Schistosomiasis in Lambaréné and the surrounding areas

Understanding disease epidemiology to pave the way to improved control

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Jean Claude Dejon Agobé

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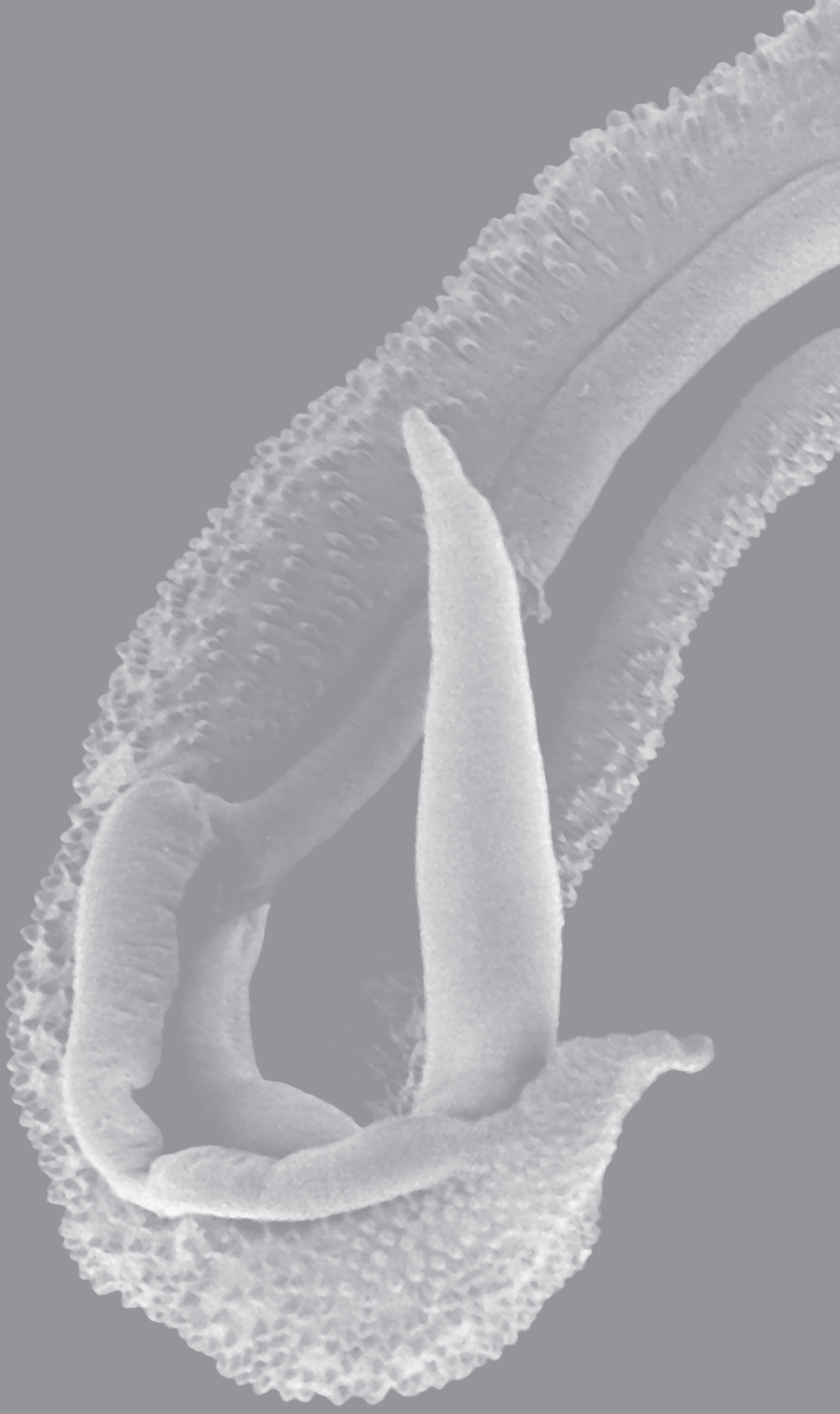

