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Manthia DIALLO

Les filarioses humaines au Sénégal : dépistage et épidémiologie

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Affidavit

Je soussigné, Manthia DIALLO, déclare par la présente que le travail présenté dans ce manuscrit est mon propre travail, réalisé sous la direction scientifique de **Dr Oleg MEDIANNIKOV** et du **Dr Cheikh SOKHNA**, dans le respect des principes d'honnêteté, d'intégrité et de responsabilité inhérents à la mission de recherche. Les travaux de recherche et la rédaction de ce manuscrit ont été réalisés dans le respect à la fois de la charte nationale de déontologie des métiers de la recherche et de la charte d'Aix-Marseille Université relative à la lutte contre le plagiat.

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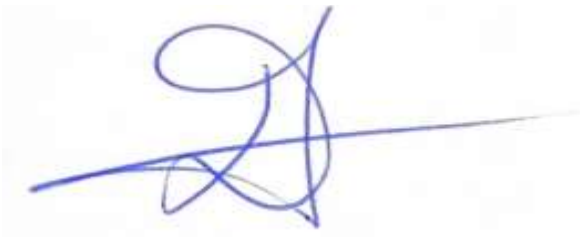

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I, undersigned, Manthia DIALLO, hereby declare that the work presented in this manuscript is my own work, carried out under the scientific supervision of **Dr Oleg MEDIANNIKOV** and **Dr Cheikh SOKHNA**, in accordance with the principles of honesty, integrity and responsibility inherent to the research mission. The research work and the writing of this manuscript have been carried out in compliance with both the French national charter for Research Integrity and the Aix-Marseille University charter on the fight against plagiarism.

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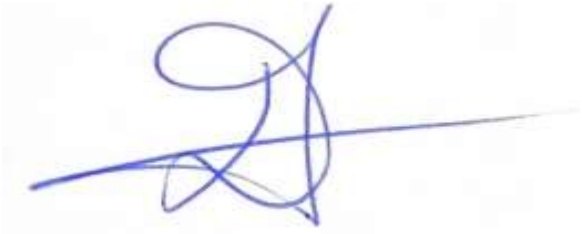

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Liste des publications et participation aux conférences et congrès

I. **Liste des publications réalisées dans le cadre de ma thèse** (**premier auteur**) :

1. **Diallo Manthia**, Hubert Bassene, Georges Diatta, Florence Fenollar, Cheikh Sokhna, Oleg Mediannikov.
   Human filariasis in Senegal: screening and epidemiology. (Review in progress)

2. **Diallo Manthia**, Bassene Hubert, Diatta Georges, Noel Magloire Manga, Ba Ousseynou, Fenollar Florence, Sokhna Cheikh, Mediannikov Oleg.
   Elephantiasis and *Mansonella perstans* in Senegal (West Africa)

3. **Diallo Manthia**, Bassene Hubert, Diatta Georges, Masse Sambou, Fenollar Florence, Sokhna Cheikh, Mediannikov Oleg.
   Role of Tabanidae in the transmission of filarial (In progress)

II. **Autre publication** :

Pascal Dembélé, **Manthia Diallo**, Papa Mouhamadou Gaye, Mady Cissoko, Mahamadou A Thera and Stéphane Ranque*
Epidemiology of *Mansonella perstans* in Mali

III. **Participation aux conférences et congrès au cours de la période de thèse** :

Manthia DIALLO, (2022), Journée scientifique de la Fondation Méditerranée Infection et Infectiopôle Sud, Marseille, (présentation sous forme de poster)

Manthia DIALLO, (2023), 1er Congrès de la Société Sénégalaise de Parasitologie, Mycologie et Entomologie (SOSEPAME). (Présentation orale).

Manthia DIALLO, (2024), Journée scientifique de la Fondation Méditerranée Infection et Infectiopôle Sud, Marseille, (présentation sous forme de poster)

Résumé

Les filarioses sont des infections parasitaires courantes, causées par des vers nématodes filaires dans l'organisme. Parmi les filarioses humaines, on distingue la filariose lymphatique, causée par les espèces *Wuchereria bancrofti*, *Brugia timori* et *Brugia malayi* ; l'onchocercose, ou cécité des rivières, causée par *Onchocerca volvulus* ; la loase, provoquée par le parasite *Loa loa* et la mansonellose, due aux espèces *Mansonella perstans, Mansonella streptocerca* et *Mansonella ozzardi*. Au Sénégal, les programmes de lutte ont permis d'interrompre la transmission des parasites dans la majorité des districts sanitaires notamment pour l'onchocercose et la filariose lymphatique. Cependant, la filariose lymphatique reste encore endémique dans les zones rurales du pays.

Ce travail de thèse porte sur les filarioses humaines au Sénégal. Il se concentre principalement sur le dépistage et l'épidémiologie des filaires impliquées dans la transmission des maladies filariennes dans le sud du Sénégal. Notre étude a pour objectif principal d'explorer l'étiologie et l'épidémiologie des filarioses dans les zones étudiées, et/est scindé en deux parties :

La première partie présente une revue de la littérature sur les filarioses en Afrique de l'Ouest, abordant les pathologies existantes, leur épidémiologie, leur pathogénicité, les méthodes de diagnostic, le traitement et les différentes mesures de prévention ciblant les vecteurs arthropodes.

Dans la seconde partie, la technique de la PCR en temps réel (RT-PCR) et le séquençage ont été utilisée pour détecter *M. perstans* à partir d'échantillons de sang capillaire, de cassettes TDR *Plamodium falciparum* négatifs et de moustiques culicoïdes vecteurs. *M. perstans* a été détecté chez 2 patients sur 23 atteints d'éléphantiasis collectés en 2021, chez 12 sur 279 échantillons de sang de sujets fébriles de Mlomp (2018-2020) et chez 11 sur 3714 cassettes TDR du paludisme négatives. Des moustiques (n=1487) ont été capturés avec des pièges lumineux CDC, OVI et à partie de la Faune Matinale Résiduelle. En plus, des spécimens de culicoïdes (n=991) ont été capturés uniquement avec les pièges lumineux. Après analyses moléculaires basées l'ADN, aucun spécimen de moustique et de culicoïdes n'a été retrouvé infecté par *M. perstans*.

Mots-clés : Filarioses, *Wuchereria bancrofti*, *Brugia timori*, *Brugia malayi*, *Onchocerca volvulus*, *Loa loa*, *Mansonella perstans*, *Mansonella streptocerca*, *Mansonella ozzardi*, Arthropodes.

Abstract

Filariasis is a common parasitic disease caused by the presence of filarial nematode worms in the body. Human filariasis includes pathologies: lymphatic filariasis, caused by *Wuchereria bancrofti*, *Brugia timori*, and *Brugia malayi*; onchocerciasis or river blindness, caused by *Onchocerca volvulus*; loiasis, caused by the parasite *Loa loa*; and mansonellosis, caused by the species *Mansonella perstans*, *Mansonella streptocerca*, and *Mansonella ozzardi*. In Senegal, control programs have successfully interrupted the transmission of the parasite in most health districts for onchocerciasis and lymphatic filariasis. However, lymphatic filariasis remains endemic in rural areas of Senegal.

This thesis work focuses on human filariasis in Senegal, particularly screening and epidemiology of filariasis involved in the transmission of human filarial diseases in southern Senegal. Our study aims to investigate the etiology and epidemiology of filariasis in the studied areas and is divided into two parts. The first part presents a literature review on filariasis in West Africa, addressing existing pathologies, their epidemiology, pathogenicity, diagnostic methods, treatment, and various prevention measures targeting arthropod vectors. In the second part, the real-time PCR (RT-PCR) technique and sequencing were used to detect *M. perstans* from capillary blood samples, *Plasmodium falciparum*-negative RDT cassettes, and vector Culicoides mosquitoes. *M. perstans* was detected in 2 out of 23 patients with elephantiasis collected in 2021, in 12 out of 279 blood samples from febrile subjects in Mlomp (2018-2020), and in 11 out of 3714 negative malaria RDT cassettes. Mosquitoes (n=1487) were captured using CDC light traps, OVI traps, and from the morning residual fauna. Additionally, Culicoides specimens (n=991) were captured exclusively with light traps. Following DNA-based molecular analyses, no mosquito or Culicoides specimens were found to be infected with *M. perstans*.

Keywords: Filariasis, *Wuchereria bancrofti*, *Brugia timori*, *Brugia malayi*, *Onchocerca volvulus*, *Loa loa*, *Mansonella perstans*, *Mansonella streptocerca*, *Mansonella ozzardi*, Arthropods.

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INTRODUCTION

Les filarioses sont un groupe de maladies infectieuses causées par des nématodes appelés filaires, transmises à l'Homme par l'intermédiaire d'insectes vecteurs (arthropodes hématophages femelles : moucherons, moustiques, simulies ou taons) (Walther et Muller 2003; Mendoza et al. 2009). Les filarioses sont largement répandues dans le monde, touchant principalement les populations pauvres vivant dans les pays en développement, particulièrement dans les zones à climat tropical et subtropical. Environ 1 milliard de personnes sont exposées, et plus de 200 millions de personnes sont actuellement atteintes par au moins une forme de filariose (Desoubeaux, Chesnay, et Chandenier 2020). Ces maladies sont associées à un faible niveau de développement, à de mauvaises conditions d'hygiène et à certaines conditions écologiques (Pérez-Molina *et al*. 2010). Bien que les filarioses aient des manifestations cliniques, celles-ci n'apparaissent généralement qu'une fois que les parasites ont atteint leur stade adulte, et dépendent des espèces ainsi que de la localisation des adultes et des microfilaires (Bouchery et al. 2013). En outre, ces parasites peuvent entraîner des incapacités sévères ou des lésions permanentes. Les filarioses ont un impact socio-économique majeur, provoquant une forte morbidité et des maladies graves qui entraînent une stigmatisation sociale, une marginalisation et une perte d'emploi pour les personnes affectées (Lustigman, Grote, et Ghedin 2017).

Plusieurs espèces de filaires sont connues pour leur intérêt médical, notamment *Onchocerca volvulus*, *Loa loa*, *Wuchereria bancrofti*, *Brugia malayi*, *Brugia timori*, *Mansonella perstans*, *Mansonella streptocerca* et *Mansonella ozzardi*. Les vers adultes ou macrofilaires produisent des embryons ou microfilaires qui circulent dans le derme (pour *O. volvulus* et *M. streptocerca*) ou dans le sang (M. Boussinesq 2007). Ces microfilaires sont récupérées par les insectes vecteurs lors de leurs repas sanguins et se développent en larves L3 après plusieurs mues. Les larves L3 sont ensuite transmises à un autre individu lors des repas sanguins suivants (Walther et Muller 2003; Alhassan *et al*. 2015). Les adultes peuvent se localiser à différents endroits du corps humain en fonction de l'espèce impliquée. Dans certaines régions d'Afrique, la filariose lymphatique, l'onchocercose, la mansonellose à *M. perstans* et/ou la loase sont co-endémiques, et certains patients peuvent présenter des infections multiples. Les filaires chez l'homme sont classées selon leur localisation dans le corps, leur mode de contamination, leurs manifestations cliniques et leur répartition géographique. La **filariose lymphatique (FL)**, communément appelée **Eléphantiasis**, fait partie des maladies tropicales négligées (MTN) les plus anciennes. C'est l'une des maladies les plus débilitantes et

défigurantes chez l'Homme avec des conséquences socio-économiques énormes matérialisées par la stigmatisation sociale, la baisse ou la perte totale de productivité et le coût élevé des traitements (Devi et Raju 2018). Selon l'OMS, 51,4 millions de personnes sont infectées (World Health Organization 2022) dans le monde. La FL est principalement causée par trois espèces de nématodes appartenant à la famille des *Filarioidea*, dont *Wuchereria bancrofti* (Cobbold, 1887), responsable de 90 % des cas dans le monde (Ottesen 2006; Katiyar et K. Singh 2011), principalement dans les régions tropicales et subtropicales et dans le Pacifique. Les deux autres espèces, *Brugia malayi* (Brug, 1927) et *Brugia timori* (Partono, 1977), sont endémiques en Asie. En Afrique, où la maladie est entièrement causée par *W. bancrofti*, plus de 45 millions de personnes sont touchées (Hotez et Kamath 2009). Au Sénégal, la FL est endémique avec une prévalence globale de 4,7 % chez les personnes âgées de plus de 45 ans (Faye et al. 2009). Bien que l'espèce *W. bancrofti* ait été morphologiquement décrite au Sénégal, elle n'a jamais été confirmée sur la base de l'ADN. L'infection survient lorsque les parasites filaires sont transmis à l'homme par des moustiques des genres *Culex*, *Aedes*, *Anopheles* et *Mansonia* (Manguin et al. 2010; Diwakar et al. 2018). La FL présente des symptômes chroniques et visibles, tels que l'hydrocèle, un lymphœdème secondaire des membres inférieurs (Ramaiah et Ottesen 2014), ou une éosinophilie pulmonaire tropicale qui ressemble à l'asthme (Tahita et al. 2024).

L'**onchocercose** est une filariose cutanée causée par le nématode *Onchocerca volvulus* (Leuckart, 1893), qui se développe dans le tissu sous-cutané de l'Homme. Ce parasite est transmis par la piqûre de la femelle du genre *Simulium*. C'est une maladie invalidante, un obstacle majeur à l'établissement de la population. L'infection par *O. volvulus* est responsable de manifestations cutanées et oculaires graves (Adewole et Ayeni 2009; Katawa et al. 2015; Njim, Ngum, et Aminde 2015), et est également associée à l'épilepsie et au syndrome du hochement de tête (Colebunders et al. 2018; Vinkeles Melchers et al. 2018). C'est une maladie liée à la pauvreté et à la productivité des ménages (Ibe et al. 2015; Opoku et al. 2018) et constitue un réel problème de santé publique. L'onchocercose touche environ 21 millions de personnes, dont 99 % des cas signalés dans 31 pays d'Afrique subsaharienne (World Health Organization (WHO) 2020). En 2019, une évaluation entomologique réalisée dans les 8 districts concernés a révélé un taux d'infectivité des simulies inférieur à 0,05 %. Ce résultat confirme l'interruption de la transmission de l'onchocercose dans ces zones. Ces données témoignent des avancées significatives dans le contrôle de la maladie (World Health Organization (WHO). 2022).

L'infestation par ***Loa loa*** (Cobbold, 1894) est une maladie parasitaire connue sous le nom de **ver oculaire africain**. La loase est endémique dans les zones tropicales d'Afrique de l'Ouest et d'Afrique centrale, notamment au Cameroun, au Congo, au Gabon et au Nigéria (Zouré et al. 2011).

Bien que la loase ne soit pas endémique au Sénégal, un cas a été détecté, avec la présence du ver adulte en migration dans l'œil, chez un patient originaire de la République centrafricaine (Diagne et al. 2018).Le filaire *Loa loa* est transmise à l'Homme par des tabanidés du genre *Chrysops* (Anderson 2000). La loase est bien souvent considérée comme bénigne et dans la plupart des cas asymptomatique, mais peut présenter de nombreux aspects cliniques (A. Aubry et al. 2022). Les manifestations cliniques les plus frappantes sont la migration du ver adulte sous la conjonctive bulbaire et les œdèmes migratoires transitoires, appelés tuméfaction de Calabar (Gardon et al. 1997; A. Aubry et al. 2022).

Bien que négligée, la **mansonellose** est une filariose humaine bénigne, mais reste une infection filarienne négligée malgré le fait que 600 millions de personnes sont exposées à un risque élevé en Afrique et dans certaines parties de l'Amérique centrale et du Sud (Simonsen, Onapa, et Asio 2011; Ta-Tang et al. 2021). À ce jour, la mansonellose est causée par trois espèces de nématodes filaires : *Mansonella perstans* (Manson, 1891), *M. ozzardi* (Manson, 1897) et *M. streptocerca* (Macfie & Corson, 1922), qui ne provoquent que des symptômes bénins et généralement non spécifiques (Medeiros et al. 2017; Ta-Tang et al. 2018; Ehrens, Hoerauf, et Hübner 2022). Récemment, des études génétiques ont montré la présence d'une autre variante génétique, *Mansonella sp. Deux*, infectant les humains en Afrique, notamment au Gabon (Mourembou et al. 2015; Sandri et al. 2021; Rodi et al. 2023). *M. perstans* est la plus fréquente en Afrique, avec une prévalence souvent élevée dans les zones endémiques. Elle infecte environ 114 millions de personnes en Afrique, principalement dans 33 pays d'Afrique subsaharienne. Cette espèce est responsable de l'infection au Sénégal, où la prévalence est de 14,5 % dans la région rurale de Kédougou (Bassene et al. 2015).

Des études antérieures ont montré qu'il existe de nombreuses méthodes de diagnostic pour détecter les filarioses, utilisées en première intention en cas de suspicion clinique. Ces recherches peuvent être effectuées :

- **Dans le sang** : par observation à l'état frais, par frottis mince ou goutte épaisse, ou à l'aide de techniques de concentration comme la leucoconcentration, la technique de Knott ou la filtration membranaire.

- **Dans une biopsie cutanée exsangue (BCE)** : soit directement à l'état frais, soit après coloration ou par examen histologique.

En outre, des techniques telles que la précipitation (électrosynérèse, coélectrosynérèse, immunoélectrophorèse), l'immunofluorescence indirecte et des techniques immunoenzymatiques de type ELISA sont employées pour rechercher les anticorps et antigènes circulants. Ces méthodes s'appliquent aux diagnostics des filarioses lymphatiques, de la loase, des mansonelloses (*M. perstans*, *M. ozzardi*) et de l'onchocercose (*O. volvulus*, *M. streptocerca*). Au Sénégal, la réaction en chaîne par polymérase (PCR), qui est une méthode moléculaire très spécifique et sensible, est également utilisée pour détecter ces filaires.

Selon l'OMS, plusieurs stratégies de contrôle ont été mises en place pour stopper la propagation de ces maladies. Ces stratégies reposent principalement sur la **chimiothérapie préventive** par l'administration à grande échelle de médicaments (Mass Drug Administration, MDA). Ces médicaments agissent soit sur les microfilaires, soit sur les adultes. Classiquement, les principaux antifilaricides disponibles sont la **diéthylcarbamazine (DEC)**, l'**ivermectine (IVM)** et l'**albendazole (ABL)**, utilisés seuls ou en combinaison. Le choix du traitement dépend de la confirmation du type de filaire.

Le Sénégal est endémique à l'onchocercose et à la FL. Cependant, après plusieurs campagnes de traitement de masse avec l'IVM, l'onchocercose a été totalement éliminée et la prévalence de la FL a été réduite de 95 % dans plusieurs districts sanitaires endémiques (World Health Organization (WHO). 2022). À noter que *M. perstans* montre une certaine résistance, voire une insensibilité, à certains médicaments filaricides, notamment la DEC et l'IVM (S. M. Asio, Simonsen, et Onapa 2009; Chelsea et William 2022).

Ce travail vise à étudier l'étiologie et l'épidémiologie des filarioses au Sénégal, en mettant particulièrement l'accent sur les nématodes parasites et leurs vecteurs dans le sud du pays. L'étude est scindée en plusieurs parties :

1. **Revue de la littérature** : Analyse des filarioses en Afrique de l'Ouest, incluant leurs généralités, leur épidémiologie, leur pathogénicité, leurs méthodes de diagnostic et les moyens de lutte employés dans les programmes de contrôle.
2. **Étiologie et dépistage** : Cette partie porte sur la détermination des causes des cas d'éléphantiasis, l'évaluation de l'utilité des bandelettes nitrocellulose des tests diagnostiques rapides (TDR) pour le dépistage de la filariose, et l'identification des

moustiques et culicoïdes comme vecteurs potentiels de *M. perstans* en milieu rural au Sénégal.

Nous avons ainsi réalisé la détection des parasites filaires dans des prélèvements sanguins de patients, à partir de TDR négatifs pour le paludisme, ainsi que sur des spécimens de moustiques et de culicoïdes. Pour cela, des analyses basées sur l'ADN, notamment la **PCR en temps réel (RT-PCR)** et le **séquençage Sanger**, ont été utilisées.

Ces filaires, bien que peu nombreuses parmi les espèces parasitant l'homme, représentent une diversité importante et jouent un rôle crucial dans les efforts de contrôle des maladies infectieuses.

PARTIE 1: REVUE DE LA LITTERATURE

Filariasis in West Africa

ARTICLE 1

Filariasis in West Africa

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Filariasis in West Africa

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Résumé

Les maladies parasitaires liées à la filariose sont très fréquentes à travers le monde. Elles sont provoquées par des nématodes, ou filaires, appartenant à la famille des Filaroidae. En Afrique, la filariose se manifeste avec des tableaux cliniques variés, en fonction de l'espèce parasitaire impliquée. Ces maladies constituent un problème de santé publique majeur, entraînant des pertes socio-économiques significatives dans de nombreux pays tropicaux et subtropicaux. En Afrique de l'Ouest, la filariose occupe une place prépondérante en termes de santé publique. Selon leur impact sanitaire, les espèces filariennes sont classées en filarioses pathogènes (filariose lymphatique, onchocercose, loase) et filarioses non pathogènes (mansonellose). Toutes ces espèces partagent un cycle de vie similaire, nécessitant un hôte intermédiaire indispensable à leur développement.

Cette revue se concentre sur les principales filarioses présentes en Afrique de l'Ouest, notamment leur épidémiologie, leur pathogénicité, les méthodes diagnostiques employées, les stratégies de prévention, ainsi que les traitements recommandés par l'OMS. La capacité à détecter les parasites chez l'hôte est cruciale pour mettre en œuvre des stratégies de contrôle efficaces. Cette revue examine les différentes méthodes de détection et de prévention des infections filariennes, incluant l'utilisation de médicaments antifilariens efficaces ou partiellement efficaces, qui agissent à différents stades du cycle parasitaire. Face au défi que représente l'absence de médicaments à la fois sûrs et performants, il est essentiel d'envisager des mesures approfondies et durables, permettant d'optimiser l'efficacité des traitements antifilariens, utilisés seuls ou en association.

