UNIVERSITÄTSKLINIKUM HAMBURG-EPPENDORF

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Treatment and diagnosis of *Loa loa* and *Schistosoma haematobium* infections in Gabon

Dissertation

zur Erlangung des Grades Doktorgrades PhD

an der Medizinischen Fakultät der Universität Hamburg.

vorgelegt von:

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Hamburg 2023

Angenommen von der Medizinischen Fakultät am: 04.06.2024

Veröffentlicht mit Genehmigung der Medizinischen Fakultät der Universität Hamburg.

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1. Rationale for the workplan

Infectious diseases remain the main health burden for many rural communities in sub-Sahara Africa. Whereas malaria, HIV/AIDS and tuberculosis benefit from a high level of investment for the development of new diagnostic and therapeutic tools, other infectious diseases are more out of focus of the international community. Urogenital schistosomiasis is a vector borne helminth infection, caused by *Schistosoma haematobium*, that shows high prevalence in affected communities in Gabon. Due to the absence of active control program in Gabon, there is a large unmet need for improved management. This relies on the development and evaluation of new tools to diagnose, treat, and prevent schistosomiasis.

Loiasis is a filarial infection caused by *Loa loa*, endemic in rural communities residing in the rainforest regions of West and Central Africa. Up to 75% of adult residents are chronically infected and suffer from loiasis related morbidity and excess mortality. Yet there is no appropriate treatment available and no control programs to control transmission are implemented in any of the endemic countries of central or west Africa.

In the present PhD project, we therefore focussed on two important aspects of these neglected fields of biomedical research. Firstly, the thesis focuses on the treatment of schistosomiasis using conventional antimalarial combination therapies commonly used for the treatment of malaria. Malaria and schistosomiasis are known to be co-endemic in many regions in Gabon. Whereas malaria treatment is readily available and children are treated for acute disease several times per year, treatment of the co-endemic schistosomiasis is rarely performed. It is known from previous work that certain antimalarial drugs may exert a therapeutic effect on schistosomiasis, however this has not been evaluated systematically for new antimalarial combination therapies (ACTs). Therefore, we set out to assess the eventual added benefit of the use of ACTs when used for the treatment of malaria on concurrent schistosomiasis in the local population.

Secondly, we focused on the diagnosis and treatment of loiasis. The epidemiology of loiasis and its burden have recently been described in two areas of Gabon; Moyen-Ogooue and Ngounie provinces indicating the high prevalence and burden that loiasis causes in these regions. Whereas diethylcarbamazine (DEC) – the standard of care for loiasis in non-endemic regions – is not a therapeutic option in most endemic regions due to safety concerns, the broad-spectrum anthelmintic drug albendazole has been reported to exert substantial activity against

L. loa. Similarly, ivermectin is known to be highly active against microfilariae of *L. loa* but safety concerns limit its indiscriminate use. We therefore systematically evaluated different albendazole based regimens alone or with ivermectin to assess their potential to reduce *L. loa* transmission in these highly endemic regions.

Finally, we performed a review of the clinical and therapeutic aspects of loiasis based on the many new insights that have emerged over the past decade. In this review we provide updated estimates for the epidemiology of loiasis indicating a substantially higher number of patients suffering from this chronic infection. We propose a new concept to classify loiasis based on the clinical penetrance and parasitological characteristics and suggest a new classification of the signs and symptoms of loiasis into typical and non-specific symptoms and rare but clinically important complications. An updated treatment algorithm is presented taking into account all evidence of treatment studies on loiasis over the past decade.

In summary, the aim of this thesis was thus to evaluate therapeutic and diagnostic tools for schistosomiasis and loiasis, to summarise our understanding of loiasis as a disease with the final goal to assist in ameliorating the management and control of these two important but neglected infectious diseases. Specifically, we have:

- assessed the effectiveness of antimalarial combination therapy when administered for uncomplicated malaria on concomitant urogenital schistosomiasis in an area where malaria and schistosomiasis are co-endemic. Here, the objectives were to assess the egg excretion reduction 4- and 6-weeks following antimalarial treatment, the cure rate 4 and 6-weeks post malaria treatment and the proportion of subjects with microhaematuria before and after malaria treatment.
- assessed the efficacy and safety of albendazole and ivermectin based regimens for the treatment of microfilaraemic loiasis to decrease *L. loa* microfilaraemia below 100mf/µl at 6-month post treatment, to assess the proportion of amicrofilareamic subjects at 6-months post treatment, the median reduction each month and the proportion of subject with adverse events of grade 3 or at least possibly related to treatment.
- assessed potential diagnostic differences in microfilaraemia of *L. loa* and *M. perstans* in samples of capillary and venous blood as appropriate diagnostics are a prerequisite for implementation of appropriate treatment strategies.

- assessed Field’s stain as a rapid staining technique in comparison to Giemsa stain for the detection of microfilariae in peripheral blood to avoid time-consuming laboratory methodology which precludes its use in population-based screening programs.
- condensed the new evidence that emerged over the past decade into a new conceptual framework of loiasis as a disease of substantial importance for populations in endemic regions.

2. Helminth infections and their importance in sub-Sahara Africa

Helminths (from the Greek word "Helmins", meaning worm) are parasitic worms. They are the most common infectious agents of humans in sub-Sahara Africa and produce a global prevalence of disease that exceeds better-known conditions including malaria and tuberculosis [1,2]. Nearly one-quarter of the global population is infected with helminth parasites [3,4], rendering them among the most prevalent infectious agents in the world. Helminth induced mortality is low compared to other tropical diseases, however, their chronic presence can have a major impact on overall health which makes them an important public health concern.

There are three major phyla of helminths of importance to human health. These include the cestodes, trematodes (grouped into the group of platyhelminths - also known as flatworms – including among others the trematodes such as *Schistosoma spp*. and cestodes such as among others the pork tapeworm that causes cysticercosis [5]) and the large family of nematodes. The nematodes (also known as roundworms) include the major intestinal worms (also known as soil-transmitted helminths) and the filarial worms that cause lymphatic filariasis, onchocerciasis and loiasis (besides less important filarial pathogens including *Dirofilaria spp*. and *Mansonella spp.*). Globally, the most prevalent helminthiases are intestinal worms (soil-transmitted helminths), schistosomiasis and filariasis. While soil-transmitted helminths (STH) and schistosomiasis are officially recognised in the WHO-list of neglected tropical diseases (NTD) as a major public health problem in the developing world [6], loiasis awaits still to be recognised as a neglected tropical disease [7] despite its proven burden of disease and magnitude as a public health problem.

Soil-transmitted helminth infections are found mainly in areas with warm and moist climates where sanitation and hygiene are poor, including in temperate zones during warmer months. These STHs are considered neglected tropical diseases because they inflict important burden with considerable social and economic impact but at the same time adequate tools are available to control or eliminate this infection. Sub Sahara Africa is among the parts of the world where the highest number of STH infection occurs and where preschool-aged and school-aged children are particularly exposed to STH. Soil-transmitted helminth infections are relatively easily treatable with medication prescribed by health care providers. The WHO's target to provide regular anthelmintic treatment to at least 75% of children in settings where infection prevalence exceeds 20% was recently nearly met in sub-Saharan Africa [8]. Considering this great improvement in the control of STH, the new WHO 2021-2030 Neglected Tropical Disease Roadmap sets out to eliminate STH as a public health problem.

As for STH, schistosomiasis is highly related to the environment and population behaviours. Schistosomiasis is especially prevalent in poor rural communities with limited access to safe water and adequate sanitation in SSA. For the control of schistosomiasis, WHO recommended several approaches, which include prevention by access to safe water and improvement of sanitation and hygiene education in addition to vector control.

Praziquantel is the only currently WHO-recommended drug for the prevention and treatment of schistosomiasis. However, although efficacy of the drug is reported satisfactory, the re-infection rate and recrudescence due to its inefficacy on juvenile forms of the worm leads to sustained disease transmission in endemic regions. Since the control of schistosomiasis remains difficult, the 2021- 2030 WHO NTD Roadmap recommends several approaches to optimise the control of the disease including development of new therapeutic interventions that may become alternatives to praziquantel. To achieve this goal, the local epidemiology of the disease with concomitant co-infection and treatment have to be well evaluated. Schistosomiasis is frequently co-endemic with other helminths including STH and loiasis.

Loa loa, the African eye worm, is a filarial pathogen of central and west African rainforest regions. Traditionally considered benign, these filarial infections have been more or less neglected by health organizations, making them even neglected among the neglected tropical diseases. Compared to other filarial infections, less attention has been paid to the control of loiasis even though programs against onchocerciasis and lymphatic filariasis had to be stopped in regions where *L. loa* and other filariasis are co-endemic due to the occurrence of serious adverse reactions. These included the death of subjects with high *L. loa* microfilaraemia following ivermectin administration. There is a lack of data for loiasis control including test and treatment strategies for loiasis in individual or on community level.

This thesis will focus on alternative complementary drug regimens for schistosomiasis in an endemic area of Gabon, and on the improvement of the diagnostic modalities and the treatment for loiasis control. Finally, we took the advantage of this work to summarise our knowledge and understanding of loiasis disease.

2.1 Schistosomiasis

Schistosomiasis infects an estimated of 230 to 250 million people annually and 779 million people are at risk of infection [9].This disease causes 280,000 deaths annually [10]. Schistosomiasis is considered the second most important parasitic infection after malaria [9]. Sub-Sahara Africa bears the brunt of the burden of disease. The disease is poverty-related, particularly in rural regions where parasite exposure through contact with infested freshwater is frequent. In such areas, continuous reinfection is common [11]. Children are known to be the most affected patient group due to their play habits in addition to potential immunological factors. Women are also disproportionately affected among adults during their household activities leading to reproductive health problems. Anaemia which can lead to growth retardation in childhood, bladder carcinoma and female genital schistosomiasis are classic complications of *Schistosoma* infection. Moreover, there is an association between female genital schistosomiasis and the risk for sexual transmission of human immunodeficiency virus (HIV), with evidence indicating an increased risk for HIV infection in *S. haematobium* infected women [12,13].

2.1.1 Schistosoma parasite life cycle

Schistosomiasis is caused by infection with blood flukes of the genus *Schistosoma*. Six species of Schistosoma are responsible for the two major forms of diseases: *Schistosoma haematobium* responsible for urogenital schistosomiasis and *S. mansoni*, *S. guineensis*, *S. japonicum*, *S. intercalatum* and *S. mekongi* being responsible for intestinal schistosomiais.

The *Schistosoma* life cycle occurs between the host snails and humans, in which asexual or sexual reproduction occur, respectively. (Figure 1). Asexual reproduction occurs in freshwater snails with the development of miracidia into a sporocyst. Sporocysts multiply and grow into cercariae. In the human hosts, parasites grow to become mature, mate, and produce eggs.

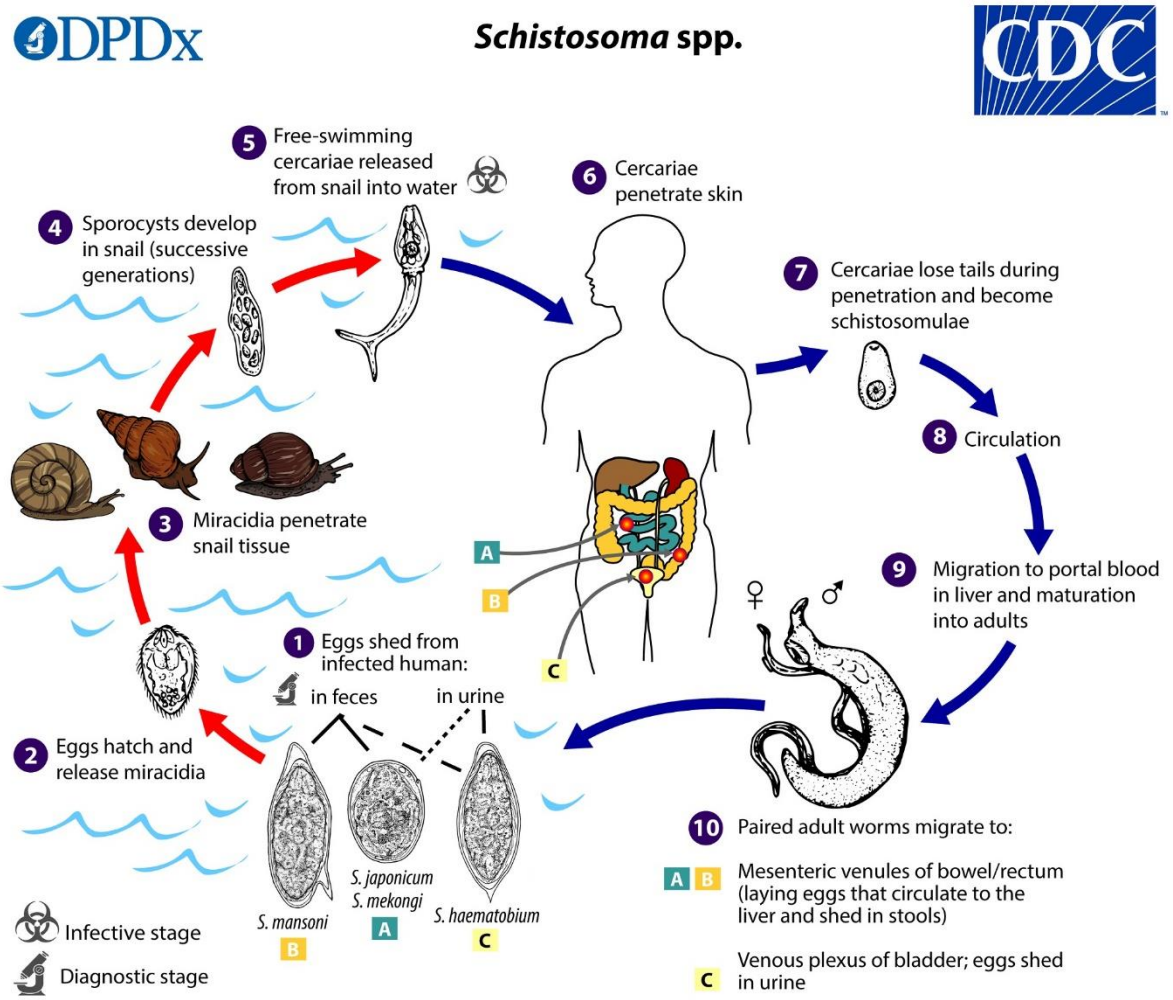

Figure1: *Schistosma spp.* life cycle illustration from the Centre for Disease Control and Prevention (Center for Disease Control, US)

2.1.2 Schistosomiasis treatment

Few drugs are available for the treatment of schistosomiasis. Oxamniquine and praziquantel are the only drugs used for the treatment of schistosomiasis. Oxamniquine, a 2-aminomethyltetrahydroquinoline derivative, is effective only against *Schistosoma mansoni* and has no notable effect on other *Schistosoma spp.* that parasitize humans. Praziquantel, a pyrazinoisoquinoline derivative is effective against *S. mansoni* and the other clinically relevant *Schistosoma* species. Praziquantel is therefore the only drug recommended for the treatment of schistosomiasis and it is used for individual treatment and for large scale control programs [10]. Praziquantel is safe and effective against adult schistosome worms [14,15], but ineffective against juvenile developmental stages [16,17]. Therefore, praziquantel is not suitable for prevention of early reinfection.

In addition to the above cited drugs, some other antiparasitic drugs have been reported to exert some antischistosomal activity *in vitro* or in preclinical animal models. Artemisinin derivatives were found to be active on juvenile schistosome stages and therefore could play a role in the prevention of early reinfection. The antimalarial class of the 4-amoniquinolines, Mannich bases, and arylaminoalcohols including mefloquine, amodiaquine and pyronaridine have been demonstrated to exert some activity. Importantly, these drugs are used in combination with artemisinin derivatives in antimalarial combination therapy, thus potentially leading to synergistic activity against schistosomiasis.

2.2 Loiasis

Loiasis is also called the African eye worm disease. It is caused by the filarial pathogen *Loa loa* - a nematode belonging to the family of *Onchocercidae*. *Loa loa* is exclusively occurring in sub-Sahara Africa – more precisely in most of central Africa and parts of west Africa. An estimated 24 million people are infected and roughly 42 million live in high or moderate transmission zones [18]. The highest endemicity of loiasis is reported in rainforest areas, which is the classical habitat of its vector *Chrysops dimidiata* and *Chrysops silacea*. Characteristic manifestations of loiasis are eye worm migration through the conjunctiva and Calabar swelling which is the transient angioedema swelling. From a parasitological point of view loiasis can be classified as occult loiasis – indirect signs and symptoms of adult worm migrations (eye worm migration, Calabar swelling) in the absence of microfilaraemia – and loiasis with peripheral microfilaria regardless of signs for adult worm migration. For a long time loiasis has been considered an infection with limited clinical importance easily tolerated by infected individuals in endemic regions. It therefore attracted relatively little interest for control and research until the activities of African Programme for Onchocerciasis Control based on mass drug administration of ivermectin were stopped in highly loiasis-endemic regions because subjects with high *L. loa* microfilarial density may develop a potentially fatal encephalopathy following ivermectin treatment.

2.2.1 *Loa loa* life cycle

Loa loa is transmitted from human to human via bites of tabanid deer flies of the genus *Chrysops*, with *C. silacea* and *C. dimidiata* being the most important species for transmission. *Loa loa* infected flies transmit third-stage larvae during the blood meal into the wound of the

human host. These third-stage larvae migrate through the subcutaneous tissue of the host and mature into female and male adult filariae which mate and produce asexual larval stages, called microfilariae. *Loa loa* microfilaria exhibit diurnal periodicity that coincide with feeding habits of anthropophilic *Chrysops spp.*. When another fly takes a blood meal on the infected patient, it takes up the microfilariae which then develop into first-stage larvae and further into infective third-stage larvae (figure 2).