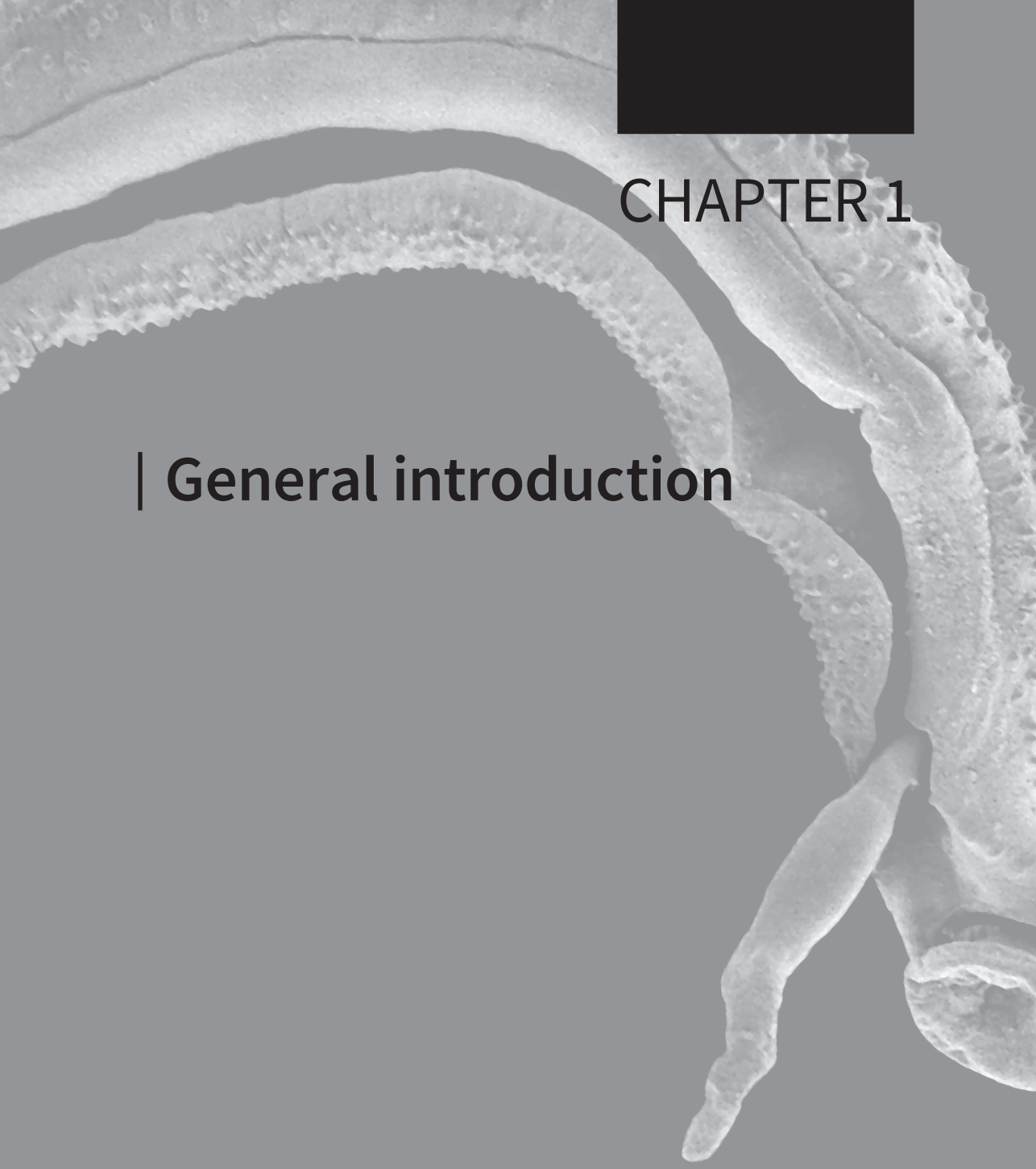

CHAPTER 1

General introduction

SCHISTOSOMIASIS: THE PROBLEM

Sir Francis Bacon recognised some 500 years ago that "*knowledge is power*". We understand that this is particularly true when aiming to fight infectious disease in its areas of endemicity. Knowledge on the distribution of the disease in the population, on its burden, but also on its risk factors should therefore provide the capacity and power to develop and implement adequate interventions to address the disease, permitting its control, its elimination, and ideally even its complete eradication. According to the Centers for Disease Control and Prevention (CDC), schistosomiasis is one of the two most devastating parasitic diseases in the world [1], and thus a public health issue of great interest. Schistosomiasis is prevalent in tropical and subtropical areas and affects the various layers of the population differently. Praziquantel (PZQ) is the principal drug recommended for the killing of adult, egg-producing *Schistosoma* spp. pathogenic to man, but is neither suitable for preventing infection or re-infection, nor for mitigating long-term sequelae of infection due to granuloma formation around disintegrated eggs. Moreover, to date, no vaccine against schistosomiasis exist. As re-infections are frequent in exposed populations, the World Health Organization (WHO) recommends exposure prevention and the use of PZQ for treatment and reduction of the disease burden through control programs. For the implementation of an efficient control program, epidemiological data are needed. Indeed, the affected population and risk factors for the disease need to be determined for optimal intervention design. The burden of disease should be assessed over time to determine the effect of the different interventions, and different treatment protocols should be evaluated to assess their relevance.

Schistosoma spp. infection and parasite life cycle