Mots-clés : Filariose, Filariose lymphatique, Onchocercose, Loase, Mansonellose, Pathogénicité, Diagnostic, Prévention, Traitement, Afrique de l'Ouest.

Abstract

Parasitic diseases caused by filariasis are widespread across the globe. They result from infections by nematodes, or filariae, belonging to the Filaroidae family. In Africa, filariasis exhibits varied clinical presentations depending on the species of parasite involved. These diseases represent a major public health issue, leading to significant socio-economic losses in many tropical and subtropical countries. In West Africa, filariasis holds significant importance to public health. Based on their impact on public health, filarial species are classified into pathogenic filariasis (lymphatic filariasis, onchocerciasis, loiasis) and non-pathogenic filariasis (mansonellosis). These species share a similar life cycle, with an obligatory intermediate host playing a key role in their development.

This review focuses on the main filarial diseases present in West Africa, including their epidemiology, pathogenicity, diagnostic methods, prevention strategies, and treatments recommended by the WHO. The ability to detect parasites in the host is essential for implementing effective control measures. This review explores various methods of parasite detection and infection prevention, including the use of antifilarial drugs, both effective and less effective, which act at different stages of the parasite's life cycle. Given the challenge posed by the lack of safe and highly effective drugs, it is crucial to implement deeper and more sustainable measures, allowing antifilarial treatments to be optimized, either used individually or in combination, to enhance their efficacy.

Keywords: Filariasis, Lymphatic Filariasis, Onchocerciasis, Loiasis, Mansonellosis, Pathogenicity, Diagnosis, Prevention, Treatment, West Africa.

I. Generality of filariasis

Filariasis is a tropical parasitic disease prevalent worldwide. It is caused by nematodes called filariae (roundworms), which belong to the Filarioidae families. These pathologies present varied clinical manifestations depending on the species involved and are endemic in several countries, particularly in West Africa. Filariasis is classified according to the location of the macrofilariae (adult worms) in the body. These diseases show a great diversity of filariae, although very few species parasitize humans. The infections can be categorized as lymphatic (lymphatic filariasis), cutaneous or dermal (onchocerciasis and loiasis), or serous (mansonellosis), depending on the tropism of the viviparous nematodes that cause them and their location in the human body. The infections can be categorized as lymphatic (lymphatic filariasis), cutaneous or dermal (onchocerciasis and loiasis), or serous (mansonellosis), depending on the tropism of the viviparous nematodes that cause them and their location in the human body (Bhalla, Dumas, et Preux 2013).

Transmission to humans occurs via the bite of infected mosquitoes, midges, blackflies, or horseflies, which act as intermediate hosts for the infective larvae. Humans serve as the definitive host. Filariasis constitutes a global public health problem, due to its impact on population health and the enormous burden it imposes on endemic countries. Worldwide, more than 200 million people are affected (Kwarteng, Ahuno, et Akoto 2016), and almost a billion live in areas at risk of filariasis (Risch et al. 2021).Worldwide, more than 200 million people are affected (Kwarteng, Ahuno, et Akoto 2016), and almost a billion live in areas at risk of filariasis (Risch et al. 2021). These diseases are defined as conditions caused by invasive or filarial tissue nematodes. The adult worms typically reside under the skin, in lymphatic vessels, or in lymph nodes, while their microfilaria circulates in the blood, the dermis, or the lymphatic pathways, depending on the species of filaria. They also result in enormous economic losses in many African countries. Although many species of filariae exist, only a few infect humans. These are divided into major filariasis (pathogenic) and minor filariasis (little or no pathogen) (Table 1). All these species use humans as definitive hosts and are distributed globally, particularly in West Africa, depending on their preferred location in the body.

Pathogenic filariasis includes lymphatic filariasis caused by *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*, cutaneous filariasis: onchocerciasis caused by *Onchocerca volvulus*, loasis caused by *Loa loa* and serous filariasis caused by *Mansonella perstans*, *Mansonella streptocerca* and *Mansonella ozzardi* (Gentilini, Pinon, et Danis 1973).

They all have comparable reproductive cycles and parasitological characteristics (Frédéric 2018) according to their geographical distribution, modes of transmission, and clinical manifestations. They all have comparable reproductive cycles and parasitological characteristics (Frédéric 2018) according to their geographical distribution, modes of transmission, and clinical manifestations.

These filarial species have a significant social and economic impact on affected populations. They cause serious morbidity, leading to social stigma, exclusion from society, and loss of employment for those affected, resulting in a heavy disease burden. These filarial species have a significant social and economic impact on affected populations. They cause serious morbidity, leading to social stigma, exclusion from society, and loss of employment for those affected, resulting in a heavy disease burden (Lustigman, Grote, et Ghedin 2017).

Table 1. Characteristics of filarial species.

	Species	Locations: Adults / microfilariae	Vectors	Clinical manifestations	Geographical distribution	References
Highly pathogenic filariae	*Wuchereri bancrofti*	Lymphatic / Blood (Nocturnal)	*Anopheles* sp, *Aedes* sp, *Culex* sp	Lymphedema, hydrocele, elephantiasis	Tropical and intertropical regions	(Taylor, Hoerauf, et Bockarie 2010; Nutman 2013; World Health Organization (WHO) 2023)
	Brugia timori	Lymphatic / Aperiodic blood	*Mansonia* sp		Timor	
	Brugia malawi				Malaysia	
	Onchocerca volvulus	Cutano-dermal / Dermal Suc	*Simulie*	Lesions (lymphatic-ocular-cutaneous), pruritus, nodules	Sub-Saharan Africa, Central and South Africa, Yemen	(Coffeng et al. 2014)
	Loa loa	Cutano-dermal / Blood (Diurnal)	*Chrysops*	Squid edema and adult worm under conjunctiva	West Equatorial Africa	(Zouré et al. 2011)
Filariae with low pathogenicity	*Mansonella perstans*	Serous / Aperiodic blood	*Culicoides* sp	Pruritus, headache, arthralgia, fever, oedema, rash, keratitis, lymphadenopathy, pulmonary symptoms	Sub-Saharan Africa	(Simonsen, Onapa, et Asio 2011a; Enrico Rino Bregani et al. 2006)
	Mansonella streptocerca	Subcutaneous / dermal			Sub-Saharan Africa	
	Mansonella ozzardi	Subcutaneous / Aperiodic blood			America, Caribbean	
	Dirofilaria immitis	Pulmonary artery and sometimes in the right ventricle of the heart	*Anopheles* sp, *Aedes* sp, *Culex* sp	Benign asymptomatic pulmonary nodules	It is more widespread in tropical and temperate regions, particularly in the south-eastern United States,	(Alsarraf et al. 2021; Simón et al. 2012; Pampiglione, Canestri Trotti, et Rivasi 1995)

		(Immature adult) / Blood			many South American countries, Asia, Australia and southern Europe.	
	Dirofilaria repens (Subcutaneous and ocular heartworm disease)	subcutaneous connective tissue (Adult and pre-adult worms) / Blood		- Affects different ocular regions (orbital area, eyelids and subconnective and intravitreal tissues). - Severe cases, with vision disturbances and complications such as retinal detachment, glaucoma, vitreous humor opacity, lens.	The Old World, with areas of high prevalence in Southern and Eastern Europe, Asia Minor, Central Asia and Sri Lanka.	

II. Epidemiology of filariasis in West Africa

Known filarial infections that parasitize humans include *W. bancrofti*, *Brugia* sp., *O. volvulus*, *L. loa*, *M. perstans*, *M. streptocerca*, and *M. ozzardi*. In this review, we focus only on filarial species endemic to West African countries (Figure 1).

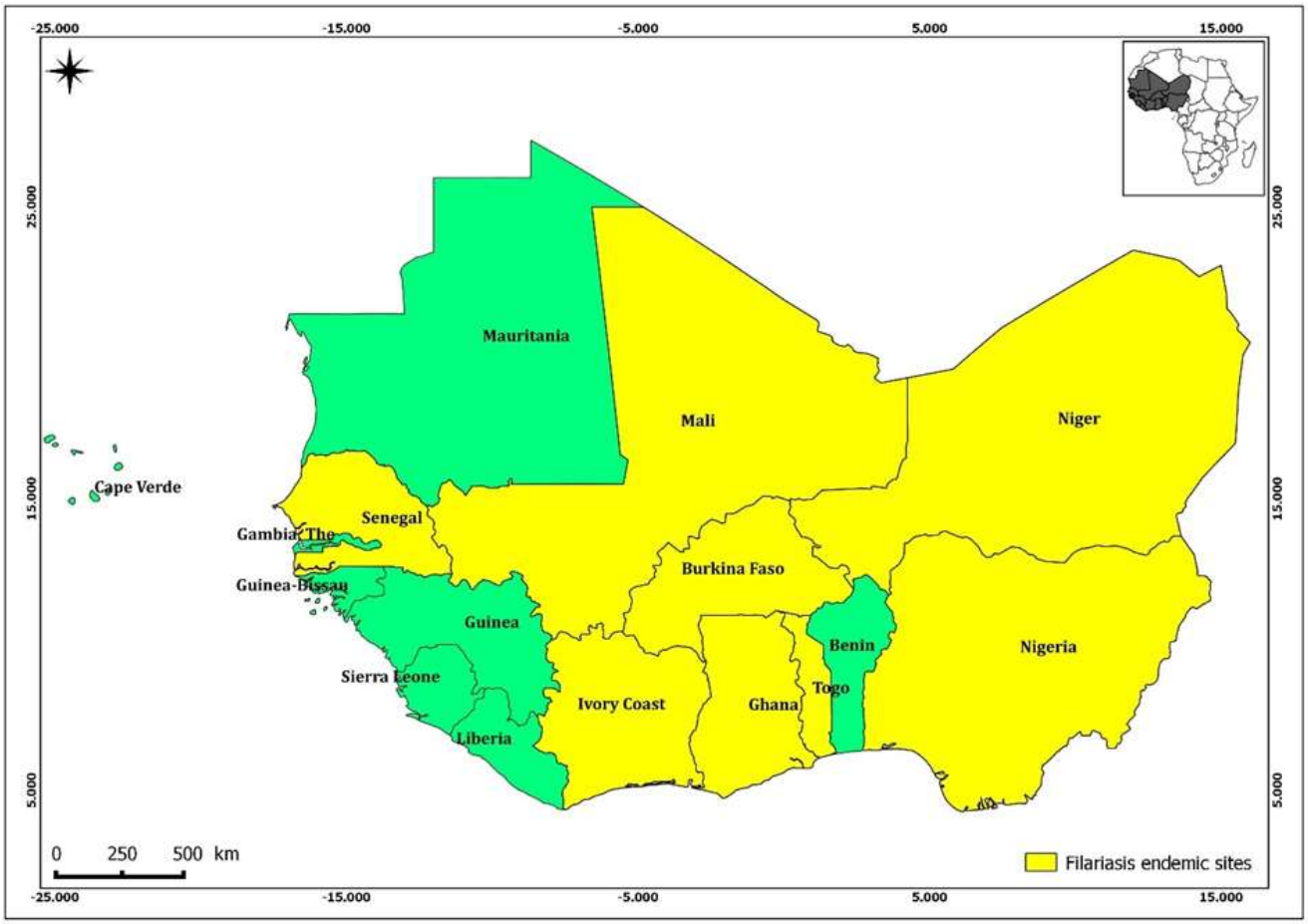

Figure 1: Countries endemic for lymphatic filariasis, onchocerciasis, loiasis, and mansonellosis. (**Senegal is not endemic to loasis**)

1. Lymphatic filariasis

A. Description

Lymphatic filariasis (LF), also called elephantiasis, continues to be a major cause of morbidity and permanent disability in poor, rural, and marginalized populations, as well as in endemic areas (Hotez et al. 2007; Davis et al. 2019). It is caused by parasitic of the genera *Wuchereria* and *Brugia* sp transmitted by mosquitoes (Yaya I. Coulibaly et al., 2016; Dewi et al., 2015). This disease encompasses pathological manifestations resulting from parasitism of the human body by filariae. More than 90% of infections worldwide are caused by the filarial species *W. bancrofti*, which is widely present in tropical and subtropical zones in Africa

(Manguin et al. 2010; Small, Tisch, et Zimmerman 2014), Asia Pacific, and America. *B. malayi* accounts for most of the remaining cases (Taylor, Hoerauf, et Bockarie 2010; Bockarie et Deb 2010). The visible, painful, and disfiguring manifestations of the disease lymphedema (elephantiasis) and hydrocele negatively impact the lives and socio-economic well-being of those affected, as well as their families (Ouedraogo et al. 2016). These symptoms often appear later in life. Lymphatic filariasis also affects the lymphatic system, leading to psychosocial difficulties such as social disgrace, reduced marriage prospects, and sometimes the dislocation of couples or families (Wynd 2007; Molyneux et Taylor 2001). Many individuals feel socially excluded in their own communities due to the stigma associated with the disease (Coreil et Mayard 2009). Infection occurs when filariae responsible for the disease are transmitted to humans through the bites of blood-sucking female mosquitoes belonging to the genera *Aedes*, *Anopheles*, *Mansonia*, and *Culex* (World Health Organization (WHO) 2021) (This disease encompasses pathological manifestations resulting from parasitism of the human body by filariae. More than 90% of infections worldwide are caused by the filarial species *W. bancrofti*, which is widely present in tropical and subtropical zones in Africa (Manguin et al. 2010; Small, Tisch, et Zimmerman 2014), Asia Pacific, and America. *B. malayi* accounts for most of the remaining cases (Taylor, Hoerauf, et Bockarie 2010; Bockarie et Deb 2010). The visible, painful, and disfiguring manifestations of the disease lymphedema (elephantiasis) and hydrocele negatively impact the lives and socio-economic well-being of those affected, as well as their families (Ouedraogo et al. 2016). These symptoms often appear later in life. Lymphatic filariasis also affects the lymphatic system, leading to psychosocial difficulties such as social disgrace, reduced marriage prospects, and sometimes the dislocation of couples or families (Wynd 2007; Molyneux et Taylor 2001). Many individuals feel socially excluded in their own communities due to the stigma associated with the disease (Coreil et Mayard 2009). Infection occurs when filariae responsible for the disease are transmitted to humans through the bites of blood-sucking female mosquitoes belonging to the genera *Aedes*, *Anopheles*, *Mansonia*, and *Culex* (World Health Organization (WHO) 2021) (https://iris.who.int/handle/10665/346577). These mosquitoes are intermediate hosts of the parasite, while humans are the definitive host. which are the intermediate hosts of the parasite while humans are the definitive host.

B. Epidemiology

Lymphatic filariasis is one of the neglected tropical diseases (NTDs) and is recognized as the second most debilitating mosquito-borne disease after malaria (Manguin et al. 2010;

Bockarie et Deb 2010; Shenoy 2008). It is particularly impactful due to the physical disabilities and socio-economic consequences it causes. Lymphatic filariasis is one of the neglected tropical diseases (NTDs) and is recognized as the second most debilitating mosquito-borne disease after malaria (Manguin et al. 2010; Bockarie et Deb 2010; Shenoy 2008). It is particularly impactful due to the physical disabilities and socio-economic consequences it causes. In 2018, 893 million people in 49 countries were at risk of lymphatic filariasis and required preventive chemotherapy to halt the spread of infection (Nizeyimana 2021). By 2021, 51.4 million people were infected in 72 endemic countries (P. P. Aubry et Docteur Bernard-Alex Gaüzère 2023), with 36 million developing chronic manifestations such as hydrocele or secondary lymphedema (Ramaiah et Ottesen 2014; Cromwell et al. 2020).In 2018, 893 million people in 49 countries were at risk of lymphatic filariasis and required preventive chemotherapy to halt the spread of infection (Nizeyimana 2021). By 2021, 51.4 million people were infected in 72 endemic countries (P. P. Aubry et Docteur Bernard-Alex Gaüzère 2023), with 36 million developing chronic manifestations such as hydrocele or secondary lymphedema (Ramaiah et Ottesen 2014; Cromwell et al. 2020).

Globally, initial estimates for lymphatic filariasis indicated that 25 million men were affected by hydrocele, while more than 15 million people suffered from debilitating lymphedema (Adhikari et al. 2015). Globally, initial estimates for lymphatic filariasis indicated that 25 million men were affected by hydrocele, while more than 15 million people suffered from debilitating lymphedema (Adhikari et al. 2015). In Senegal, the presence of lymphatic filariasis has been known for decades. Early survey studies conducted in the Mbour department on the southern coast identified a focus of the disease (Diallo et al. 1977). However, since then, few studies have been conducted, and filarial infection remains a low national priority due to the prominence of diseases like malaria and bilharzia. The latest study in Senegal showed an overall prevalence of 4.7% among people aged over 45 years, with significant regional variations (Faye et al. 2009). Lymphatic filariasis is also endemic in other West African countries, such as Mali, particularly in the Sikasso district, where *W. bancrofti* is transmitted primarily by *Anopheles gambiae sensu lato* and *Anopheles funestus* (Brengues et al. 1968; Yaya Ibrahim Coulibaly et al. 2013), In a study of two neighboring villages in Mali, located in a *W. bancrofti* endemic area, the overall prevalence of circulating antigen was greater than 50% among 373 participants aged 14 to 65 years (Dolo et al. 2018). Lymphatic filariasis affects children as young as 6 years old, with an antigenemia prevalence of 0.24% detected using the FTS test for the parasite *W. bancrofti* (Kima et al. 2021). In Niger, low levels of *W. bancrofti*

transmission have been observed in previously non-endemic areas. Among participants aged 5 to 105 years, 29 individuals (0.7%) tested positive using the filariasis test strip (FTS) (Badia-Rius et al. 2023). In Côte d'Ivoire, pre-mass drug administration (MDA) surveys showed a prevalence of *W. bancrofti* infection ranging from 3.38% to 5.50% in border health districts, including Aboisso, Bloléquin, Odienné, and Ouangolodougou, based on FTS (Yokoly et al. 2020).

2. Onchocerciasis

A. Description

Onchocerciasis is an endemic disease caused by a nematode called *O. volvulus* (O'Hanlon et al. 2016; Vinkeles Melchers et al. 2021; Crawford et al. 2024), for which humans are the only reservoir. The vector breeds in fast-flowing, oxygen-rich rivers in affected areas, with disease transmission and prevalence typically extending along these river basins, hence the name river blindness (Herricks et al. 2017). It is a serious disease with slow progression, caused by the accumulation of filariae in the human body. These parasites are transmitted by a small black fly, the intermediate host, which belongs to the genus *Simulium*. The Damnosum complex is most often responsible for onchocerciasis in Africa. Vector species are mainly present in humid areas and reproduce in fast-flowing waterways (Robert et al. 2022).

B. Epidemiology

O. volvulus causes significant skin and eye diseases. The vector is a widely distributed dipteran of the genus *Simulium*, a black fly measuring 1–3 mm with a painful bite, and is most active in Africa (Brattig, Cheke, et Garms 2021). Black flies lay their eggs on plants or rocks in cool, fast-flowing, nutrient-rich, and highly oxygenated waters, such as waterfalls and rapids. The location of larval breeding grounds explains why the distribution of onchocerciasis is closely tied to hydrographic networks. Black flies typically have a lifespan of no more than one month. In 2017, onchocerciasis affected 20.9 million people worldwide, including 14.6 million cases of dermatological manifestations and 1.15 million cases of vision loss (James et al. 2018). In West Africa, female black flies live up to 9 days after their first blood meal, with 9% in forest areas and 47% in savannah areas (M. Boussinesq 2003). Approximately 160 million people are at risk of onchocerciasis, spread across 31 African countries (Porter et Hopkins 2015). In Burkina Faso, the disease decreased by 15% in 2002 after the implementation of the Onchocerciasis Control Program in West Africa. However, in 2011, a resurgence of the disease was observed, with prevalence ranging from 0.7% to 71%

(Sawadogo 2019). In Nigeria, before ivermectin treatment, prevalence ranged between 23.1% and 84.9%, with a median prevalence of 52.0%. After 15 to 17 years of treatment, prevalence dropped to 0% in all communities (Tekle et al., 2012). However, O. volvulus infections remained at 15.2% in skin samples (Ojurongbe et al., 2015). According to Herricks, Nigeria bears one of the highest burdens of onchocerciasis worldwide (Herricks et al. 2017). A recent study in Togo identified ocular diseases in children and young adults in villages, including papillitis, cataracts, chorioretinitis, conjunctivitis, and tropical limbo-conjunctivitis (LCET). Other findings included iridocyclitis, sclerosing keratitis, and blindness in one eye, despite ongoing MDA programs that reduced microfilariae levels (Komlan et al. 2018). Currently, 99% of cases are found in 31 sub-Saharan African countries (World Healh Organization (WHO) 2020). In the villages along the Gambia River in Senegal, a study conducted by De Sole et al. in 1991 revealed high microfilarial loads, exceeding 10 microfilariae per skin (mf/s) among adults, indicating intense onchocerciasis transmission (De Sole et al. 1991). However, due to targeted control efforts and interventions, an entomological evaluation conducted in 2019 across the eight affected districts showed a blackfly infectivity rate below 0.05%. This result confirms the interruption of disease transmission in these areas, highlighting significant progress in the fight against onchocerciasis (World Health Organization, 2022).

3. Mansonellosis

A. Description

Mansonellosis is a tropical disease, often considered even more neglected than Neglected Tropical Diseases (NTDs). Although it is arguably the most common filarial infection worldwide, it is the least studied and classified as a neglected parasitic infection (Lima et al. 2016; Ta-Tang et al. 2018). Human infection is caused by filarial nematodes of the genus Mansonella, including *M. perstans*, *M. streptocerca* and *M. ozzardi* (Tatuene et al. 2014a). These species are widespread in West Africa and other parts of Africa (Ta-Tang et al. 2018). Outbreaks of *M. perstans* and *M. streptocerca* are limited and occur mainly in sub-Saharan Africa, with sporadic cases in South America (Tavares da Silva et al. 2017; Ta-Tang et al. 2018). *M. ozzardi*, on the other hand, is found exclusively in the western hemisphere, from southern Mexico to north-western Argentina (Lima et al. 2016; Ta-Tang et al. 2018). *M. streptocerca* is primarily a human parasite but has been reported to infect wild chimpanzees in rare cases. A fourth species, *Mansonella* sp. 'TWO', has recently been described from Gabon, although its clinical relevance remains uncertain (Rodi et al. 2023a). Biting midges of the genus

Culicoides are the main vectors of *M. perstans* and *M. streptocerca*. For *M. ozzardi*, transmission is mainly by black flies (Simulium) (Gaillard et al. 2020; Ta-Tang et al. 2021b). After a blood meal, intermediate hosts ingest microfilariae, which develop into adult worms residing in pleural or peritoneal cavities or in the subcutaneous dermal layer (Medeiros et al. 2017; Ferreira, Crainey, et Luz 2021). Adult Mansonella females are viviparous and release M. perstans and *M. ozzardi* microfilariae into the peripheral blood, while *M. streptocerca* larvae are found in the skin (Ta-Tang et al. 2021b; Medeiros et al. 2017).