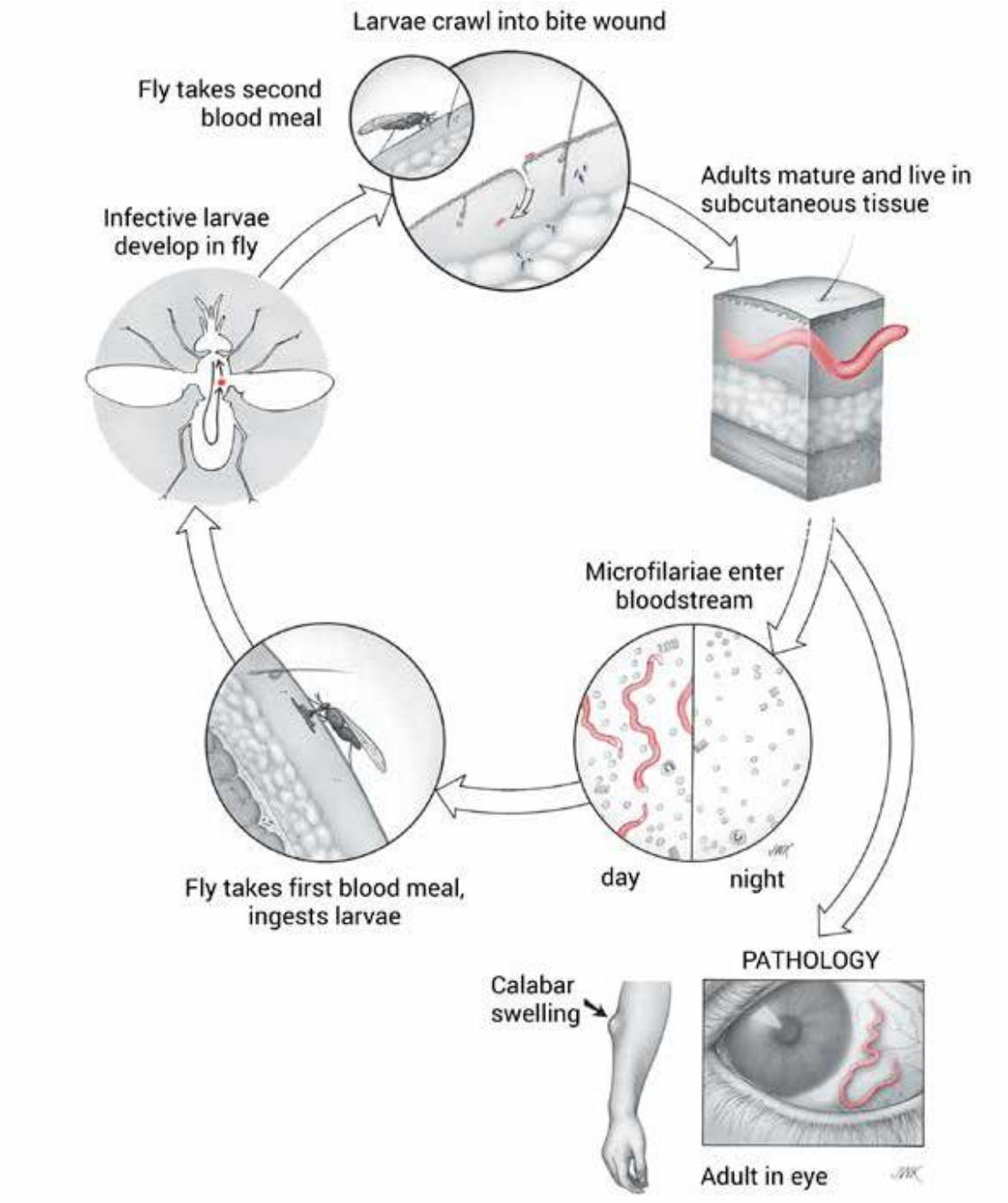

Figure 2: Life cycle of *loa loa* illustration. From Parasitic Diseases Seventh edition ebook [19]

2.2.2 Loiasis diagnostics

Diagnosis of loiasis faces several difficulties and different methods might be regarded as more or less useful depending on the clinical setting. To date, there is no specific biomarker for the adult stage of *L. loa* which resides and migrates within the subcutaneous and intermuscular fascial layers. In this case, diagnosis is made by the presence of characteristic signs such as eye worm migration or presence of Calabar swelling or by the history of the above mentioned signs. Besides these indirect markers such as eosinophilia, elevated IgE levels, and specific antibodies to nematode antigens may serve as diagnostic hints.

A highly specific diagnostic methodology for microfilaraemic patients is the direct microscopic detection of microfilariae in peripheral blood collected between 10 am and 4 pm to account for *L. loa* periodicity. It is routinely done by microscopic examination of a thick blood smear or by concentrations techniques increasing the sensitivity of detection stained with Giemsa stain. The presence of microfilariae is therefore a visible, reliable criterion to prove infection and to evaluate the effect of drugs on the parasite. Other diagnostics possibilities include PCR, antigen test and serology, which are mostly not accessible in routine care in endemic regions.

2.2.3 Loiasis treatment

The treatment of loiasis is complex and depends on the clinical forms and the level of *L. loa* microfilaraemia in the blood. The drug of choice for the treatment of loiasis is diethylcarbamazine (DEC) a piperazine class anthelminthic drug - which is active against adult worms and *L. loa* microfilariae. Its use is associated with the risk for acute anaphylactic reactions, fatal encephalopathy or other severe adverse neurologic events related to the microfilarial load. Treatment with DEC often requires hospitalization of patients for close supervision while the doses of DEC are slowly increased. Ivermectin, used on a large scale for the control of onchocerciasis and lymphatic filariasis, is effective against the microfilariae. Ivermectin does however only minimally affect the adult worm. Ivermectin can lead to safety issues when used in individuals with high *L. loa* microfilaraemia density. It is estimated that the risk for severe encephalopathy following ivermectin administration increases from 1% to 30% with peripheral *L. loa* microfilarial density of 20,000 to 100,000/ml, respectively [20].

Albendazole, an anthelminthic drug of the benzimidazole class, is effective against the adult worms and can thus decrease the *L. loa* microfilaraemia. Albendazole may be used for individuals loiasis with higher levels of microfilaraemia [21,22]. Despite single case reports, albendazole does not appear to be prone to cause encephalopathy, like the two others drug. Albendazole can be used to treat individuals with very high microfilarial loads (>50 000 mf/mL of blood) and still has shown to be safe in terms of clinical adverse events, hepato- and nephrotoxicity.

Finally, adult worms in the eye can be removed surgically. Although this constitutes a curative treatment approach, it is usually of limited therapeutic value due to the high number of adult worms in patients residing in endemic regions.

2.3 Schistosomiasis and loiasis in Lambaréné, Gabon

In Gabon, schistosomiasis and loiasis are prevalent in almost the whole country except for the capital region Libreville [23,24]. The country – located in the west coast of central Africa – Is 800 km long and 20 to 300 km wide, consists of 80% rainforest, and is bordered to the west by the Atlantic Ocean. The country counts nine provinces and is irrigated and drained by several rivers (Figure 3). Our studies were conducted in Lambaréné – department of Ogooue et lacs – located in Moyen-Ogooue province and in Fougamou – department of Tsamba-Magotsi located in Ngounie province. The most epidemiological data on schistosomiasis and loiasis are from Lambaréné and Fougamou and its surrounding villages [25–28] where research centers have been established [29,30].

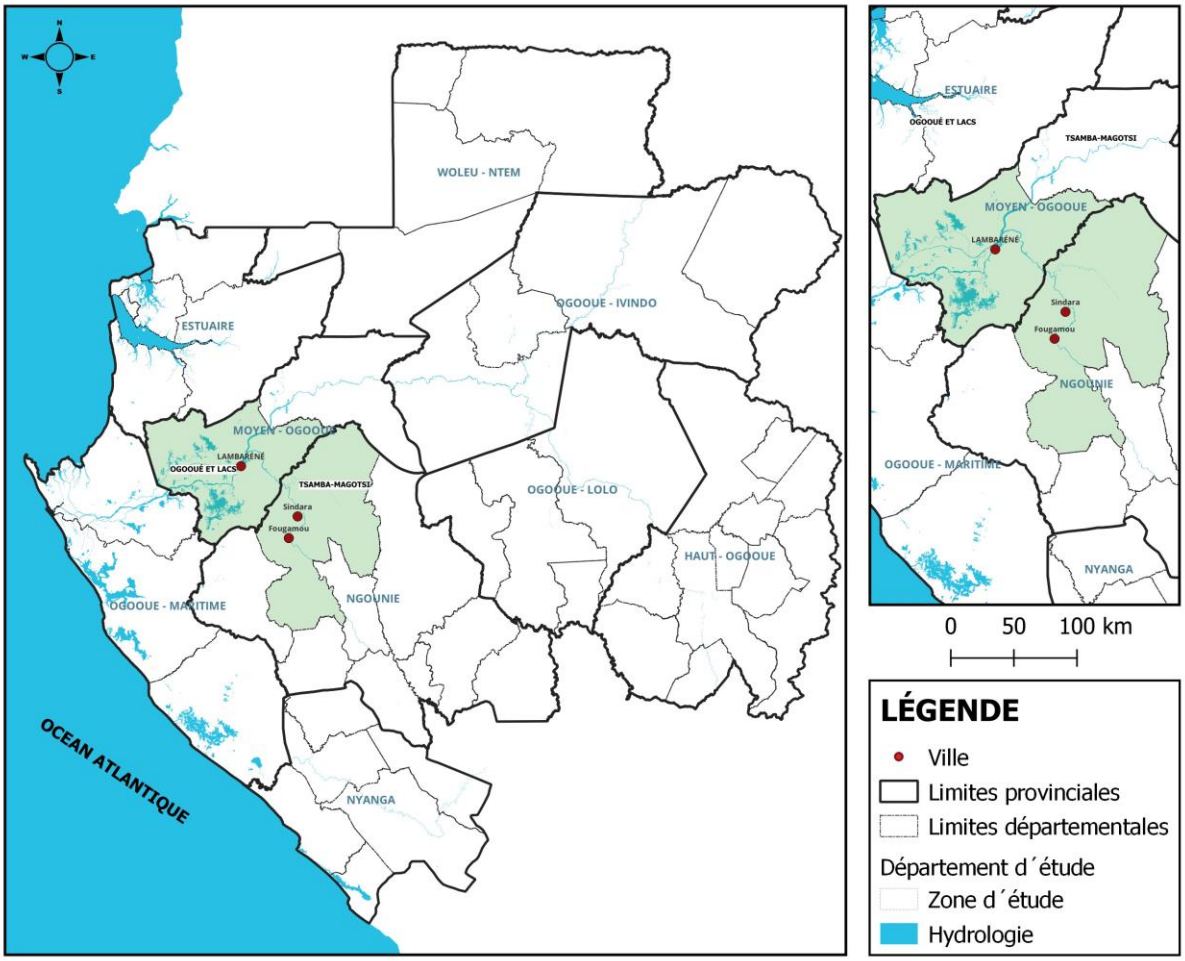

Figure 3: Map displaying the nine provinces of Gabon with their capital. Study area is highlighted in green with towns market by a red dot.

Lambaréné – the capital of the Moyen-Ogooue province - is a semi-urban town located in the central region of Gabon within the equatorial rainforest. Lambaréné and its surrounding areas are irrigated by several tributary of the Ogooue river and are characterised by the presence of several lakes and temporary ponds making the area favourable for the efficient development of *Schistosoma spp.* and anopheline larvae. Lambaréné and its surrounding region is known for its endemicity of *S. haematobium* and other parasitic infections such as malaria. A prevalence of 30 % of schistosomiasis and 58 % for malaria has been reported for Lambaréné and its surroundings with about 9% of co-infection [31,32]. Lambaréné and surroundings were reported as areas with lower prevalence of loiasis at around 20%, whereas Fougamou and its sourrounding regions are characterised by a prevalence of loiasis of up to 80% in the adult population [24]. Fougamou is a rural municipality located in Tsamba-Magotsi department, Ngounie province, about 100 km south of Lambaréné. Fougamou and surrounding villages are

located in a forest region which is crossed by the Ngounie river. The main activities of the local population include subsistence farming, game hunting, and fishing.

2.4 Problems in the treatment and diagnostics of schistosomiasis and loiasis

Although WHO recommends mass drug administration with praziquantel for the control of schistosomiasis, its implementation in many SSA regions including Gabon still poses a challenge. Treatment approaches using praziquantel for the treatment of schistosomiasis have a good efficacy of around 80%. However, it has only limited activity on juvenile forms of schistosomiasis and rapid reinfection rates or recrudescence is about 40%. This makes it difficult to effectively control schistosomiasis by the use of praziquantel as a curative drug alone. To optimise disease control and reduce morbidity associated to schistosomiasis alternative drugs are needed besides complementary control tools such as safe water supply and adequate sanitation facilities.

Curative treatment of loiasis is most commonly attempted in travellers returning after incidental exposure or in chronically-exposed patients who have migrated to non-endemic regions. Current therapeutic options for loiasis are either not feasible for use in endemic regions due to the requirement for hospitalization of patients with adequate level of care for the management of side effects (DEC), or are associated with rare but potentially serious adverse drug reactions (ivermectin). Albendazole appears as a safe option for the treatment of loiasis irrespective of microfilaraemia level. However, appropriate regimens and the sustainability of albendazole's effect remain to be described appropriately.

Accessible and reliable diagnostic tools are an important aspect while aiming for the control of this disease. This is particularly true for loiasis where levels of microfilaraemia are an important determinant for the choice of an appropriate treatment regimen. Diagnostics of loiasis is based on detection of microfilaria in stained thick blood smears by microscopy in routine care in tropical setting. Improved and more rapid diagnostic methods are needed for the detection of *L. loa* microfilaraemia to support future treatment and control programs.

3. Materials and Methods

The thesis consists of the following independent studies:

- Schistosomiasis treatment study (effects of antimalarial drugs given to treat malaria in adults and children on schistosomiasis)
- Loiasis treatment study (parasitological efficacy of albendazole alone or associated with a single dose of ivermectin for the reduction of *L. loa* microfilaraemia in adult patients)
- Loiasis diagnostic study I (differences in microfilaraemia of *L. loa* and *M. perstans* in samples of capillary and venous blood)
- Loiasis diagnostic study II (Field's stain as a rapid staining technique in comparison to Giemsa stain for the detection of microfilariae *L. loa* and *M. perstans* in peripheral blood)
- Review of loiasis (summary of the major advances in our understanding of loiasis as a relevant disease including its treatment and control and highlighting the many gaps that await to be addressed urgently)

3.1 Data collection and Study procedures

Two prospective clinical trials (schistosomiasis treatment study and loiasis treatment study) and two cross-sectional studies (loiasis diagnostic study I and loiasis diagnostic study II) were conducted at the Centre de Recherches Médicales de Lambaréné (CERMEL), Lambaréné, Gabon [30] between 2015 and 2020. All these studies were approved by the institutional ethics committee of CERMEL (reference numbers: CEI-CERMEL 006/2018, CEI-CERMEL 010/2018, CEI-013/2018). Procedures and objectives of each study were presented to participants or legally authorized representatives in case of minors and informed consent forms were signed before start of any study procedures. Clinical trials were registered at the clinical trial register clinicaltrials.gov under the identifier NCT04264130 and at the Pan-African Clinical Trials Registry under the identifier PACTR201807197019027. A review of loiasis was performed with a search of scientific literature up to 2023.

Schistosomiasis treatment study

The first prospective clinical study focusing on schistosomiasis treatment was a non-randomized, longitudinal and prospective study designed to assess the efficacy of antimalarial combination therapy given for the treatment of uncomplicated malaria on concomitant urogenital schistosomiasis. The study was conducted between 2018 and 2019 in Lambaréné and surrounding villages – a region which is considered of moderate endemicity for loiasis and high endemicity for schistosomiasis. Participants were adults and children of both sexes with uncomplicated malaria treated with antimalarial combination therapy. Eligibility criteria were urine sample positive for *S. haematobium* prior the initiation of antimalaria treatment and no intake of praziquantel documented during the six previous weeks. Participants were followed up until 6 weeks post treatment of malaria and were treated with praziquantel at the end of the follow-up period. All patients were either participants of antimalarial drug trials (clinical trials with artesunate–pyronaridine or artefenomel–ferroquine were ongoing during the study period) or were offered first-line standard treatment for uncomplicated malaria with either artemether–lumefantrine or artesunate–amodiaquine. Artesunate–pyronaridine, artesunate-amodiaquine and artemether–lumefantrine are conventional artemisinin-based combination therapies while artefenomel–ferroquine may be classified as a second-generation synthetic artemisinin-based combination therapy. Artefenomel is a synthetic trioxolane closely related to the artemisinin class of antimalarials. Study medication was administered orally in accordance with the label information or the specific study protocol. Four and six weeks after malaria treatment coinciding with a patient's visit in the antimalarial drug trial, urine samples were collected during two consecutive days to assess participants' eggs excretion status.

Loiasis treatment study

The second prospective clinical trial which was focusing on loiasis' treatment was an open label randomized assessor blinded controlled clinical trial evaluating the efficacy and safety of three albendazole-based regimens versus a control group in adults with *L. loa* microfilaraemia. This trial was conducted between May 2018 and April 2020. Study participants were mainly from regions around Fougamou located in the department of Tsamba- Magotsi, Ngounie province. This region is characterized as a highly endemic region for *L. loa*, and low endemicity for *Schistosoma spp.* [28,33]. Adult Participants were eligible based on the following inclusion

criteria: presence of *L. loa* microfilaraemia between 5,000 and 50,000 mf/ml in peripheral blood during daytime sampling (10 a.m. – 3 p.m.) assessed microscopically by thick blood smears of peripheral venous or capillary blood and willingness to participate in this clinical trial for the entire 6-month follow-up period. Eligible patients were randomized with an allocation ratio of 1:2:2:2 to either the inactive control group (CONTROL), or one of the following three albendazole-based fixed sequential treatment regimens: albendazole (ALB), albendazole+albendazole (ALB-ALB), or albendazole+ivermectin (ALB-IVM). Participants in the control group (CONTROL) received symptomatic treatment with the antihistaminic drug loratadine 10 mg oral once daily, for seven days. All participants enrolled in one of the three intervention groups received twice-daily oral therapy with 400 mg albendazole after food intake for 21 days. Participants randomized to the ALB-ALB group received an additional 14-day treatment course of twice-daily 400 mg albendazole. Participants allocated to the ALB-IVM group received a single dose of 150 µg/kg ivermectin. Ivermectin was administered only after assurance that *L. loa* microfilarial loads was below 4000 mf/ml. If microfilaria were above this threshold, participants were transferred back to the ALB-ALB treatment group. Blood samples were taken for haematology and biochemistry. A urine pregnancy test was performed for women under the age of 55 years. Assessments of treatment adherence, clinical status, adverse events, and concomitant medications were recorded at each visit during the treatment and follow-up period up to day 168. Two thick blood smears of 20µl peripheral venous or capillary blood were prepared at baseline, before study drug administration and on days 1, 3, 7, 10, 14, 17, 21, 23, 24, 26, 28, 30, 33, 35, 37, 42, 49, 56, 70, 84, 98, 112, 126, 140, 154 and 168.

Loiasis diagnostic study I

Study I on loiasis diagnostics aimed to investigate the diagnostic performance characteristics of capillary and venous blood to detect *L. Loa* and *M. perstans* in infected persons. The study was conducted from 2015 to 2019. Participants adults and children attending CERMEL for loiasis screening were invited to participated. For each participant enrolled in the study loiasis diagnostic I, two thick smears of 10 µl of blood were prepared according to the Lambaréné method [34]. One thick smear from capillary blood labelled "CAP" and one thick smear from venous blood labelled "VEN" were prepared at the same time for further evaluation and

quantification. Sample collected for a similar assessment focused on Plasmodium infection [35] were re-assessed for the detection and quantification of *L. loa* and *M. perstans* as well.

Loiasis diagnostic study II

Study II was performed from October to December 2019 and aimed to evaluate Field's stain as a rapid staining technique in comparison to Giemsa stain for the detection of microfilariae in peripheral blood. Participants of both sexes including adults and children, residing in the region of Lambaréné and Fougamou were invited to participate if signs and symptoms for loiasis were suspected. Two thick blood smears from capillary blood were prepared according to the Lambaréné method [34]. One thick blood smear was labelled "A" and the other thick blood smear was labelled "B" for further evaluation and quantification of *L. loa* and *M. perstans*.

Review of clinical characteristics and management of loiasis

A review of the literature was conducted up to June 2023. A literature search was performed on PubMed, Scopus, Web of Science and Google Scholar databases using keywords "Loa loa", "loiasis", "African eye worm", "filaria" and "loase" in English, German and French. All primary publications, case reports, and review articles irrespective of study design were assessed for relevance and full text papers were obtained where the title and abstract were inconclusive for inclusion.

3.2 Laboratory procedures

Schistosomiasis treatment study

Regarding the study on schistosomiasis treatment, urine test strips and urine filtration techniques were performed on each fresh urine sample collected at baseline, 4- and 6-weeks post malaria treatment. A urine test strip was performed to assess for the presence of haematuria while the urine filtration technique was used to detect the presence of Schistosoma eggs. The urine filtration technique consisted of passing 10 ml of fresh urine through a micro-filter

membrane of 10–12 µm (MF, Whatman, New Jersey, USA) using a syringe. The membrane was then transferred onto a glass slide, mounted on a microscope and read using a objective (40×) of a light microscope . Samples were considered *S. haematobium* positive if at least one egg was detected in at least one urine sample out of at least two urine samples provided by each subject and as negative if all two urine samples did not have any eggs present.

