run in duplicate and in parallel with appropriate positive and negative controls. Ultrapure water was used as the no-template control (NTC).

A sample was considered positive for filaria parasite if the melting temperature (Tm) curve of the amplified fragments was 76 °C ± 0.5 °C (Figure 2) and the species identification was conducted according to the amplified product size performed using automatic gel electrophoresis (QIAxcel Advanced, QIAGEN GMBH, Germany) or agarose gel electrophoresis stained with Pronasafe (Figure 3). The ITS1 sizes of 312, 301, and 286 base pairs indicated infection with *M. perstans*, *W. bancrofti*, and *L. loa*, respectively.

In addition, the quality of the DNA obtained was checked using human small subunit ribosomal RNA gene sequences as the internal positive control [26].

2.7. DNA sequencing and sequence analysis

The confirmation of the filarial species was performed by sequencing the amplified fragment using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, MA, USA) on an ABI PRISM 3700 DNA analyzer (Applied Biosystems, Massachusetts, USA). Previously, PCR products were purified using Speedtools PCR Clean Up Kit 250 rxns (Biotools, B&M Lab, SA, Madrid, Spain). All amplified products were sequenced twice in both directions, with forward and reverse primers. The sequences obtained were checked with the sequences database from GenBank of the National Center for Biotechnology Information (NCBI), whose accession numbers are as follows: *M. ozzardi* (EU272180), *M. perstans* (EU272181, EU272182, MN432520), *M. mariae* (AB362562), *O. volvulus* (EU272179), *L. loa* (EU272176) and *W. bancrofti* (EU272178). They were also aligned using CLUSTAL W [27].