Five species of the genus *Schistosoma* are responsible for two major forms of the disease: *Schistosoma haematobium* is responsible for urogenital schistosomiasis; and *S. mansoni*, *S. guineensis, S. japonicum*, and *S. mekongi* are responsible for intestinal schistosomiasis. *S. intercalatum*, a variant of *S. guineensis*, is also responsible for intestinal schistosomiasis. As illustrated in Figure 1, *Schistosoma* eggs shed into freshwater by infected humans hatch in the following hours, particularly during daylight. In the case of *S. mansoni*, Maldonado and Acosta-Matienzo reported that 55 and 79% of eggs hatch during the first eight and 16 hours of their release into freshwater, respectively [2]. Hatching eggs will release miracidia, the free-swimming ciliated larvae, which are able to penetrate freshwater snail tissues in the five-to-six hours following their release. After two or three weeks of infection, snails will release cercariae, the larval form of the parasite, into freshwater. As reviewed by Braun et al., cercariae can live from ten to 40 hours under natural conditions, and can exceed 100 hours when the temperature is below 30°C [3]. Infection of humans occurs through contact with infested freshwater. Cercaria penetrate the human body through the skin, and turn into schistosomulae. This process is sometimes hallmarked by a 'swimmers itch'. The schistosomulae will mature into adults, mate, and become able to lay eggs approximately four-to-six weeks after migration into the blood stream (often leading to the seroconversion syndrome known as Katayama fever). The female adult worm will migrate preferentially into either the small blood vessels of the bladder for urogenital schistosomiasis; or into those of the intestine for intestinal

schistosomiasis, where the eggs will be laid. The laid eggs migrate passively through the wall of the respective small vessel and viscera to the organ lumen [4]. The patient will then release worm eggs either into the urine and/or into the stool. A period of approximately six weeks or more between cercarial penetration and the onset of egg production is generally observed. Transmission of the disease occurs when an infected human either urinates or defecates the eggs into freshwater, which forms the starting point of a new lifecycle of the parasite. In the human host, *Schistosoma* worms will live for an average of three to ten years, but in some cases alledgedly as long as 40 years [5], retaining their fertility and hence egg production capacity for theoretically at least a decade.