Loiasis treatment study

Blood smears were dried and stained with 20% Giemsa stain for 20 minutes according to standard protocols used at CERMEL [38]. Stained microscopic slides were examined at x10 magnification for detection and x100 magnification for species level identification by two microscopists qualified in the detection of microfilariae and unaware of treatment allocation. *L. loa* microfilarial density was expressed as the number of microfilariae per ml of blood. A blood smear was considered negative when no microfilarial parasite was detected in 20 µl of blood by any of the two microscopists.

Loiasis diagnostic study I

Thick smears of venous and capillary blood were dried and stained with 4% Giemsa for 60 minutes according to procedures used to stain thick smears of blood samples previously collected for similar malaria-focused evaluation. Thick blood smears were examined by two independent blinded microscopists unaware of the study code.

Loiasis diagnostic study II

The thick smears were dried and either stained with 20% Giemsa stain for 15 minutes for thick smear with label “A” or with Field's stain A and B prepared according to manufacturer manual for the thick smear label “B”. Briefly, each thick smear with label “B” was immersed in Field’s B for 5 to 6 seconds, after rinsing with tap water, Field's A stain (blue) was applied for 10 to 30 seconds followed by subsequent air-drying of the slide. Study codes on each thick smear were masked for the microscopist for blinding of the identity of the patient. The entire surface

of the slide was read by blinded microscopists unaware of slide label code. Microfilariae were searched at x10 magnification and species identification was done at x100 magnification.

3.3 Outcomes/endpoints

Schistosomiasis treatment study

The clinical trial on schistosomiasis treatment was a study aiming to descriptively demonstrate the effect of antimalarial combinations on *S. haematobium* egg excretion with no comparative statistical hypothesis to be tested. The primary endpoint was the egg excretion reduction (EER) assessed 6 weeks after initiation of antimalarial treatment with an anti-malarial combination therapy. The secondary endpoints included EER at 4 weeks posttreatment and cure rate (CR) at 4- and 6-week post-treatment calculated as the percentage of volunteers cured among those treated and proportions of subjects with micro-haematuria before and after treatment.

Loiasis treatment study

Regarding the clinical trial on loiasis treatment, the following endpoints were defined: the primary efficacy outcome was defined as the proportion of subjects reaching *L. loa* microfilarial density ≤100 mf/ml within 6 months. Secondary efficacy outcomes were the proportion of individuals in each treatment group being amicrofilaraemic at the last observation on day 168; the median reduction of *L. loa* microfilaraemia at each month after initiation of treatment and the median time from initiation of treatment to the individual nadir of *L. loa* microfilaraemia. Principal safety outcomes were defined as the occurrence of any related or unrelated grade 3 adverse event or serious adverse event and occurrence of all adverse events at least possibly related to study medication assessed from the time of first administration of study drugs up to day 168.

Loiasis diagnostic study I

On study I, the aim was to quantify a potential difference of microfilaraemia of *L. loa* and *M. perstans* between capillary and venous blood samples assessed by microscopy and to assess

whether microfilariae are more likely to be found in either blood source by evaluating odd ratio for paired data.

Loiasis diagnostic study II

On study II, the aim was to evaluate the performance of Field's stain compared to conventional Giemsa stain for the detection of blood microfilariae by evaluating the diagnostic sensitivity, specificity and feasibility of species diagnosis for *L. loa* and *M. perstans*.

Review of clinical characteristics and management of loiasis

The aim of this review was to summarise our current understanding of loiasis as a clinically relevant disease itself, including its treatment and control, without mention of its interactions with the control of other concomitant filarial infections.

3.4 Statistical analysis

Data were transcribed from paper case report forms to the REDCap electronic data capture system version 8.3.1. 206 hosted at CERMEL. Analyses were done using Stata IC version 13.1 (StataCorp LLC, College Station, Texas, USA). For data from study II on loiasis diagnosis, statistical analyses were performed using R (version 4.0.5, R Foundation for Statistical Computing, Vienna, Austria). TREND guidelines for reporting non-randomized clinical trials were followed in the study on schistosomiasis treatment and CONSORT guidelines for randomized controlled clinical trial for the clinical trial on loiasis treatment. Descriptive statistics are presented as numbers and proportions for categorial variables and median and range or means and standard deviation variables depending on the distribution of the data.

Schistosomiasis treatment study

The analysis of the schistosomiasis treatment study was done according to treatment given. *Schistosoma haematobium* infection intensity was classified as light when eggs count per 10ml of urine was less than 50 and heavy when it was equal to or more than 50 according to WHO

criteria [39]. A sub-analysis was performed to explore whether there was any alteration that could be imputed to the infection intensity. A post-hoc analysis comparing baseline characteristics of cured and not-cured subjects during the follow-up period was performed in.

Loiasis treatment study

The analysis of the loiasis treatment study was done according to study group. The primary efficacy analysis included all participants who completed the treatment period as per protocol and were follow up to day 168. The proportions of participants with *L. loa* microfilariae ≤ 100mf/ml were presented using a frequency table with a 95% confidence interval of proportions calculated by the Wald test. Odds ratios indicating whether microfilaraemia below 100/ml are more likely to be found in either study group were computed. The microfilaraemia reduction in each group was calculated as the median of the relative reduction in microfilaraemia for each participant. The relative reduction after treatment was thus calculated as follows: [*L. loa* microfilaraemia at baseline – *L. loa* microfilaraemia after treatment] / *L. loa* microfilaraemia at baseline. was used to define the time to the lowest of the median of the *L. loa* microfilaraemia in each interventional group was described using the graphical depiction of the evolution of *L. loa* microfilaraemia. Area under the curve of log-10 transformed *L. loa* microfilaraemia until day 168 has been calculated by trapezoidal rule with 95% confidence intervals. The number and percentage of adverse events in the safety population were tabulated by study group.

Loiasis diagnostic study I

Log2-transformed of microfilaraemia was performed due to the skewed distribution of microfilaraemia and venous microfilaraemia was subtracted from capillary microfilaraemia. Paired t-tests or Wilcoxon signed rank tests were used for comparisons of microfilaraemia in capillary and venous blood samples. Pearson's r coefficients were calculated to quantify correlation of capillary (CAP) and venous (VEN) microfilaraemia and intraclass correlation coefficient was created to assess reproducibility of both type of sample.

Contingency tables were tabulated to visualise the prevalence of microfilareamia in capillary and venous samples. Odds ratios for paired data were calculated to assess the odds of

microfilaraemia detection in capillary versus venous blood samples. The McNemar test was used for comparison of paired proportions. Furthermore, diagnostic sensitivity of capillary and venous samples was computed by using a light microscopy gold standard. An individual was regarded positive for microfilariae if either a capillary or a venous sample was positive in microscopy. Diagnostic sensitivity was computed for the overall study samples and for a sub-population of individuals with a low-level microfilaraemia (i.e. <200 microfilariae/mL blood).

Loiasis diagnostic study II

To compare performance characteristics of Field's Stain with Giemsa in study diagnostic II, the Spearman rank correlation coefficient ρ (rho) was calculated to quantify correlation between both staining method while Cohen's kappa was measured to indicate the agreement between the qualitative results of both staining methods with the strata poor (below 0.00), slight (0.00–0.20), fair (0.21–0.40), moderate (0.41–0.60), substantial (0.61–0.80), and almost perfect (0.81–1.00), as described elsewhere [40]. Two-sided p-values were presented, and an α of 0.05 was determined as the cut-off for significance.

3.5 Funding

The study on schistosomiasis treatment and the studies on loiasis diagnostic were funded by institutional funds from the Centre de Recherches Médicale de Lambaréné and the Bernhard Nocht Institute for Tropical Medicine while the study on loiasis treatment was co-funded by the Austrian Ministry of Science and Research and the CERMEL. The review on loiasis was financially supported by a grant from GIZ (Deutsche Gesellschaft für Internationale Zusammenarbeit; grant number: 81281913) and the German Center for Infection Research (DZIF).

4. Results

From 2015 to 2020, four studies were conducted at the Centre de Recherches Medicales de Lambaréné. Study populations in the study on schistosomiasis were mostly school-aged children from Lambaréné, Moyen-Ogooue province, while studies on loiasis treatment and diagnosis had adult populations from Tsamba-Magotsi, Ngounié province. A review on the loiasis was performed with a search on the literature conducted until June 2023.

Schistosomiasis treatment study

A total of 351 participants with confirmed uncomplicated malaria were consent to be screened for urinary schistosomiasis and to be followed up for 6-weeks. Out of them, 321 provided urine at baseline before initiation of antimalarial treatment and 52 (16%) of them were found positive for *S. haematobium*. Of the 52 *Schistosoma haematobium* positive patients, 24 were treated with artesunate–pyronaridine, 23 with artemether–lumefantrine, two with artesunate–amodiaquine, and three with artefenomel–ferroquine. Eight participants did not provide any urine sample during the follow-up visits and were excluded from the analysis (figure 4).

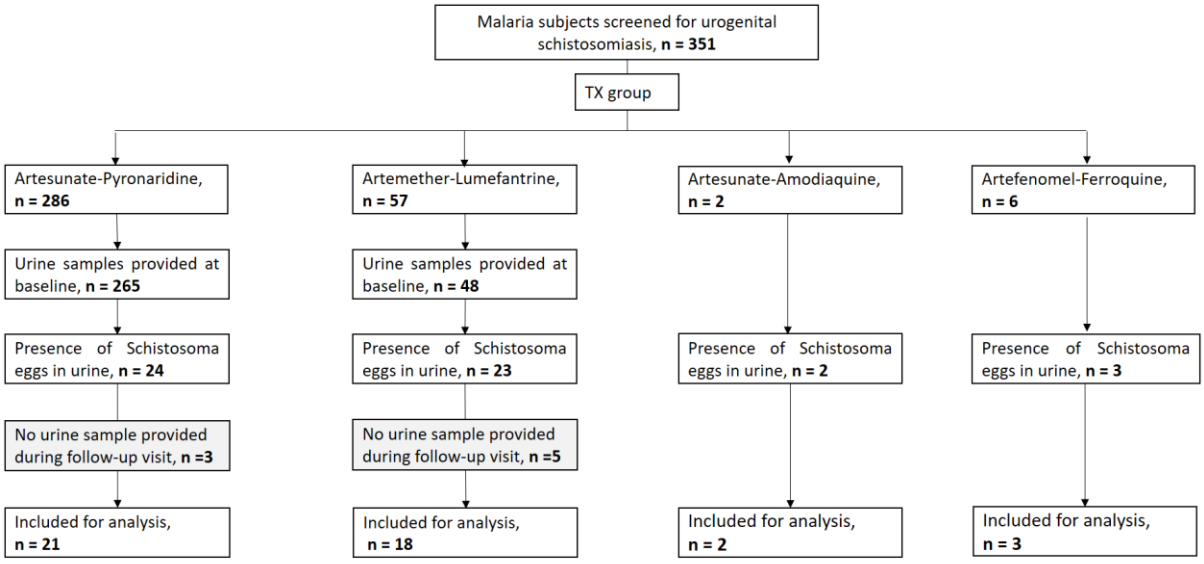

Figure 4: Participants flow [41]
Abbreviation: Tx: treatment

A total of 39 out of 47 patients treated with either artesunate – pyronaridine or artemether – lumefantrine completed at least one follow up visit and was included in the outcome analysis. Most patients, 61 % (23/39) were school aged children. The median egg load at baseline was lower in the artesunate – pyronaridine group compared to the other treatment groups, and there were more light infections in the artesunate – pyronaridine group. Baseline characteristics of the study population are described in table 1.

Table1: Baseline characteristics of study participants treated with ACTs, N=52

haracteristics	**Overall study population**	**AP**	**AL**	**ASAQ**	**OZFQ**
N (%)	52 (100.0)	24 (46.8)	23 (44.2)	2 (3.9)	3 (5.8)
Age in years, median (IQR)	11.7 (7.46- 17.2)	13.8 (9.4 - 18.6)	11.2 (7.2– 13.9)	17.2	3.5 (3- 25.3)
School age children (<14 years), n (%)	32 (61.45)	13 (54.2)	17 (73.9)	0	2 (66.7)
Adolescents and adults (≥14 year), n (%)	20 (38.5)	11 (45.8)	6 (26.1)	2 (100)	1 (33.3)
Sex					
Female, n (%)	16 (30.8)	5 (20.8)	7 (30.4)	1 (50.0)	3 (100)
Male, n (%)	36 (69.2)	19 (79.2)	16 (69.6)	1 (50.0)	0
Median egg count /10ml (IQR)	74 (16 - 614)	26 (6.0 - 158)	517 (53 - 1785)	261 (1-521)	124 (46 - 238)
Infection intensity					
Light infection (< 50 eggs/10ml), n (%)	22 (42.3)	15 (62.9)	5 (21.7)	1 (50.0)0	1 (33.3)
Heavy infection (≥ 50 eggs/10ml), n (%)	30 (57.7)	9 (37.5)	18 (73.8)	1 (50.0)	2 (66.7)
Hematuria, n (%)	47 (90.4)	20 (83.3)	23 (100)	2 (100)	2 (66.7)
Location					
Lambaréne, n (%)	41 (78.9)	17 (70.8)	21 (91.3)	1 (50.0)	2 (66.7)
Vicinity, n (%)	11 (21.1)	7 (29.2)	2 (8.7)	1 (50.0)0	1 (33.3)

Abbreviations: AP: artesunate-pyronaridine; AL: artemether-lumefantrine; N or n: number, IQR: interquartile range. The table is adapted from Zoleko et al [41]

Regarding the artesunate – pyronaridine treatment group, the median reduction of egg excretion was 100% (IQR; 17 – 100) at 4-week and 65% (IQR; -133 – 100) at 6-week post-treatment. Nine patients (56.3%) out of 16 at 4-weeks and seven patients (36.8%) out of 19 at 6 weeks, were classified as cured as they did not excrete any egg in any of the samples provided during two consecutive days. In this treatment group, haematuria was observed in 9 (56.3%) out of 16 and in 12 (66.7%) out of 18, at 4 weeks and 6 weeks post-treatment, respectively, compared to 83.3% (20/24) at baseline (Table 2).

For the artemether – lumefantrine treatment group, there was a decrease in egg excretion from 517 eggs/10ml of urine at baseline to 164 eggs/10ml of urine at 4-week post-treatment. Moreover, there was a median reduction in egg excretion of 64% (IQR: -91 – 79) after 6-week of follow-up. None of the patients in that treatment group completely cleared their eggs to be classified as cured. Egg excretion reduction, cure rate and haematuria are described in table 2.

Table 2. *Schistosoma* egg excretion reduction and cure rate and four and six weeks post malaria treatment among the 21 and 19 participants treated with artesunate-pyronaridine (AP) and artemether-lumefantrine (AL), respectively.

	AP		AL	
	Median	Range	Median	Range
	4-Weeks post-treatment			
N	16	-	15	-
Baseline Egg excretion/10ml	17	6 – 76	120	36 – 1704
Egg excretion/10ml	0	0 – 30	164	62 – 500
Egg excretion reduction, %	100	17 – 100	35	-250 – 70
Cure rate; n (%)	9, (56)		-	-
Hematuria; n (%)	9, (56)		13, (100)**	
	6-Weeks post-treatment			
N	19		18	
Baseline Egg excretion/10ml	19	5 – 103	105	39 – 1293
Egg excretion/10ml	9	0 – 304	56	14 – 209
Egg excretion reduction, %	65	-133 – 100	65	-65 – 79
Cure rate, n (%)	7, (37)		-	-
Hematuria , n (%)	12, (67)*		18, (100)	

Abbreviations: N or n: number, *: 1 missing data; **: 2 missing data; N: total number of participants
The table is adapted from Zoleko et al [41]

For the artesunate-amodiaquine group, 2 patients were evaluated at 4-weeks and one at 6-weeks post-treatment. There was a decrease in egg excreted at 4-weeks from 261 eggs/10ml to 61 eggs/10ml followed by an increase at 6-weeks post-treatment. No case of complete cure was observed and patients continued to have haematuria.

For the artefenomel – ferroquine treatment group, two and three patients were evaluated at 4-weeks and 6-weeks posttreatment, respectively. There was an increase in egg excretion at 4-weeks post-treatment (-98% [IQR: -235.1 to 39]) and at 6-weeks post-treatment (-61% [IQR: -65 to -61]). None of the patients were cured and all had haematuria present at follow-up.

Considering the infection intensity regarding subjects treated with artesunate – pyronaridine or artemether – lumefantrine, 33 participants were from Lambaréné among which 19 were classified as heavy infections (Table 1). Out of the 21 participants with heavy infection, 12 (67%) were treated with artemether–lumefantrine. Considering Schistosoma infection intensity, egg excretion rate at 4-weeks was higher in artesunate – pyronaridine (97% [IQR: 90 to 100]) group compared to artemether – lumefantrine group (51% [IQR: -29 to 86]) for subjects with heavy schistosoma infection (Table 3).

Table 3: *Schistosoma* infection intensity and *Schistosoma* egg excretion 4- and 6-weeks post malaria treatment

	AP			AL		
	Baseline egg excretion/10ml	**Egg excretion at 6 weeks/10ml**	**Egg excretion reduction %**	**Baseline egg excretion/10ml**	**Egg excretion at 6 weeks/10ml**	**Egg excretion Reduction %**
			4-Weeks post-treatment			
Infection intensity						
Light infection,	6	0	100	17	62	-250
(< 50 eggs/10ml), Median (IQR)	(4 – 19)	(0 – 37)	(-23 – 100)	(9 – 36)	(21 – 183)	(-370 – -72)
Heavy infection	235	2	97	517	251	51
(≥ 50 eggs/10ml), Median (IQR)	(98 – 688)	(0 – 20)	(91 – 100)	(89 – 1704)	(130 – 565)	(-29 – 86)
			6-Weeks post-treatment			
Infection intensity						
Light infection,	6	4	53	17	24	-13
(< 50 eggs/10ml), Median (IQR)	(4 – 19)	(0 – 38)	(-13 – 100)	(9 – 36)	(14 – 27)	(-167 – 25)
Heavy infection	235	160	69	517	133	70
(≥ 50 eggs/10ml), Median (IQR)	(103 – 322)	(14 – 368)	(-43 – 96)	(89 – 1704)	(25 – 526)	(52 – 87)

The table is adapted from Zoleko et al [41]

For the artesunate – pyronaridine treatment group, a sub-analysis comparing baseline characteristics of participant who cleared their eggs with those who still excreted egg during follow-up was performed. Overall, among the 21 patients followed-up in the artesunate–pyronaridine group, five (25%) were found with no eggs in urine at both 4- and 6-weeks post-treatment, while 7 (35%) still had eggs throughout the follow-up period. The baseline median egg excretion was 5 eggs/10ml urine and 40 eggs/10ml urine in the cured and non-cured groups, respectively (Table 4). Participants with high egg counts at baseline were less likely to completely clear egg excretion.