B. Epidemiology

Mansonellosis affects an estimated 114 million people across Africa, mainly in 33 countries, (B. Downes et Jacobsen 2010; Simonsen, Onapa, et Asio 2011). Prevalence is often high in endemic areas, even among children (Simonsen, Onapa, et Asio 2011a; Bassene et al. 2015). In Senegal, the prevalence was reported at 14.5% in febrile and afebrile children in the Kedougou region. *M. perstans* infections were more frequent among individuals aged over 29 years (33.3%) (Bassene et al. 2015a). In Ghana, a 2014 study reported a high burden of M. perstans among individuals co-infected with Buruli ulcers, with a prevalence of 23% in affected patients versus 13% in controls (Phillips et al. 2014). The overall prevalence in the studied communities was 32%, with variation between 2% and 75%, depending on vector species (L. B. Debrah et al. 2017). In Nigeria, among 755 nomadic Fulani, 8.7% were infected with *M. perstans*. The infection rate was higher in men (9.7%) than in women (6.5%) (Anosike et al. 2005). In addition, the prevalence of *M. perstans* increases with age in volunteers aged between 18 and 65. *M. perstans* is endemic in the two districts of Kolokani and Kolondieba in Mali, with prevalences of 11.79%, 4.35% and 9.50% respectively in the villages of Tieneguebougou, Bougoudiana and Boundioba (Sangare et al. 2018)

4. Loiasis

A. Description

Loiasis caused by *Loa loa* and commonly known as the African eye worm, mainly affects the forested areas of Central Africa (Jacobsen et al. 2022; Zouré et al. 2011). The disease is transmitted to humans by the bites of tabanid flies of the genus Chrysops, in particular *Chrysops silacea* and *Chrysops dimidiata*, with humans being the only known reservoir (Knopp et al. 2012; Kelly-Hope et al. 2017). In most cases, loasis is asymptomatic or presents as a mild infection (Adam et al. 2020; A. Aubry et al. 2022). Parasites develop in the lungs at night to escape clearance by the spleen (Ramharter et al. 2023). The adults migrate to the subcutaneous

tissues, passing under the skin and the transparent mucous membrane of the eyes, and are visible in the subconjunctival space. *L. loa* parasites are round, whitish worms measuring 3 to 7 cm, but can exceed 10 cm (Mandal et al. 2012; Jacobsen et al. 2022). Transmission occurs all year round in endemic areas, with peaks during the rainy season (Demanou, Pion, et Boussinesq 2001; Akue et al. 2011).

B. Epidemiology

Loasis is endemic in the tropical forest regions of Central and West Africa, with an estimated 10 million people infected in 10 endemic countries (Zouré et al. 2011; Metzger et Mordmüller 2014). Areas of high prevalence include southern Nigeria, Cameroon, Gabon, Congo, Equatorial Guinea, northern Angola and parts of the Democratic Republic of Congo (Zouré et al., 2011). *Loa loa* filariasis is not endemic in Senegal or Niger, although sporadic cases have been reported. These include a student from central Africa in Senegal (Diagne et al. 2018) and a 25-year-old boy in Niger (Adam et al. 2020). In Nigeria, loasis has been reported in people aged 65 to 82 years, with a prevalence of 14.3% in men from venous blood samples (Ojurongbe et al. 2015). A 2016 study in southern Nigeria found a 6.3% prevalence of *L. loa* microfilariae in villages hypoendemic for onchocerciasis, with a mean of 326 mf/ml among positives (Emukah et al. 2018).

III. Pathogenicity and clinical signs

The clinical signs of filariasis only appear when the parasites have reached the adult stage. They depend on the species involved and the location of the adult worms and microfilariae (Bouchery et al. 2013).

a) Lymphatic filariasis

The causal agent of lymphatic filariasis is the roundworm *Wuchereria bancrofti* (Famakinde 2018). Mosquitoes of the genera Aedes, Anopheles, Culex and Mansonia ingest microfilariae during a blood meal when they bite humans (Erickson et al. 2013). During this process (Figure 2), an infected mosquito deposits third-stage (infective) larvae on the skin of the human host, where they enter through the bite wound.

Adult viviparous females are larger than males, measuring 80 to 100 mm in length and 0.24 to 0.30 mm in diameter, while males measure around 40 mm in length and 0.1 mm in diameter. Females produce microfilariae that circulate permanently in the lymph and periodically in the blood (either nocturnally or diurnally (P. P. Aubry et Docteur Bernard-Alex Gaüzère 2023).

These microfilaria are mobile and pass into the blood at night. They live for 1 to 2 months before transforming into adult worms.

Adult filariae are mainly found in the lymphatic vessels or sinuses of the lymph nodes of the lower limbs and scrotum (Chakraborty et al. 2013). Macrofilariae (adult worms) have a circadian periodicity linked to the vector's lifestyle and can survive 5 to 8 years in their host (Taylor, Hoerauf, et Bockarie 2010; Khan et al. 2015). Microfilariae present in blood have specific characteristics that are easily identified using thick Giemsa-stained smears. In mosquito vectors, microfilariae undergo several transformations: coiled forms (stage 1), intermediate forms (stage 2) and infectious forms (stage 3). infectious forms (stage 3).

Lymphatic filariasis is often asymptomatic in many individuals. It is generally contracted during childhood and causes invisible lesions of the lymphatic system. Clinical manifestations range from acute symptoms - such as adenolymphangitis, filarial fever and tropical pulmonary eosinophilia - to chronic symptoms, including hydrocele, lymphoedema and elephantiasis (Figure 3) in severe cases (Chandy et al. 2011).

According to Haddix et al, hydrocele and lymphoedema can lead to substantial reductions in productivity, which can last a lifetime (Haddix et Kestler 2000). Hydrocoele: this condition, caused by *W. bancrofti* in male patients, is characterised by the accumulation of fluid between the two layers of the tunica vaginalis in the scrotum. It is associated with the presence of adult parasites in the lymphatic vessels of the scrotum (Noroes et al. 2003; Babu et Nutman 2012).

Lymphatic filariasis can also lead to mental health problems and social and financial difficulties, contributing to both stigma and poverty.

In West Africa, particularly Senegal, elephantiasis is one of the most debilitating health problems associated with lymphatic filariasis. The condition causes permanent swelling of a lower limb but can also affect the arms and genitals. The affected limb is at risk of secondary streptococcal infections, which can worsen the disease (Shenoy 2008).

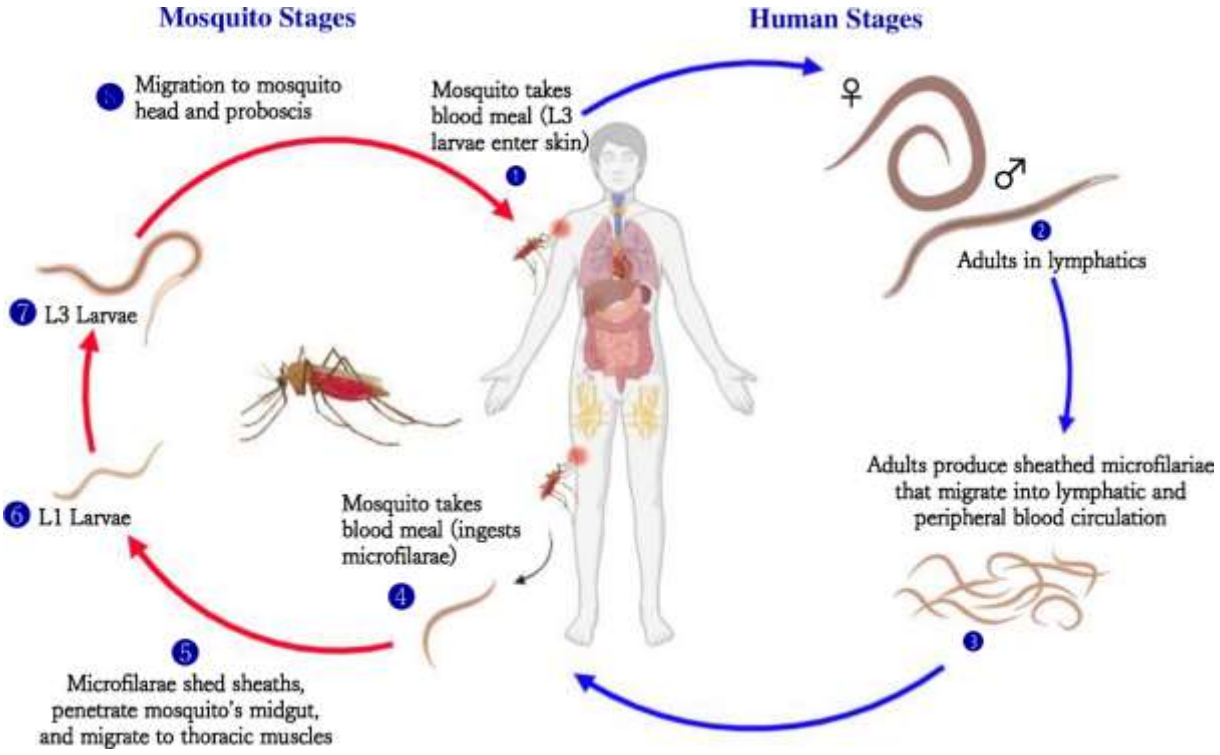

Figure 2. Life cycle of *Wuchereria bancrofti*. Created with BioRender.com.

Figure 3. Clinical signs of lymphatic filariasis (a and b) = cases from our study in Kolda and Sedhiou

b) Loiasis

Loasis is a cutaneous-dermal parasite that occurs exclusively in Africa. The vector flies live in the forests of equatorial Africa and take their blood meal during the day. After infection (Figure 4), the filaria penetrates the subcutaneous tissues and rapidly mature. They migrate in the subcutaneous tissues for more than 20 years, with females producing thousands of

microfilariae that can circulate in the blood for 6 to 12 months (S. D. S. Pion et al. 2006; Díaz-Menéndez et al. 2011). The microfilariae produced by females circulate in peripheral blood with a diurnal periodicity (S. D. Pion et al. 2020) and probably reside in lung tissue at night, although this has not been formally demonstrated (M. Boussinesq 2006). Unlike other filaria, *Loa loa* does not harbour a *Wolbachia* endosymbiont (Büttner et al. 2003; McGarry et al. 2003), and it does not appear to have developed new metabolic capacities to compensate (Desjardins et al. 2013).

Clinical signs of the disease may appear years after infection, often after the age of 21 (Richardson et al. 2012). Infected individuals may remain asymptomatic for long periods, with symptoms often mild and transient. Common manifestations include allergic, temporary and migratory oedema localised to the limbs, known as 'Calabar oedema'(Jain et al. 2008), as well as subcutaneous crawling of the worm. Generalised symptoms may include Pruritus (itching), Urticaria (hives), Myalgia (muscle pain), Arthralgia (joint pain), Fatigue, Visible migration of adult worms under the skin.

In severe cases, serious complications such as encephalopathy may occur in heavily infested patients. Rarely, subconjunctival manifestations (worms visible in the eye) are observed in affected individuals (Meda et al. 2009; Varenne et al. 2016).

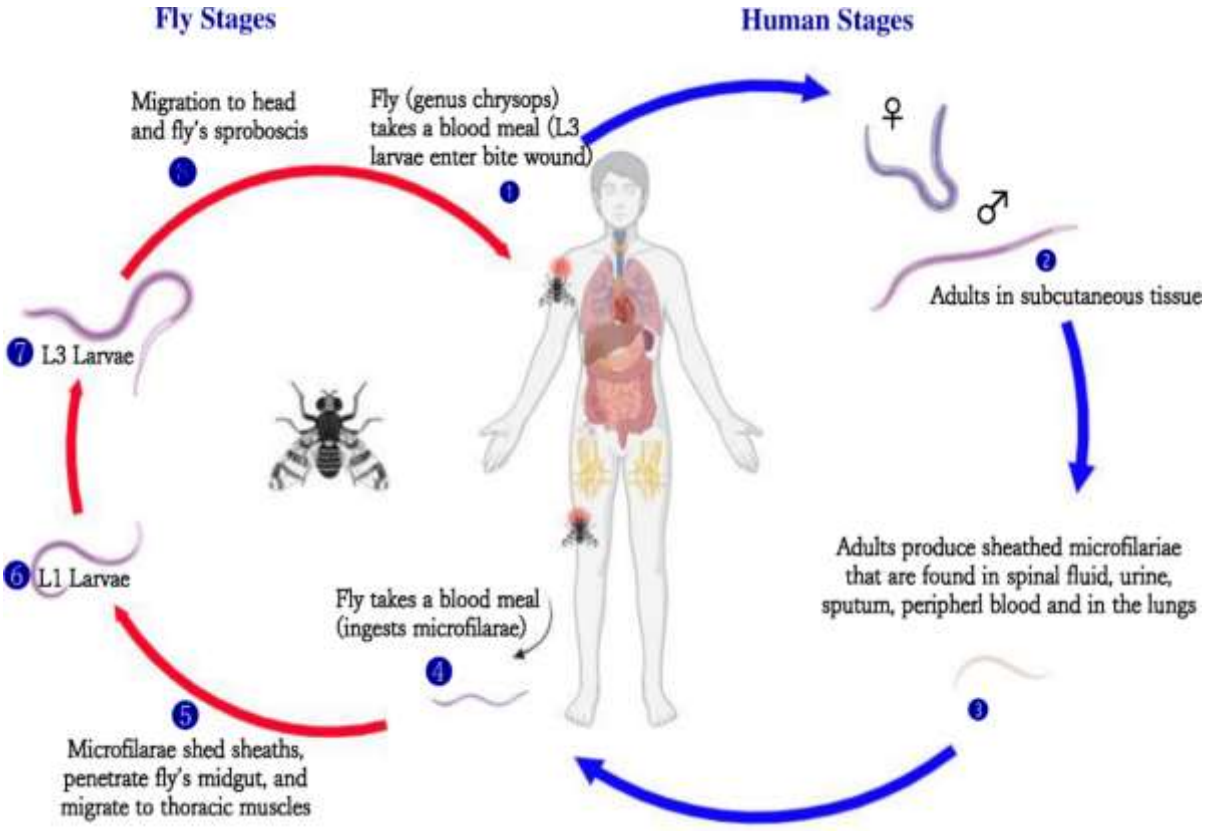

Figure 4. Life cycle of *Loa loa*. Created with BioRender.com.

c) Onchocerciasis

The primary organ affected by *Onchocerca volvulus* infection is the skin, but it can also cause severe visual impairment, making it the world's second leading cause of blindness after trachoma (Gyasi, Okonkwo, et Tripathy 2024). Infectious microfilarial larvae (stage L3) are introduced into the human body during a blood meal taken by a female black fly (Figure 5). Over a period of 6 to 12 months, these larvae develop into adult worms (macrofilariae) that reside in subcutaneous fibrous nodules, typically located around the iliac crest, head, and torso. Female worms are fertilized by males within the nodules and produce large quantities of microfilariae. These nodules are irrigated by blood and lymphatic vessels (Attout et al. 2009). Microfilariae are unsheathed and measure approximately 300 µm in length. Some of these microfilariae are found in lymphatic vessels, which they may use to reach the dermis, where they become accessible to Simulium vectors (Attout et al. 2009; Allen et al. 2008). Microfilariae can also be detected in the subconjunctival tissues of the eye.

The pathogenesis of onchocerciasis is thought to result from the chronic inflammatory immune response triggered by the death of microfilariae in the skin or eyes. This reaction is likely exacerbated by their Wolbachia endosymbionts, which contribute to the inflammatory process (Ngwewondo, Scandale, et Specht 2021). Symptoms include debilitating pruritus linked to microfilariae in the dermis (Desoubeaux, Chesnay, et Chandenier 2020) and may progress to permanent blindness. Onchocerciasis has also been associated with neurological disorders, including epilepsy (Chesnais et al. 2020), nodding syndrome (Geelhand De Merxem et al. 2019), and growth retardation. Adult worms have a lifespan of up to 15 years, during which the infection may remain asymptomatic or progress to various clinical manifestations such as papules and inflammatory lesions, skin changes (leopard skin, loose skin, skin atrophy and fibrosis), punctate keratitis, sclerosing keratitis, subcutaneous nodules, systemic signs and psychological and social complications (Hartley 2021; CDC 2024).

The incubation period ranges from 1–2 weeks on average but can extend up to 4 years (Antinori et al. 2017). In sub-Saharan Africa, onchocerciasis remains a leading cause of visual disturbances and blindness (Thylefors 2004).

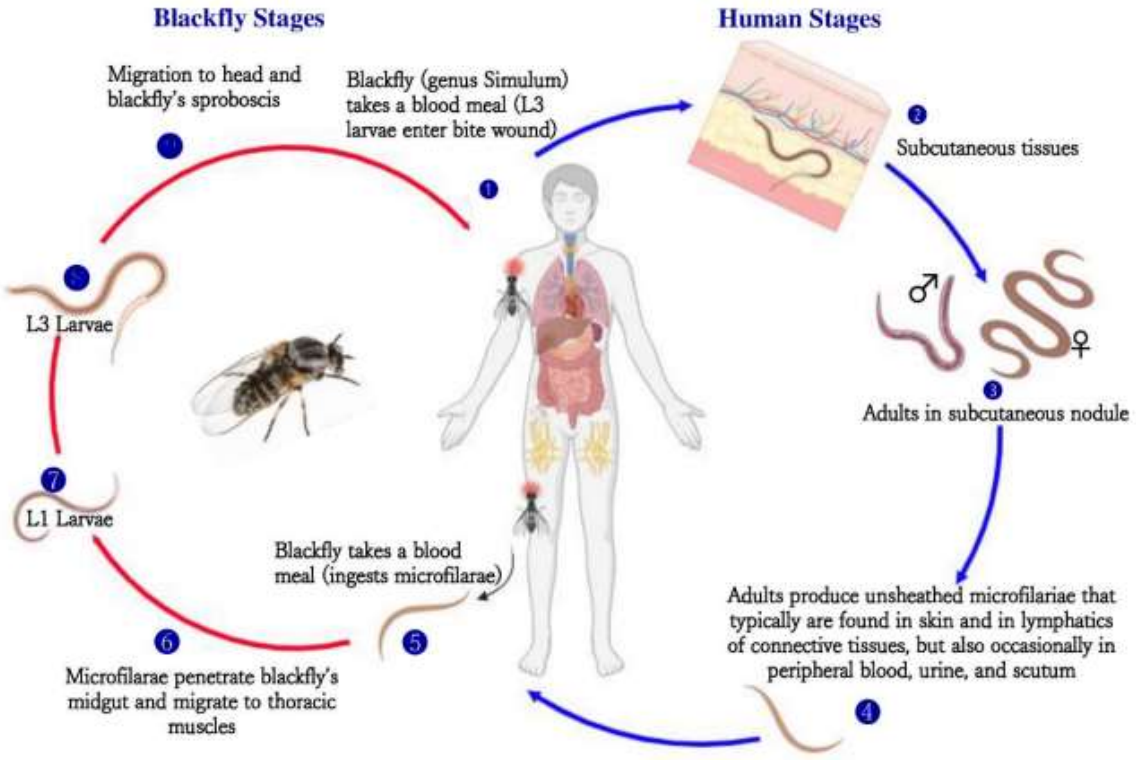

Figure 5. Life cycle of *Onchocerca volvulus*. Created with BioRender.com.

d) Mansonellosis

An infected *Culicoides* or *Simulium* female introduces infective filarial larvae (L3) into the human host during a blood meal. The larvae develop into adult filariae, which typically reside in the pleural cavity, peritoneal cavity, or subcutaneous dermal layer (Figure 6). Female worms produce microfilariae, which are found in: Peripheral blood (*M. perstans* and *M. ozzardi*), Skin (*M. streptocerca* and *M. ozzardi*). When an arthropod ingests microfilariae during a blood meal, the larvae undergo two molts within the vector to become infectious L3 larvae (Tamarozzi et al. 2022). These three parasite species (*M. perstans*, *M. ozzardi*, and *M. streptocerca*) have similar life cycles and epidemiology. They generally cause mild, non-specific symptoms or remain asymptomatic (Ta-Tang et al. 2018; Medeiros et al. 2017). Adult *M. perstans* worms are primarily found in serosal cavities, visceral adipose tissue, or skin tissue, while microfilariae develop in the blood and lymphatic system (Ta-Tang et al. 2018; Mediannikov et Ranque 2018a).

The pathogenicity of *Mansonella* species remains under debate. Infections are often asymptomatic, but certain clinical manifestations are associated with specific species:

M. perstans and *M. ozzardi*: linked to pruritus, headaches, edema of the lower limbs, arthralgia, inguinal lymphadenopathy, hydrocele, and ocular pathologies (Medeiros et al. 2017; Carme et Esterre 2012). *M. streptocerca*: primarily associated with skin lesions and inguinal damage. Although *M. perstans* is often asymptomatic, it may cause symptoms such as: Pruritus (itching), Urticaria (hives), Fatigue, Skin rashes, arthralgia, myalgia, fever, and neurological disorders (Tamarozzi et al. 2022).