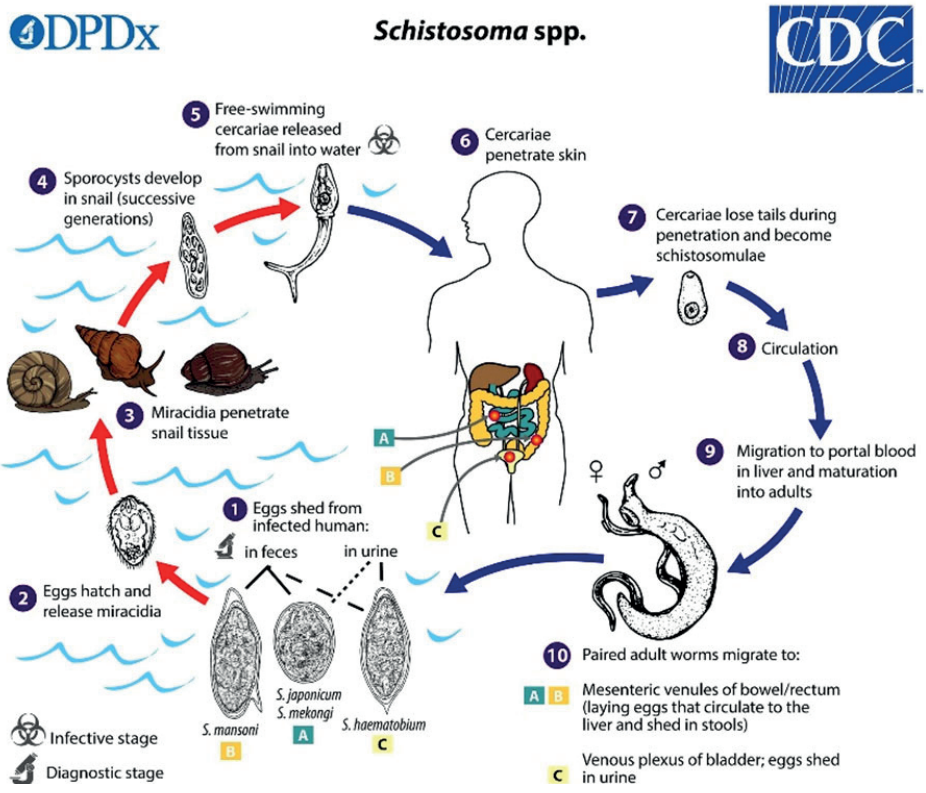

Figure 1: Schistosoma life cycle illustration from the Centers for Disease Control and Prevention (CDC).

Malacology

Biomphalaria and *Bulinus* are the two snail genera responsible for schistosomiasis in Africa [6]. The *Biomphalaria* genus responsible for *S. mansoni* is widely distributed in Africa, particularly *Bi. pfeifferi*, [7]. *Bulinus* for its part is reported to be the intermediate host of *S. haematobium* responsible for urogenital schistosomiasis, but also of *S. guineensis* and the related *S. intercalatum*, both responsible for intestinal schistosomiasis. Several *Bulinus* species act as intermediate hosts for the larval stage of those *Schistosoma* parasites. However, the role of each species in the transmission of the disease differs considerably between and within sub-Saharan countries [8,9]. The geographic distribution of the different snail species over the continent depends on the preferential biotope and climate of each snail species, and thus governs the distribution of the different forms of the disease across the continent. Indeed, if *S. guineensis* and the related *S. intercalatum* are mainly present in the rain forest areas of central Africa, *S. haematobium* and *S. mansoni* are widely but differently distributed throughout the continent. Snail control is one of the three pillars advised by the WHO for the control of schistosomiasis [10]. The control of snail populations using molluscicides or plants can indeed contribute to reducing the transmission of the disease among exposed populations. However, their use is complicated by their impact on the environment. *Phytolacca dodecandra* for instance, a native plant of sub-Saharan Africa and Madagascar traditionally used for various ailments and which has shown molluscicidal activity, is more toxic to other aquatic invertebrates and fish than to snails [11].

Epidemiology of schistosomiasis

Schistosomiasis ranks second only to malaria in the world as the most devastating parasitic disease [1]. Schistosomiasis transmission has been reported in 78 countries, particularly in tropical and subtropical areas [10]. In 2019, the WHO reported that more than 230 million people were at risk of the disease, with 90% of them are living in Africa [10], showing that the African region bears the brunt of the disease burden. In endemic areas, low socio-economic level communities without or with limited access to safe water and adequate sanitation are especially affected due to their exposition to the parasites. Children, particularly school-aged children, are known to be most affected due to their play habits. Adults, particularly women, are mainly infected during their household and/or agricultural activities [10].