Table 4: Baseline characteristics of cured and non-cured individuals among 21 participants treated with artesunate-pyronaridine

	Subjects with eggs present during FU	Subjects with egg clear at		
		D42	**D28**	**D28 & D42**
N	**7**	**7**	**9**	**5**
Age				
Median, IQR	14.6 (13.6– 20.7)	17.4 (9.6 – 22.5)	11.7 (9.6 – 17.4)	11.6 (9.6 – 17.4)
Sex				
Male; n (%)	6 (85.7)	5 (71.4)	6 (66.7)	3 (60.0)
Female; n (%)	1 (14.3)	2 (28.6)	3 (33.3)	2 (40.0)
Residence				
Lambarene; n (%)	3 (42.9)	6 (85.7)	7 (77.8)	4 (80.0)
Vinicity; n (%)	4 (57.1)	1 (14.3)	2 (22.2)	1 (20.0)
Egg count /10ml:				
Median; (IQR)	40; (4.0 – 213.0)	6.0; (4.0 – 30.0)	6.0; (5.0 – 19.0)	5.0; (4.0 – 6.0)

2 patient who cleared eggs at 4-weeks missed visit at 6-weeks; 2 patients cleared eggs only at 6 weeks; 2 patients who cleared eggs at 4 weeks had reappearance of eggs at 6 weeks; IQR: interquartile range; FU: follow-up; n: number. The table is adapted from Zoleko et al [41]

Loiasis treatment study

Participants recruitment and follow-up were conducted between May 2018 to April 2020. A total of 78 adult participants were invited to participate and gave their consent to be screened for *L. loa* microfilaraemia between 5000 to 50 000mf/ml. The details of participants' flow are depicted in figure 5. Of the 49 eligible patients randomized into the different study groups, three withdrew their consent prior to treatment initiation and 4 patients in the ALB+ALB group discontinued treatment before day 21. These 7 participants were replaced by the next available eligible patient each. Overall, 36 randomized patients (12 in each of the intervention groups) completed their 21 days of ALB treatment. One patient randomized in the ALB+ALB group was inadvertedly treated with ivermectin after 21 days of albendazole treatment and was therefore included for statistical analysis in the ALB+IVM group. Two patients randomized in the ALB+IVM group received follow-up treatment with an additional 14 days of albendazole, instead of a dose of ivermectin, because their microfilaraemia was still above the protocol-specified threshold of 4,000/ml. These patients were reallocated to the ALB+ALB group for statistical analysis. Six patients were randomized in the control group and received loratadine for 7 days. All of them completed their follow up visit up to day 168. One patient in the ALB group was lost to follow-up after Day 154 and two patients in the ALB+IVM group were lost to follow-up after visits on Day 37 and Day 154. All other patients were followed-up for 168 days (figure 5). All 46 participants who received at least one dose of study treatment were included in the safety population. A total of 39 participants completed their treatment period and were successfully followed up to day 168. This group were included in the per protocol population for the efficacy analysis.

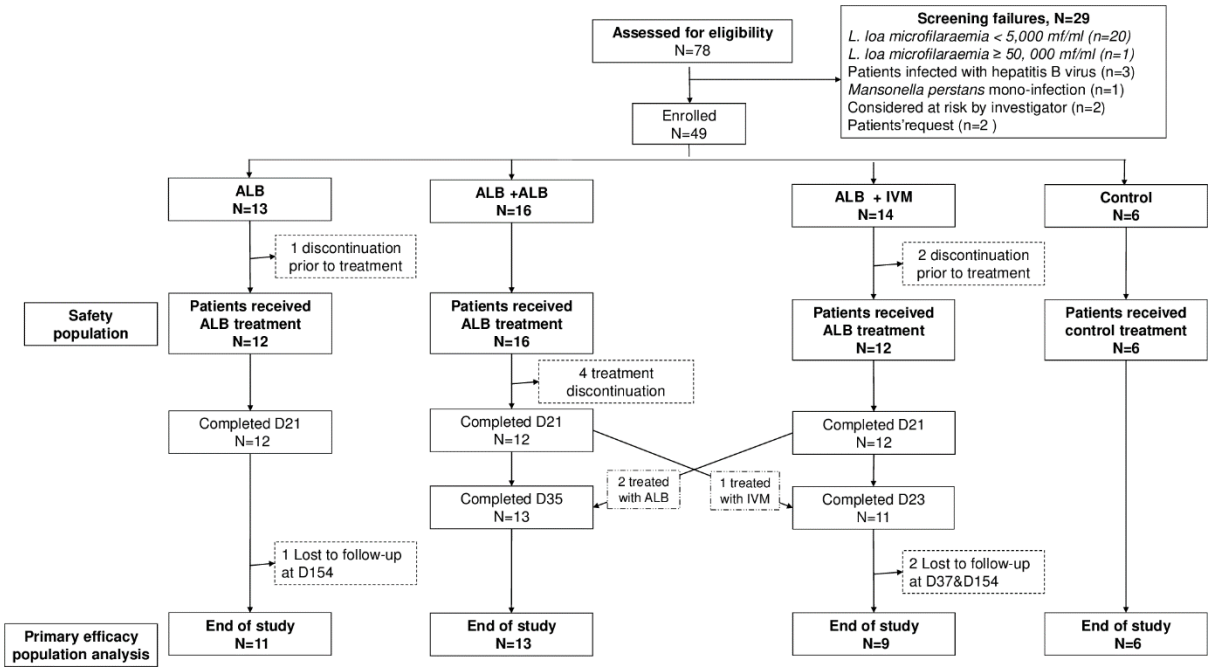

Figure 5: study participants flow [42]

Abbreviations: ALB: albendazole; IVM: ivermectin

Overall, considering the safety population, the median age was 63 years (IQR: 51-72). There were slightly more male than female participants (22/24) participating the the study. The median load of microfilariae was at 9950 mf/µl which was similar between groups at baseline (Table 5).

Table 5. Baseline demographic, clinical and biological characteristics of the safety population (N=46)

	ALB	ALB+ALB	ALB+IVM	Control	Total
N	12	16	12	6	46
Age (years), median (IQR)	68 (62-78)	60 (47-71)	62 (57-72)	52 (47-63)	63 (51-72)
Sex (Female/Male)	7/5	8/8	5/7	2/4	22/24
Median Baseline LLM (parasite/ml), IQR)	9625 (8050-12850)	9225 (7300-16025)	12725 (6600-17550)	11900 (7500-17350)	9950 (7500-15550)

Abbreviations: ALB: albendazole; IVM: ivermectin; LLM: *Loa loa* microfilaraemia. The table is adapted from Zoleko et al [42]

A total of 39 participants with contributing data until the end of follow-up on Day 168 were included in the primary analysis endpoint which was the proportion of subjects with *L. loa* microfilaraemia. 39% (5/13) and 22% (2/9) of participants in the ALB+ALB group and the ALB+IVM group respectively reached microfilariae loads ≤ 100 mf/ml at 6-month follow-up. None of the patients in the control group had a microfilarial load below 100mf/ml (Table 6).

Table 6: Proportion of participants with *L. loa* ≤ 100mf/ml at 6-month [42]

	n/N	**Proportion (95%CI)**	**OR (95%CI)**
ALB	1/11	9 (0 – 26)	1
ALB+ALB	5/13	39 (12 – 65)	6 (1 - 65)
ALB+IVM	2/9	22 (0 – 49)	3 (0 – 38)
control	0/6	0	--

Abbreviations: ALB: albendazole; IVM: ivermectin; OR: odd ratio; CI: confident interval; n/N: ration of number of subject with event by number of subject in group

In total, 4 patients were found with no detectable microfilariae in the blood at 6-month follow-up, 1 out of 11 (9%) in the ALB group and 3 out 13 (23%) in the ALB+ALB group.

Time to the lowest median microfilaraemia and change in median microfilarial load were described in each treatment group by graph depiction. Figure 6 shows for each treatment arm the time point following the first treatment and the median of value of *L loa* microfilaraemia. The median of the lowest microfilarial load was reached at Day 21, Day 30 and Day 37 following initiation of treatment in the intervention groups ALB group, ALB+IVM group and ALB+ALB group, respectively (Figure 6). While median microfilarial loads for all treatment groups at baseline were around 10,000 per ml as shown in Table 5, the median microfilarial load remained constantly below 2,500 per ml for the treatment groups ALB+IVM and ALB+ALB following individuals' nadir while it went up to around 5,000 per ml for the ALB group. The control group showed a fluctuation in microfilarial load that remained above 5,000 per ml throughout the study period.

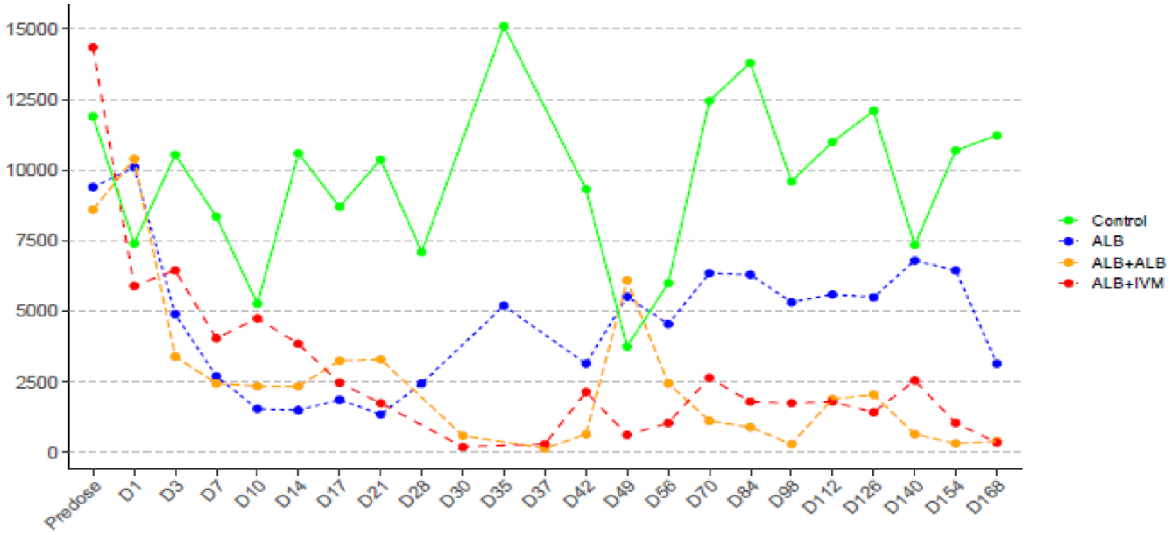

Figure 6: Median *Loa loa* microfilaraemia load variation from baseline to the end of follow-up according to study group [42]

Abbreviations: ALB: albendazole; IVM: ivermectin;

To compare the activity of each treatment regimen on microfilarial load for each respective group, the relative reduction in median microfilarial load (IQR) was computed. The relative reduction from baseline was above 90% in the ALB+ALB and the ALB+IVM groups starting from one-month post-treatment initiation with some fluctuations during the 6-month follow-up. The ALB group showed a slightly lower relative reduction of about 80% from one-month post-treatment - also observed at the end of the 6-month follow-up period - while there were also considerable variations throughout the follow-up period. The control group did not show any clinically significant relative reduction in median microfilarial load throughout the follow-up period. Details are shown in Table 7.

Table 7: Median (IQR) of relative reduction in *L. loa* mf per month by study group [42].

Month	ALB	ALB+ALB	ALB+IVM	Control
M1	0.80 (0.59 - 0.88)	0.91 (0.44 - 0.99)	0.96 (0.93 - 0.99)	0.23 (0.15 - 0.61)
M2	0.60 (0.12 - 0.82)	0.71 (-0.42 - 0.71)	0.87 (0.69 - 0.99)	0.13 (-0.01 – 0.20)
M3	0.47 (0.03 - 0.77)	0.96 (0.58 - 0.99)	0.84 (0.47- 1)	0.10 (0.05 - 0.10)
M4	0.47 (0.46 - 0.92)	0.88 (0.62 - 0.97)	0.90 (0.70 - 0.98)	-0.16 (-0.06 - 0.54)
M5	0.27 (-0.20-0.85)	0.98 (-0.12-0.99)	0.86 (0.68-0.95)	0.15 (0.06-0.72)
M6	0.69 (0.57 - 0.85)	0.93 (0.87 - 0.99)	0.94 (0.85 - 0.97)	0.02 (-0.38 - 0.35)

Abbreviations: ALB: albendazole; IVM: ivermectin; M: month

Area under the curve (AUC) of microfilaraemia was calculated from baseline until D168 in the respective treatment groups as in Table 8. The lowest AUC were seen in the ALB+ALB group and the ALB+IVM group with 448 (327 to 570) and 498 (386 to 611), respectively.

Table 8. Area under the curve of microfilaraemia in the respective treatment groups over 6 months follow-up [42].

Treatment groups	n	AUC (Mean; (95% CI))
Control	6	663 (623 - 703)
ALB	11	566 (504 - 627)
ALB+ALB	13	448 (327 - 570)
ALB+IVM	9	498 (386 - 611)

Abbreviations: ALB: albendazole; IVM: ivermectin; n, number, AUC: area under the cuve, CI: confident interval

46 patients were included in the safety population that either received one or more doses of albendazole (40 patients) or were enrolled in the control group (6 patients). Up to 168 days after albendazole or placebo administration 15 (32.6%) participants reported one or more adverse events at least possibly related to study drugs, including just one patient in the control group, six in the ALB group and four each in the ALB+ALB and the ALB+IVM groups. Across all participants, the most commonly reported adverse events were headache, dizziness and

fatigue, followed by pruritus, rash and nausea as shown in Table 9. Out of 4 patients randomized to the ALB+ALB group who were lost during the treatment period, only one of them reported an adverse event before leaving the study, which was headache, grade I and unlikely related to the study drug.

Table 9: Grade 1-2 adverse events at least possibly related to study treatment [42]

Adverse event, n (%)	**ALB (N=12)**	**ALB+ALB (N=16)**	**ALB+IVM (N=12)**	**Control (N=6)**	**Total (N=46)**
Patient with any at least possibly related AE	6 (50.0)	4 (25.0)	4 (33.3)	1 (16.6)	15 (32.6)
Number of AEs at least possibly related to study drug	8	7	6	1	22
Headache	0	4 (57.1)	0	0	4 (18.2)
Dizziness	1 (12.5)	1 (14.3)	2 (33.3)	0	4 (18.2)
Vertigo	0	1 (14.3)	0	0	1 (4.6)
Nausea	0	0	2 (33.3)	0	2 (9.1)
Abdominal pain	0	0	0	1 (100)	1 (4.6)
Diarrhoea	1 (12.5)	0	0	0	1 (4.6)
Fatigue	3 (37.5)	0	1 (16.7)	0	4 (18.2)
Pruritus	1 (12.5)	1 (14.3)	1 (16.7)	0	3 (13.6)
Rash	2 (25.0)	0	0	0	2 (9.1)

Abbreviations: ALB: albendazole; IVM: ivermectin; AEs: adverse event

Among all patients in the safety analysis, all AEs were of mild or moderate intensity except one severe adverse event which met the criteria for SAE. One participant, 73-years-old man with a history of alcohol abuse was randomized to the ALB group with an initial *L. loa* micofilaraemia of 10,050 mf/ml. Baseline haematology and biochemistry parameters were normal, except for gamma glutamine transferase at 1.5-time upper normal limit. One day before

the end of the three weeks albendazole drug intake, the patient became asthenic with psychomotor retardation without fever. Five days later, the decision was taken to hospitalize the patient due to a new onset of vertigo and aggravation of asthenia. At hospital admission, the patient was afebrile, and asthenic. Neurological examination was normal. Haematology and biochemistry assessments were unremarkable, except for C-reactive protein and gamma glutamine transferase which were 3 and 2 times upper normal limit, respectively. *Loa loa* microfilariae count was 1,400mf/ml. Symptomatic supportive treatment was given and the patient was discharged four days later in full remission with the suspicion of alcohol withdrawal syndrome as the underlying cause of the clinical symptoms. This serious adverse event was classified as being unlikely related to the study treatment.

A slight increase in liver transaminases (alanine aminotransferase and aspartate aminotransferase) was observed following 21 days of albendazole intake in all active treatment groups. Nevertheless, no further increase was observed after an additional course of 14 days of albendazole in the ALB+ALB group, nor after administration of a single dose of ivermectin in ALB+IVM.

Loiasis diagnostic study I

In Study I, a total of 713 participants gave their consent to be included between 2015 and 2019. Of them, 41% (289/710) were below 20 years of age. There were more female than male participants (386:325 sex ratio female-male). Among the 713 included participants, 152 (21%) had a microscopically-confirmed infection with *L. loa* and 102 (14%) with *M. perstans*. The positive rate for *L. loa* was similar in CAP blood (18.9%) and VEN blood (18.4%), while for *M. perstans* the positive rate in VEN blood (4.1%) was almost the half of the positive rate observed in CAP blood (10.7%) (Table 10).

Table 10: Proportion of microfilariae in CAP and VEN samples

Species		**Qualitative assessment**	
		Positives (%)	Negatives (%)
Loa loa	CAP	135 (18.9)	578 (81.1)
	VEN	131(18.4)	582 (81.6)
Mansonella	CAP	76(10.7)	637 (89.3)
perstans	VEN	59 (4.1)	654 (95.9)

For individuals with detectable *L. loa* in thick blood smear, median microfilaraemia (IQR) was 3,650 (275 – 11100) mf/ml and 2,775 (200 – 8875) mf/ml in CAP and VEN sample respectively. There was a significant difference between the microfilaraemia quantity measured in CAP and VEN blood (p<0.0001). The microfilareamia in CAP blood was 34.5% (95% CI: +11.0 to +63.0) higher compared with the quantity measured in VEN blood (p = 0.0027) (Table 11). For individual with *M. perstans* microfilaraemia, median microfilaraemia (IQR) was similar in CAP and VEN blood at 100 (0 – 200) mf/ml. The average microfilaraemia was 24.8% (95% CI: +0.0 to +60.5) higher in CAP samples compared with VEN samples with insufficient statistical evidence for a true difference (p = 0.08).

Table 11: Microfilaraemia in capillary (CAP) and venous (VEN) blood samples

	n	Microfilaraemia; Median (IQR)			Microfilaraemia; log2CAP – log2VEN		
		CAP	VEN	P*	Difference, (95%CI)	Excess, (95%CI)	P**
Loa loa	152	3,650 (275 – 11100)	2,775 (200 – 8875)	<0.001	+0.43 (+0.15 – +0.70)	+34.5% (+11.0 – +36.0)	0.0027
Mansonella perstans	102	100 (0 – 200)	100 (0 – 200)	0.44	+0.32 (-0.04 – +0.68)	+24.8% (0.0 – +60.5)	0.08

*Wilcoxon signed-rank test, **t-test. The table is adapted from Michlinger et al [43]

The relationship between CAP and VEN blood considering subjects with detectable microfilaraemia was high as indicated by Pearson's r of 0.94 and 0.90 for *L. loa* and *M. perstans*, respectively. Also, reproducibility of CAP microfilaraemia in VEN microfilaraemia was excellent for both *L. loa* and *M. perstans* with intra-class correlation coefficients of 93.4% (98.4 - 95.8; p<0.0001) and 94.7% (89.3-97.4; p<0.0001), respectively.

For subject with *L. loa*, the number of discordant pair was higher in the of 'CAP+ & VEN- ' ('CAP+ & VEN- ': 21) than in the pair of 'CAP- & VEN+' ('CAP- & VEN+': 17) (Table 12). Concordantly, the odd indicates that *L. loa* microfilaraemia was more prevalent in CAP blood compare to VEN blood (OR:1.24; 95% CI: 0.65–2.34).

Different result was observed for subject with *M. perstans*. The number of discordant pair was more higher in the of 'CAP+ & VEN- ' ('CAP+ & VEN- ': 43) than in the pair of 'CAP- & VEN+' ('CAP- & VEN+': 26) (Table 11). *M. perstans* was significantly more detected in CAP blood than in VEN blood (OR1.65 (95% CI: 1.0–2.68); p = 0.041).