In some cases, *M. perstans* infections present with non-specific manifestations similar to loiasis or other filarial infections, such as: localized or generalized pruritus, transient edema in various parts of the body, Headache, lymphadenopathy, and rash (Bottieau et al. 2022; Puente et al. 2020). In non-endemic areas, M. perstans infections have been associated with abdominal pain, asthma-like symptoms, pericarditis, pleuritis, and granulomatous nodules surrounding dead adult worms (Puente et al. 2020; Ferreira, Crainey, et Gobbi 2023; Gobbi et al. 2017).

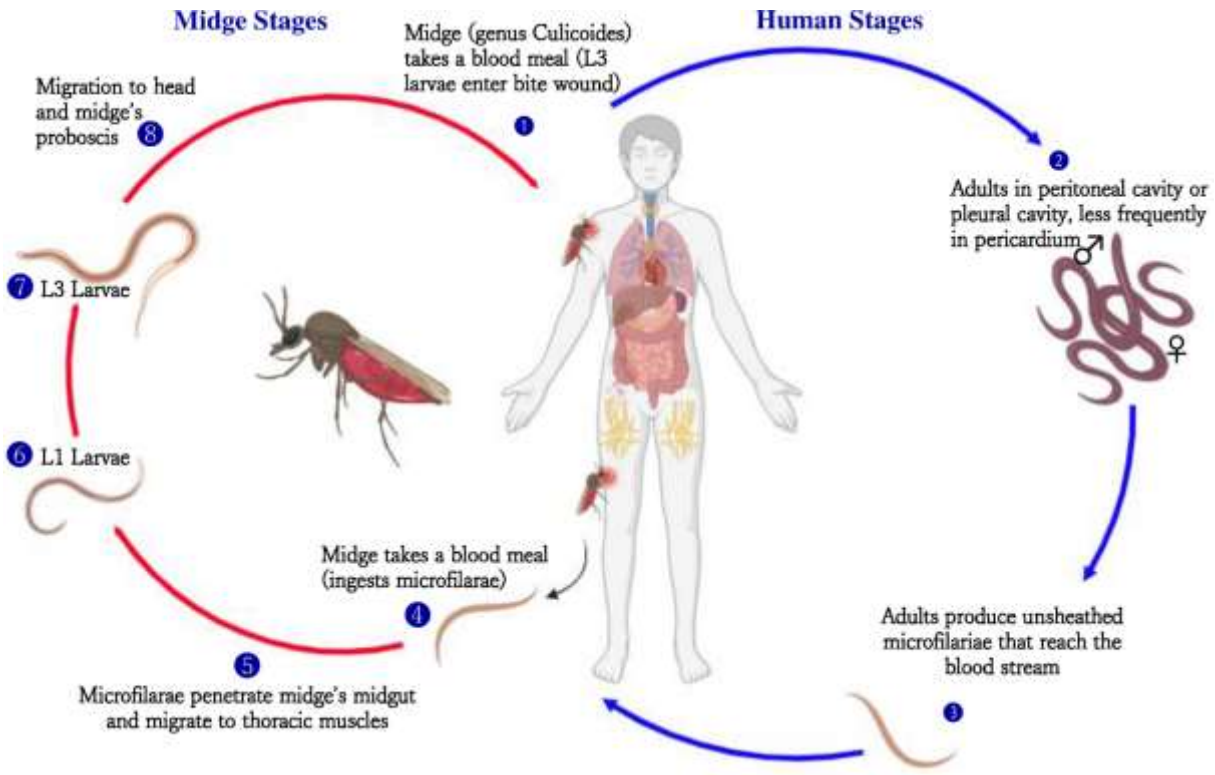

Figure 6. Life cycle of *Mansonella perstans*. Created with BioRender.com.

IV. Diagnostic methods

Classically, the diagnosis of filariasis is based on the detection (direct or indirect) of adult worms or microfilariae produced by adult females. Several diagnostic methods are available to detect these parasites :

- Blood microscopy: Used for species found in humans (*L. loa*, *W. bancrofti*, *Brugia malayi*, and *M. perstans*).
- Skin biopsy: Used for *O. volvulus* and *M. streptocerca*.
- Both methods: Utilized for *M. ozzardi* species (Kamgno et al. 2017).

In some cases, microfilariae can even be found in urine (Gaillard et al. 2020). Various other techniques, including antigen/antibody detection and polymerase chain reaction (PCR), are used for individual diagnoses and to assess infection endemicity in communities (Drame et al. 2016).

i. Lymphatic Filariasis

The diagnosis of lymphatic filariasis involves the detection of microfilariae in the blood or adult worms through direct and indirect examination.

1. Microscopy

Fresh blood examination: A drop of blood is collected after finger disinfection using a vaccinostyle (10–20 µL). The sample is placed on a slide and examined under low magnification (x50 or x100). Mobile microfilariae can be visualized among red blood cells (Desoubeaux, Chesnay, et Chandenier 2020; Duong et Richard-Lenoble 2008).

- Leucoconcentration technique: This involves enrichment by hemolysis using a 2% saponin solution, followed by centrifugation. The pellet is smeared, stained, and examined under a microscope (Herbeuval, Herbeuval, et Duheille 1962; Walther et Muller 2003). This technique increases sensitivity and detects parasite loads as low as 1 mf/µL (Melrose 2002).

Thin and thick smears: Blood volumes of 5–20 µL are used to prepare smears, stained with May-Grünwald Giemsa. This technique helps in identifying species based on morphological characteristics:

- Microfilariae length: 250–300 µm.
- Thickness: 7–8 µm with a sheath and visible internal body (Frédéric 2018; Duong et Richard-Lenoble 2008).

- Posterior end: Tapered.
- Cephalic end: Large or small.

The limit for microfilariae detection using this method is 15 mf/mL, making it less sensitive in low-density infections (Melrose 2002; Frédéric 2018).

- Knott's technique: This involves adding 1 mL of blood to 9 mL of 1% formalin, followed by centrifugation. The sediment is then examined under a microscope. This method allows precise measurement and preserves microfilariae morphology (Duong et Richard-Lenoble 2008).

2. Imaging Techniques

- Lymph node biopsy: In rare cases, *W. bancrofti* macrofilariae can be identified during histopathological examination.
- Lymphatic ultrasound: Detects adult worms through the “filaria dance sign” movements. This is a highly sensitive method for detecting worms in asymptomatic or amicrofilaremic patients (Desoubeaux, Chesnay, et Chandenier 2020). However, it becomes ineffective in advanced stages of lymphedema, as adult worms are no longer present (Mendoza et al. 2009).

3. Immunological Tests

- Antigen and antibody detection:
  - Techniques include ELISA, indirect immunofluorescence (IFI), and immunoelectrophoresis (IELP), using crude antigens from animal filarial species (*Dirofilaria immitis*, *Dipetalonema viteae*, etc.) (Desoubeaux, Chesnay, et Chandenier 2020).
  - These tests can detect pan-filarial antibodies, but they lack specificity and may cross-react with other nematodes (Melrose 2002).
- ICT (Immunochromatographic Cart Test) and FTS (Filariasis Test Strip): These tests detect circulating filarial antigens in blood samples collected from the fingertip at any time of day. They are highly sensitive and effective for diagnosing

lymphatic filariasis but cannot differentiate between past and active infections (Knopp et al. 2012; Cromwell et al. 2020).

4. Molecular Techniques

- Polymerase chain reaction (PCR): PCR is highly sensitive and specific, capable of detecting *W. bancrofti* and *B. malayi* in blood, urine, and tissue samples. It is primarily used in research to monitor infection prevalence and evaluate control (Walther et Muller 2003; Supali et al. 2006).

ii. Loiasis

The diagnosis of loiasis relies on the detection of microfilariae in blood samples. Key methods include:

Microscopic Examination: Detection is performed on fresh blood or after staining with thin or thick smear techniques. For cases of low microfilaremia or negative initial examinations, advanced methods such as the Knott technique and nucleopore membrane filtration are employed (CAMPILLO et al. 2023).

Sample Timing: Due to the diurnal periodicity of *Loa loa* microfilariae, blood samples should be collected around midday.

Adult Worm Detection: Adult helminths can sometimes be identified migrating under the skin or in the conjunctiva. Their extraction may require tools like a vaccinostyle or clawless forceps, or through scarification (Díaz-Menéndez et al. 2011; Varenne et al. 2016).

Morphological Identification: Microfilariae have a sheath, poorly stained by Giemsa, and exhibit visible ovoid nuclei overlapping to the tail but sparing a 5 µm space at the head (Bourée 2005).

Antibody Detection: Methods include ELISA, indirect immunofluorescence (IFI), and co-electrosyneresis (COES), with a focus on Pan-filarial antigens.

Advanced techniques like Luciferase Immunoprecipitation Systems (LIPS) detect IgG4 antibodies specific to *L. loa* (Burbelo et al. 2008).

Molecular Techniques: PCR is used for specific L. loa DNA detection and as an epidemiological tool in control programs (Knopp et al. 2012).

iii. Onchocerciasis

For onchocerciasis, several diagnostic techniques exist:

1. Microfilariae Visualization:

- Skin Snip Test: Small skin biopsies (from iliac crests, shoulder blades, or other regions) are placed in saline to observe microfilaria emerging from the dermis.

Staining: Using May-Grünwald Giemsa, microfilariae with elongated nuclei and curved tails are identified (Desoubeaux, Chesnay, et Chandenier 2020).

2. Histological Examination:
   - Biopsies or nodules are examined post-staining with hematoxylin and eosin to identify adult worms (Carme et Esterre 2012).
3. Immunological Tests:
   - ELISA tests detect IgG4 antibodies against recombinant *O. volvulus* antigen Ov-16.
   - Rapid diagnostic tests (RDTs) or LIPS tests are also available ((Vlaminck, Fischer, et Weil 2015).
4. Molecular Techniques:
   - PCR amplifies repetitive O-150 DNA sequences from skin snips or nodules, significantly increasing diagnostic sensitivity (Alhassan et al. 2015).
5. Specialized Tests:
   - Patch Test: Using diethylcarbamazine to provoke localized erythema, indicating onchocerciasis presence.
   - Ultrasound: Detects adult worms in nodules through their characteristic movement (Unnasch et al. 2018).

iv. Mansonellosis

The diagnosis of mansonellosis is species-specific:

1. Microscopy:
   - *M. perstans*: Detected in blood samples.
   - *M. streptocerca*: Identified through skin biopsies.
   - *M. ozzardi*: Found in both blood and dermal samples (Frédéric 2018).
2. Molecular Techniques:
   - PCR allows the differential diagnosis of *M. streptocerca* from *O. volvulus*, *W. bancrofti*, and *L. loa* (Walther et Muller 2003).

- LAMP (Loop-mediated Isothermal Amplification) offers a more rapid alternative for species detection (Poole et al. 2017; Deng et al. 2019).

3. Other Sample Sources:

- Microfilariae of *M. perstans* and *M. ozzardi* have been detected in urine, cerebrospinal fluid, and other body fluids (Gaillard et al. 2020).

v. Prevention

- **Lymphatic Filariasis**

Lymphatic filariasis (LF) is a neglected tropical disease whose prevention focuses on raising community awareness about transmission and the importance of preventive measures. Key strategies include:

1. Community Awareness and Hygiene: Promoting personal and environmental hygiene to reduce exposure to mosquito vectors.
2. Mass Drug Administration (MDA):
   - The Global Programme to Eliminate Lymphatic Filariasis (GPELF), launched in 2000 by WHO, aims to eliminate LF as a public health problem.
   - MDA involves annual administration of albendazole in combination with either diethylcarbamazine (DEC) or ivermectin for at least 5 years in regions with ≥1% prevalence of filarial antigens (Ottesen 2006; World Health Organization 2011).
   - The goal is to kill circulating microfilariae, thereby reducing transmission (Simonsen et al. 2014).
   - There is no macrofilaricidal drug for MDA, which would eliminate adult worms.
3. Vector Control:
   - Intensive use of long-lasting insecticide-treated nets (LLINs) and xenomonitoring (PCR detection of LF parasites in mosquito vectors) has proven effective (Bockarie, Taylor, et Gyapong 2009; Njoku et al. 2013).
   - Addressing stagnant water sources and improving drainage systems also helps control mosquito breeding sites.
4. Psychosocial Support:
   - Community programs to combat stigma and provide psychosocial assistance for patients, improving their well-being and reintegration into society.

- **Onchocerciasis**, or river blindness, is the second leading infectious cause of preventable blindness. Preventive strategies include:

1. Vector Control Programs:
    - The Onchocerciasis Control Program (OCP) in West Africa and the African Programme for Onchocerciasis Control (APOC) used aerial spraying of insecticides and mass drug distribution to reduce blackfly populations (Boatin 2008).
2. Mass Drug Administration:
    - Ivermectin is distributed annually to affected communities to control skin and eye symptoms and reduce microfilariae levels (Amazigo 2008).
3. Challenges in *L. loa*-Endemic Areas:
    - In areas co-endemic with *Loa loa*, ivermectin can cause severe adverse events. Measures are needed to detect high microfilaremia levels before administering the drug (Vinkeles Melchers et al. 2018).
4. Personal Protection:
    - Use of insect repellents and protective clothing when traveling through endemic areas.
    - There is currently no vaccine for onchocerciasis.

- **Loiasis**

Loiasis, caused by *Loa loa*, lacks a global eradication program. Preventive measures focus on:

1. Vector Control:
    - Reducing exposure to Chrysops vectors using insect repellents (DEET), permethrin-treated clothing, and mosquito nets, although the latter are less effective due to the diurnal activity of vectors.
2. Chemoprophylaxis:
    - Weekly doses of diethylcarbamazine (DEC) can reduce risk but carry the potential for side effects, particularly in high microfilaremia cases (Frédéric 2018).
    - Initial treatment with albendazole, a less potent microfilaricide, is recommended to minimize adverse reactions before stronger drugs like ivermectin or DEC are administered.
3. Monitoring:
    - Identifying and monitoring high-risk individuals with elevated microfilaremia to minimize complications.

- **Mansonellosis** remains poorly studied and lacks targeted control programs. Preventive efforts include:

Insecticide Use:

Indoor residual spraying (IRS) has shown promise in reducing Culicoides populations, although its effectiveness for mansonellosis control depends on vector behavior (González et al. 2020).

Limitations of Bed Nets:

Bed nets, effective for malaria and LF, are less impactful for mansonellosis because Culicoides are small and typically bite outdoors (Brugman et al. 2017; Harrup et al. 2016).

Research Needs:

More studies are needed to understand vector ecology and optimize control strategies globally.

VI. Treatment

- Lymphatic Filariasis

Since the launch of the Global Programme to Eliminate Lymphatic Filariasis (GPELF) by the WHO in 2000, the main strategy has been to stop the spread of the disease through Mass Drug Administration (MDA). This involves large-scale preventive chemotherapy, which is primarily aimed at eliminating microfilariae in the bloodstream rather than adult worms.

Treatment Regimens:

1. Ivermectin (150-200 µg/kg) + Albendazole (400 mg): Used in regions co-endemic with onchocerciasis.
2. Diethylcarbamazine (6 mg/kg) + Albendazole (400 mg): Recommended in areas free of onchocerciasis.
3. Albendazole alone (400 mg): Administered twice a year in regions where loiasis is endemic.

These treatments do not kill the adult parasites but remove microfilariae from the blood, thereby preventing further transmission. For the treatment to be effective, it must be administered annually for at least five years with complete coverage of the at-risk population (Rebollo et Bockarie 2017).

- Doxycycline (200 mg/day for 4–6 weeks) targets Wolbachia bacteria, which live in symbiosis with adult worms, leading to the gradual death of the parasites (Ottesen 2000).

❖ Mansonellosis is difficult to treat due to the resistance of *M. perstans* to standard antifilarial drugs. Some treatments have shown varying levels of success:

1. Combination therapy with diethylcarbamazine and mebendazole is effective in reducing microfilaremia.
2. Thiabendazole alone has been shown to significantly reduce microfilariae in the blood.
3. Ivermectin and praziquantel are not effective for *M. perstans* infections.
4. Mebendazole alone is an alternative, more effective than diethylcarbamazine alone in clearing M. perstans infections (Medeiros et al. 2017; Enrico Rino Bregani et al. 2006).

Additionally, doxycycline has been found effective for *M. perstans* infections, especially in West Africa, where *M. perstans* carries Wolbachia bacteria. However, its efficacy is uncertain in regions where Wolbachia is absent in *M. perstans* populations (Yaya I. Coulibaly et al. 2009a).

- Ivermectin has shown effectiveness in reducing microfilariae of *M. ozzardi* in short and long terms (Shelley et Coscarón 2001).
- Diethylcarbamazine and ivermectin are also effective against M. streptocerca, with ivermectin suppressing microfilariae for up to one year (Pérez-Molina et al. 2010).

❖ Treating onchocerciasis is essential to prevent skin damage and long-term blindness (Antinori et al. 2017).

1. Ivermectin kills microfilariae and temporarily halts their production by adult worms.
    - It is administered annually to populations at risk, but it does not eliminate adult worms.
    - Doxycycline combined with ivermectin has shown promise in significantly reducing both microfilarial loads and adult worm burden, by targeting Wolbachia bacteria that are essential for the survival of adult worms (A. Y. Debrah et al. 2015).
2. Moxidectin, a newer single-dose antiparasitic, has also shown efficacy against onchocerciasis in a study across Ghana, Liberia, and the Democratic Republic of Congo (Opoku et al. 2018).

❖ The treatment of loiasis must be done under medical supervision due to the risk of serious adverse effects:

Diethylcarbamazine is typically used, but it can cause dangerous side effects in patients with high microfilarial densities (encephalopathy).

Albendazole is prescribed first when the microfilarial load exceeds 8000/mL; it is followed by ivermectin or diethylcarbamazine for lower microfilarial densities (Michel Boussinesq 2012).

Ivermectin and diethylcarbamazine are used in the treatment of high microfilarial density, but they may trigger encephalopathy in patients with very high microfilarial counts (Whittaker et al. 2018).

Challenges in Treatment

Albendazole-induced encephalopathy has been reported, particularly in the context of high L. loa microfilarial densities, emphasizing the need for caution when administering these drugs (Volpicelli et al. 2020; Métais, Michalak, et Rousseau 2021).

Table 2. Main characteristics of recommended medications for *Loa loa* infection.

	Action mechanism	**Recommended use**	**Dosage**	**Benefits**
Diethylcarbamazine	Microfilaricide and Macrofilaricide	Number of Mf < 2000 mf/ml	1 mg/kg/day, increasing over 3 days to 9 mg/kg/day for 3 weeks	Rapid macrofilaricidal effect
Ivermectine	Microfilaricide	Number of Mf between 2000/ml and 8000/ml	150-200 µg/kg, single or multiple dose	Rapid microfilaricidal effect, high efficiency in combination with ALB
Albendazole	Macrofilaricide (Embryotoxic effect), Slow effect	Mf rate greater than 8000/ml	200-400 mg twice daily for 21-28 days	Fewer adverse events, high efficacy in combination with IVM

Conclusion

This review highlights the crucial importance of filariasis as a public health problem in tropical areas, particularly in West Africa, where it imposes a heavy health and socio-economic burden on affected populations. The wide spectrum of filariasis, including lymphatic infections such as lymphatic filariasis, skin infections such as onchocerciasis, and serous infections such as mansonellosis, illustrates the diversity and complexity of this pathology. Current diagnostic and control strategies, although they have made significant progress, show limits in the sustainable elimination of transmission, partly due to the complexity of the biological cycles of filariae and the diversity of vectors involved. Massive treatment interventions with drugs like

ivermectin have been key to reducing morbidity rates, but challenges persist, particularly in co-endemic areas where some treatments can cause serious adverse effects.

For more effective and long-term control of filariasis in West Africa, several perspectives and recommendations are necessary. It is essential to: Strengthen diagnostic capacities: Developing more specific and less expensive diagnostic methods, including rapid field tests, will help better target interventions and reduce transmission in endemic areas. Develop integrated prevention strategies: This includes controlling vectors through means such as insecticide-treated bed nets and introducing community awareness programs to encourage the adoption of personal protection measures. Support research into new treatments: Exploring new therapeutic agents less likely to cause adverse effects in areas co-endemic with Loa loa, for example, could strengthen adherence to mass treatment programs and improve outcomes sanitary facilities.

Improve epidemiological surveillance: Continuous and detailed monitoring of the prevalence of infections will make it possible to quickly detect resurgences and adjust interventions, accordingly, thus ensuring the effectiveness of long-term control programs. Strengthen international partnerships: Collaboration between governments, international organizations, and local communities is essential to support efforts to eliminate filariasis in West Africa and mobilize the resources necessary to achieve this ambitious goal. Adopting these integrated approaches could transform filariasis control efforts, reducing transmission and easing the socio-economic burden in affected regions.

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Informed Consent Statement

Not applicable.

Data Availability Statement

All data are provided in the manuscript.

Conflicts of Interest

The authors declare that they have no competing interests.

Abbreviations

NTD	Neglected Tropical Diseases
FTS	Filariasis Test Strip
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-Linked Immuno Assay
MDA	Mass Drug Administration
PCR	Polymerase Chain Reaction
LAMP	Loop-mediated amplification
GE	thick drop
IFI	Immunofluorescence
COES	Co-Electrosyneresis
IELP	Immunoelectrophoresis
ICT	Immunochromatographic
LIPS	Luciferase Immunoprecipitation System
WHO	World Health Organization
BCE	Bloodless skin biopsy
MGG	May Grunwald Giemsa
GPELF	Global program for the elimination of lymphatic filariasis
OCP	Onchocerciasis in West Africa
APOC	Africa onchocerciasis control program
DEET	NN-diethyl-3-methylbenzamide

PARTIE 2

Diagnosis of filariasis in patients and identification of potential vectors *Mansonella perstans* in southern Senegal

ARTICLE 2

Elephantiasis and *Mansonella perstans* in Senegal (West Africa)

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Key Words:	Elephantiasis, Mansonella perstans, rapid diagnostic tests, Mosquitoes, biting midges

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Résumé

Les filarioses sont des maladies parasitaires fréquentes qui touchent des millions de personnes dans les régions tropicales et subtropicales engendrées par différentes espèces de vers ronds connus sous le nom de filaires. L'éléphantiasis est une affection clinique, grave due à l'obstruction du système lymphatique par les vers adultes. *Mansonella perstans* est plus négligé car il est considéré comme bénigne causant principalement des infections asymptomatiques. L'infection par l'une de ces maladies se produit lorsque les parasites filaires responsables sont transmis à l'Homme par des arthropodes hématophages des genres Moustiques et Culicoïdes. Le but de cette étude était de rechercher l'étiologie des cas d'éléphantiasis, d'explorer la faisabilité de l'utilisation des cassettes tests de diagnostic rapide (TDR) négatifs pour le paludisme et d'identifier si les arthropodes hématophages sont vecteurs potentiels de la filariose au Sénégal.