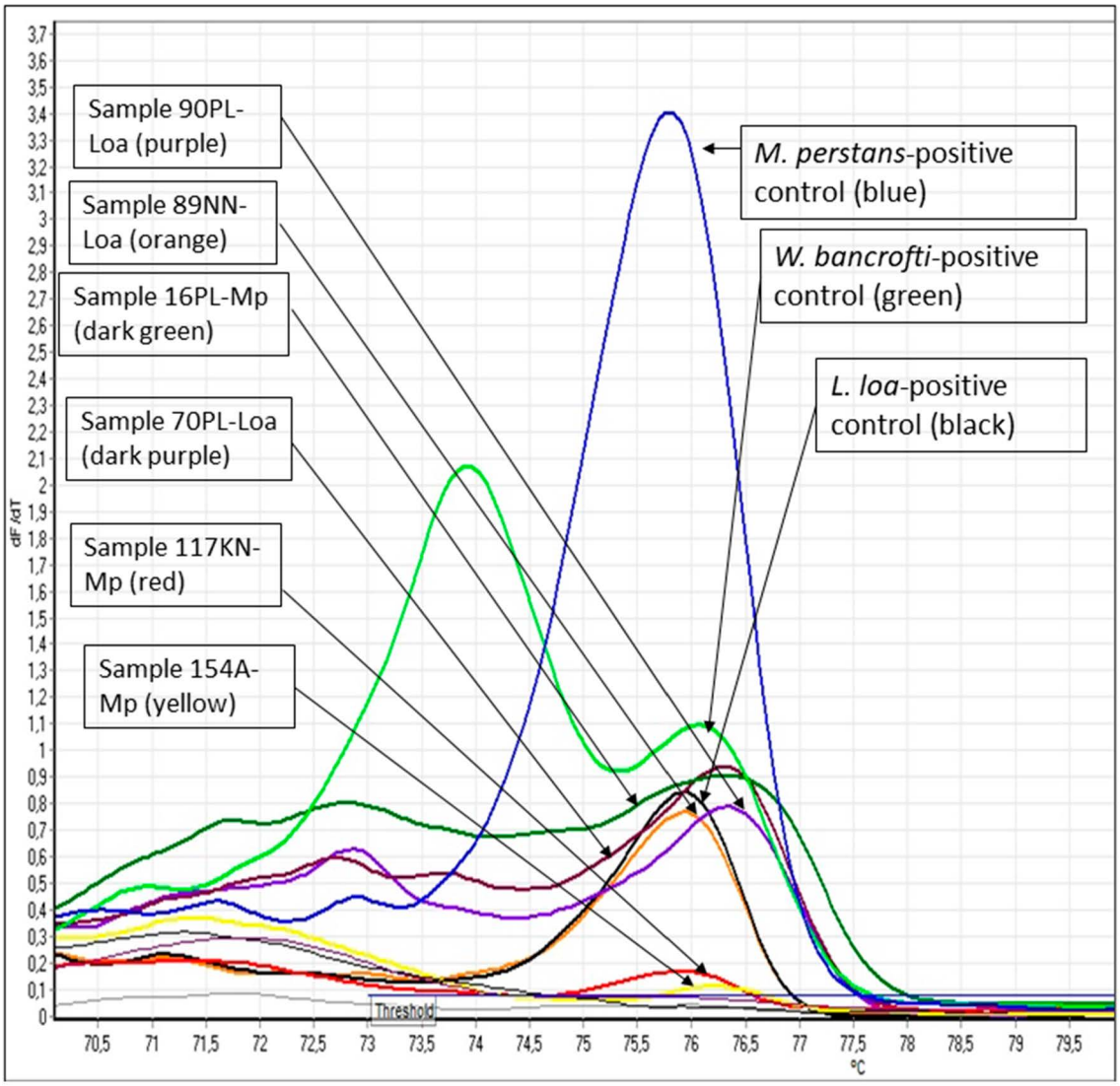

Figure 2: Graphic showing the melting temperature (Tm) curve of the filarial-positive samples tested. The height of the Tm curve correlates with the microfilaria load of the samples. Note: The Rotor-Gene Q 5plex software gives the temperature decimals according to Spanish rules, using comma instead of period.

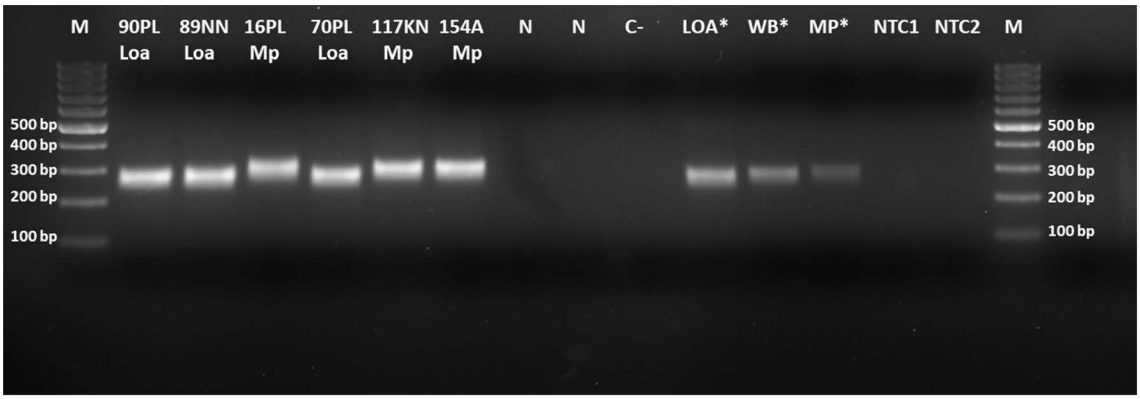

Figure 3: Some of the F-RT-PCR-positive samples run in the agarose gel electrophoresis stained with Pronasafe (current name Condasafe. Condalab). The identification of the infecting filarial species was dependent on the size of the amplified fragment. Sizes of 312, 301, and 286 base pairs indicated infection with *M. perstans*, *W. bancrofti*, and *L. loa*, respectively. Note: the difference in the amplified size product was more significantly differentiated using sophisticated equipment with a higher resolution power, like QIAxcel capillary electrophoresis. Loa: *L. loa*; Mp: *M. perstans*; N: clinical samples negative for filarial disease: C-: negative control for any filarial parasite; LOA, WB, and MP: artificial positive controls created in the laboratory for *L. loa*, *W. bancrofti*, and *M. perstans*; NTC 1 and 2: non-template duplicated control. M: size marker 100 bp (Biotools, B&M Lab, SA).

3. Results

3.1. Study population characteristics

Overall, 1018 participants attending ANC were included in this study and underwent the F-RT-PCR diagnostic test. The socio-demographic characteristics of pregnant women (age, gestational period, educational status) are provided in Table 1. Almost 75% of the study participants had an age between 18 and 34 years old. The median age was 26 years old, within a range of 15–45 years old. More than 80% were in their second and third trimesters. The illiteracy rate was 70% (712/1018). Signs and symptoms suggestive of malaria were absent in most of the cases. Approximately 60.81% (619/1018) had anemia with an average hemoglobin level of 10.54 g/dL and a standard deviation of 1.8 (Table 1).