Table 12: Cross-tabulation of capillary and venous microfilaraemia by microscopy and odds ratios to quantify the odds to detect a microfilaraemia in capillary blood than in venous blood, N = 713.

		Capillary blood		OR** (95%CI)	*P-value**
		Positive	Negative		
		Loa loa			
Venous blood	Positive	114	17	1.24 (0.65 – 2.34)	0.52
	Negative	21	561		
		Mansonella perstans			
Venous blood	Positive	33	26	1.65 (1.0 – 2.68)	0.041
	Negative	43	611		

*McNemar test. ** Odds ratio for paired data. The table is adapted from Michlinger et al [43]

The diagnostic sensitivity was higher in CAP blood at 88% and 74.5% respectively for *L. loa* and *M. perstans* than in VEN blood where sensitivity was at 86.2 % and 57.8 % respectively for *L. loa* and *M. perstans* (Table 13). Similar result was observed among participants with a low level of microfilaraemia defined as microfilareamia <200 mf/ml. The diagnostic sensitivity in CAP blood was at 58.8% and 50.0% for *L. loa* and *M. perstans* respectively, while sensitivity in VEN blood was at 66.7% and 47.2% for and for *L. loa* and *M. perstans* respectively.

Table 13: Diagnostic sensitivity of capillary (CAP) and venous (VEN) blood to detect a microfilaraemia using microscopy among the 713 study participants.

	Overall sample, % (95% CI)		**Low-level microfilaraemia*, % (95% CI)**	
	CAP	**VEN**	**CAP**	**VEN**
Loa loa	88.8 (86.5 – 99.1)	86.2 (83.7 – 88.7)	58.8 (54.9 – 62.8)	50.0 (46.0 – 54.0)
Mansonella perstans	74.5 (71.3 – 77.7)	57.8 (54.2 – 61.5)	66.7 (63.1 – 70.2)	47.2 (43.5 – 51.0)

*defined as<200 microfilariae/ml for *L. loa* (n = 595) and *M. perstans* (n = 683). The table is adapted from Michlinger et al [43]

Loiasis diagnostic study II

Study II on loiasis diagnostic was conducted from October 31 to December 31, 2019, and a total of 175 subjects were recruited. The median age was 35 years (IQR 28-49 years) and there were slightly more male compared to female patients recruited (94:81 male-to-female ratio). Almost half of all samples were positive for *L. loa* microfilaraemia (49.7% of Giemsa-stained and 46.9% of Field's stain treated samples). The positive rate for *M. perstans* was lower with 24.6% of Giemsa-stained and 21.1% of Field's stain treated samples (Table 14).

Table 14: Proportion and intensity of microfilariae in Giemsa-stained and Field's stain treated samples [44].

Species		**Qualitative assessment**		**Quantification**
		Positives (%)	Negatives (%)	Median (IQR), Mf/ml
Loa loa	Giemsa	87 (49.71)	88 (50.29)	600 (200-1962.5)
	Field	82 (46.86)	93 (53.14)	537.5 (200-1462.5)
Mansonella	Giemsa	43 (24.57)	132 (75.43)	100 (100-200)
perstans	Field	37 (21.14)	138 (78.86)	100 (100-200)

In statistical analysis for the presence of microfilariae of each species there was an almost perfect agreement between *L. loa* detection and a substantial agreement between *M. perstans* detection for both staining methods (Cohen's κ 0.92 [CI 0.86-0.98] for *L. loa* and 0.74 [CI 0.61-0.86] for *M. perstans*).

Considering only subjects with detectable microfilaraemia on thick blood smear, median *L. loa* microfilaraemia was 600 (IQR 200-1963) and 538 (IQR 200-1463) mf/ml for Giemsa-stained and Field's stain treated samples, respectively. This resulted in a strong positive linear relationship (Spearman rank correlation coefficient ρ=0.91, p-value<0.001) (Table 15 and Fig. 7). The relationship of both staining methods was only moderately positive for *M. perstans*, with 100 (IQR 100-200) mf / ml; median microfilaraemia for both methods (Spearman rank correlation coefficient ρ=0.54, p-value=0.002).

Table 15: Quantitative assessment of the number of microfilariae in Giemsa-stained and Field's stain treated samples.

Staining method	Median (IQR), Mf/ml	Spearman rank correlation coefficient ρ
	Loa loa	
Field	600 (200 – 1962.5)	0.905, *p-value* < 0.001
Giemsa	537.5 (200 – 1462.5)	
	Mansonella perstans	
Field	100 (100 – 200)	0.538, *p-value* = 0.002
Giemsa	100 (100 – 200)	

Abbreviations: IQR: interquartile range; Mf: microfilariae; ml: millilitre. The table is adapted from Ekoka Mbassi et al [44]

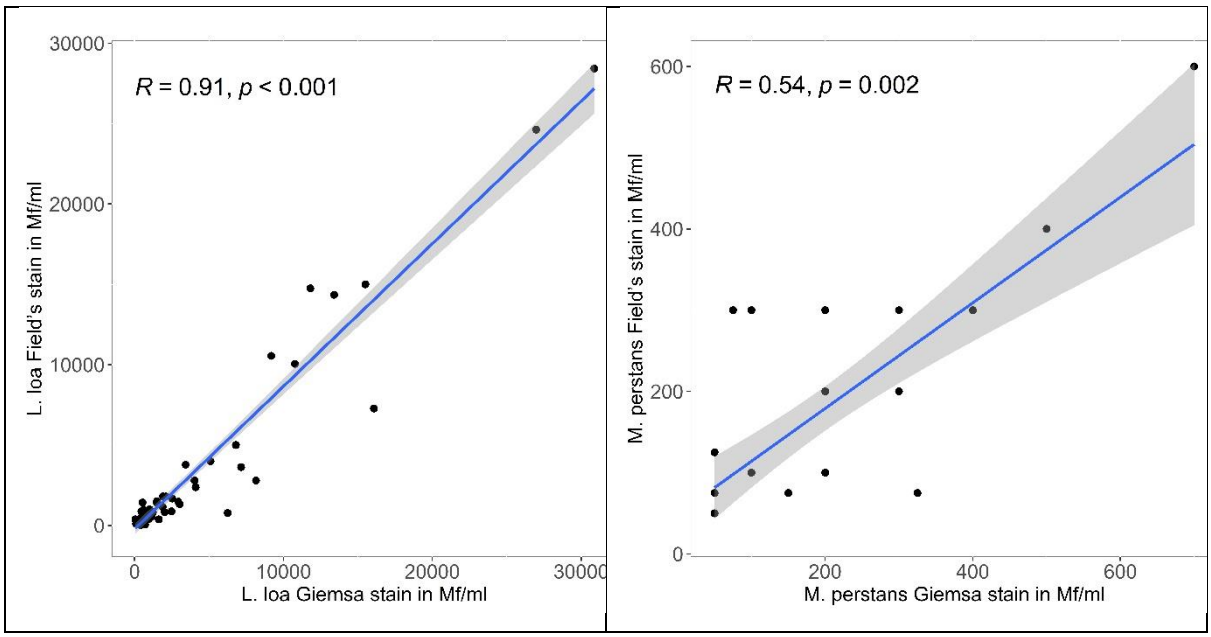

Figure 7. Quantitative agreement of microfilaraemia quantification. R = Spearman's ρ [44]

Review of clinical characteristics and management of loiasis

Epidemiology and burden of loiasis

The main study underlying our understanding of the epidemiology of loiasis used the WHO-recommended Rapid Assessment Procedure for Loiasis tool (RAPLOA), which is a short-standardized questionnaire on the history of eye worm migration in known or suspected areas to be endemic for loiasis [45]. There is no representative data available for the prevalence of loiasis based on both clinical and parasitological investigations for all endemic regions. In 2010, around 30 million of people lived in high (prevalence of eye worm history above 40%) and intermediate (prevalence of eye worm history between 20% and 40%) transmission region of loiasis and nearly the whole country of Gabon was classified as high-risk region [45]. In 2023, based on an estimated 3% annual population growth in the Central African region around 42 million peoples are estimated to live in high and intermediate transmission regions of loiasis. The majority of affected people lives in rural regions of Gabon, Equatorial Guinea and parts of Cameroon, the Central African Republic, the Democratic Republic of Congo, and the Republic of Congo [18]. Loiasis transmission intensity is associated with local ecology and professional activities of rural populations. while prevalence is low in urban areas where deforestation and urbanisation are common and lead to disappearance of vector habitat. Contrarily, in rural rainforest regions prevalence is very high because of the abundance of the vector and residents' main activity agriculture, fishing, and hunting.

Recent reports on the morbidity and mortality directly associated with loiasis have significantly changed our understanding on loiasis as it has long been regarded as a benign infection. A retrospective population-based cohort study conducted in Cameroon reports an attributable fraction of death associated with presence of *L. loa* microfilaraemia at about 14.5% [46]. This finding of increased mortality in microfilaraemic individuals was confirmed in a retrospective assessment of mortality conducted in the Republic of Congo [47].

The attributable disability-adjusted life years lost to loiasis was estimated around 413 per 100,000 inhabitants in highly endemic transmission regions in Gabon [28] and 82 when normalized to the entire country [18]. This finding indicates that loiasis leads to a comparable magnitude of DALY estimates as other neglected tropical diseases.

Life cycle of *L. loa* and clinical features of loiasis

Loiasis is a parasitic disease caused by the filarial worm *Loa loa* and transmitted from human to human by bites of tabanid flies. The adult worms reside in the patient's subcutaneous tissues where they mate and produce large numbers of microfilariae. Microfilariae are found in peripheral blood. During the blood meal of the Chrysops fly, microfilariae enter the midgut of the insect to initiate a consecutive developmental from L1 larvae to L3 larval stages, which migrate into the proboscis and head region of the Chrysops fly. During the next blood meal, the L3 larva is transmitted to the next host which form the starting point of a new life cycle of the parasite (figure 2). These L3 larvae migrate through the subcutaneous tissue of the host and mature into female and male adult filariae. The incubation period is not less than 3-6 months [48]. The time from infection to appearance of microfilariae in peripheral blood – is thought to be around one year (the shortest documented period being two months) but may last up to 15 years [49–51]. Adult worms are remarkably long-lived in the human host with a maximum documented lifespan of up to 20 years [52,53].

Historically, it has been reported that most cases of *L. loa* infection are asymptomatic whereas loiasis has been described to be among the three most common reasons for medical consultations in some endemic regions [54,55]. *Loa. loa* infection is characterised by classical and atypical signs and symptoms (figure 8).

Classical signs and symptoms of loiasis

The typical clinical sign of loiasis occurring in about 80% of *L. loa* infected individuals in endemic settings is the transient eye worm migration [28] which may constitute the only apparent clinical sign [56]. Unspecific signs and symptoms of loiasis frequently also occurs in others diseases included generalised fatigue and asthenia, reappearing headaches, transient paresis or paraesthesia, prutitis and transient oedemas [28].

Rare but clinically important complications of loiasis may lead to severe organ damage. Loiasis affects the heart, kidneys or brain. Encephalopathy is a well known complication most often occurring after administration of antifilarial drugs, especially ivermectin and diethylcarbamazine. However, sometimes it can occur spontaneously. Encephalopathy may lead to a death or result in sequelae. Nephropathy frequently manifests as haematuria or proteinuria in patients with loiasis [57]. In rare cases, loiasis related renal changes may lead to nephrotic syndrome and renal failure [58]. Endomyocardial fibrosis and pulmonary complications related to loasis have been reported in some patients [59–63]. The overall

incidence of cardio-pulmonary complications of loiasis in endemic regions are currently unknown and requires further epidemiological investigations.

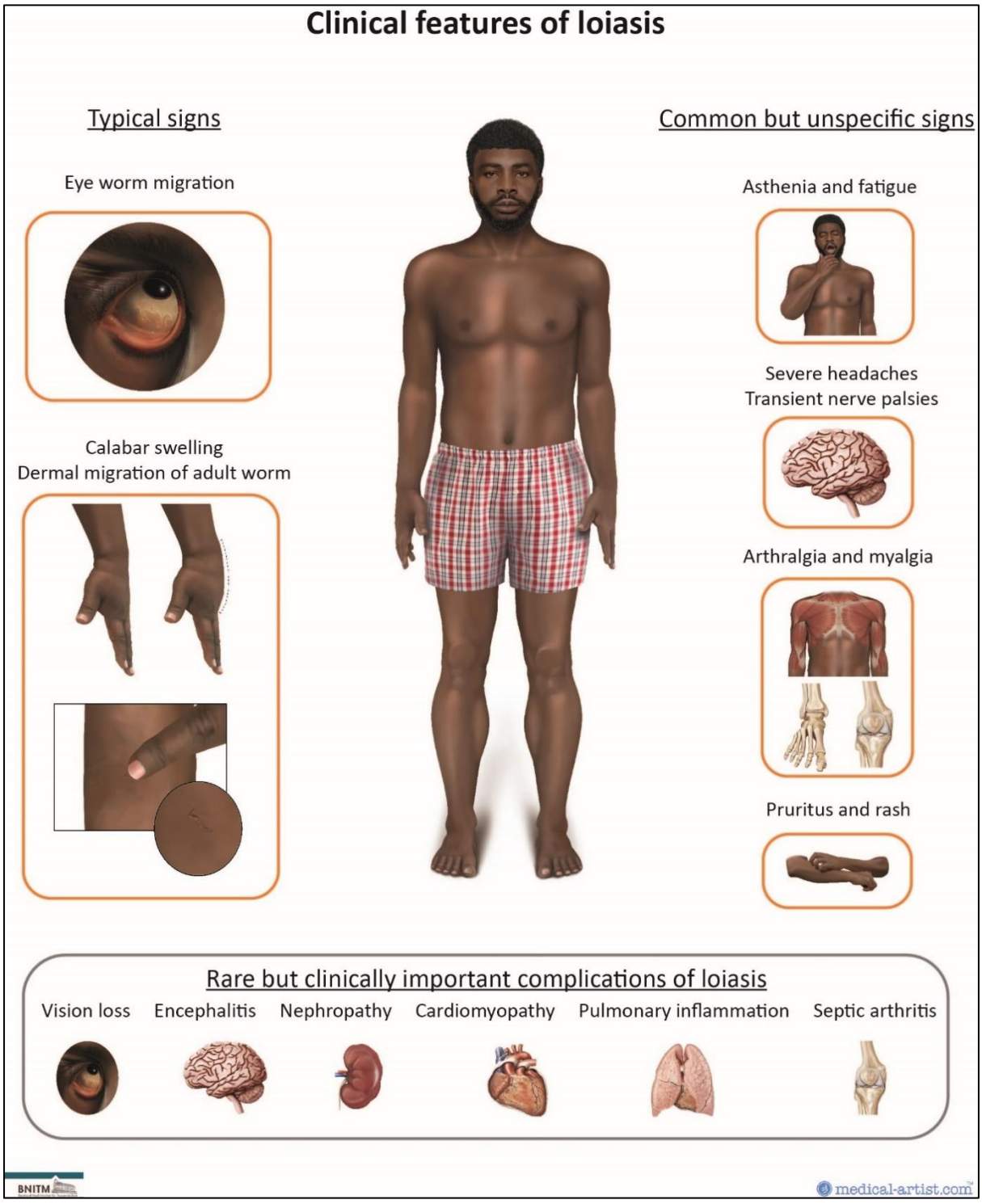

Figure 8: Illustration of typical, common but unspecific signs, and rare but clinically important complications of loiasis. [18]

Classification of loiasis

Loiasis has been traditionally classified from a parasitologist's point of view in occult and microfilaraemic infection which does not necessarily reflect the clinical penetrance of loiasis. From the clinical point of view, loiasis can be classified as "migratory loiasis" characterized by the presence of signs and symptoms of adult worm migration (eye worm migration, Calabar swelling, and transient nerve palsies, pruritis, urticaria) regardless of whether or not microfilaria are present in peripheral blood [69] and "non-migratory loiasis" defined by the presence of microfilariae but the absence of clinical signs of adult worm migration or Calabar swelling. Non-migratory loiasis is mostly characterised by the absence of clinical signs and symptoms. A third group consists of patients with rare life-threatening complications such as encephalopathy, cardiopulmonary complications, or renal failure.

Diagnostics of *Loa loa* infection

Classical microscopy is the gold standard diagnostic in clinical routine and directly detects peripheral microfilaraemia. Concentration techniques with lysing solutions or filtration techniques importantly improve sensitivity in individuals with low levels of microfilaraemia [70–72]. The smartphone-based automated reader (LoaScope) has shown to be a useful test for ruling out *L. loa* hyper-microfilaraemia in onchocerciasis control programs [73–75]. However, its diagnostic accuracy for individual case management has not yet been established.

Treatment of loiasis

There are several drugs with known activity against *L. loa*. Albendazole is thought not to directly kill microfilariae but rather to suppress embryogenesis and release of microfilariae. This leads to a relatively slow, gradual and safe reduction of microfilaraemia. Albendazole has been shown to be of limited efficacy when used for less than one week. However when given for three to six weeks, albendazole has a profound effect on microfilaraemia [76,77]. Due to these parasitological effect, albendazole therapy is recommended for the reduction of microfilaraemia prior to administration of DEC for curative effect or ivermectin [78,79].

Treatment with ivermectin in a single dose of 150 to 200 µg/kg has a rapid microfilaricidal effect leading to an approximately 80% reduction in *L. loa* microfilaremia. However, ivermectin use is associated with treatment-related encephalopathy in subjects with a high level of *L. loa* microfilarial burden [20,80–82]. Due to its specific action on microfilariae, ivermectin is thought not to exert effect on the signs and symptoms linked to the migration of adult worms.

However, data suggest a limited effect on clinical symptoms [83], but this need to be further evaluated.

Diethylcarbamazin (DEC) – the gold standard for curative treatment of loiasis – is the only drug that has specifically been developed to treat filarial infections. DEC exerts microfilaricidal and aduticidal activity against *L. loa* leading to a cure rate of around 40-80% of patients after three weeks of treatment. Commonly, more than one course of DEC therapy is needed for complete cure [113,114,115,116]. A significant proportion of patients with high microfilaraemia experiences neurological adverse events such as encephalopathy. Thus, CDC recommends its use only in individuals with microfilaraemia below 2,000/ml. Due to the potential safety issues, DEC treatment is rarely employed in endemic regions for the treatment of loiasis where reinfection is common.

Other therapeutic interventions include mechanical extraction of adult worms [88] which do not lead to a cure due to the multiplicity of infections and the significant risk of reinfection. For subjects with loiasis hyper-microfilaraemic, aphresis has been used to safely reduce mechanically microfilarial loads before the initiation of antifilarial drug [89–94]. Another therapeutic approach for excessive hyper-microfilaraemia is the use of host-directed therapies including adjunct glucocortocoide therapy.

Prevention of loiasis

There is to date no effective strategy or tool available to control Chrysops populations in regions of loiasis transmission (144). It is unclear whether classical repellents, including DEET and icaridine, confer protection against bites from *Chrysops spp.*. Limiting outdoor exposure during daytime and use of protective clothing are therefore currently the only effective ways to prevent individuals from infectious bites.