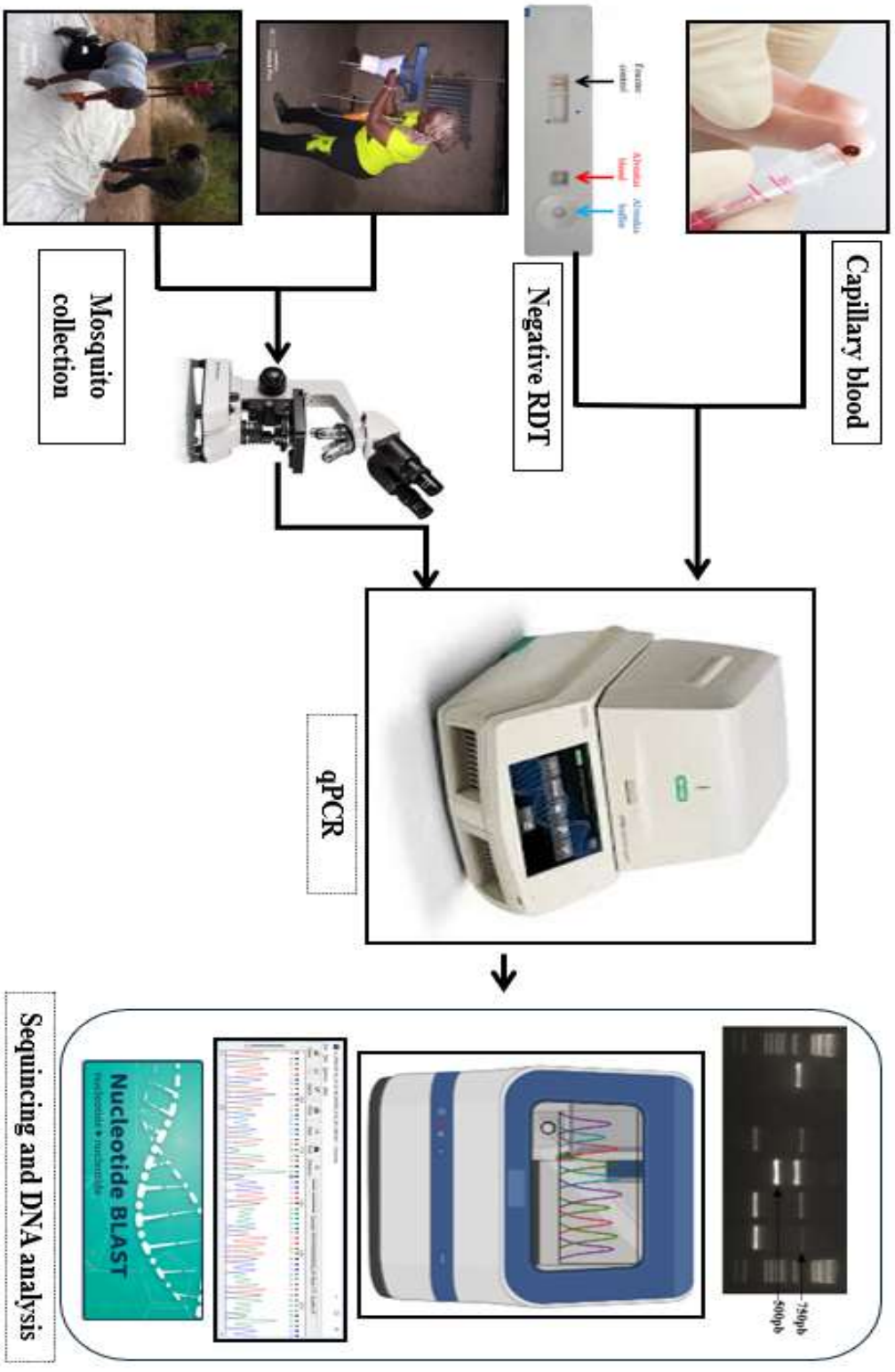
La collecte des échantillons a été réalisée au Sud du Sénégal qui est endémique par la filariose. Nous avons analysé 23 échantillons de sang des patients atteints d'éléphantiasis résidant à Kolda et Oussouye en 2021, 279 échantillons de sang de sujets fébriles à Mlomp entre 2018 - 2020 et 3714 cassettes TDR *Plasmodium falciparum* (*P.f*) négatives collectées dans les postes et centres de santé de 2020 à 2021. En plus, 254 moustiques collectés à Kolda, Oussouye et Sédhiou en 2021 à l'aide de pièges lumineux CDC, OVI et faune matinale résiduelle et 1233 moustiques collectés en 2007 et 2012, et 991 spécimens de culicoides ont été analysés.

La présence de *M. perstans* a été détectée chez 8,7 % (2/23) des patients malades et chez 4,3 % (12/279) des sujets fébriles de Mlomp. Parmi les 3714 cassettes TDR négatives *P.f* testées, 0,24 % (9/3714) se sont révélées positives.

Nos résultats montrent la présence de *M. perstans* dans les TDR, chez les sujets fébriles et chez les individus atteints d'éléphantiasis. Aucun arthropode n'a été trouvé infecter par *M. perstans*. Il est nécessaire de poursuivre la recherche du vecteur responsable de la filariose à *M. perstans* dans les sites endémiques du sud du Sénégal.

Keywords: Elephantiasis, *Mansonella perstans*, TDR, Arthropodes, filarioses, qPCR, Sénégal

Résumé graphique du projet



Abstract

Background: Filariases are parasitic diseases that infect millions of people living mostly in less developed areas, mainly in West Africa. Infection occurs when the filarial larvae are transmitted to humans by mosquitoes or other dipterans. Elephantiasis is a clinical condition, often severe, usually associated with mosquito-transmitted filarial worms of *Wuchereria* or *Brugia* genera. *Mansonella perstans* is considered as clinically mild, mainly asymptomatic filariasis and not usually associated with elephantiasis. The aim of this study was to investigate the etiology and epidemiology of elephantiasis cases and to screen the filaria hosting in population in Senegal.

Methods: This study was carried out in July-November and December 2021 in endemic sites in southern Senegal. We analyzed 23 blood samples from elephantiasis patients residing in Kolda and Oussouye in 2021, 279 blood samples from febrile subjects in Mlomp examined at the dispensary between 2018 - 2020 and 3714 nitro-cellulose strips from malaria rapid diagnostic tests collected in health posts and centres from 2020 to 2021. 254 mosquitoes and 991 *Culicoides* biting midges were collected in residences of elephantiasis patients in Kolda, Oussouye and Sédhiou in 2021 using light traps CDC, OVI and residual morning fauna. In addition, 1233 mosquitoes collected in other regions of Senegal in 2007 and 2012 were analyzed.

Results: The presence of Mansonella perstans was detected in 8.7% (2/23) of ill patients and 4.3% (12/279) of febrile subjects from Mlomp. Among the 3714 negative P. falciparum RDT cassettes tested, 0.24% (9/3714) were found to be positive. None of the mosquitoes and *Culicoides* tested were infected with *M. perstans,* but other probably animal filarial species were detected.

Conclusion: Our results show the presence of only *M. perstans* in both elephantiasis and febrile patients in Senegal, no other filarial nematodes were detected. No arthropods were infected with human pathogen filaria. It is necessary to continue studies of etiology of elephantiasis in Senegal and the search for the vector responsible for *M. perstans* filariasis in endemic sites in southern Senegal.

Keywords: Elephantiasis, *Mansonella perstans*, RDT, mosquitoes, biting midges, filariasis, qPCR, Senegal

INTRODUCTION

The World Health Organization (WHO) estimates that about 1.7 billion people suffer from neglected tropical disease and that 49 African countries bear 40% of the global burden. Elephantiasis or lymphatic filariasis is one of the oldest and most debilitating neglected tropical disease affecting more than 51.4 million people worldwide in 72 endemic countries [1]. According to the WHO, in 2023, more than 882 million people in 44 countries are at risk of lymphatic filariasis [2]. Since the beginning of the Global Program for the Elimination of Lymphatic Filariasis (GPELF) in 2000, the goal was to stop the parasite transmission by mass drug administration, management of morbidity and prevention of disabilities [3]. Between 2007 and 2019, 34 African countries had lymphatic filariasis, with 26 countries of them reporting lymphoedema and hydrocele with 148,114 and 137,696 cases, respectively. Lymphatic filariasis results from infestation by *Wuchereria bancrofti* and *Brugia* sp. [4]. These are transmitted to humans by the bite of mosquitoes belonging to the genera *Culex*, *Anopheles*, *Mansonia* and *Aedes* [1]. Hydrocele, lymphedema and elephantiasis are the visible chronic consequences that do not appear until later in life [1,5]. Lymphatic filariasis causes an apparent alteration of the lymphatic system and can lead to an abnormal increase in the volume of certain parts of the body, resulting in pain, severe disability, and social stigma.

Elephantiasis has been historically reported in Senegal. Published in 1821 detailed description of the frigate “Méduse” shipwreck mentioned the existence of elephantiasis and probably onchocerciasis in Senegal [6]. Interesting, for the best of our knowledge, *W. bancrofti* was neither characterized nor even detected by molecular methods in Senegal. Based on very few studies, it is considered to be present in certain areas, but diagnostic relies only on microscopic studies [7–10].

Recent studies on children in Uganda have revealed co-infections between *W. bancrofti* and *Mansonella perstans*, the agent responsible for mansonellosis, in 33 individuals (0.3%) in six schools. [11].

Despite the wide distribution of mansonellosis, very few studies have investigated its epidemiology and clinical picture [12]. Simonsen estimated that 114 million people were infected. In Africa alone, 581 million people in 33 countries are at risk of *M. perstans* [13]. Prevalence is often very high in endemic areas and children are not spared [12]. Mansonellosis is globally caused by three species of filarial nematodes, generally producing chronic human microfilaremia and with variable geographical distribution: (1) *M. perstans*, generally found in

sub-Saharan Africa, but also in South America; (2) *Mansonella streptocerca* endemic to sub-Saharan Africa; (3) *Mansonella ozzardii*, found exclusively in America and the Caribbean islands [13–15]. Recently, genetic studies showed that at least one other genetic variant *Mansonella* sp. DEUX infect humans in Africa (Gabon) [16–18]. Whole genome analysis clearly showed that Mansonella sp. "DEUX" is a separate species, [16] but it is yet not clear if it is a new, yet undescribed species or one of old ape-associated *Mansonella* species that were described only morphologically [19]. These are *Mansonella vanhoofi* from bonobos and gorillas, *Mansonella rodhaini* from bonobos and chimpanzees, *Mansonella lopeensis*, *Mansonella leopoldi* and *Mansonella gorilla*, all three from gorillas.

The recent study in rural Senegal (Kedougou) showed an overall prevalence of 14.5% of *M. perstans* in febrile and afebrile subjects [20]. Although infection with *M. perstans* is considered a low pathogenic, often asymptomatic, various clinical manifestations have been associated with its presence, such as asthenia, skin swelling, itching, ocular or palpebral pruritus, rashes, angioedema, headache, arthralgia, abdominal pain, chest pain, pericarditis, pleurisy, neurological manifestations, visual impairment, inflammatory granulomatous nodules surrounding dead adult worms, and fever in some cases [12,20,21]. Eosinophilia can also be observed.

M. perstans is considered to be transmitted by *Culicoides* biting midges [22]: *Culicoides milnei* and *Culicoides austani* in Cameroon [23,24] and *Culicoides fulvithorax* and *Culicoides grahamii* in Nigeria [25,26]. However, in Senegal, no *Culicoides* vector species has yet been identified [20].

The aim of this study carried out in rural Senegal was to determine the etiology of cases of elephantiasis, to demonstrate the usefulness of nitro-cellulose strips of malaria rapid diagnostic tests (RDTs) to the screening of filariasis, and to identify whether mosquitoes could be potential vectors of *M. perstans* in the regions studied.

Methods

Ethical statement

This study was approved by the National Committee on Health Research Ethics of Senegal (CNERS) and the Research Ethics Committee (CER) of UCAD under the numbers SEN21/81 and O75/2O21/CER/UCAD. Local authorizations were also obtained by the chief medical officers of the districts of Kolda, Sedhiou, Kedougou and Ziguinchor (Oussouye).

Individual written informed consent was obtained from adult participants and, for minors, from their parents or legal guardians, and all were informed of the procedure. Data was recorded and stored to preserve patient anonymity.

Study areas

The study was conducted in 4 regions (Ziguinchor, Kolda, Sedhiou and Kedougou) in Senegal (**Fig. 1**). In the Ziguinchor region, the study was conducted in the department of Oussouye (12°29'06'N - 16°32'49'E), in the villages of Kagnout, Diakene diola, Djibelene, Niambalang and Diakene ouolof. Oussouye is one of the 3 departments of the Ziguinchor region, located in Lower Casamance on the left bank of the Casamance River. The average temperature in Ziguinchor is 26.0°C in the coldest month (January) and 29.8°C in the hottest month (June). Rainfall reaches 1,400 millimeters per year, making it quite abundant. During the least rainy months (February, March, April, December), it is 0 mm, while during the wettest month (August), it reaches 481 mm [27].

In the Kolda region (12°53'00"N - 14°57'00"E), the study was conducted in the villages of Bagadadji, Demba korbali, Sare pathe, Missira kamarang, Guiro yero alpha, Sare sandiong, Missara Ousmane and Kampissa. Kolda is in southeastern Senegal on the southern border of Gambia. Its climate is Sudano Guinean with an average temperature of 28°C, with a minimum of 18.3°C and a maximum of 37.8°C. Annual rainfall is 1185 mm [28]

In the Sedhiou region (12°42'29'N – 15°33'25'E), the study was conducted in the villages of Tambanaba and Badiary. Sedhiou is bounded to the north by Gambia, to the south by the republics of Guinea and Guinea Bissau, to the east by the region of Kolda, to the west by the region of Ziguinchor. Temperatures range from 25 - 30°C with an average precipitation of 1000 mm per year [29].

In the Kedougou region (12°33'N – 12°11'E), the study was conducted in the localities of Mako, Ndiormi, Fadiga, Dalaba and Tomboronkoto. Kedougou is limited to the West and North by the Tambacounda region, to the South-East by the Republic of Guinea and Mali. The rainfall is very important which makes it one of the rainiest regions of Senegal with at least 1300 mm per year. The climate is Sudano-Guinean, and temperatures are relatively high ranging from a maximum of 42°C to a minimum of 21°C [30].

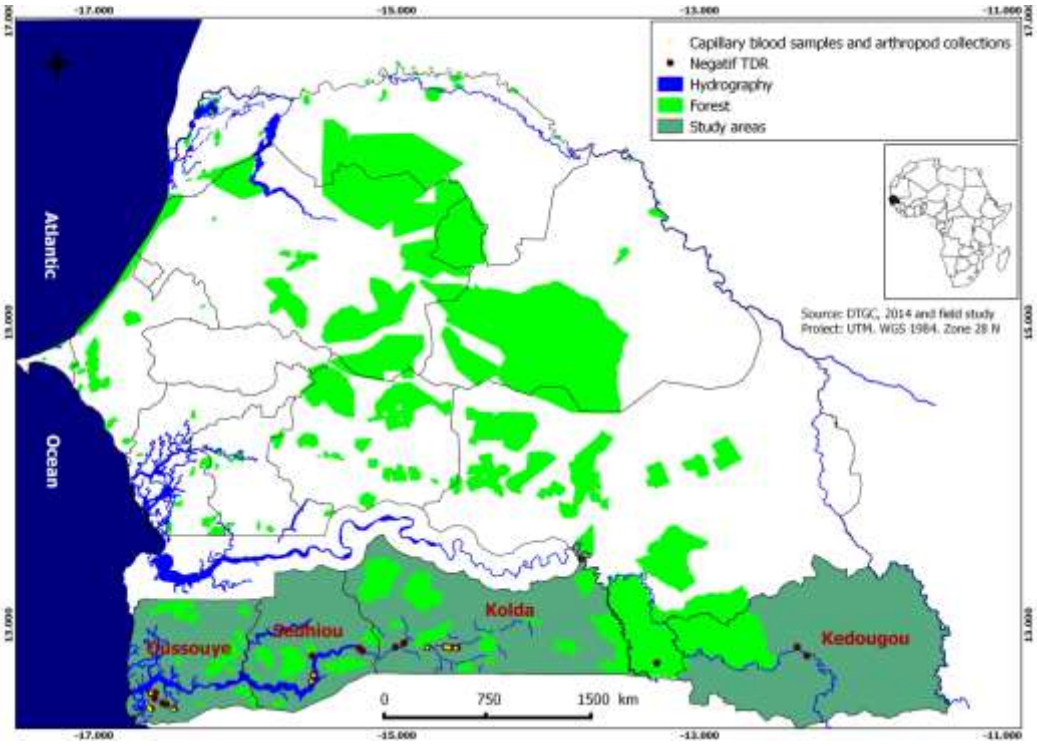


Figure 1. Localization of villages and health posts in study areas (QGIS Desktop 2.2.0).

Samples collection

Capillary blood sampling in patients with elephantiasis

Capillary blood samples were collected in July 2021 in patients with symptoms of elephantiasis and residing in 8 localities of Kolda (Bagadadji, Demba korbali, Sare pathe, Missira kamarang, Guiro yero alpha, Sare sandiong, Missara Ousmane and Kampissa) and 4 localities of Oussouye (Kagnout, Diakene diola, Djibelene, Niambalang and Diakene ouolof). (**Table 1**) A questionnaire designed to collect the patient's personal and clinical information was completed by the team's doctors prior to sample collection. Wherever possible, two capillary blood samples were taken from patients by finger prick at 10 a.m. (day) and 10 p.m. (night) in EDTA tubes. The samples were then stored in a cooler at 4°C and transferred to Dakar (Senegal's capital) under the same conditions.

Table 1: Clinical data on certain cases observed in participants in the study areas

										qPCR Pan Filaria		qPCR M. perstans	
Sites	**Villages**	**Codes**	**Sex**	**Ages**	**Symptoms**	**Duration of illness (YEARS)**	**Hospitalization**	**Specific treatment**		**Diurnal**	**Nocturnal**	**Diurnal**	**Nocturnal**
Kolda	Bagadadji	KL 1	F	68	Leg edema with recurrent inflammations, pruritus, vomiting, headache	~ 40	YES	Extencillin 1.2 MUT, mycostere 1 application, Local care		0	0	0	0
	Missira kamarang	KL 2	M	71	Hydrocele, vomiting, headache, recurrent inflammation	~ 18	NO	NO		0	0	0	0
	Missira ousmane	KL 3	M	58	Leg/foot edema, pruritus, headache	25	NO	Betadine dermique, Mycoster pomade, Fleming (Amoxy Acide Clavulanique)		0	0	0	0
	Kampissa	KL 4	M	67	Leg edema with recurrent inflammation, pain in the liver area, muscle pain	~ 60	YES	Extencillin, Amoxicillin, Acide clavulanique, Permaganate, SAT, VAT, Mycoster		0	0	0	0
	Demba korbali	KL 5	M	76	Leg/foot edema, periorbital edema, bilateral inguinal hernia	55	NO	Flucanozole, Mycoster, Vaselinesalicyle		31,08	28,13	32,58	32,08
	Saré Sandiong	KL 6	F	~ 95	Leg edema, recurrent erysipelas	~ 5	YES	NS*		0	0	0	0
	Bagadadji	KL 7	M	51	Leg/foot edema with recurrent infection, pruritus, omitting, headache	25	NO	NO (Surgery recommended)		0	0	0	0
	Guiro yero alpha	KL 8	F	70	Edema of groin area-Pruritus-Muscle Pain-Headache-Arthralgia	50	NO	Anti-fungal cream		0	0	0	0
	Saré Pathé	KL 9	F	65	Leg edema, muscle pain, headache, arthralgia, recurrent erysipelas	> 20	NO	NO		0	0	0	0
	Saré Pathé	KL 10	F	65	Quincke's edema, periorbital edema, arm/hand edema with recurrent inflammations, muscle pain, pruritus, stomach pain	More 40	YES	Hospital treatment in Gambia		0	0	0	0
	Saré Pathé	KL 11	F	90	Quincke's edema, hypogastric pain, myalgia, pruritis, arthralgie, lower limb swelling with recurrent inflammation	> 60	NO	NO		0	0	0	0
Oussouye	Kagnout Ebrouaye	KL 12	F	69	Leg edema, pruritus, stomach pain, arthralgia, itching, frequent erysipelas	> 40	NO	NO		0	0	0	0
	Kagnout Ebrouaye	KL 13	M	79	Leg/foot edema, periorbital edema, myalgia, pruritus, arthralgia, erysipelas	8	YES	NS*		0	26,73	0	31,14
	Diakène Diola	KL 14	F	55	Leg edema, muscle pain, pruritus, headache, arthralgia, erysipelas sometimes	35	NR	NS*		0	0	0	0
	Diakène Diola	KL 15	M	70	Leg edema, periorbital edema, myalgia, stomachache, arthralgia, headache, erysipelas	8	NO	1 time Ivermectin / Albendazol		0	0	0	0
	Diakène Diola	KL 16	F	61	Muscle pain-Arthralgia-Phlebitis odor	35	NO	2 times Ivermectin / Albendazol		0	0	0	0
	Djibetène / Mlomp	KL 17	F	67	Leg swelling, muscle pain, arthralgia	birth	NO	NO		0	0	0	0
	Diakène	KL 18	M	76	Leg edema, muscle pain, pruritus, arthralgia, cough for 7 years	1	NO	NO		0	0	0	0

	Diakène Diola	KL 19	M	64	Leg edema, muscle pain, pruritus, arthralgia, loss of sensation in the feet	3	NO	2 times mass treatment with Ivecmectin	0	0	0	0
	Niambalang	KL 20	M	66	Leg/foot edema, myalgia and arthralgia	28	NO	NO	0	0	0	0
	Niambalang	KL 21	M	57	Hydrocele, periorbital edema, muscle pain, pruritis	29	NR	NS*	0	0	0	0
	Niambalang	KL 22	M	76	Hydrocele, periorbital edema, headache	28	NR	NS*	0	0	0	0
	Diakène ouolof	KL 23	M	61	Leg edema, myalgia, pruritus, headache, erysipelas	41	NO	NO	0	0	0	0

*not specified

Capillary blood sampling in febrile patients attending the Mlomp health post

Capillary blood samples were taken daily from 2018 to 2020 in febrile patients consulting in the health post of Mlomp. A fever was defined by an axillary temperature of ≥37.5°C. The samples were kept at 4°C and then the nucleic acids were extracted in the laboratory of the Mlomp dispensary.