The disease and its pathogenesis

Schistosomiasis is a parasitic disease caused by blood flukes of the genus *Schistosoma*. Acute schistosomiasis occurs after the primary infection and is characterised by Katayama syndrome, occurring in many patients within 14 to 84 days after infection, and is encountered with infections by all species, but most frequently by *S. mansoni* and *S. japonicum*. The Katayama syndrome, which includes fever, cough, abdominal pain, diarrhea, hepatosplenomegaly and eosinophilia, is a systemic hypersensitivity reaction against the migrating schistosomulae and eggs. In chronic schistosomiasis, the symptomatology is often less overt and mainly due to eggs laid by adult female worms in the blood stream and present in the tissue of the bladder for urogenital schistosomiasis and the intestine for intestinal schistosomiasis. Anaemia and its

associated consequences, particularly in children, can be explained by the blood lost during the excretion of the eggs into the lumen of the respective viscera (bladder or intestine) in both forms of the disease, but the remaining morbidity of schistosomiasis depends on the form of the disease. In intestinal schistosomiasis, the liver is the primarily affected organ. Eggs trapped in the liver tissue will die and ooze protein into their environment, a process which leads to granuloma formation responsible for scarring and fibrosis. Portal hypertension, esophageal varices and cardiac impairment can result and very often will lead to death. Urogenital schistosomiasis encompasses urinary and genital schistosomiasis. In urinary schistosomiasis, the urinary system is the main system affected, with haematuria as the main symptom. Spleen impairment and bladder cancer are reported as late consequences of the disease. However, genital schistosomiasis and particularly female genital schistosomiasis (FGS) seems to cause more morbidity. Considered as particularly neglected [12], FGS is caused by *Schistosoma* eggs trapped in the genital tissues. Its clinical manifestations include vaginal discharge, bloody discharge, bleeding after intercourse or 'spotting' (petechial bleeding), genital itching or a burning sensation and pelvic pain or pain during or after intercourse [13]. Contact bleeding which increases the risk of HIV transmission, infertility, abortion or ectopic pregnancy, involuntary urination when coughing, laughing or jumping, genital ulcers and tumors or swelling (vulva, vagina, cervix) are common complications of the disease [13] with social impact on the affected women. Indeed, social exclusion, marital discord, depression and stigma have been reported to be associated with the disease. In male genital schistosomiasis (MGS), schistosome eggs can be found in the semen [14]. Similarly to FGS, eggs entrapped in genital organs are responsible for the clinical manifestations of MGS. These include pelvic; coital, or ejaculatory pain, haematospermia, erectile dysfunction, abnormal swelling of genital organs and infertility.

Schistosomiasis as a co-infection

Schistosomiasis is endemic in areas generally also endemic for many other parasitic and viral infections. Schistosomiasis is a disease with a strong effect on the immune system, particularly during the chronic phase. In the chronic phase, schistosomiasis is very often found in conjunction with acute diseases such as malaria, or other chronic diseases such as other helminth (soil-transmitted helminths, filariases) or viral (HIV) infections. This immune response to schistosomiasis explains the clinical interaction between schistosomiasis and other diseases in case of co-infection. Similarly, the immune response to schistosomiasis also explains the deleterious effect of schistosomiasis on vaccine efficacy as recently reported in our community [15]. In case of co-infection, schistosomiasis is very often a pre-existing infection and can decrease or increase the severity of the subsequent infection, as reviewed by Abruzzi and Fried [16]. In schistosomiasis-malaria coinfection, schistosomiasis has been reported in children to either confer protection against severe malaria [17] or to increases the prevalence of *Plasmodium* parasites [18], for instance.