5. Discussion

Mapping, identification of at-risk subjects and evaluation of therapeutic interventions are crucial for effective disease control. To improve our understanding of these important aspects the effect of antimalarial therapeutic combinations on concomitant urogenital schistosomiasis was evaluated in semi-urban areas of Gabon and the effectiveness of different therapeutic regimens based on albendazole alone or in combination with ivermectin for the reduction of *L. loa* microfilaraemia in adults was assessed in rural areas of Gabon. We were also interested in the diagnostic aspect of *L. loa* microfilaraemia given that the choice of treatment for loiasis depends on the level of *L. loa* microfilaraemia and the need for a reliable, rapid diagnostic assay. An additional aspect of this work was to provide epidemiological data on the helminth species that prevail in the Gabon region which is an important information for the effective conduct of treatment and control programs.

Schistosomiasis treatment study

Here, we were foremost interested in the potential benefit of the use of artemisinin-based combination therapies (ACTs) on the reduction of *Schistosoma* egg excretion. We found that schistosomiasis and malaria are co-endemic in Lambaréné and surrounding villages with a prevalence of 16% for schistosomiasis among malaria patients. Moreover, most of the malaria patients were pre- and school-aged children. This indicates that malaria and urogenital schistosomiasis are not only co-endemic but mainly affect as well the same paediatric patient population. This finding is in agreement with other data previously published [95].

In our study, the real burden of urogenital schistosomiasis among subjects with uncomplicated malaria may even have been underestimated since the diagnostic method used in this project – urine microscopy – might have not been sufficiently sensitive. There are other methods which are more sensitive including molecular diagnostic techniques which could be used in future studies to improve the precision of measuring the true prevalence of schistosomiasis.
The result of our analysis also demonstrated moderate efficacy of ACTs (egg reduction rate of approximately 65 % for artemether–lumefantrine and artesunate–pyronaridine) at 6 weeks post malaria treatment. Importantly, some patient treated with artesunate–pyronaridine completely cleared their egg excretion at 6-weeks post malaria treatment. Since urogenital schistosomiasis accounts for high morbidity, especially in children, and on the other hand, malaria is a major

cause of childhood morbidity and mortality in the SSA region, this result seems to show that the use of ACT for the treatment of uncomplicated malaria could assist in reducing the morbidity associated not only with malaria but also with urogenital schistosomiasis. Indeed, it is reported that children can developed more than three malaria episodes per year in endemic areas of Gabon [Dejon et al; *Schistosoma haematobium* infection seems to be associated with an increased risk for new malaria episodes in children living in rural areas around Lambaréné, Gabon; oral communication]. These findings are in line with previous reports but add important information for the new ACT artesunate-pyronaridine [96,97].

The artesunate-amodiaquine and artefenomel-ferroquine treatment groups did not contribute a lot to this analysis as the number of patients was very limited. Further data are therefore necessary to appreciate their comparative anti-schistosomal potential *in vivo*. The design of this study was inadequate to answer this question, as a conclusion of the comparative activity of respective ACTs could not be supported by a statistical test due to the lack of randomization and the relatively small number of subjects. However, based on our data the use of ACTs could have a collateral benefit on urogenital schistosomiasis. Thus, further data are needed on the impact of repeated use of ACTs on the transmission of schistosomiasis in area where the two diseases are co-endemic to assess this collateral benefit. There is a lack of knowledge about the effect of malaria itself on *Schistosoma* egg excretion mainly due to the fact that malaria constitutes an acute illness resulting in symptoms that requires prompt treatment. Further research is needed to understand whether malaria may directly influence *Schistosoma* egg excretion. Other limitations are the lack of schistosome egg viability assessment after drug exposure, robust diagnostic tools and absence of some physical exercise before collecting the urine samples – which may further increase the sensitivity of egg detection in urine. Other laboratory techniques including detection of specific antigens such as circulating anionic antigen (CAA) and the circulating cathodic antigen (CCA) could help in the future to understand the potential stage specificity of the activity of drugs on juvenile or adult developmental forms.

Loiasis treatment study

The main aim of this clinical trial was to evaluate whether an albendazole-based treatment regimen could be used to safely reduce microfilaraemia below a level necessary to sustain onwards transmission in patients residing in a high transmission setting. We therefore assessed the parasitological outcome of three albendazole based regimens alone or associated with ivermectin in comparison with a control group. Albendazole treatment for five weeks and albendazole administered for three weeks followed by a single dose of ivermectin were the most efficacious treatment regimens to reduce *L. loa* microfilaraemia below 100 mf/ml at 6 months after the initiation of treatment. This finding indicated that albendazole could be used in most of the cases to reduce *L. loa* microfilaraemie before initiation of other drugs such as ivermectin. Furthermore, the AUC of the level of microfilaraemia indicated that the 5-week albendazole and the 3-week albendazole plus a single dose of ivermectin regimens led to the most consistent reduction of microfilaraemia over time.

Similar findings were reported by other authors previously, although in different settings and with different treatment objectives [76,78,79]. Three weeks albendazole alone had a similarly strong action as a long course while the treatment was still administered. However, in our study microfilaraemia increased in the shorter course regimen subsequently. A similar finding was also reported by Kamgno et al. and could indicate that a short course of albendazole below 4-5 weeks is not able to permanently sterilise or kill adult worms. The addition of a single dose of ivermectin has a similar effect as prolongation of albendazole therapy alone for another 2 weeks and was similarly not associated with complete suppression of microfilaraemia. Contrarily to this finding a cohort of patients with imported loiasis in Italy, where reinfection of individuals was no longer possible, showed complete cure with an albendazole-ivermectin based regimen [79].

All treatment regimens were safe and well tolerated with a few grade I adverse events possibly related to study drugs reported. One patient was hospitalised due to fatigue and dizziness twenty days after the first administration of albendazole. This incident was however judged as not related to the study drug due to underlying alcohol withdrawal syndrome. However, a potential role of the drug cannot be completely excluded as well as an influence of the spontaneous course of disease as spontaneous encephalopathy in the absence of any treatment have been described previously [28,98,99].

In our study a transient increase of liver enzymes was observed during the treatment period with a return to normal levels after the end of the treatment period. This is in line with previous

reports for the use of albendazole for other indications [76,77,100–102]. A similar observation was made for eosinophil counts which is ascribed to the natural course of disease after initiation of effective anthelmintic therapy.

This proof-of-concept study was limited by its design – descriptive as it was designed with no pre-defined statistical hypothesis to be tested at a frequentist's level of statistical significance. This approach was chosen to limit the overall sample size for this first proof of concept study which had as its main objective to systematically describe the effect of the treatment regimen over a 6-months period. No further conclusions may be drawn for a longer duration of the effect beyond the 6-month follow-up period due to lack of longer follow-up. Interestingly, only a fraction of participants has reached the pre-specified threshold of 100mf/ml, which was set as a measure to reduce onwards transmission of loiasis. Further epidemiological and experimental data on the infectivity of loiasis at respective microfilarial loads are required to corroborate robust endpoints for clinical trials aiming at reducing loiasis transmission in highly endemic regions [103]. The assessment of *L. loa* microfilaraemia from both venous and peripheral blood samples is another limitation of this study as we have shown previously that capillary measurements are higher than those for venous blood samples. However, based on the natural diurnal variation of microfilarial load and the consistent blood drawing schedule between treatment groups, no systematic confounding was introduced between treatment regimens. Finally, the evaluation of treatment response from a patient's perspective should be performed in future evaluations of individual treatment outcomes as there is a complete lack in our understanding how these treatments affect individual wellbeing and alleviation of signs and symptoms.

<u>Loiasis diagnostic study I</u>

In this study, which aimed to investigate the role of blood sampling on the diagnostic features of microscopy, we observed that microscopically-determined microfilaraemia is higher in capillary blood samples than in venous blood samples for *L. loa* infection and *M. perstans* infection. This indicated that capillary blood was shown to be more sensitive for detection of *L. loa* and *M perstan* microfilaraemia than venous blood specially for the low level of microfilaraemia. Similar findings were reported by other authors previously, although in different disease pathogen and with different among of blood collected [43,104].

A sensitive and accurate diagnosis of loiasis is the key to choosing an adequate treatment or for exclusion of hypermicrofilaraemic individuals in onchocerciasis controls programs using

ivermectin mass drug administration. Considering an 35% increase in quantification of *L. loa* microfilaraemia by using capillary blood, an important proportion of patients may be mistreated if venous blood is used for laboratory analysis. For patient safety, it may be necessary to define a level of parasitemia depending on the blood collection route given that many studies on antimicrobial chemotherapy often rely on the exact quantification of microbial blood density in study participants. In addition, the use of capillary blood rather than venous blood avoids venipuncture and any discomfort at the puncture site. Furthermore, for community-based diagnostic, using capillary blood is less time-consuming and resource-intensive.

It needs to be mentioned, that the probability of microfilaraemia detection by light microscopy does not only potentially depend on the type of blood sample (i.e. capillary or venous) used for diagnostic purposes, but also on the quantity of the investigated sample volume. This study only investigated 10 microliters of blood according to the standard operational procedure used at CERMEL for diagnosis of malaria. This may be a limitation, as higher blood quantities (e.g. 50 microliters) lead to higher diagnosis sensitivity. Recent studies evaluating diagnostic performance of thick blood smear of 50 microliters compared to 10 microliters using fresh blood did however not confirm this hypothesis [105]. A strength of the study was that the exact amount of blood and same procedure were used for both CAP and VEN blood samples. In addition, the microscopic analysis of slides was performed by masked microscopists.

Loiasis diagnostic study II

The main aim of this study was to assess whether rapid staining techniques could assist to simplify the diagnosis of blood filarial pathogens by shortening the turnaround time of a diagnostic test. Field's stain is a staining technique that has been used for the microscopic detection of malaria parasites for several decades. We therefore investigated whether this staining technique could also be used for detection of filarial pathogens. Microscopic diagnostics based on Field's stain compared to conventional Giemsa staining method showed similar performance for *L. loa* microfilaraemia detection and quantification. This high concordance with conventional Giemsa staining makes Field's stain an attractive option for rapid diagnosis, which may be particularly useful for population-based screening programs. Importantly, microscopic screening for high microfilarial loads as required for treatment decisions for loiasis or for exclusion of hypermicrofilaraemic individuals in onchocerciasis

controls programs using ivermectin mass drug administration, shows almost perfect concordance thus making Field's stain a viable option in this setting.

A somehow different result was observed for *M. perstans* microfilaraemia. The detection rate for *M. perstans* was lower for blood slides stained with Field's stain compared with Giemsa staining. This finding may be explained by the shorter length and particularly the thinner diameter of *Mansonella spp.* compared with *L. loa.*

In conclusion Field's stain and conventional Giemsa staining lead to good visualization of the morphology of microfilariae, allowing species differentiation and both staining techniques allow differentiation of microfilariae with certainty.

This diagnostic study had some limitations. Patients were selected for the presence of signs and symptoms suggestive for loiasis rather that randomly selected from the community therefore increasing the pre-test probability of positive test results. However, this setting is representative of most clinical routine settings where diagnostic tests are performed based on clinical suspicion for a particular disease. At least in theory, it may also be possible that the probability of microfilaraemia detection by light microscopy potentially depends not only on the type of staining method but also on the order of slide sampling. This was not evaluated in our study and we assume that the order of slide preparation was randomly distributed thus minimizing a potential influence on the outcome of this study.

Review of clinical characteristics and management of loiasis

We performed a narrative review on the current state of our understanding of loiasis in both endemic regions and when detected as an imported infection outside of sub-Sahara Africa. There is not a lot of research activities conducted on the topic of loiasis without connection to others filariasis diseases. Loiasis affects mostly rural population in west and central Africa. The data reveal a significant disease burden and the disability-adjusted life years lost due to loiasis is similar to others helminth infections such as schistosomiasis. Moreover, high levels of microfilaraemia are associated with the occurrence of organ damage and an increased risk of death. Despite its limited geographic distribution, the burden of loiasis is high and we argue that loiasis needs to be recognised as disease with public health importance on its own and added to the WHO's list of Neglected Tropical Diseases.

The clinical presentation of loiasis was traditionally grouped into the parasitological categories occult and microfilaraemic loiasis. However, our n ewly proposed distinction of “migratory loiasis” characterised by history of eye worm migration or Calabar swelling with or without accompanying microfilaraemia and “non-migratory loiasis” characterised by the presence of microfilaraemia without a history of eye worm migration or Calabar swelling correctly describes more aptly the subjective clinical penetrance of loiasis.

Diagnostics of loiasis remains a matter of concern. Conventional microscopy constitutes the diagnostic standard in clinical routine for detection of peripheral microfilaraemia but it is not capable of detecting adult worms, and thus to prove infection in occult loiasis. A suitable tool for the diagnosis of occult loiasis is therefore still needed. Moreover, to date there is not accurate tool for the rapid detection of loiasis in community case management. The control of loiasis is also greatly associated to treatment and vector control. Several publications examined the issue of loiasis treatment. Although the treatment protocol of loiasis described by CDC seems to be sufficiently efficacious for travellers returning from endemic areas, this treatment protocol has not yet shown its efficacy in endemic regions where re-infection is common. Indeed, the treatment of loiasis is associated with adverse reactions which can be life-threatening or which may even lead to death. The prevention of loiasis based on control of *Chrysops spp* did not yet show clear prospects. However, in general some progress has been made to understand loiasis, although there are still gaps to be filled, mapping the disease epidemiology with standardized tools, improving the control of related disease morbidity, and the control of loiasis itself.

6. Conclusion

At the end of this thesis, we are confident that our work will contribute to providing new knowledge on the therapeutic and diagnostic aspects of schistosomiasis and loiasis. Artemisinin-based combination therapies are effective in reducing *S. haematobium* egg excretion following the treatment of uncomplicated malaria suggesting therefore some collateral benefit of antimalarial drugs in treating malaria as both malaria and schistosomiasis are co-endemic and affect the same population. However, additional work in larger sample size and adequately designed clinical trials still needs to be done to further characterize the effect of artemisinin-based combination alone or associated with praziquantel on subjects infected with *S. haematobium*. Moreover, the effect of the repeated use of artemisinin-based

combination therapies on *Schistosoma* egg excretion requires evaluation in areas where both diseases are co-endemic.

The treatment regimens, albendazole 5-weeks or 3-weeks followed by a single dose of ivermectin were most efficacious to reduce *L. loa* microfilaraemia without any safety concern potentially allowing for its use for loiasis control. The prolonged treatment regimen may however constitute an important obstacle in population-based control programs. Future studies investigating the potential of other drugs have to be conducted using regimens with shorter treatment duration.

Knowing that the level of *L. loa* microfilaraemia is an important determinant to choose the adequate treatment regimen, comparison of microfilaraemia level in blood from capillary and venous blood shows that higher microfilaraemia counts are found in capillary than in venous blood. Furthermore, Field's stain as rapid staining method has shown excellent diagnostic performance for *L. loa* microfilariae detection compared to conventional Giemsa staining. Diagnostic methods for *L. loa* detection still need to be evaluated as some subjects only harbour adult worms and to date there is no available diagnostic method for detection of adult worms.

In summary, loiasis is a complex infectious disease infecting patients residing mostly in rural of west and central Africa. The clinical feature of loiasis is variable and may present with the classical signs of eye worm migration or transient Calabar swellings but may include common, unspecific symptoms or rare but potentially life-threatening complications. Loiasis remains difficult to diagnose, treat, and control due to a lack of reliable point-of-care diagnostic assays, safe and efficacious drugs, and cost-effective prevention strategies.

7. Abbreviations

ACT	Artemisin Base Combination
AE	Adverse Event
ALB	Albendazole
AUC	Area Under the Curve
CAP	Capillary
CDC	Center for Disease Control
D	Day
DEC	DiEthylCarbamzine
CERMEL	Centre de Recherches Medicales de Lambaréné
CI	confidence interval
DALYs	Disability Adjusted Life Years
IQR	Inter Quantile Range
IVM	Ivermectin
M	Month
Mf	Microfilariae
ml	milliliter
NTDs	Neglected Tropical Diseases
OR	odds ratios
RAPLOA	Rapid Diagnostic Test for Loa loa
SAE	Serious Adverse Event
SSA	sub-Sahara African
VEN	Venous
WHO	World Health Organisation

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9. Publications

9.1 Publication I

Effectiveness of antimalarial drug combinations in treating concomitant urogenital schistosomiasis in malaria patients in Lambaréné, Gabon: A non-randomised event-monitoring study.
Zoleko-Manego R, Okwu DG, Handrich C, Dimessa-Mbadinga LB, Akinosho MA, Ndzebe-Ndoumba WF, Davi SD, Stelzl D, Veletzky L, Kreidenweiss A, Nordmann T, Adegnika AA, Lell B, Kremsner PG, Ramharter M, Mombo-Ngoma G. PLoS Negl Trop Dis. 2022 Oct 31;16(10):e0010899. doi: 10.1371/journal.pntd.0010899. PMID: 36315579; PMCID: PMC9648843.

9.2 Publication II

Efficacy, safety, and tolerability of albendazole and ivermectin based regimens for the treatment of microfilaraemic loiasis in adult patients in Gabon: A randomized controlled assessor blinded clinical trial.

Rella Zoleko-Manego, Ruth Kreuzmair, Luzia Veletzky, Wilfrid Ndzebe- Ndoumba, Dorothea Ekoka Mbassi, Dearie G. Okwu1, Lia B. Dimessa-Mbadinga-Weyat, Roselyne D. Houtsa-Temgoua, Johannes Mischlinger, Matthew B. B. McCall, Peter G. Kresmner, Selidji T. Agnandji, Betrand Lell, Ayola A. Adegnika, Ghyslain Mombo-Ngoma, Michael Ramharter. PLoS Negl Trop Dis 17(8): e0011584. https://doi.org/10.1371/journal. pntd.0011584

9.3 Publication III

Diagnostic performance of capillary and venous blood samples in the detection of Loa loa and Mansonella perstans microfilaraemia using light microscopy.
Mischlinger J, Manego RZ, Mombo-Ngoma G, Ekoka Mbassi D, Hackbarth N, et al. (2021). PLoS Negl Trop Dis. 2021 Aug 16;15(8):e0009623. doi: 10.1371/journal.pntd.0009623. PMID: 34398886; PMCID: PMC8389422.

9.4 Publication IV

Performance of Field's Stain Compared with Conventional Giemsa Stain for the Rapid Detection of Blood Microfilariae in Gabon.

Mbassi FE, Mombo-Ngoma G, Ndoumba WN, Yovo EK, Eberhardt KA, Mbassi DE, Adegnika AA, Agnandji ST, Bouyou-Akotet MK, Ramharter M, Zoleko-Manego R.. Am J Trop Med Hyg. 2022 Jul 5;107(2):383–7. doi: 10.4269/ajtmh.22-0061. Epub ahead of print. PMID: 35895407; PMCID: PMC9393457.

9.5 Publication V

The African eye worm: current understanding of the epidemiology, clinical disease, and treatment of loiasis.

Ramharter M, Butler J, Mombo-Ngoma G, Nordmann T, Davi SD, Zoleko Manego R. Lancet Infect Dis. 2023 Oct 16:S1473-3099(23)00438-3. doi: 10.1016/S1473-3099(23)00438-3. Epub ahead of print. PMID: 37858326.

10. Deutsche Zusammenfassung

Die Kontrolle von Wurminfektionen, unter anderem der Schistosomiasis und der Loiasis, bleibt eine Herausforderung in vielen Ländern in Sub-Sahara-Afrika (SSA). Schistosomiasis und Malaria infizieren dieselben Patienten in vielen endemischen ländlichen Gebieten gleichzeitig, und es wurde gezeigt, dass Antimalariamedikamente zur Behandlung unkomplizierter Malaria eine gewisse Wirkung gegen *Schistosoma haematobium* haben. Bei der Loiasis ist die Kontrolle der *Loa loa*-Infektion derzeit durch die wenigen Medikamente mit nicht genau beschriebener Wirksamkeit und das Risiko schwerwiegender Nebenwirkungen bei Patienten mit hoher Mikrofilarienbelastung begrenzt. Derzeitige angepasste und sichere Behandlungsschemata zur Reduzierung der Mikrofilarämie in Endemiegebieten wurden in Endemiegebieten nicht etabliert. Darüber hinaus hängt die Wahl des Behandlungsschemas zur Behandlung von *Loa loa* vom Grad der Mikrofilarämie ab.