Negative RDT samples for *P. falciparum* in targeted stations

With the agreement of the National Malaria Control Programme (PNLP), all RDT cassettes from malaria-negative patients attending 18 health facilities were analysed. RDTs were therefore sorted at each site, and those that were negative were placed in plastic bags at room temperature. Once at the laboratory, each sample was assigned an internal code to preserve patient anonymity.

Arthropods collection and identification

In addition to Residual Morning Fauna (RMF), two types of light traps were used: Center for Diseases Control (CDC) modified (Bio Quip Products, Compton, CA, USA) and Onderspoort Veterinary Institute (OVI) developed by (Onderstepoort Veterinary Institute, Pretoria, South Africa). The RMF allowed arthropods to collect at rest in the rooms. The light traps were used in July, November and December 2021 in 12 villages in the districts of Kolda, Sedhiou and Oussouye. In the households where patients with elephantiasis lived, we placed one inside the patient's room that slept under a mosquito net and another outside. The traps were set at dusk at 6 p.m and removed in the morning at 8 a.m for two consecutive days. All collected arthropods were sorted, morphologically identified using dichotomic keys [31] and placed in individual 1.5 ml Eppendorf tubes containing silica gel [32,33] and carded cotton. Once they arrived at the laboratory, the mosquitoes were dissected, the head, thorax and abdomen were placed in an Eppendorf tube for filarial research, the legs and wings in the original tube. *Culicoides* species were not dissected, they were identified morphologically using identification keys based primarily on the characters described in the identification keys [34,35].

DNA extraction

According to the manufacturer's instructions, samples were extracted using different protocols:

1. EZ1®DNA tissue kit (Qiagen, Hilden, Germany) for blood samples collected from elephantiasis patients, and mosquitoes and culicoides captured in patients' homes in 2021,

2. E.Z.N.A® tissue DNA kit (Omega Bio-Tek, Norcross, GA, USA), blood samples from febrile Mlomp subjects.

3. 2% cetyltrimethylammonium bromide (CTAB) method to extract DNA from blood deposits on nitrocellulose membrane from negative TDRs [36] and mosquitoes collected in 2007 and 2012 in Oussouye and Kedougou.

Molecular screening of filariae by real-time quantitative PCR

Real-time quantitative PCR (qPCR) assays were used to screen for the presence of filariae. The 28S gene (pan-filarial 28S qPCR system) was first targeted to screen for the presence of all filarial species [37].

Then, when the sample was positive, the internal transcribed spacer 1 (ITS) of *M. perstans* was specifically targeted [20] as well as cytochrome c oxidase I mitochondrial subunit I (COX1) (**Table 2**).

Table 2. Primers and probes used in this study.

PCR types	Target sequences	Name of Primers and Probes	Sequences (5'-3')	Ref
qPCR	ITS	M. p-F	5′-AGGATCATTAACGAGCTTCC-3′	[20]
		M. p-R	5′-CGAATATCACCGTTAATTCAGT-3′	
		M. p-P	6-FAM-TTCACTTTTATTTAGCAACATGCA-TAMRA	
	28S	qFil-28S-F	5'-TTGTTTGAGATTGCAGCCCA-3'	[37]
		qFil-28S-R	5'-GTTTCCATCTCAGCGGTTTC-3'	
		qFil-28S-P	6-FAM-CAAGTACCGTGAGGGAAAGT-TAMRA	
	COX1(nuclear copy)	Nfil - F	5'-TACAGCGAGTCAACCAGAGG-3'	(this study)
		Nfil - R	5'-TGAAGAATCGGCCAAAACAGG-3'	
		Nfil - P	6-FAM-AATATGCGGTCTGTTGCTGC- TAMRA	
Sequencing	ITS1	ITS1-F	5'-GGTGAACCTGCGGAAGGATC-3'	[17]
		ITS1-R	5'-CTCAATGCGTCTGCAATTCGC-3'	
	5S	5S-S2	5'-GTTAAGCAACGTTGGGCCTGG-3'	
		5S-S16	5'-TTGACAGATCGGACGAGATG-3'	
	COI	COIint-F	5'-TGATTGGTGGTTTTGGTAA-3'	[38]
		COIint-R	5'-ATAAGTACGAGTATCAATATC-3'	
	12S	Fwd-12S-Nem-1	5'-AAGTTTGATTTTGGTTTTGGTTG-3'	[37]
		1Rwd-12S-Nem-681	5'-CCATTGACGGATGGTTTGTA-3'	
		R-2198	5'-TASACTTCAGGGTGSCCRAARAAYCA-3'	

Amplification was performed using a CFX96 thermocycler (BioRad Laboratories, Foster City, CA, USA) in a final reaction volume of 20 µl, containing 10 µl of Master Mix Roche (Eurogentec, Seraing, Belgium), 0.5 µL of each primer at a concentration of 20 µM, 0.5 µL of the probe at a concentration of 5 µM, 0.5 µL of UDG, 3 µL of water DNase/RNase free and 5 µL of the DNA sample to be tested. The amplification program consisted of an initial cleaning step of 2 minutes at 50°C followed by a denaturation of 5 minutes at 95°C, then 40 denaturation cycles at 95°C for 5 seconds and a hybridization and elongation at 60°C for 30 seconds. For each series, negative (the mix) and positive controls were used. All samples positive for pan-filarial 28S qPCR system and for *M. perstans* and Nfil qPCR assays were sequenced to confirm.

Sequencing

Standard PCRs targeting COX1, ITS1, 5S and 12S genes using the primers listed in (Table 2) were performed using a thermal cycler (Applied Biosystems, 2720, Foster City, USA) and AmpliTaq Gold® 360 Master Mix (Applied Biosystems, Waltham, MA, USA). The amplification protocols consisted of a 15-minute incubation step at 95°C, 40 cycles (30 seconds at 95°C, 30 seconds at the hybridization temperature depending on the target gene, one minute at 72°C elongation and a final step of extension from 5 minutes to 72°C. Migration of the amplicons was carried out on a 2% agarose gel for 25 to 30 minutes at 180V. The bands were visualized under UV light. PCR products were purified using NucleoFast 96 PCR plates (Macherey Nagel, Hoerdt, France) according to the manufacturer's instructions. Then, they were sequenced using the Big Dye Terminator Cycle sequencing kit (Perkin Elmer Applied Biosystems, Foster City, CA, USA) with an automated ABI sequencer (Applied Biosystems). The resulting sequences were assembled and analyzed using Chromas Pro v.1.77 software (Technelysium Pty. Ltd, Tewantin, Australia) in order to compare them to the reference sequences of filarial species available on the National Center for Biotechnology Information (NCBI) online database (http://blast. ncbi.nlm.nih.gov) through a Basic Local Alignment Search Tool (BLAST) search. We have deduced evolutionary relationships between wireline types identified by phylogenetic analysis using the neighbor junction method by Molecular Evolutionary Genetics Analysis MEGA version 7 (https://www.megasoftware.net/).

Statistical analysis

In the absence of up-to-date data on the prevalence of *M. perstans*, the sample size for screening of RDT needed to estimate the prevalence of the disease in each site at 5% of its true value, and with a 95% confidence interval was determined by adopting a proportion (p) of

14.5% for the Kedougou area and 50% (maximum prevalence) for the other areas, to calculate sample sizes. Sample size (n) was calculated using the following formula with sample size to be determined (n), z = 1.96; interval width (2δ), last known prevalence 14.5%

$$n = (z1\text{-}\alpha/\delta)2\ p\ (1\text{-}\ p)$$

Data were entered into a Microsoft Excel (version 13) spreadsheet.

Results

Selected patients with elephantiasis

In 2021, according to information obtained from the head nurse, 13 villages were visited, 8 in Kolda and 5 in the department of Oussouye. Patients were selected and sampled according to their local presence, with 11 patients living in the Kolda region and 12 patients in the department of Oussouye. Capillary blood samples were collected twice (at day and at night) from 23 patients with symptoms of elephantiasis (**fig. 2**) living in the regions of Kolda (11) and Oussouye (12). In Kolda region, 9 patients had leg or arm lymphedema (right or left) swelling and 2 cases of hydrocele. In Oussouye, 8 patients had leg or arm lymphedema (right or left) swelling, 3 cases hydroceles and 1 case who had lymphedema of both legs and hydrocele. Patient ages ranged from 51 to 95 years. The male/female sex ratio was 1.3% (13/10). These patients complained of symptoms such as groin pain, muscle pain, pruritus, headaches, arthralgia, recurrent erysipelas and pain in the liver area and others. All 23 patients were sampled during the day between 9 and 10 a.m., while only 14 were sampled at night between 10 and 11 p.m.

Fig. 2 Patients with clinical manifestations of elephantiasis in the study areas (Kolda and Oussouye). Among the study participants, (**KL8**) Swollen left arm, (**KL 9,4**) lymphedema affecting one leg (right and left), and (**KL 11,12,5**) lymphedema of both legs.

At the Mlomp dispensary, 279 capillary blood samples were collected from acute febrile patients between 2018 - 2020. The sampling has been performed within the framework of diagnostic activities. A total of 3,714 malaria-negative RDTs were retrieved from health facilities in the study sites and analyzed according to the sample size calculations, including 762 (20.5%) in Kedougou, 762 (20.5%) in Kolda, 863 (23.3%) in Oussouye and 1,327 (35.7%) in Sedhiou. The detailed numbers of RDTs tested per health post and center are shown in **Table 3**.

Table 3. RDTs cassettes collected and prevalence by study area.

Study areas	Sanitary facilities	Number of RDTs collected	Number of RDTs tested	Pos qfil 28S (%)	Pos *M. perstans* (%)	Prevalence of *M. perstans* by area
Kedougou	Mako*	675	124	2 (1.6%)	1 (0.08%)	1.0%
	Ndiormi§	1676	307	5 (1.6%)	5 (1.6%)	
	Fadiga*	443	81	0	0	
	Dalaba*	580	106	0	0	
	Tomboronkoto*	786	144	0	0	
Kolda	Bagadadji*	922	252	1 (0.39%)	1 (0.39%)	0.1%
	Sare Bilaly*	299	81	0	0	
	Sare Kemo*	1,571	429	0	0	
Oussouye	Oussouye§	1,743	352	1 (0.28%)	1 (0.28%)	0.1%
	Carounate*	258	253	0	0	
	Louda Ouoloff*	55	55	0	0	
	Diakene Djola*	154	54	0	0	
	Mlomp*	145	149	0	0	
Sedhiou	Diana Malary*	1,492	300	1 (0.33%)	1 (0.33%)	0.2%
	Oudoucar*	1,614	238	1 (0.4%)	1 (0.4%)	
	Dembo Coly*	904	300	1 (0.33%)	1 (0.3%)	
	Djende*	354	300	0	0	
	Dianna Ba*	207	189	1 (0.5%)	0	
Total		**13,878**	**3,714**	**13 (0.35%)**	**11 (0.30%)**	

* Health post

§ Health center

Arthropods

Overall, 254 mosquitoes were collected and analyzed for filariae in 2021 in 11 villages (6 in Kolda region [Missira ousmane, Missira mamadou, Demba korbali, Sare sandiong,

Bagadadji and Missira kamarang], 3 in Oussouye region [Diakene diola, Niambalang and Kagnout] and 2 in Sedhiou region [Tambanaba, Badiary]. Using dichotomous keys [31], the following proportions were determined: 35% *Anopheles gambiae* s.l. (89/254), 5.1% *Anopheles funestus* (13/254), 1.1% *Anopheles rufipes* (3/254), 0.3% *Anopheles squamosus* (1/254), 1.1% *Culex* sp (3/254), 55.9% *Mansonia uniformis* (142/254) and 1.5% *Mansonia africana* (4/254). The same traps also captured 991 biting midges of *Culicoides* genus. Using the identification keys from Cornet et al, all midges were identified *C. fulvithorax*, *C. imicola*, *C. milnei*, *C. moreli*, *C. enderleini* and *C. oxystoma.*

In addition, 1,233 mosquitoes collected using CDC traps were selected for molecular analysis of filariae: Kedougou 36% (444/1233) were *Aedes* collected in 2007 and 64% (789/1233) represented *An. gambiae*, *An. ziemanni*, *Culex* sp, *An. rufipes*, *Mansonia* sp, *Aedes* sp, *An. squamosus*, *An. pharonsis* in Oussouye in 2012.

Molecular detection of filariae

Patients

A total of 37 blood samples were collected (23 daytime and 14 night-time) from the 23 elephantiasis patients and analyzed by qPCR. The pan-filarial 28S qPCR system yielded two positive results in different patient samples, a positive elephantiasis patient (day and right) in Kolda with both legs swollen and another patient who has hydrocele is positive only at night in Oussouye, representing an infection prevalence of 9% (2/23). These two samples were also positive for qPCR targeting the *M. perstans*-specific ITS gene (**Table 2**).

Of the 279 samples taken from febrile Mlomp patients, 16 were positive by pan-filarial 28S qPCR, a prevalence of 6%. Among these samples, 12 were positive to qPCR targeting ITS sequences specific to *M. perstans* and standard PCR targeting mitochondrial cox1 gene giving a prevalence of *M. perstans* of 4.3% (12/279). (**Table 2**). Other four samples (all with elevated ct) did not produce positive results neither with *M. perstans* qPCR nor with pan-filarial standard PCR targeting cox1 and were considered as negative.

Of 3,714 RDTs analyzed, 13 were positive for the 28S pan-filarial qPCR system, for an overall prevalence of 0.35%. Among them, 11 were also positive for qPCR targeting *M. perstans*, for a prevalence of 0.3% (11/3,714). Prevalence by regions were as follows: 1% (6/762) in Kedougou, 0.1% (1/762) in Kolda, 0.1% (1/863) in Oussouye and 0.2% (3/1327) in Sedhiou (**Table 3**).

Probable mitochondrial cytochrome oxidase copy of *M. perstans* in nuclear genome

Amplification targeting the cox 1 gene using primers COIintF (5′-TGATTGGTGGTTTTGGTAA-3') and COIintR (5′-ATAAGTACGAGTATCAATATC-3') was performed on *M. perstans* positive samples. Most samples produced double bands visualized during gel migration.

All bands obtained were re-sequenced individually. The heavy bands appear to be the *M. perstans* species and for the light bands, BLAST showed the similarity of 82-83% with different filarial species, including *Loa loa, Brugia timori, Brugia malayi* and *W. bancrofti* (**Fig. 3**). Based on the obtained sequence we have developed the specific qPCR; all samples positive for *M. perstans* were also positive for this second "neofilarial" cox1 filarial variant. Moreover, analyzing the 617 bps cox1 fragment alignments we have detected at least 4 single nucleotide deletions causing multiple stop codons.

These results suggested that the detected "neofilarial" DNA represent NUMT [39] gene, a non-functional copy of the mitochondrial genome present in the nuclear genome.

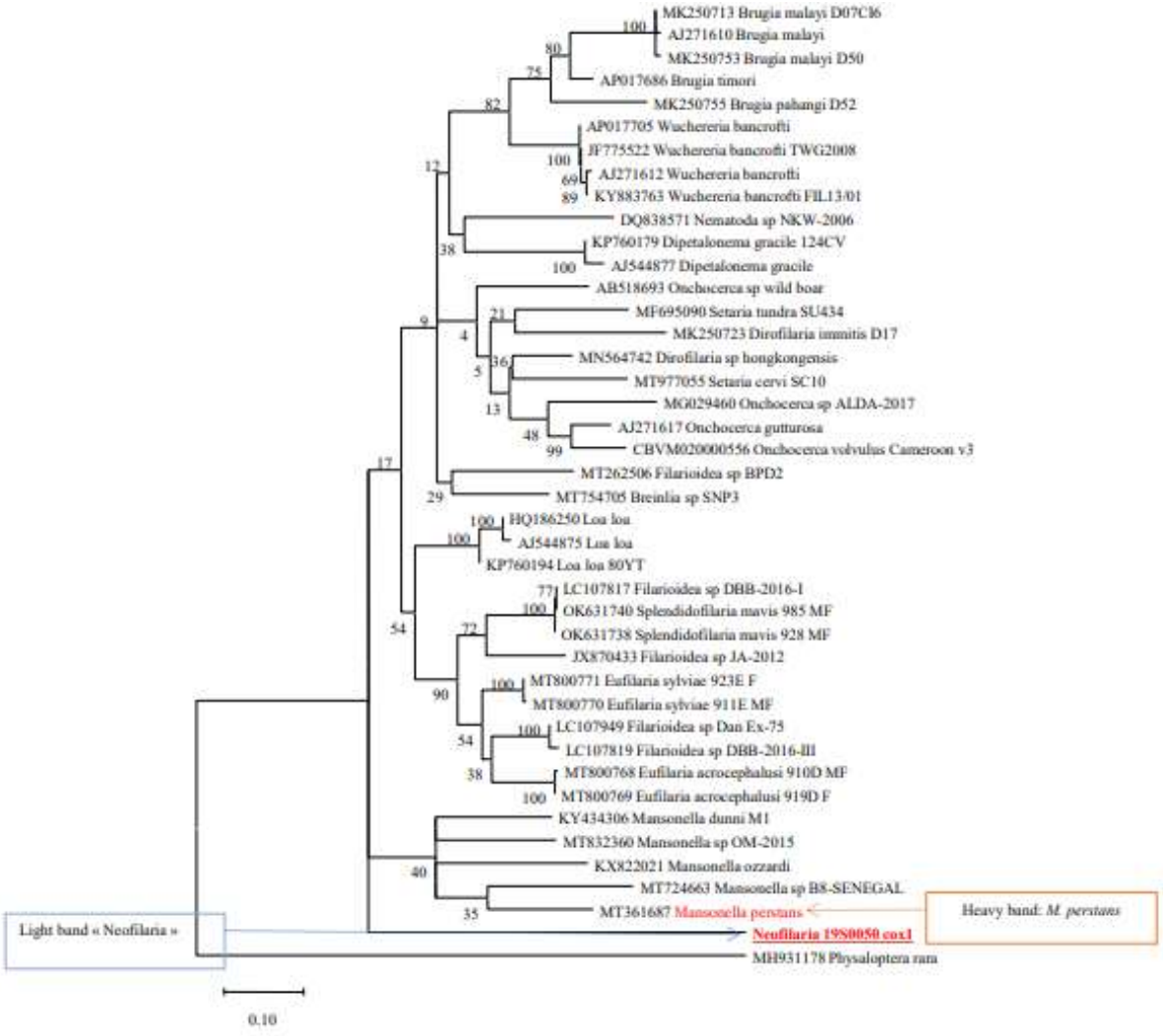


Fig. 3 Phylogenetic tree based on the functional mitochondrial and NUMT copies of cox1 sequences of *Mansonella perstans* identified in blood samples from febrile subjects and other filarial species. The sequences were aligned using ClustalW. The tree was deduced using the maximum likelihood method based on 100 bootstraps. The tree is drawn to scale, with branch lengths in the same units as the evolutionary distances used to infer the phylogenetic tree. Evolutionary distances were calculated using the Tamura-Nei method and are expressed in units of the number of base substitutions per site. Evolutionary analyses were performed using MEGA7.

Arthropods

Among 254 mosquitoes collected in 2021 in the bedrooms of elephantiasis patients we detected 5 (5/254, 2%) individuals positive with 28S pan-filarial qPCR system. All 5 specimens were identified as *Mansonia uniformis*, that resulted in the infection rate of *M. uniformis* at 3.5% (5/142).

5 specimens of *Aedes* sp. among 444 (1.1%) in Kedougou collected in 2007 and 8 mosquitoes including 5 *An. ziemanni*, 2 *Culex* sp. and 1 An. gambiae among 789 (1%) collected in Oussouye in 2012 were also positive in pan-filaria qPCR.

None of 991 *Culicoides* spp. collected in 2021 in patient's bedrooms was detected positive for *M. perstans* by specific qPCR.

Sequencing confirmation of filariae

Patients

The presence of *M. perstans* in the 2 elephantiasis patients were confirmed by sequencing the following genes ITS1 (443pb; PQ041263-PQ041267), COX1 (643pb; PQ097864-PQ097865) and 5S (379pb; PQ043272-PQ043273) that were identical among each other and shared 100% identity with different *M. perstans* samples in Genbank.

In febrile patients from Mlomp health dispensary, sequencing also confirmed the presence of *M. perstans* with 100% identity targeting the ITS1 (443pb; PQ041264) and 5S (379pb; PQ043274) genes. With regard to the RDTs, sequencing of the ITS1 gene confirmed the presence of *M. perstans* (PQ041650) in 9 samples with 100% identity (**Fig. 4**).