3.2. Molecular analysis: Filaria Real-Time PCR (F-RT-PCR)

Out of the 1018 samples examined from pregnant women, ten samples tested positive for microfilaria parasites using the F-RT-PCR method, representing 0.98% of the total women studied as follows: two tested positive for *W. bancrofti* (0.2%), four for *L. loa* (0.39%) and four for *M. perstans* (0.39%) (Table 2). No concomitant filarial infections were detected. In addition, none of the ten F-RT-PCR-positive samples were co-infected with malaria parasites when comparing the F-RT-PCR and malaria-microscopic results and considering microscopy as the gold standard method for malaria diagnosis. Most filarial-positive participants in this study were localized in the Nanoro and Pella areas.

Table 1: Socio-demographic characteristics of the pregnant women studied (n = 1018) attending antenatal care.

Variables	*n*	%
Maternal age (years)		
[15–18]	65	6.39
[19–34]	824	80.94
[35–45]	129	12.67
Gestational period		
1st trimester	72	7.07
2nd trimester	491	48.23
3rd trimester	455	44.70
Schooling		
Yes	190	18.66
No	828	81.34
Parity		
Nulliparous	167	16.40
1–3	543	53.34
⩾4	308	30.26
ITN use		
Yes	762	74.85
No	162	15.92
ND	94	9.23
Anemia		
Yes	619	60.81
No	399	39.19

ITN: insecticide-treated nets; ND: no data.

Although blood smears were collected for the examination of malaria parasites, these stained blood smears could have also been used to visualize the microfilariae of *W. bancrofti*, *L. Loa*, or *M. perstans*. However, none of the microscopists screened the slides for microfilariae. Therefore, the corresponding microfilaremia via microscopy for this filarial parasite is unavailable. The threshold cycle or C_t value was used to estimate the relative concentration of filarial organisms per milliliter present in the DBS samples by comparing the obtained values to an appropriate standard curve derived from a series of dilutions of known filarial organism concentrations.

The relative microfilaria intensity for the ten F-RT-PCR-positive samples is described in Table 2. These relative quantifications represent, in general, low microfilaremia for any of the microfilariae detected. The microfilaremia of sample #3B could not be calculated. This sample had detectable *M. perstans* of genomic DNA via F-RT-PCR, but the microfilariae burden was too low to be estimated correctly using F-RT-PCR based on the standard curve; this sample likely had a smaller number of microfilariae than sample #154A. There was no possibility of obtaining a new DNA isolation from sample #3B due to the lack of blood samples for conducting a new calculation of the microfilaremia.

Table 2: Characteristics of the ten F-RT-PCR-positive samples.

Sample	Year	Age	District Health Center	m-RDT	Haemoglobin Level (g/dL)	F-RT-PCR	Microfilarial Intensity by F-RT-PCR (mF/mL)
154A	2010	38	Nanoro	Negative	10.8	*M. perstans*	85
170A	2010	21	Nanoro	Negative	9.5	*L. loa*	100
3B	2010	25	Nanoro	Positive	10.3	*M. perstans*	NA
138B	2010	28	Nazoanga	Negative	9.5	*W. bancrofti*	114
197C	2010	30	Nanoro	Negative	10.9	*W. bancrofti*	200
117KN	2020_21	35	Kindi	Negative	11.8	*M. perstans*	574
89NN	2020_21	25	Nanoro	Negative	10.1	*L. loa*	1180
16PL	2020_21	39	Pella	Negative	9.2	*M. perstans*	725
70PL	2020_21	24	Pella	Negative	7.3	*L. loa*	1205
90PL	2020_21	24	Pella	Negative	7.7	*L. loa*	961

m-RDT: malaria rapid diagnostic test; F-RT-PCR: filaria real-time PCR; KN: Kindi; NN: Nanoro; PL: Pella; NA: not available; mF/mL: microfilariae per milliliter. The WHO considers anemia in pregnancy as hemoglobin values below 11 g/dL.

3.3. Sequencing and analysis of the F-RT-PCR-amplified fragments

The amplified products generated using F-RT-PCR were sequenced and compared to *W. bancrofti*, *L. loa*, and *M. perstans* 18S ribosomal RNA gene sequences deposited in the GenBank/NCBI. A BLAST search showed 100% identity with *W. bancrofti*, *L. loa*, and *M. perstans* sequences, thereby confirming that the ten positive patients were infected with blood filarial parasites. These sequences were also aligned using CLUSTAL W [27] to confirm the diagnosis and detect possible mutations; no mutation in the amplified fragment was detected.