Ziel dieser Arbeit war es daher, die Wirkung von Malariamedikamenten, die bei unkomplizierter Malaria bei Erwachsenen und Kindern verabreicht werden, auf die gleichzeitige urogenitale Schistosomiasis zu bewerten, die Wirksamkeit verschiedener Therapieschemata zur Reduzierung der *Loa-loa*-Mikrofilarämie zu untersuchen und erstmals verschiedene diagnostische Methoden zum Nachweis und zur Quantifizierung der *L. loa*-Mikrofilarämie zu evaluieren.

In Gabun wurden zwei klinische Studien zur Therapie und zwei Querschnittsstudien zur Diagnose der Loiasis durchgeführt und am Ende eine Übersichtsarbeit zur Klinik und Therapie der Loiasis publiziert. 38 Malaria-Teilnehmer mit gleichzeitiger urogenitaler Schistosomiasis wurden mit Antimalaria-Wirkstoffkombinationen (Artesunat-Pyronaridin und Artemether-Lumefantrin) behandelt und 6 Wochen lang zum Nachweis der Ausscheidung von Schistosoma-Eiern beobachtet. 39 Teilnehmer mit *L. loa*-Mikrofilarämie wurden mit einer auf Albendazol basierenden Therapie oder einem Placebo behandelt und anschließend 6 Monate lang nachbeobachtet. Antimalariabehandlungen mit Artesunat-Pyronaridin und Artemether-Lumefantrin konnten die Ausscheidung von *S. haematobium*-Eiern um 65 % reduzieren. Die 5-wöchige Behandlung mit Albendazol oder eine 3-wöchige Behandlung mit Albendazol gefolgt von Ivermectin waren mit 90 % nach Verabreichung der Arzneimittel am wirksamsten bei der Reduzierung der Mikrofilarämie.

Für diagnostische Studien wurde ein dicker Tropfen von 10 Mikrolitern Kapillar- und Venenblut angefertigt und parallel mit der Field-Färbung als Schnellfärbetechnik und der herkömmlichen Giemsa-Färbung gefärbt. Ein qualitativer und quantitativer Vergleich der Mikrofilarämie wurde in Proben durchgeführt, die mit Field-Färbung und Giemsa-Färbung gefärbt wurden. In die jeweiligen Studien wurden 713 bzw. 175 Teilnehmer einbezogen. Die durchschnittlichen Werte der Mikrofilarämie von *L. loa* waren in kapillaren Blutproben signifikant höher als in venösen Blutproben. Die Field-Färbung zeigt im Vergleich zur Giemsa-Färbung hervorragende diagnostische Leistungsmerkmale für *L. loa* Mikrofilarämie.

Die Verwendung von ACTs zur Behandlung unkomplizierter Malaria zeigte einen zusätzlichen Nutzen bei der Reduzierung der Eizellen von *Schistosoma haematobium* und kann als ergänzendes Instrument zur Bekämpfung der Schistosomiasis eingesetzt werden. Behandlungsschemata auf Albendazol-Basis können in Kontrollprogrammen für Loiasis eingesetzt werden. Die im Kapillarblut im Vergleich zu venösem Blut festgestellten höheren Werte der L. loa-Mikrofilarämie könnten Auswirkungen auf die Behandlungsalgorithmen der Loiasis haben, für die eine genaue Quantifizierung der L. loa-Mikrofilarämie wichtig ist. Die Field-Färbung bietet eine schnelle Alternative zur Giemsa-Färbung zum Nachweis von L. loa-Mikrofilarien in dicken Blutausstrichen. Loiasis ist eine komplexe Infektionskrankheit, die erhebliche Morbidität verursacht und die Lebenserwartung verkürzt. Die klinischen Merkmale der Loiasis sind unterschiedlich; Unzureichende Diagnoseinstrumente und unbefriedigende Behandlungsmöglichkeiten erschweren das klinische Management. Weiterhin haben wir keine Interventionen, um Loiasis aus Regionen mit hoher Übertragungsrate sicher kontrollieren und schließlich eliminieren zu können.

11. English Summary

Control of helminth infections, including schistosomiasis and loiasis, remains a challenge in many sub-Sahara African (SSA) countries. Schistosomiasis and malaria co-infect the same patients in many endemic rural areas, and antimalarial drugs used to treat uncomplicated malaria has been shown to have some activity against *Schistosoma haematobium*. For loiasis, the control of *Loa loa* infection is currently limited by the few drugs with not well characterized efficacy and the risk of serious adverse effects in patients with high microfilarial loads. Current adapted and safe treatment regimens to reduce microfilaraemia in endemic areas have not been established in endemic areas. In addition, the choice of treatment regimen for treatment against *Loa loa* depends on the level of microfilaraemia.

The aim of this work was therefore to evaluate the effect of antimalarial drugs administered for uncomplicated malaria in adults and children on concomitant urogenital schistosomiasis, to evaluate the effectiveness of different therapeutic regimens to reduce *Loa loa* microfilaraemia and, for the first time, to evaluate different diagnostic methods for the detection and quantification of *L. loa* microfilaraemia and then to summarize our understanding on loiasis disease.

Two therapeutics clinical trials, two cross-sectional studies (diagnostic studies) have been conducted in Gabon and a review of literature on loiasis was performed at the end. 38 malaria participants with concomitant urogenital schistosomiasis were treated with antimalarial drug combinations (artesunate-pyronaridine and artemether-lumefantrine) and were followed for 6 weeks for detection of Schistosoma eggs excretion. 39 participants with *L. loa* microfilaraemia were treated with albendazole based regimen or placebo and then followed for 6 months. Antimalarial treatments with artesunate-pyronaridine and artemether-lumefantrine were able to reduce *S. haematobium* eggs excretion by 65%. The 5-week regimen of albendazole or a 3-week regimen of albendazole followed by ivermectin were most efficacious to reduce microfilaraemia at 90% following administration of drug. For diagnostic studies, either a thick smear of 10 microliters of capillary and venous blood was prepared, or each participant`s capillary thick smears were stained in parallel with Field`s stain as a rapid staining technique and conventional Giemsa stain. Qualitative and quantitative comparison of microfilaraemia was made in sample stained with field`s stain and Giemsa staining. 713 and 175 participants respectively were included in the respective studies. The average levels of microfilaraemia of *L. loa* was significantly higher in capillary blood samples than in venous blood samples. Field's stain shows excellent diagnostic performance characteristics for *L. loa* microfilariae compared with Giemsa staining.

ACTs use for the traitment of uncomplicated malaria shown a collateral benefit on schistosoma haematobium egg reduction, and can be use as complementary tool for schistosomiasis control. Albendazole based treatment regimens may be used in control programs for loiasis. The higher levels of *L. loa* microfilaraemia detected in capillary blood compared to venous blood may have an implication for treatment algorithms of loiasis, for which accurate quantification of *L. loa* microfilaraemia is important. Field's stain offers a rapid alternative to Giemsa stain for detection of *L. loa* microfilariae in thick blood smears. Loiasis is a complex infectious disease which causes important morbidity and reduce life expentancy. Clinical features of loiasis are variable; inadequate diagnostic tools and unsatisfactory treatment options complicate clinical

management. Importantly, we still have no tool to control and eventually eliminate loiasis from high transmission regions safely.

12. Declaration of own contribution to the publications

I, declare hereby that I contributed as described below to the following publications:

1-Effectiveness of antimalarial drug combinations in treating concomitant urogenital schistosomiasis in malaria patients in Lambaréné, Gabon: A non-randomised event-monitoring study.
Zoleko-Manego R, Okwu DG, Handrich C, Dimessa-Mbadinga LB, Akinosho MA, Ndzebe-Ndoumba WF, Davi SD, Stelzl D, Veletzky L, Kreidenweiss A, Nordmann T, Adegnika AA, Lell B, Kremsner PG, Ramharter M, Mombo-Ngoma G. PLoS Negl Trop Dis. 2022 Oct 31;16(10):e0010899. doi: 10.1371/journal.pntd.0010899. PMID: 36315579; PMCID: PMC9648843.

I have been involved in this project since I drafted the study protocol. Followingly, I have been in charge of the project during preparation and data collection. I organized logistics (materials and organization at CERMEL), developed and wrote study documents, trained team members, lead the team, conducted the field work, including informed consent, provision of treatment, performed data clearance, developed the database, did data entry and quality control, performed statistical analysis and wrote the publication. This work was done under the supervision of Professor Ghyslain Mombo-Ngoma.

2- **Efficacy, safety, and tolerability of albendazole and ivermectin based regimens for the treatment of microfilaraemic loiasis in adult patients in Gabon: A randomized controlled assessor blinded clinical trial**
Rella Zoleko-Manego, Ruth Kreuzmair, Luzia Veletzky, Wilfrid Ndzebe- Ndoumba, Dorothea Ekoka Mbassi, Dearie G. Okwu1, Lia B. Dimessa-Mbadinga-Weyat, Roselyne D. Houtsa-Temgoua, Johannes Mischlinger, Matthew B. B. McCall, Peter G. Kresmner, Selidji T. Agnandji, Betrand Lell, Ayola A. Adegnika, Ghyslain Mombo-Ngoma, Michael Ramharter. PLoS Negl Trop Dis 17(8): e0011584. https://doi.org/10.1371/journal. pntd.0011584

I have been involved in this project since I drafted the study protocol. Followingly, I have been in charge of the project during preparation and data collection. I organized logistics (materials and organization at CERMEL), developed and wrote study documents, trained team members, lead the team, conducted the field work, including informed consent, provision of treatment, follow-up visit, performed data clearance, developed the database, did data entry and quality

control, performed statistical analysis s and wrote the publication. This work was done under the supervision of Professor Michael Ramaharter.

3-**Diagnostic performance of capillary and venous blood samples in the detection of Loa loa and Mansonella perstans microfilaraemia using light microscopy.**
Mischlinger J, Manego RZ, Mombo-Ngoma G, Ekoka Mbassi D, Hackbarth N, et al. (2021). PLOS Neglected Tropical Diseases 15(8): e0009623. doi.org/10.1371/journal.pntd.0009623

In this project, I have been in charge of the project during data collection. I developed and wrote study documents and trained team members, lead the team. I was involved in the fieldwork, including inclusion of participants, blood withdrawal, preparation of thick blood smears, providing malaria treatment. Further I reviewed the manuscript. This work was done with collaboration of Johannes Mischlinger.

4- **Performance of Field's Stain Compared with Conventional Giemsa Stain for the Rapid Detection of Blood Microfilariae in Gabon.**
Mbassi FE, Mombo-Ngoma G, Ndoumba WN, Yovo EK, Eberhardt KA, Mbassi DE, Adegnika AA, Agnandji ST, Bouyou-Akotet MK, Ramharter M, Zoleko-Manego R. Am J Trop Med Hyg. 2022 Jul 5;107(2):383–7. doi: 10.4269/ajtmh.22-0061. Epub ahead of print. PMID: 35895407; PMCID: PMC9393457.

In this project, I have been involved in this project since I drafted the study protocol. I have been in charge to supervise a student (Franck Ekoka Mbassi) who joined our team in order to write his thesis. We developed and wrote study documents. I lead the team and supervised all the work. Further I reviewed the manuscript and provided major input. This work was done under the supervision of Professors Ghyslain Mombo-Ngoma and Michael Ramaharter.

5- **The African Eyeworm: Current Understanding of the Epidemiology, Clinical Disease, and Treatment of Loiasis.**
Ramharter M, Butler J, Mombo-Ngoma G, Nordmann T, Davi SD, Zoleko Manego R. Lancet Infect Dis. 2023 Oct 16:S1473-3099(23)00438-3. doi: 10.1016/S1473-3099(23)00438-3. Epub ahead of print. PMID: 37858326.
In this review, I reviewed the manuscript and provided major input. The final manuscript was approved by Professor Michael Ramaharter.

13. Acknowledgments

My greatest thanks go to all the participants included in these studies. In addition, I would like to underline my gratitude to those who accompanied me in the conduct of these projects: Hornella Ekoume Ngombibang, Willia carlene Bouanga, Roger Brothier Mouckoga, Suzane

Mengue. They collected the samples and proceeded to the treatment administration. They did not know how to count the time, only the accomplishment of the tasks which were theirs counted in their eyes, even the long distance to travel to patient house could not stop them. I had the chance to work so closely with you and I hope that we can still count on you for future projects.

A special thanks to Ruth Kreuzmair, who put so much passion into the loiasis treatment project I will never forget the effort and perseverance during the long nights of microscopy in addition to the work in the field.

I am deeply grateful to Michael Ramharter, who has been my supervisor throughout this period and gave me the opportunity to work on the therapeutic aspect in the context of clinical trials of these diseases that caught my attention. I am very grateful to my local supervisor, Ghyslain Mombo-Ngoma, for his support, the trust placed in me, the advice and the availability he has shown since my beginnings in his research group. Thanks for the support.

My thanks to all the co-directors of CERMEL for their commitment to maintain an environment to the development of researcher. A special thanks to all colleagues and particularly those from my research group led by Prof Ghyslain Mombo-Ngoma: To Wilfrid Nzebe Ndoumba, Dearie Okwu, thank you for your support and contribution to the realisation of this project.

Special thanks also go to Matthew McCall and Johannes Mischlinger for the cooperation and common interest in these projects.

Special thanks also go to all my family, especially to my parents (Zoleko André and Azamo Thérése) who motivated me and taught me that I can achieve what I want. To the Houtsa family who welcomed me and encouraged me to work in a research center. Finally, and above all, my deep gratitude goes to my sons who did not always understand why I have to work for so long, but it was the warmth of your presence that motivated me to continue.

To all of you and to so many others that I cannot name, thank you, it wouldn't have been possible without you.

Rella Zoleko-Manego, Hamburg 1st December 2023

14. Curriculum vitae

Personal details

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Education

2010, MD, University of health sciences, Libreville, Gabon

2015, University diploma, Method and practices in epidemiology, University of Bordeaux Segalen, Bordeaux-France
2016, University diploma, Statistical method in health, University of Bordeaux Segalen, Bordeaux-France
2017, Master of public health, University of Bordeaux Segalen, School of public health, Bordeaux-France

2019, PhD candidate, University Medical Center Hamburg-Eppendorf, Hamburg-German

Research position

2023 – ongoing Responsible to a research team at Sindara-Gabon, CERMEL - satellite site

2020 – ongoing Research fellow at Bernard Nocht Institute for Tropical Medicine, Department of Clinical ressearch, Hamburg, German

2017 – ongoing principal investigator at Centre de Recherches Médicales de Lambaréné, (CERMEL), Lambaréné, Gabon

2011 – 2017 investigator at Centre de Recherches Médicales de Lambaréné, (CERMEL) Lambaréné, Gabon

Funding

- EDCTP Grant RIA2017T-2018- WANECAM II project - Co-applicant
- EDCTP Grant RIA-101103053-eWHORM project - Co-applicant
- EDCTP Grant RIA 101103204-Integrate project- Co-applicant

Conferences attended and presentations given

- European Congress for Tropical Medicine and International Health (ECTMIH2) Utrecht 2023. Development of the combination KAF156 Plus Lumefantrine and contribution of CERMEL. Oral presentation

- 11th EDCTP ForumParis 2023. Chair of session A paradigm shift towards local and gender balanced leadership, promoting equitable partenerships and next generation African leaders, PamAfrica-Sindofo cases studies
- Trimester Scientific meeting held at Centre de Recherches Medicales de Lambaréné (CERMEL). Oral Presentation
- Weekly scientific meeting held at institute of Tropical Medicine University of Tübingen. Oral Presentation
- Weekly scientific meeting helf in Medicine for Pride (med4Pride) Group (AG Ghyslain Mombo-Ngoma) at centre de Recherches Medicales de Lambaréné (CERMEL). Oral presentation
- Eight SAPI congress, Libreville Gabon 2023. Effect of different albendazole-based treatment regimens on Loa loa microfilaraemia in an endemic region of Gabon: an open label randomised controlled clinical trial. Oral presentation
- 67th ASTMH annual meeting, Seatle 2022. Effect of different albendazole-based treatment regimens on Loa loa microfilaraemia in an endemic region of Gabon: an open label randomised controlled clinical trial. Poster presentation
- Malaria meeting, Hamburg 2019. safety and Efficacy of Ivermectin for the treatment of Plasmodium falciparum infections in asymptomatic Gabonese adults: Preliminary Result. Oral Poster presentation
- 67th ASTMH annual meeting, Washinton DC, 2019. Effect of different albendazole-based treatment regimens on Loa loa microfilaraemia in an endemic region of Gabon: an open label randomised controlled clinical trial: Preliminary result. Poster presentation
- HelmvitII congress, Lambarene, Gabon 2021. Growth, nutritional status and mortality with regard to birth weight of infants living in Gabon during their first year of life. Oral presentation
- Ninth EDCTP forum; Lisbon, Portugual 2018. Highly Parasitemic and Symptomatic Plasmodium falciparum in Gabonese Adolescents and Adults. Oral poster presentation

Course or workshop

- 01/2020 Basic Immunology and helminth immunology Medical Research Council/Uganda Virus Research Institute and LSTMH, Lambaréné-Gabon
- 09-10/2021 Research methodology and research protocol development, The Geneva Foundation for Medical Education and Research, Online
- 06/2023 Financial and project management. EDCTP3, Online
- 08/2023 Clinical traial and project management. Centre de Recherches Medicales de Lambaréné, Lambaréné-Gabon
- 10/2023 Leadership and skills management. Strathmore University Bussinnes shool Nairobi-Kenya

Major research activities

Current projects

Integrate, Capacity building lead
Starting date: 1 Februray 2023 / Duration: 60 months / EDCTP Grant agreement HORIZON-JU-RIA-101103204

eWHORM, Principal Investigator
Oxfendazole as pan-nematode drug to treat several helminth diseases, i.e. onchocerciasis, loiasis, mansonellosis and trichuriasis in adult male and non-pregnant female patients. Starting date: 1 April 2023/ Duration: 60 months / EDCTP Grant agreement HORIZON-JU-RIA-101103053

CADPT13A12201, Principal Investigator
(PLATINUM): A multi-part, multi-center PLATform study to assess the efficacy, safety, tolerability, and pharmacokinetics of anti-malarial agents administered as monotherapy and/or combination therapy IN patients with Uncomplicated Plasmodium falciparum Malaria. Started February 2023/ NOVARTIS study

M5717_MS201618_0033, Principal investigator
Phase IIa Proof of Concept, Multicenter, Randomized, Open-label Study to Evaluate the Safety, Efficacy, and Pharmacokinetics of the Combination M5717 plus Pyronaridine Administered once Daily for 1 or 2 Days to Adults and Adolescents with Acute Uncomplicated *Plasmodium falciparum* Malaria. Starting date 05 April 2022

WANECAM2, Site Principal Investigator and member of board
A phase II and III clinical trial program to assess safety, efficacy and transmission-blocking properties of the new antimalarial KAF156 combined with a new formulation of lumefantrine in children and adults with uncomplicated Plasmodium sp. malaria in West and Central Africa Starting date: 1 March 2019 / Duration: 60 months / EDCTP Grant agreement: RIA2017T-2018