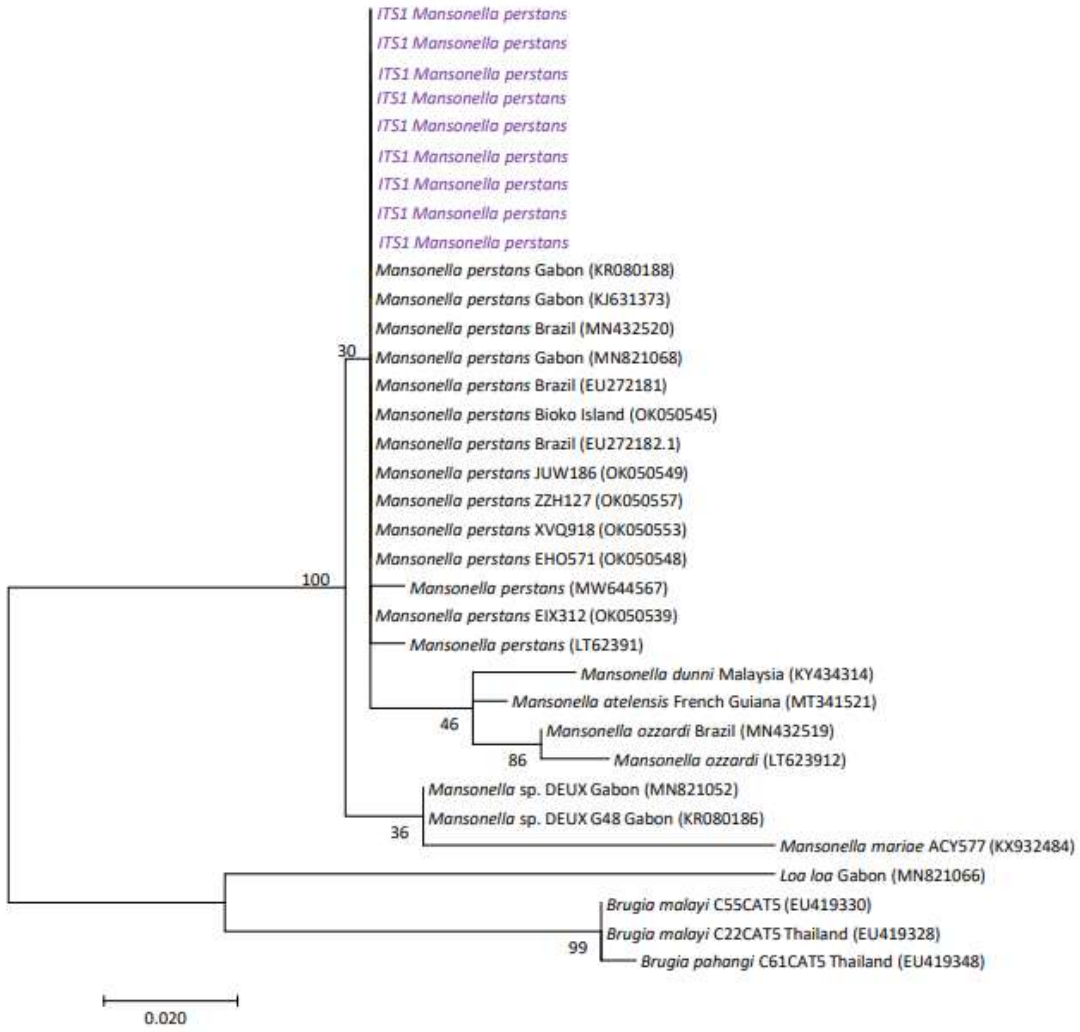


Fig. 4. Phylogenetic tree based on ITS1 sequence showing the clustering of the *Mansonella* identified in malaria-negative RDTs with other filarial species. Sequence alignment was performed using ClustalW. The tree was deduced using the maximum likelihood method based on 100 bootstraps. The tree is drawn to scale, with branch lengths in the same units as the evolutionary distances used to infer the phylogenetic tree. Evolutionary distances were calculated using the Tamura-Nei method and are expressed in units of the number of base substitutions per site. Evolutionary analyses were carried out using MEGA7.

Arthropods

We have amplified and sequenced a portion of filarial mitochondrial 12S from all mosquito samples positive by pan-filarial qPCR. Sanger sequencing of these fragments showed that different mosquito species harbored at least 5 different filarial species (Figure 5). *Setaria digitata* (PQ047250) and *Setaria labiapapillosa* (PQ047258) were identified in *Mansonia africana*, and *An. coustani*; *Onchocerca* sp. (PQ047260), *Onchocercidea* sp. (PQ047256) and

Filarioidea sp. (PQ047257) in *Culex* cf. *sitiens* and *Culex* sp. mosquitoes collected in 2021-2007 and 2012 (**Fig. 5**). All sequences obtained have been submitted to the GenBank database.

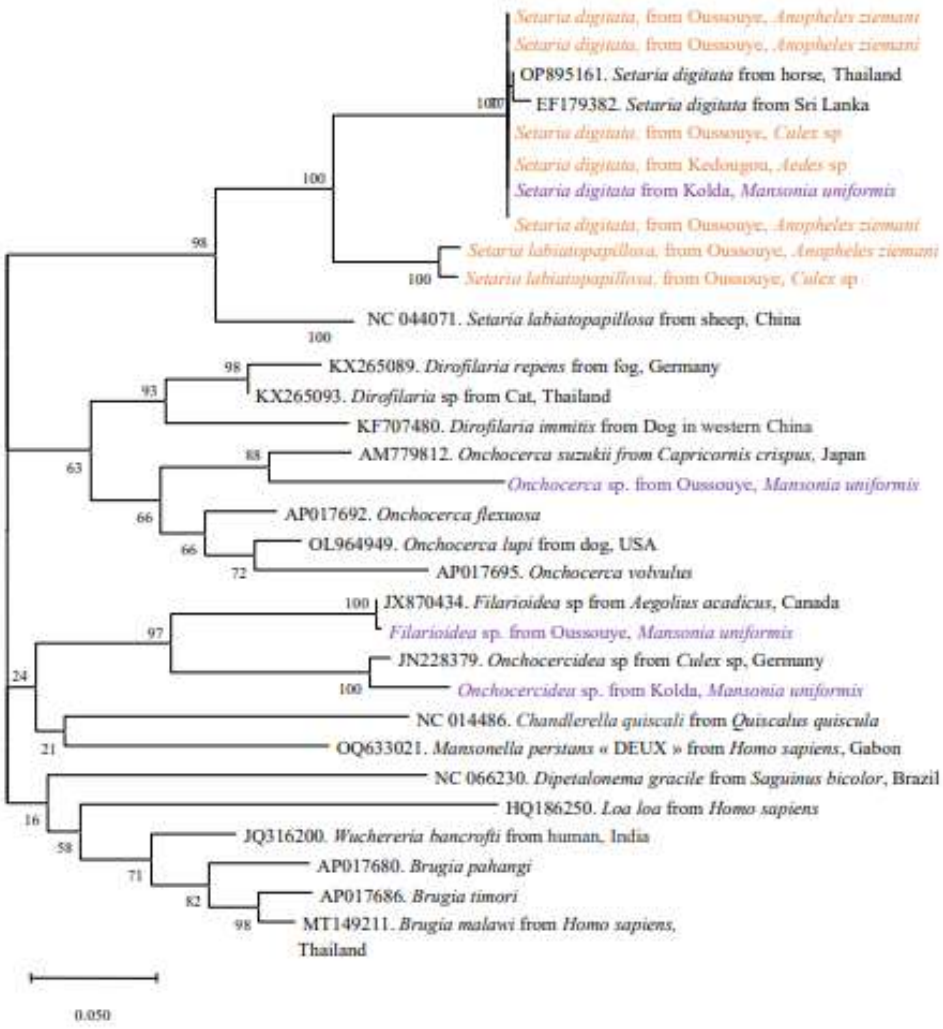


Fig. 5 Phylogenetic tree based on the 12S gene sequence of 31 specimens showing filarial species found in arthropods collected between 2021 - 2007 and in 2012. Sequence alignment was performed using ClustalW. The evolutionary history was inferred using the maximum likelihood method based on the Tamura-Nei model. Nodes are associated with bootstrap values based on 100 replicates. Bootstrap support values <70 is not shown. Initial trees for heuristic search were obtained automatically by applying the Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Composite Maximum Likelihood (CML) approach, then selecting the topology with the highest log-likelihood value. The tree is drawn to scale, with branch lengths measured as the number of substitutions per site. Evolutionary analyses were performed with MEGA7.

Discussion

Our study enabled us to detect *M. perstans* in patients suffering from elephantiasis and hydrocele in the Kolda and Oussouye zones in Senegal. Neither *W. bancrofti,* nor any other filarial parasite responsible for lymphatic filariasis were detected in patients with elephantiasis. Patients were sampled either once or twice (day and/or night, taking into account the circadian rhythms of certain filaria). In the majority of patients (21/23), no filarial parasites were identified in the blood.

It is known that in endemic areas the prevalence of lymphatic filaria in blood of the total population and even in elephantiasis patients may vary significantly. Often it is about 0.2-2% [40] but sometimes may be as high as 18.5% [41]. PCR is a very sensitive and specific test to detect filaria in blood samples; usually all cases positive in microscopy and by antigen tests are also positive in specific qPCR [42].

A possible explanation may be that the examined patients may be already treated by anti-filarial drugs. Indeed, the implementation of the 2016-2020 and 2022-2025 Strategic Plans to combat neglected tropical diseases (NTDs) in Senegal (NTD, 2016-2020) (NTD, 2022-2025), targeted lymphatic filariasis among other diseases [43,44].

However, in our cohort of patient, only two were treated by antifilarial drugs, others denied any treatment in the pass. Two patients positive for *M. perstans* also refused to receive any anti-filarial treatment. *M. perstans* infection is, however, known to be tenacious due to its insensitivity to filaricidal drugs [21].

Our results of the screening of febrile patients for filarial parasites were concordant. Neither in DNA extracted directly from patients, nor in DNA extracted from nitrocellulose strips from malaria RDT *W. bancrofti* DNA was not identified; the only filarial species identified was *M. perstans*. In febrile subjects from Mlomp *M. perstans* was detected in 4,3% of samples. A hydrocele patient with *M. perstans* microfilariae lived in several kilometers from the Mlomp dispensary.

Malaria-negative RDT cassettes were used to determine the prevalence of *M. perstans* in febrile patients with malaria symptoms in the different study areas. Overall prevalence was relatively low. In Kedougou, endemic region for *M. perstans* [20], we found 1% of RDT samples positive. The total prevalence of confirmed cases in RDT was 0.24%. However, these results may be impacted by several factors as small volume of the blood samples conserved in

nitrocellulose strip and unstable conditions and long terms of storage of RDT cassettes that may impact the DNA conservation.

Taking together, none of three sampling series (elephantiasis patients, febrile patients with DNA extracted in Mlomp dispensary laboratory and RDT collected from other dispensaries) detected filaria other than *M. perstans*.

W. bancrofti lymphatic filariasis in Senegal has been described only morphologically, without any genetic confirmation. Our data provide substrate for further research on the presence of Bancroftian filariasis in Senegal and probable role of *M. perstans* in elephantiasis. In Uganda, a similar study carried out between 2000 and 2003 on elephantiasis patients reported a low proportion (0.3%) of *W. bancrofti* and *M. perstans* co-infection in an area where the two parasites coexisted [11]. Both elephantiasis patients with confirmed *M. perstans* in our study were lder men between 76 and 79 years of age living in rural areas. These findings confirm previous studies [45–47].

Characterizing *M. perstans* positive samples we occasionally amplified and sequenced possible NUMT of cox1 gene. All samples positive for *M. perstans* were positive for this unusual "neofilarial" cox1 gene by specific qPCR, and the gene itself was not functional containing many deletions and stop-codons. The NUMTs in *M. ozzardi* were already reported [48] and in *M. perstans* were recently identified during sequencing of a whole genome of *M. perstans* from Cameroon [49]. Interesting that in case of Mansonella, the NUMTs are poorly conserved and present a week identity with functional mitochondrial genes in 86-87% [49].

We tried to identify the vectors of filarial parasites in Senegal. None of those tested were positive for *W. bancrofti* or *M. perstans*. Previously, the vector had not been detected in *Culicoides* biting midges in rural Senegal (Kedougou region). In our study we also tested biting midges collected in endemic regions for *M. perstans*; we tested also mosquitoes trying to identify another vector. We have identified three undescribed filarial genotypes that have never been identified in humans. One is obviously grouped within *Onchocerca* genus two others are identified as *Filarioidea* sp and *Onchocercidea* sp. Our genotypes are close to those previously identified in domestic cattle of the "Ndama" breed in the Guinean savannah regions of Kedougou, Senegal [50]. Other filaria identified in mosquitoes, *S. digitata* and *S. labiapapillosa* are mosquito-borne filaria found in the abdominal cavity of cattle [51]. *S. digitata* and *S. labiopapillosa* have been detected in *An. coustani* and *Mansonia africana*, an exophilic and exophagous mosquito [52]. This species prefers to bite cattle, sheep and poultry. Females are

very aggressive and can bite during the day [53]. *M. africana*, a mosquito found in swampy areas that bites outdoors at night, although some are endophilic and behave exophilically after a blood meal. Like other zoonotic vectors, *Culex sitiens* [54] is an important coastal vector of many mosquito-borne diseases [55], including Japanese encephalitis [56], found in mangroves, salt marshes and crab holes. It is also a species that bites humans at all hours, but especially at dusk and at night.

Conclusion

Overall, for the first time in Senegal, *M. perstans* was found in elephantiasis patients in the absence of "classical" lymphatic filaria. Screening of blood samples (based on DNA extracted from malaria RDT and from febrile patients in dispensary) documented wide distribution of *M. perstans* and virtual absence of other human pathogenic filaria. Our data suggest that *M. perstans* mansonellosis, although asymptomatic, remains endemic in the southern regions of Senegal. A cost-effective approach to continue studies of filariases may be to reuse *P. falciparum* RDTs as a diagnostic tool for monitoring prevalence, parasite density and other blood-borne diseases. The role of *M. perstans* a causative agent of elephantiasis should be further examined.

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ANNEXES

Publication en collaboration

ARTICLE 3

Epidemiology of *Mansonella perstans* in Mali

(Soumis dans **Parasitology & Vector-Borne Diseases**)

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Epidemiology of *Mansonella perstans* in Mali

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Abstract

Introduction: Mansonellosis remains one of the most neglected tropical diseases caused by filarial nematodes of the genus *Mansonella*, whose different species are: *Mansonella perstans, Mansonella ozzardi* and *Mansonella streptocerca*. *Mansonella perstans* is the most common species in Mali. The aim of our study was to use real-time PCR (RT-PCR) to detect *Mansonella perstans* parasites in febrile patients in Mali, using malaria rapid diagnostic tests (RDTs).

Methods: From June to December 2021, negative and positive RDTs were collected from 41 sites in the nine regions of Mali. Molecular tools were used to detect and identify the presence of pan-filaria at the base of 28S in DNA samples extracted from the RDT cassette tape. Positive samples were tested with a (RT-PCR) specific for *Mansonella perstans*.Standard polymerase chain reaction (PCR) molecular analysis of targeting the DNA internal transcribed spacer 1 (ITS1) gene and phylogenetic analysis were performed.

Results*: Mansonella Perstans* DNA was detected in 1.33% of malaria-negative RDTs (2 out of 150 randomly selected), collected in the region of Koulikoro.

Conclusion: Result show the presence of *M. perstans,* in febrile patients using negative malaria RDTs although this infection due to a vector-borne filarial nematode is sufficiently neglected in Mali, and the need to further strengthen surveillance of mansonellosis caused by *M. perstans.*

Keywords: *Mansonella Perstan*; RDT; Mansonellosis; qPCR; Mali

Introduction

Mansonella perstans infection is a little-known parasitic disease caused by a filarial nematode transmitted to humans by the bites of tiny blood-sucking flies of the Culicoides genus. It commonly known as biting midges. These insects are tiny flies that feed on the blood of their hosts, enabling the transmission of filarial parasites. Mansonella perstans is widespread in several regions of sub-Saharan Africa, where it poses an often-underestimated public health issue. It is also found in some parts of Central and South America, although its geographical distribution there is more limited (Simonsen, Onapa, et Asio 2011b).

Three main species are responsible for mansonellosis in humans: *M. perstans, M. streptocerca* and *M. ozzardi* (E. R. Bregani, Tantardini, et Rovellini 2007; B. L. Downes et Jacobsen 2010). *M. perstans* and *M. streptocerca* are mainly found in west, east and central Africa, where they often coexist with other parasitic infections. These regions are characterized by environmental conditions that favor the proliferation of biting midges, thereby increasing the risk of transmission. when, *M. ozzardi* is mainly found in Latin America, with an uneven geographical distribution. It is endemic in certain regions Central and South America, as on several Caribbean islands [3]. In addition to *Mansonella perstans*, a potential new species of *Mansonell*a sp "TWO" was recently identified in febrile children in Gabon (Rodi et al. 2023b). A large part of sub-Saharan Africa is affected by *M. perstans*, it extends from Senegal to Angola, through central Africa, north to southwest Sudan, east to Uganda, Kenya, Tanzania, and south to Zimbabwe (Agbolade et al. 2006). In the most affected countries, the species *M. perstans* mainly prevails in poor rural populations (Agbolade et al. 2006; Wanji et al. 2003). It is important to note that more than 100 million people are currently infected in Africa alone and approximately 600 million people live in 33 countries at high risk of *M. perstans* infection (Simonsen, Onapa, et Asio 2011b).

The essential data relating to mansonellosis and geographical distribution are based on the morphological characterization of microfilariae collected from blood samples by thick film or blood smear (Simonsen, Onapa, et Asio 2011b). Adult worms appear to live in various cavities of the body (pleural, peritoneal or pericardial, etc.) and the mesentery (El Setouhy et al. 2004), while the microfilariae produced by females circulate in the peripheral blood or the skin without any periodicity (*World Health Organization. Bench Aids for the Diagnosis of Filarial Infection. Geneves: 1997.Https://Iris.Who.Int/Handle/10665/37156 Consulté Le 2024-07-11*, s. d.). How long mature *M. perstans* worms survive in the human body remains unclear,

while their larval form, microfilariae, persist for approximately 4 months (Santa Maria Asio, Simonsen, et Onapa 2009a).

There are similarities in the life cycle of the three species of *Mansonella* parasites responsible for mansonellosis in humans and they vary in terms of biological, clinical and epidemiological characteristics (Simonsen, Onapa, et Asio 2011b; B. L. Downes et Jacobsen 2010; Mediannikov et Ranque 2018b). S1 Table summarizes the biological characteristics of these three filariasis (Simonsen, Onapa, et Asio 2011b; Mediannikov et Ranque 2018b).

Currently, it is recognized that *M. perstans* has mild pathogenic effects and mainly manifests asymptomatically. Eosinophilia is a common feature (Mediannikov et Ranque 2018b). However, in *M. perstans* certain clinical manifestations could be associated with the migration of worms and in particular allergic reactions such as "Calabar edema", pruritus, fever, headache, pericarditis and pleurisy (Simonsen, Onapa, et Asio 2011b). No duration between infection and the manifestation of microfilariae in peripheral blood has yet been proven [5]. Detection of microfilariae in blood smears helps confirm the diagnosis of *M. perstans* (Adolph, Kagan, et McQuay 1962; El Setouhy et al. 2004). One of the difficulties in the management of mansonellosis is the absence of a known standard treatment [5].

In a study carried out in a rural area of Senegal, the overall prevalence of *M. perstans* was 14.5% in febrile and non-febrile patients. It increases with age moreover this figure could reach up to 40% to 50% in rural areas (Bassene et al. 2015b; Santa Maria Asio, Simonsen, et Onapa 2009b). *Mansonella perstans* is the species involved in Mali, well known since 1912 (Keita 1979).

Several studies on mansonellosis caused by *M. perstans* in Mali attract our attention in particular: Thiroux had found a prevalence rate of 46% of *M. perstans* among soldiers recruited in French Sudan (present-day Mali) and that of leger (12.4%) of *M. perstans* in the capital Bamako(Keita 1979). Similarly, Rougement in 1979 found in Masantola in the circle of Kolokani a high prevalence of (65%) then in Kadiolo in February 1978, (central district), a team from the epidemiology laboratory of parasitic infections of the faculty of medicine and dentistry stomatology (FMOS) estimated that the prevalence of *M. perstans* among schoolchildren aged 6 to 17 was 6.7%(Keita 1979). In the village of Lofigué in Kadiolo *M. perstans* was 28.45%. In the Kolokani circle (Central District) in April 1978, out of 565 daytime thick blood films carried out among the populations of 10 villages, the prevalence of *M. perstans* was between 5.5% and 78% (Yaya I. Coulibaly et al. 2009b; Keiser et al. 2003; 2008). Since then, no recent information has been available on this disease in Mali. Cases of non-malarial fever may be

attributed to other parasitoses. It is necessary to estimate the prevalence in different regions and transmission zones of Masantola.

The objective of this study was to assess the prevalence of this neglected parasite in febrile patients using nucleic acids (DNA) extracted from positive and negative malaria rapid diagnostic tests collected in different regions of Mali.

Materials and Methods

Study sites

Mali is a landlocked country in West Africa located between the 10- and 25-degree north latitudes and between the 4- and 12-degree west longitudes. It covers a 1,241,238 km2 area. There are 3 climatic zones in Mali that extend from south to north: the Sudano-Guinean zone, which covers 25% of the territory and has a rainfall of approximately 1300 to 1500 mm per year; the Sahelian zone, which covers 50% of the territory and receives rainfall of 200 to 800 mm per year; and the Saharan desert zone, which represents 25% of the territory. This zone is marked by irregular rainfall, often less than 200 mm per year. For this study, we collected RDT cassettes from 41 sites in the following regions of Mali: Kayes, Koulikoro, Sikasso, Ségou, Mopti, Timbuktu, Gao, Kidal and Menaka, from June to December 2021. No sample was collected in the Taoudeni region or the capital city Bamako.

Ethical Considerations

Authorization to use malaria RDTs cassettes for research purposes was obtained from the competent authority of the National Malaria Control Program (NMCP) under number 064/MSDS-SG/NMCP, February 8, 2023), in accordance with Malian regulations on ethics and medical research.