4. Discussion

In tropical regions, the distribution of the filarial species *W. bancrofti*, *L. loa*, and *M. perstans* often overlaps, such as in Cameroon, Equatorial Guinea, or Gabon [28–32]. The prevalence of LF has decreased drastically in many health districts in Burkina Faso through the implementation of MDA campaigns that have applied albendazole and ivermectin since 2001 [33]. Infections via the filarial parasite *L. loa* mainly affect inhabitants of endemic areas; Burkina Faso is not a region associated with this infection, although travelers visiting these endemic regions can also become infected [34]. Studies on *M. perstans* infections in Burkina Faso are scarce. As of today, the incidence of mansonellosis in Burkina Faso is underestimated [19,35].

The finding of *W. bancrofti, L. loa*, and *M. perstans* in the studied area was an important discovery, as it is the first time that these infections have been evaluated in pregnant women in Burkina Faso, and therefore, in the Nanoro Health District, using a quantitative real-time PCR as the diagnostic method. Previous studies related to *W. bancrofti* were performed using a cross-sectional descriptive survey, the Filariasis Test Strip (FTS), Binax ICT (Binax, Portland, ME), or microscopy, but not molecular methods for the quantitative detection of the causative agent of LF [19,33].

Just two pregnant women were infected with *W. bancrofti*, which represents a prevalence of 0.20%, and the low microfilaria load (114 and 200 microfilariae/mL) was concordant with the study conducted by Kima et al., 2019, in which the overall prevalence of the microfilaremia was 0.62%, and the average microfilaremia density was 106 microfilariae/mL [33]. It was not a surprise that both LF-positive samples belonged to the collected period of 2010 and that none of these samples from 2020 to 2021 were positive. In the first period, the MDA campaign, with the distribution of albendazole and ivermectin, had not been established for even ten years, whereas in the second period, the distribution had already been consistent for over fifteen years [36]. This result confirms the success of the MDA program [11].

Loiasis is typically found in heavily forested areas of west-central Africa, with a limited geographical distribution determined by the vector, a tabanid fly of the genus *Chrysops* spp. [37]. Burkina Faso is not an endemic area known for *L. loa*; therefore, it is possible that the four cases of loiasis detected in this study were from women who had previously lived in *L. loa*-endemic areas before becoming married or women who became infected from traveling there, and whose diagnoses were only possible to achieve via the molecular analysis of microfilariae [38,39]. Since three out of the four *L. loa*-positive samples were from the period 2020–2021, this indicates a recently acquired infection. Regarding the *L. loa*-microfilariae intensity, this study showed a low-to-moderate microfilaria load in all cases, according to the parasitological indicators described by other authors [15,40].

Mansonellosis infection is frequently missed due to its tiny size or misidentification during slide examination, which, together with the low microfilaremia, explains why it has received limited attention [17,41,42]. The prevalence of *M. perstans* in this present study, despite appearing very low (0.39%), indicates that authorities should pay more attention to this non-pathogenic filariasis and that it is time to place this neglected tropical disease on the Ministry of Public Health's agenda and take it into consideration similar to other human filariasis. *M. perstans* filariasis was found to affect all segments of the population, independently of sex and age, affecting both school-age children and adults [43]. This study demonstrates that *M. perstans* is also present in Burkina Faso and backs up prior research in other countries [17,43]. The WHO's MDA program, which includes DEC for LF and ivermectin for onchocerciasis, is expected to clear *M. perstans* microfilariae [11]. The reality, however, is that none of these treatments efficiently clear *M. perstans* parasitemia, nor do they have an impact on its transmission [17]. This explains the four *M. perstans*-positive samples obtained in this study, both in the year 2010 and in the years 2020–2021. As of today, Burkina Faso is not widely recognized as an endemic area for *M. perstans* [44].

Currently, there are few data reporting filarial infections in pregnant women. Malaria should not be the only study target for routine public health interventions during pregnancy, but also human filariasis. The ten filarial-positive cases detected in this study did not allow a reliable association between *W. bancrofti*, *L. loa*, or *M. perstans* with anemia to be established in pregnant women. However, it would be unfair to completely rule them out as possible contributing factors to the anemia observed in these pregnant women, especially when the most frequent anemia cause, malaria disease, was absent.