ASAAP, Pharmacovigilance lead and safety monitor
assessing safety and tolerability of artemether-lumefantrine+atovaquone-proguanil tri-therapy for malaria treatment in adults and adolescents in Gabon.
Duration: 60 months / EDCTP Grant agreement: RIA2017MC-2022

MACOV, Investigator
Prevalence and impact of SARS-CoV-2 infection on maternal and infant health in African populations. Starting date:1 Jun 2020/ Duration: 24 months/EDCTP Grant agreement: RIA2020EF-2957

Completed projects

2018 – 2022 Investigator MAMAH study (funded EDCTP)

2020 – 2021 Data safety monitor board member of MULTIMAL trial, Pan African Clinical Trials Registry: PACTR202008909968293

2018 – 2021 Investigator NOVARTIS CKAF156A2202 study (Part B)

2019 – 2020 PI IVERCURE study, CERMEL, Pan African Clinical Trials Registry: PACTR201908520097051

2019 – 2020 investigator on SANOFI ACT14655 study

2018 – 2020 PI LoLotreat study, CERMEL, Pan African Clinical Trials Registry: PACTR201807197019027 (PhD project)

2018 – 2019 PI SCHISTO-ACT, CERMEL, clinicaltrials.gov registry: NCT04264130 (PhD project)

2018 – 2019 Investigator NOVARTIS KAE 609 study

2018 – 2019 Investigator Novartis CKAF156A2202 study (Part A)

2017 – 2019 Investigator phase III/IV SP-C-012-15 MMV/SHIN POONG PYRAMAX study

2018 – 2019 PI SANOFI DRI12805 study

2016 – 201 Investigator SANOFI DRI12805 study

2014 – 2015 Investigator MMV-OZ439-13-003

2014 – 2015 Investigator study JP017-FOSPIP, ClinicalTrials.gov: NCT02198807

2013 – 2014 Investigator RTSS-MAL55 study

2011 – 2013 Investigator MIPPAD study (funded EDCTP)

Scientific reviewer for the following journal

- Frontiere in Malaria

Member of the following committee

- CERMEL' institutional review Board
- SAPI (société Africain de pathologie infectieuse)

Major publications

1. Ramharter M, Butler J, Mombo-Ngoma G, Nordmann T, Davi SD, **Zoleko Manego R.** The African eye worm: current understanding of the epidemiology, clinical disease, and treatment of loiasis. Lancet Infect Dis. 2023 Oct 16:S1473-3099(23)00438-3. doi: 10.1016/S1473-3099(23)00438-3. Epub ahead of print. PMID:
2. Dorothea Ekoka Mbassi, Ghyslain Mombo-Ngoma,Jana Held, Dearie Glory Okwu, Wilfrid Ndzebe, Laura Charlotte Kalkman, Franck Aurélien Ekoka Mbassi, Lais Pessanha de Carvalho, Juliana Inoue, Malik Azeez Akinosho, Lia Betty Dimessa Mbadinga, Emmanuel Yovo, Benjamin Mordmüller, Peter Gottfried Kremsner, Ayôla AkimAdegnikai, Michael Ramharter, **Rella Zoleko-Manego**. Efficacité et sécurité de l'ivermectine pour le traitement des infections à Plasmodium falciparum chez les

adultes gabonais asymptomatiques, hommes et femmes – un essai clinique pilote randomisé, en double aveugle, contrôlé par placebo, monocentrique de phase Ib/IIa.

3. **Zoleko-Manego R**, Kreuzmair R, Veletzky L, Ndzebe-Ndoumba W, Ekoka Mbassi D, Okwu DG, Dimessa-Mbadinga-Weyat LB, Houtsa-Temgoua RD, Mischlinger J, McCall MBB, Kresmner PG, Agnandji ST, Lell B, Adegnika AA, Mombo-Ngoma G, Ramharter M. Efficacy, safety, and tolerability of albendazole and ivermectin based regimens for the treatment of microfilaraemic loiasis in adult patients in Gabon: A randomized controlled assessor blinded clinical trial. PLoS Negl Trop Dis. 2023 Aug 28;17(8):e0011584. doi: 10.1371/journal.pntd.0011584. PMID: 37639396; PMCID: PMC10491396.
4. Gansane A, Lingani M, Yeka A, Nahum A, Bouyou-Akotet M, Mombo-Ngoma G, Kaguthi G, Barceló C, Laurijssens B, Cantalloube C, Macintyre F, Djeriou E, Jessel A, Bejuit R, Demarest H, Marrast AC, Debe S, Tinto H, Kibuuka A, Nahum D, Mawili-Mboumba DP, **Zoleko-Manego R**, Mugenya I, Olewe F, Duparc S, Ogutu B: Randomized, open-label, phase 2a study to evaluate the contribution of artefenomel to the clinical and parasiticidal activity of artefenomel plus ferroquine in African patients with uncomplicated Plasmodium falciparum malaria. Malar J. 2023 Jan 3;22(1):2. doi: 10.1186/s12936-022-04420-2. PMID: 36597076 Free PMC article. Clinical Trial.
5. **Zoleko-Manego R**, Okwu DG, Handrich C, Dimessa-Mbadinga LB, Akinosho MA, Ndzebe-Ndoumba WF, Davi SD, Stelzl D, Veletzky L, Kreidenweiss A, Nordmann T, Adegnika AA, Lell B, Kremsner PG, Ramharter M, Mombo-Ngoma G: Effectiveness of antimalarial drug combinations in treating concomitant urogenital schistosomiasis in malaria patients in Lambaréné, Gabon: A non-randomised event-monitoring study. PLoS Negl Trop Dis. 2022 Oct 31;16(10):e0010899. doi: 10.1371/journal.pntd.0010899. eCollection 2022 Oct. PMID: 36315579 Free PMC article. Clinical Trial.
6. Pons-Duran C, Mombo-Ngoma G, Macete E, Desai M, Kakolwa MA, **Zoleko-Manego R**, Ouédragou S, Briand V, Valá A, Kabanywanyi AM, Ouma P, Massougbodji A, Sevene E, Cot M, Aponte JJ, Mayor A, Slutsker L, Ramharter M, Menéndez C, González R: Burden of malaria in pregnancy among adolescent girls compared to adult women in 5 sub-Saharan African countries: A secondary individual participant data meta-analysis of 2 clinical trials. PLoS Med. 2022 Sep 2;19(9):e1004084. doi: 10.1371/journal.pmed.1004084. eCollection 2022 Sep. PMID: 36054101
7. Veletzky L, Eberhardt KA, Hergeth J, Stelzl DR, **Zoleko Manego R**, Mombo-Ngoma G, Kreuzmair R, Burger G, Adegnika AA, Agnandji ST, Matsiegui PB, Boussinesq M, Mordmüller B, Ramharter M: Distinct loiasis infection states and associated clinical and hematological manifestations in patients from Gabon. PLoS Negl Trop Dis. 2022 Sep 19;16(9):e0010793. doi: 10.1371/journal.pntd.0010793. eCollection 2022 Sep. PMID: 36121900
8. Mbassi FE, Mombo-Ngoma G, Ndoumba WN, Yovo EK, Eberhardt KA, Mbassi DE, Adegnika AA, Agnandji ST, Bouyou-Akotet MK, Ramharter M, **Zoleko-Manego R**: Performance of Field's Stain Compared with Conventional Giemsa Stain for the Rapid Detection of Blood Microfilariae in Gabon. Am J Trop Med Hyg. 2022 Jul 5;107(2):383-7. doi: 10.4269/ajtmh.22-0061. Online ahead of print. PMID: 35895407

9. Dalimot JJ, Klei TRL, Beuger BM, Dikmen Z, Bouwman SAM, Mombo-Ngoma G, **Zoleko-Manego R,** Ndzebe-Ndoumba WF, Egée S, Kuijpers TW, Grobusch MP, van Bruggen R: Malaria-associated adhesion molecule activation facilitates the destruction of uninfected red blood cells. Blood Adv. 2022 Nov 8;6(21):5798-5810. doi: 10.1182/bloodadvances.2021006171. PMID: 35349634 Free PMC article.
10. Groger M, Tona Lutete G, Mombo-Ngoma G, Ntamabyaliro NY, Kahunu Mesia G, Muena Mujobu TB, Dimessa Mbadinga LB, **Zoleko Manego R**, Egger-Adam D, Borghini-Fuhrer I, Shin J, Miller R, Arbe-Barnes S, Duparc S, Ramharter M. Effectiveness of pyronaridine-artesunate against Plasmodium malariae, Plasmodium ovale spp, and mixed-Plasmodium infections: a post-hoc analysis of the CANTAM-Pyramax trial. Lancet Microbe. 2022 May 30: S2666-5247(22)00092-1. doi: 10.1016/S2666-5247(22)00092-1. Epub ahead of print. PMID: 35654079.
11. González R, Nhampossa T, Mombo-Ngoma G, Mischlinger J, Esen M, Tchouatieu AM, Pons-Duran C, Dimessa LB, Lell B, Lagler H, Garcia-Otero L, **Zoleko Manego R**, El Gaaloul M, Sanz S, Piqueras M, Sevene E, Ramharter M, Saute F, Menendez C: Evaluation of the safety and efficacy of dihydroartemisinin-piperaquine for intermittent preventive treatment of malaria in HIV-infected pregnant women: protocol of a multicentre, two-arm, randomised, placebo-controlled, superiority clinical trial (MAMAH project). BMJ Open. 2021 Nov 23;11(11):e053197. doi: 10.1136/bmjopen-2021-053197. PMID: 34815285.
12. Koehne E, Zander N, Rodi M, Held J, Hoffmann W, **Zoleko-Manego R**, Ramharter M, Mombo-Ngoma G, Kremsner PG, Kreidenweiss A: Evidence for in vitro and in vivo activity of the antimalarial pyronaridine against Schistosoma. PLoS Negl Trop Dis. 2021 Jun 24;15(6):e0009511. doi: 10.1371/journal.pntd.0009511. eCollection 2021 Jun. PMID: 34166393
13. Mischlinger J, **Manego RZ**, Mombo-Ngoma G, Ekoka Mbassi D, Hackbarth N, Ekoka Mbassi FA, Davi SD, Kreuzmair R, Veletzky L, Hergeth J, Ndoumba WN, Pitzinger P, Groger M, Matsiegui PB, Adegnika AA, Agnandji ST, Lell B, Ramharter M: Diagnostic performance of capillary and venous blood samples in the detection of Loa loa and Mansonella perstans microfilaraemia using light microscopy.. PLoS Negl Trop Dis. 2021 Aug 16;15(8):e0009623. doi: 10.1371/journal.pntd.0009623. eCollection 2021 Aug. PMID: 34398886
14. Adoke Y, **Zoleko-Manego R**, Ouoba S, Tiono AB, Kaguthi G, Bonzela JE, Duong TT, Nahum A, Bouyou-Akotet M, Ogutu B, Ouedraogo A, Macintyre F, Jessel A, Laurijssens B, Cherkaoui-Rbati MH, Cantalloube C, Marrast AC, Bejuit R, White D, Wells TNC, Wartha F, Leroy D, Kibuuka A, Mombo-Ngoma G, Ouattara D, Mugenya I, Phuc BQ, Bohissou F, Mawili-Mboumba DP, Olewe F, Soulama I, Tinto H; FALCI Study Group. A randomized, double-blind, phase 2b study to investigate the efficacy, safety, tolerability and pharmacokinetics of a single-dose regimen of ferroquine with artefenomel in adults and children with uncomplicated Plasmodium falciparum malaria. Malar J. 2021 May 19;20(1):222. doi: 10.1186/s12936-021-03749-4.
15. Koehne E, Kreidenweiss A, Adegbite BR, **Manego RZ**, McCall MBB, Mombo-Ngoma G, Adegnika AA, Agnandji ST, Mordmüller B, Held J. In vitro activity of eravacycline, a novel synthetic halogenated tetracycline, against the malaria parasite Plasmodium

falciparum. J Glob Antimicrob Resist. 2021 Mar; 24:93-97. doi: 10.1016/j.jgar.2020.11.024. Epub 2020 Dec 7. PMID: 33301999.

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17. Veletzky L, Hergeth J, Stelzl DR, Mischlinger J, **Manego RZ**, Mombo-Ngoma G, McCall MBB, Adegnika AA, Agnandji ST, Metzger WG, Matsiegui PB, Lagler H, Mordmüller B, Budke C, Ramharter M. Burden of disease in Gabon caused by loiasis: a cross-sectional survey. Lancet Infect Dis. 2020 Nov;20(11):1339-1346. doi: 10.1016/S1473-3099(20)30256-5. Epub 2020 Jun 22. PMID: 32585133.
18. **Zoleko-Manego R**, Mischlinger J, Dejon-Agobé JC, Basra A, Mackanga JR, Akerey Diop D, Adegnika AA, Agnandji ST, Lell B, Kremsner PG, Matsiegui PB, González R, Menendez C, Ramharter M, Mombo-Ngoma G. Birth weight, growth, nutritional status and mortality of infants from Lambaréné and Fougamou in Gabon in their first year of life. PLoS One. 2021 Feb 9;16(2):e0246694. doi: 10.1371/journal.pone.0246694. PMID: 33561169.
19. Matthewman J, **Manego RZ**, Dimessa Mbadinga LB, et al. A randomized controlled trial comparing the effectiveness of individual versus household treatment for Scabies in Lambaréné, Gabon. PLoS Negl Trop Dis. 2020;14(6):e0008423. Published 2020 Jun 26. doi:10.1371/journal.pntd.0008423
20. Bouwman SA, **Zoleko-Manego R,** Renner KC, Schmitt EK, Mombo-Ngoma G, Grobusch MP The early preclinical and clinical development of cipargamin (KAE609), a novel antimalarial compound. Travel Med Infect Dis. 2020 Jun 16:101765. doi: 10.1016/j.tmaid.2020.101765. Online ahead of print. PMID: 32561392
21. **Zoleko Manego R**, Koehne E, Kreidenweiss A, Nzigou Mombo B, Adegbite BR, Dimessa Mbadinga LB, Akinosho M, Matthewman J, Adegnika AA, Ramharter M, Mombo-Ngoma G. Description of Plasmodium falciparum infections in central Gabon demonstrating high parasite densities among symptomatic adolescents and adults. Malar J. 2019 Nov 21;18(1):371. doi: 10.1186/s12936-019-3002-9. PMID: 31752891
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23. Rada S, Gamper J, González R, Mombo-Ngoma G, Ouédraogo S, Kakolwa MA, **Zoleko-Manego R**, Sevene E, Kabanywanyi AM, Accrombessi M, Briand V, Cot M, Vala A, Kremsner PG, Abdulla S, Massougbodgi A, Nhacolo A, Aponte JJ, Macete E, Menéndez C, Ramharter M. Concordance of three alternative gestational age assessments for pregnant women from four African countries: A secondary analysis of the MIPPAD trial. PLoS One. 2018 Aug 6;13(8):e0199243. doi: 10.1371/journal.pone.0199243. eCollection 2018. PMID: 30080869

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25. Mischlinger J, Pitzinger P, Veletzky L, Groger M, **Zoleko-Manego R**, Adegnika AA, Agnandji ST, Lell B, Kremsner PG, Mombo-Ngoma G, Mordmüller B, Ramharter M Validity and reliability of methods to microscopically detect and quantify malaria parasitaemia. . Trop Med Int Health. 2018 Sep;23(9):980-991. doi: 10.1111/tmi.13124. Epub 2018 Jul 17. PMID: 29956431
26. Groger M, Veletzky L, Lalremruata A, Cattaneo C, Mischlinger J, **Zoleko-Manego R**, Endamne L, Klicpera A, Kim J, Nguyen T, Flohr L, Remppis J, Matsiegui PB, Adegnika AA, Agnandji ST, Kremsner PG, Mordmüller B, Mombo-Ngoma G, Ramharter M. Prospective Clinical Trial Assessing Species-Specific Efficacy of Artemether-Lumefantrine for the Treatment of Plasmodium malariae, Plasmodium ovale, and Mixed Plasmodium Malaria in Gabon. Antimicrob Agents Chemother. 2018 Feb 23;62(3):e01758-17. doi: 10.1128/AAC.01758-17. Print 2018 Mar.
27. **R. Zoleko Manego**, G. Mombo-Ngoma, M. Witte, J. Held, M. Gmeiner, T. Gebru, B. Tazemda,J. Mischlinger, M. Groger, B. Lell, A. A. Adegnika, S. T. Agnandji, P. G. Kremsner B. Mordmüller M. Ramharter and P. B. Matsiegui.Demography, maternal health and the epidemiology of malaria and other major infectious diseases in the rural department Tsamba-Magotsi, Ngounie Province, in central African Gabon. BMC public health(2017).17:130.DOI:10,11861s12
28. María Rupérez, Raquel González, Ghyslain Mombo-Ngoma, Abdunoor M. Kabanywanyi, Esperança Sevene, Smaïla Ouédraogo, Mwaka A. Kakolwa, Anifa Vala, Manfred Accrombessi, Valérie Briand, John J. Aponte, **Rella Manego Zoleko**, Ayôla A. Adegnika, Michel Cot, Peter G. Kremsner, Achille Massougbodji, Salim Abdulla, Michael Ramharter, Eusébio Macete, Clara Menéndez. Mortality, Morbidity, and Developmental Outcomes in Infants Born to Women Who Received Either Mefloquine or Sulfadoxine-Pyrimethamine as Intermittent Preventive Treatment of Malaria in Pregnancy: A Cohort Study. PlosMedicine 13(2):e 1001964. https://doi.org/10.1371/journal.pmed.1001964
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30. Ghyslain Mombo-Ngoma, Jean Rodolphe Mackanga, Raquel González, Smaila Ouedraogo, Mwaka A Kakolwa, **Rella Zoleko Manego**, Arti Basra, María Rupérez, Michel Cot, Abdunoor M Kabanywany, Pierre-Blaise Matsiegui, Seldiji T Agnandji, Anifa Vala, Achille Massougbodji, Salim Abdulla, Ayôla A Adegnika, Esperança

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36. Mesküre Capan-Melser, Ghyslain Mombo Ngoma, Daisy Akerey-Diop, Arti Basra, Heike Würbel, Mirjam Groger, Jean R Mackanga, **Rella Zoleko-Manego**, Ulla Schipulle, Julia Schwing, Felix Lötsch, Khalid Rehman, Pierre-Blaise Matsiegui, Selidji T Agnandji, Ayôla A Adegnika, Sabine Bélard, Raquel González, Peter G Kremsner, Clara Menendez, Michael Ramharter: Evaluation of intermittent preventive treatment of malaria against group B Streptococcus colonization in pregnant women: a

nested analysis of a randomized controlled clinical trial of sulfadoxine/pyrimethamine versus mefloquine. Journal of Antimicrobial Chemotherapy 02/2015; 70(6). DOI:10.1093/jac/dkv041

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41. Auer-Hackenberg L, Thol F, Akerey-Diop D, **Zoleko RM**, Rodolphe Mackanga J, Adegnika AA, Mombo-Ngoma G, Ramharter: Short report: premastication in rural Gabon--a cross-sectional survey. M. J Trop Pediatr. 2014 Apr;60(2):154-6. doi: 10.1093/tropej/fmt082. Epub 2013 Oct 3. PMID: 24097805
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15. Eidesstattliche Erklärung

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe. Ferner versichere ich, dass ich die Dissertation bisher nicht einem Fachvertreter an einer anderen Hochschule zur Überprüfung vorgelegt oder mich anderweitig um Zulassung zur Promotion beworben habe. Ich erkläre mich einverstanden, dass meine Dissertation vom Dekanat der Medizinischen Fakultät mit einer gängigen Software zur Erkennung von Plagiaten überprüft werden kann.

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