Molecular analysis

DNA extraction from RDTs cassettes

A total of 1496 RDTs cassettes was used for genomic DNA extraction as a substrate to detect the filaria parasites including *M. perstans*. The RDTs cassettes were previously used to detect *Plasmodium falciparum* (*P.f*) (Dembélé et al. 2024). DNA was extracted using the EZ1 DNA Blood Kit (Qiagen) on an EZ1 BioRobot extraction device (Qiagen, Hilden, Germany) according the manufacturer's instructions and as described by Dembélé et al. (Dembélé et al. 2024).

Real-time PCR (RT-PCR) for detection of *M. perstans*

Real-time PCR was used to detect all filaria species and *M. perstans* respectively, in all DNA extracts from RDTs cassettes (n= 1496). The RT-PCR reaction targets the 28S region for all filaria species and another specific region to detect *M. perstans*. The primers used pan-filaria 28S (qFil_F and qFil_R and probe) (Table 1), targeting all filaria species were identical to those initially described by Laidoudi et al. (Laidoudi et al. 2020). All Pan-filaria 28S positive samples were then tested with a specific *M. perstans* system using the primers and probes previously described by Bassene et al (Bassene et al. 2015b) (**Table 1**).

The RT-PCR reactions were performed using a CFX96 thermal cycler (Bio-Rad, Marnes-la-Coquette, France) in a 20 µL reaction mixture containing 5 µL of DNA,10 µL of the Master Mix, 3.5 µL of sterile distilled water, and 0.5 µL of each of the primers and 0.5 µL of the TaqMan probe (Applied Biosystems, Foster City, CA, USA). Our amplification program consisted of an initial 2-min denaturation step at 50 °C followed by a 3-min denaturation at 95 °C, then 40 cycles of 95 °C for 30 s and 60 °C for 1 min before holding the sample at 4 °C. For each RT-PCR run, negative (sterile distilled water) and positive controls (*Dirofilaria immitis* DNA was used for pan-filarial RT-PCR and a plasmid developed in our laboratory was used for *M. perstans* RT-PCR) were used.

173 **Table 1.** Primers used in the real-time PCR, Pan-filaria and *Mansonella perstans* parasite and *Mansonella* spp. sequencing protocols.

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Primer names	Marker	Target organism	Primer sequences (5'-3')	Authors
Real-time PCR				
qFil_F	28S	Pan-filariae	TTGTTTGAGATTGCAGCCCA	Laidoudi et al (Laidoudi et al. 2020)Laidoudi et al (Laidoudi et al. 2020)
qFil_R			GTTTCCATCTCAGCGGTTTC	
Probe (FAM 5'–3'TAMRA)			CAAGTACCGTGAGGGAAAGT	
Mp_Hub_F	ITS	*M. perstans*	AGGATCATTAACGAGCTTCC	Bassene et al (Bassene et al. 2015b)Bassene et al (Bassene et al. 2015b)
Mp_Hub_R			CGA ATATCACCGTTAATTCAGT	
Probe (FAM 5'–3'TAMRA)			TTCACTTTTATTTAGCAACATGCA	
Standard PCR and sequencing				
ITS1_F	ITS1	*Mansonella* spp.	GGTGAACCTGCGGAAGGATC	Mourembou et al (Mourembou et al. 2015b)Mourembou et al (Mourembou et al. 2015b)
ITS1_R			CTCAATGCGTCTGCAATTCGC	

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DNA-based sequencing

Only RT-PCR positive samples for both genes were subjected to PCR sequencing using Internal transcribed spacer 1 (ITS 1) region to identify *Mansonella* parasites. PCR reaction targeting a 240 bp region of ITS1 was performed in a thermal cycler (Applied Biosystems, 2720, Foster City, United States) with AmpliTaq Gold 360 PCR master mix (Applied Biosystems, Waltham, United States). The ITS region was amplified using primers forward ITS1_F and reverse ITS1_R Mourembou et al (Mourembou et al. 2015b) (**Table 1**). PCR was performed in a reaction mixture consisting of 5 µL of extracted DNA, 12.5 µL of AmpliTaq Gold 360 PCR master mix (Applied Biosystems, Waltham, MA, USA), 0.75 µL of each primer, and 6 µL of sterile distilled water, in a final reaction volume of 25 µL. The amplification program consisted in an initial denaturation at 95˚C for 15 min, followed by 40 cycles at 95˚C for 30 s, at 58˚C for 30 s, 72˚C for 1 min and a final step at 72˚C for 5 min.

The amplified products were purified using a Macherey Nagel plate (NucleoFast 96 PCR, Düren, Germany). Sanger sequencing was performed using the same primers as for PCR with BigDye Terminator v1.1, v3.15× sequencing buffer (Applied Biosystems, Warrington, UK) and run on an ABI 3100 automated sequencer (Applied Biosystems). The sequences obtained were assembled with ChromasPro version 1.7.7 software (Technelyium Pty. Ltd, Tewantin, Australia) and aligned with BioEdit software (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) using the ClustalW algorithm to perform multiple alignments of our sequences. The corrected sequences were compared to existing sequences in the online database of the National Centre for Biotechnology Information (NCBI) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) using basic local alignment search tool (BLAST) analysis (Chenna et al. 2003). Phylogenetic tree (**Figure 1**) was constructed using the maximum likelihood (ML) statistical method with substitution model selection determined by the Molecular Evolutionary Genetics Analysis (MEGA) v7.0.26 software (Tamura K, 1993). Statistical support for the inner branches of the trees was assessed by bootstrapping with 1,000 iterations.

Results

Molecular characterization of filarial species and *M. perstans*

A total of 1,496 malaria RDTs cassettes were collected in Mali, of which 260 were *P. falciparum* positive and 1,236 negatives in febrile patients. All RDT cassettes were tested with the Pan-filaria 28S and *M. perstans* systems specifically. Of these samples, only 2/1496 (0.1%)

were positive for both Pan-filaria 28S RT-PCR and *M. perstans* (S1 fig). *M. perstans* species were only detected on TDRs collected in the Koulikoro region.

S1 Figure. Amplification curves for real-time PCR on CFX96 targeting the qFil 28S gene of *M. perstans* species in Koulikoro (T+: positive control and the two positive samples detected in the Kangaba health district (Koulikoro region).

DNA sequence-based identification of *Mansonella* parasites

Sequencing identification was carried out on the RT-PCR positive samples of *M. perstans* (n= 2) using the ITS1 region. The sequences obtained were subjected to BLAST analysis against reference sequences from the GenBank database (GB). BLAST analysis indicated that our M. perstans sequences matched 100.00% of their homologous sequences (GB accession number: PQ041650 and MN821068) described in Senegal and Gabon respectively and available in GenBank.

Our sequences have been deposited in the GB database under accession numbers XX and XX.

Phylogenetic Tree based ITS was constructed using our *Mansonella perstan*s sequences in addition to other sequences downloaded from GenBank. The tree revealed that our sequences are close to those already found in Senegal, Brazil and Gabon and that they form the same clade. The phylogenetic tree also showed interspecific discrimination (**Figure 1**).

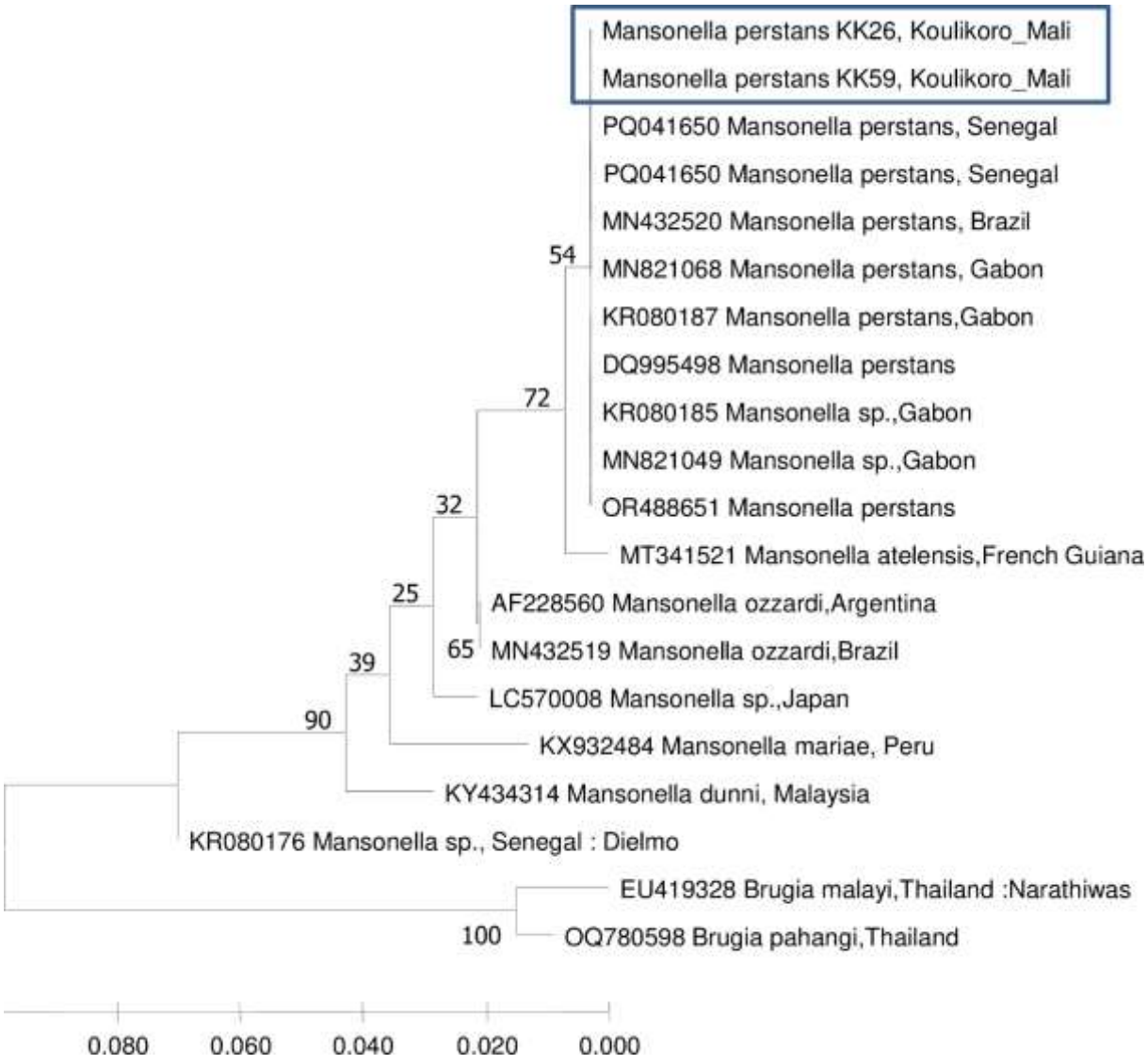

Figure 1. The tree is based on the nucleotide sequence of the ITS region, constructed using the maximum likelihood method with MEGA 7. Branch values are bootstrap support values based on 1000 replicates.

Discussion

Studies aimed at detecting and identifying filarial parasites including *M. perstans* from DNA extracted from malaria RDT cassettes collected in 9 regions of Mali.

Our findings demonstrate for the first time the presence of *M. perstans* Mansonellosis in Mali by using innovative approaches to detect the presence of the parasite from *P.f.* malaria RDT cassettes in febrile patients attending health facilities in the Koulikoro region.

Several studies on mansonellosis caused by *M. perstans* in Mali have been carried out in the south and north of the country in particular, surveys carried out by the FMOS Laboratory of Epidemiology of Parasitic Affections in the cercle of Kadiolo (Central Arrondissement, in February 1978) showed that, out of 782 thick diurnal drops in schoolchildren aged 6 to 17, the prevalence of *M. perstans* was around 6.7%, including 3.9% in children aged 7 to 10 and 10.9% in children aged 11 to 17 (Keita 1979) *perstans* was around 6.7%, including 3.9% in children aged 7 to 10 and 10.9% in children aged 11 to 17. Also, out of 123 thick nocturnal drops taken from adults in the village of Lofigué, the prevalence of *M. perstans* was 28.45%, with *M. perstans* + *W. bancrofti* associations accounting for 8.13% (Keita 1979).

In the cercle de Kolokani (Arrondissement Central, April 1978), *M. perstans* prevalence ranged from 5.5% to 78% in 565 thick diurnal drops from 10 villages (Keita 1979). In Gourma (April - May 1979), out of 608 thick diurnal drops made among nomadic populations north of parallel 15º30'N on either side of the 300 mm isohyet, none showed *M. perstans* microfilaria (Keita 1979).These results were obtained by microscopy. Then into 2003 , Paul B Keiser et al ont reported a prevalence of 75% *M. perstans* in Mali (Keiser et al. 2003).

Although *M. perstans* infection is one of the well-known human filariasis in Mali, it must be noted that very few studies are still carried out on its epidemiology and its negative effects on the health of endemic populations.

We identified by real-time PCR, *M. perstans* in two DNA samples extracted from negative and positive *plasmodium falciparum* malaria (RDTs) in febrile patients, which represents a prevalence of 0.1% (2/1496). The low prevalence obtained in our results could probably be explained by the positive effects of the molecules used (albendazole and ivermectin) during mass drug distribution campaigns against neglected tropical diseases, a strategy considered effective and which is recommended by the World Health Organization (WHO) to reduce the burden of neglected tropical diseases in the community, interrupt

transmission towards their elimination and control by 2030 (« OMS MALI Rapport annuel 2018.pdf », s. d.). For example, during the mass treatment campaign against neglected tropical diseases with preventive chemotherapy in 2018, that is to say 3 years before the collection of the malaria RDT cassettes, the therapeutic coverage (Ivermectin + Albendazole) and Ivermectin in a single dose were 86.34% and 96.84% respectively (« OMS MALI Rapport annuel 2018.pdf », s. d.). Ivermectin distributed during mass campaigns against lymphatic filariasis and onchocerciasis may affect prevalence of *Mansonella perstans* species (Ta-Tang et al. 2021a).

Contrary to our results, in Senegal the overall prevalence of *M. perstans* in a rural area of Senegal was 14.5% (29/201) in febrile and non-febrile patients (Bassene et al. 2015b). In a previous study on the comparison of *M. perstans* detection techniques, the authors showed that qPCR could be successfully used to identify microfilariae in the samples used (Bassene et al. 2015b). It was demonstrated in a study conducted in two endemic communities in Uganda that the prevalence of *M. perstans* filariasis increased rapidly with age, reaching 89.2% and 81.4% in the age group of 15 at 19 years old. The overall prevalence of microfilariae (mf) was significantly higher in Mukono (76.5%) than in Luwero (57.7%). From the age of 1 to 4 years, 40.6% and 20.5% of children were positive for microfilariae (Santa Maria Asio, Simonsen, et Onapa 2009b).

Several studies of co-infection have sometimes been reported in some endemic countries. Consequently, it is appropriate to put in place adequate diagnostic tools whose sensitivity and specificity are indisputable in order to remove all ambiguities in the treatment of neglected filariasis. The study by Tatuene et al in the Akonolinga health district located in central Cameroon corroborates that the prevalence of *M. perstans* infestation was relatively high 12.4% and that of Loa co-infection. loa – *M. perstans* was 5.1% (Tatuene et al. 2014b). Furthermore, a similar study carried out in 16 isolated villages in southern Cameroon revealed that onchocerciasis and mansonellosis were highly endemic in all villages surveyed with prevalence ranging from 28.44% to 87.17% for O. volvulus and from 52.48% to 100% for *M. perstans* [9].

The fight and eradication of filarial infections in humans requires the implementation of prevention strategies, appropriate treatment and reinforced surveillance of the disease.

Limitations

In view of the interesting results obtained in this filarial endemic region of Mali, it is important to note that the conservation of RDT cassettes, and the lack of knowledge of patient demographics and clinical symptoms could be potential limitations of our approach.

Conclusion

For the first time, the use the malaria RDT cassettes enabled molecular screening of filarial species including *M. perstans* and revealed a low infestation rate in Mali located in the south. Further molecular and genetic studies of this little-known human filariasis are needed to properly assess its importance in terms of public health, and to decipher the molecular interactions between the different species of filarial parasites with a view to strengthening mansonellosis control strategies in Mali.

Author contributions: P.D., MD., M.A.T. and S.R. designed the study and wrote the first version of the manuscript.

P.D., M.D., P.M.G., and M.C., analyzed and interpreted the results.

M.D., P.M.G., M.A.T. and S.R. Contributed to the critical revision of the manuscript. All authors read and approved the final version.

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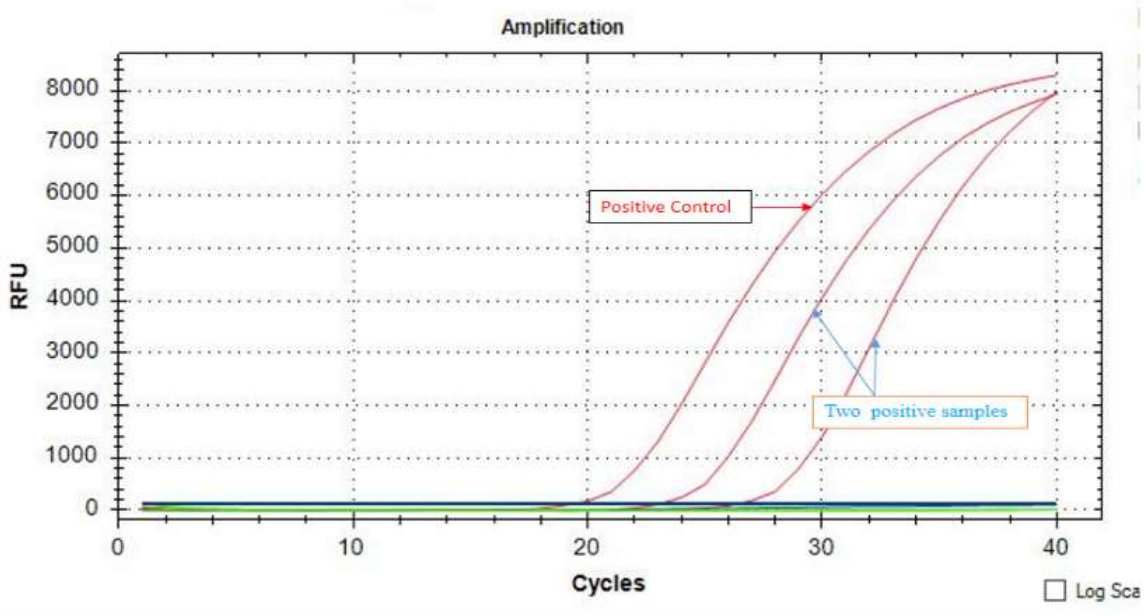
Conflicts of interest:

The authors declare no conflict of interest.

S1 Table. Main characteristics of human mansonellosis.

Characteristic	*Mansonella streptocerca*	*Mansonella ozzardi*	*Mansonella perstans*
Endemic areas	West and Central Africa	Central and South America, Caribbean	Sub-Saharan and Northern Africa, South America
Arthropod vectors	*Culicoïdes* spp.	*Culicoïdes* spp; Simulium amazonicum	*Culicoïdes* spp.
Location of adult worms	Dermis of the trunk and upper shoulder girdle	Lymphatic vessels, pericardium, pleura and peritoneum	Pericardium, pleura and peritoneum; mesentery and retroperitoneum
Location of microfilariae	Skin	Blood and skin	Blood



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CONCLUSIONS ET PERSPECTIVES

Notre projet de thèse, dans son ensemble nous a permis d'approfondir nos connaissances sur l'épidémiologie, la transmission de la filariose au Sénégal et la recherche du vecteur impliqués dans la transmission de la maladie. Nous avons scindé nos travaux en trois parties en relation avec les aspects cités précédemment.

Dans la première partie de cette thèse, nous avons réalisé une revue de la littérature qui se concentre sur les filarioses en Afrique de l'Ouest. Nous avons développé les différentes filarioses en Afrique de l'Ouest, leur épidémiologie, leurs pathogénicité, les méthodes de diagnostic le traitement et les moyens de prévention ciblant les arthropodes vecteurs. Ces données disponibles montrent que la filariose humaine est largement répandue en Afrique de l'Ouest. La mise en évidence des filaires dans leur rôle de transmission, ainsi que la détection du parasite adulte ou au stade de microfilaire, sont des éléments clés pour mieux comprendre l'infection. Cependant un diagnostic fiable est nécessaire pour discriminer les principales filaires permettant d'affirmer l'infection avec certitude. D'où, l'utilisation des différentes méthodes de détection des parasites chez les hôtes intermédiaires et définitifs qui revêtent une importance cruciale pour optimiser les stratégies de lutte contre les maladies parasitaires.

Dans la seconde partie de ce travail, nous avons porté notre attention sur la détection des filaires chez les patients, à partir des cassettes TDR du paludisme négatif et chez les arthropodes vecteurs. Nous avons utilisé un outil moléculaire qui a mis en évidence l'espèces *M. perstans* dans les prélèvements sanguins chez les patients atteints d'éléphantiasis et les sujets fébriles. Cette même espèce a été détecté dans les cassettes TDR paludisme négatifs à *Plasmodium falciparum* qui constituent de bonnes sources d'ADN filarien pour la détection des infections filariennes.

Nos résultats confirment la transmission de la mansonellose à *M. perstans* au Sénégal. Cette étude démontre, pour la première fois au Sénégal, la nécessité de réutiliser les TDR négatifs, pour une meilleure stratégie de surveillance de la transmission et définir son épidémiologie. Tous les moustiques et culicoïdes testés aucun n'est responsable de la transmission de la filariose.

Cette étude suggère des recherches plus approfondies sur les vecteurs de la filariose au Sénégal. Bien que la PCR en temps réel et le séquençage permettent d'identifier la présence de *M. perstans*, il est recommandé de développer des tests plus rapides, accessibles et spécifiques

pour une utilisation de routine dans les structures sanitaires en zone rurale. De plus, Étant donné que *Mansonella perstans* a montré une certaine insensibilité aux traitements antifilaricides courants tels que la diéthylcarbamazine et l'ivermectine, il est crucial de poursuivre des recherches pour identifier des alternatives thérapeutiques efficaces et adaptées.

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