It is well known that molecular methods are much more sensitive for the detection of microfilariae than microscopic examinations [45–47]. In the present study, a quantitative real-time PCR developed by Ta-Tang et al. [24] was used. Only ten F-RT-PCR-filaria-positive samples were obtained, and although it is unlikely that this finding has an impact on the epidemiology of the disease, it shows that *W. bancrofti, L. loa*, and *M. perstans* are present in this region. In addition, a periodic update of the filariae geographical distribution in Burkina Faso is necessary because of the spontaneous or induced disappearance of certain foci and the emergence of other foci as a result of the WHO's MDA program or migrations of infected populations from other endemic areas [11].

Unfortunately, some of the malaria microscopy slides (year 2010) cannot be re-examined in order to find microfilariae. In these cases, F-RT-PCR was found to be very interesting and useful for the retrospective study of human filariasis in this area using long-term stored samples and calculating an approximated microfilaremia [48]. There is a good correlation between the extent of microfilaremia

predicted by F-RT-PCR and the level confirmed by microscopy [24,25]. The low parasite densities obtained and their low prevalence indicate that the population of the study is under the 1% endemicity threshold for *W. bancrofti*, *L. loa*, and *M. perstans*. Moreover, it reflects, on the one hand, the success of the WHO's programs against LF and, on the other hand, the little abundance of infected vectors in those areas. However, further entomological investigations are needed to determine the real prevalence of infected vector transmitters of human filariasis.

The F-RT-PCR used in this study allowed the identification and differentiation of these filariae in the DBS samples. According to the Road Map for Neglected Tropical Diseases 2021–2030 [11], the actions required by the WHO to meet their 2030 targets include devising confirmatory diagnostic tests for use in low-prevalence settings that can assist with mapping, MDA-stopping decisions, and surveillance. The WHO also encourages the development and use of multiplex tests, rather than single tests, which can detect only a filarial species. Furthermore, the diagnostic method developed does not have to cross-react with *L. loa* and can be used both in vectors and in humans. The F-RT-PCR assay proposed in the present study is a pan-filarial molecular assay that is able to detect different human filariae in the same reaction with high sensitivity and specificity. In addition, it can be applied to clinical samples from any origin (blood, skin biopsies, organic fluids), and it is valid in both humans and vectors [24,45].

In addition, given the easy storage conditions and long-term storage capacity of the DBS, this method can be standardized for surveillance studies in endemic regions [49]. In this context, DBS collected over the years represented a precious filarial parasite DNA source for this study. The blood samples collected as DBSs is a method that is simple, practicable, has low-cost, is easy to transport and store. It should be a universal method for sample collection in epidemiological investigations and field trials [50,51].

5. Conclusion

The prevalence of human filariasis demonstrated in our study was low for any of the microfilariae detected. The progress achieved through the MDA program should be maintained by continuously monitoring these regions, anticipating a resurgence in LF, and ensuring the detection of LF cases with field-reliable diagnostic tests. The F-RT-PCR can be a confirmatory test for diagnosis in remote areas due to its capacity to detect and differentiate, both sensitively and specifically, a wide range of filarial parasites. In addition, further research should be conducted to determine the epidemiological impact of *M. perstans* among the country's population and the implications that such results could have for the country's health policy in relation to this disease and filarial disease control. Given the low number of filarial-positive samples, a reliable association between anemia and filariasis diseases cannot be established. Finally, DBS represents a cheap and easily accessible source of genetic material, not only for filarial DNA but also for other kinds of microorganisms for retrospective molecular analysis.

Limitations of the study

The present study was conducted only in pregnant women and, therefore, cannot be generalized to the whole population. To ascertain that the LF transmission is still occurring in BF, children under 10 years old should be included in future studies. Additionally, the lack of blood available did not allow the F-RT-PCR to be repeated, as was the case for sample #3B. However, these results are of utmost importance for confirming the presence of *W. bancrofti*, *L. loa*, and *M. perstans* in the studied area, even at low prevalence levels.

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Ethical Approval: The study was conducted according to the guidelines of the Declaration of Helsinki and was ethically approved by the Institutional Ethic Committee of Centre Muraz (registration no. 005-2010/CE-CM) and the Institutional Review Board of the Institut de Recherche en Science de la Santé, Burkina Faso (N/REF. A15-2020 CEIRES). Following an explanation of this study's purpose, benefits, and potential risks, pregnant women attending the maternity ward provided written consent.

Informed Consent Statement: Informed consent was obtained from all subjects involved in this study. Written informed consent was obtained from the patients to publish this paper.

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