Schistosomiasis treatment

PZQ is the currently recommended drug for the treatment of all forms of schistosomiasis, but is active only against the adult stage of the parasite. The known serum half-life of the drug is 0.8 to 1.5 hours in adults with normal renal and liver function, and 4 to 5 hours for its metabolites. The WHO considers the efficacy of PZQ as satisfactory if the egg reduction rate is equal to, or more than 90% [19]. For the treatment of schistosomiasis, the WHO recommends a dosage of 60 mg per kilogram of body weight once for the treatment of infection with *S. japonicum*, and 40 mg per kilogram of body weight once for infection with other species. As re-infection is frequent amongst populations exposed to the disease, the WHO recommends a MDA campaign of PZQ once or twice a year, depending to the local prevalence of the disease, with the main objective of reducing this prevalence and of controlling the morbidity of the disease [10]. Indeed, a recent spatiotemporal modelling study on the preventive chemotherapy effects of PZQ on schistosomiasis reported a considerable decrease in disease prevalence from 23% in 2010-2015 to 9.6% in 2015-2019, particularly for *S. haematobium* infection among school-aged children in sub-Saharan Africa, which, according to the authors, is most likely explained by the scale-up of preventive chemotherapy [20].

Gabon

Gabon is a country on the west coast of central Africa, located on the equator, and with just over two million inhabitants in 2021. The country borders with three countries: Equatorial Guinea to the northwest, Cameroon to the north and Republic of Congo to the east and south, as well as to the Atlantic in the west. The country counts nine administrative provinces, including Moyen Ogooué province, with Lambaréné as its capital, which was our area of interest in the frame of the present work. The country is irrigated and drained by several large rivers constituting three main basins which cover almost the whole country: the Ogooué basin, the Nyanga basin, and the Komo basin. The Ogooué's hydrographic basin (223.856 km^2) constitutes the largest one, draining about 80% of the country's territory into the 1200 km long Ogooué river, the main river of the country. The Ivindo (500 km long) and the Ngounié (300 km long) rivers are its main tributaries. The Ogooué river crosses the entire country at its centre from east to west, cutting through five provinces and past several towns, such as Lambaréné, where the river is over half a kilometer wide [21]. Lambaréné is a semi-urban area located partly on an island in the Ogooué river, and along of the banks of the river in the west of the country, 78 km south of the equator. Lambaréné is surrounded by rural settlements, all located along the N1 national road either north or south of the town. Lambaréné and surrounding areas are irrigated by several tributaries of the Ogooué river, and are characterised by the presence of many lakes, streams, and temporary ponds, making the area a favourable ecosystem for snail development. These hydrographical conditions render the region favourable for schistosomiasis and could explain the distribution of the disease across the country.

Figure 2: Map displaying the nine provinces of Gabon (in red) with their capitals indicated by red dots. The red squares represent the capital Libreville and Port-Gentil which are the political and economic hubs of the country, respectively. In bleu, the main rivers and lacks of the country with their respective name.

Schistosomiasis in Gabon and Lambaréné

Gabon is known to be endemic for schistosomiasis, with the first cases having been reported already in the 1960s [22]. While mainly *S. haematobium* is reported in Gabon, some cases of intestinal schistosomiasis have recently been reported from across the country. One case of *S. mansoni* and one case of *S. guineensis* were reported in the northern region of the country in 2018 [23], while four cases of *S. mansoni* were reported that same year in a neighborhood of Libreville, the capital city of the country [24], for instance. In Lambaréné, some cases of *S. mansoni* have been reported while the town and surrounding areas are known to be mainly endemic for urogenital schistosomiasis [25]. Although there is longstanding evidence for the presence of schistosomiasis in the country, the epidemiology of the disease is not clearly established and its burden has not been investigated very thoroughly. Nevertheless, some activity for the control of the disease has been implemented in the country, as Gabon has adhered to part of the WHO recommendations for the control of schistosomiasis. As the main activity to be implemented, MDA campaigns with PZQ were conducted across the country among school children during the past decade. No other actions have been implemented with respect to other recommendations for the control of the disease, neither snail control nor

WASH (water, sanitation and hygiene) activities, which form a set of WHO's recommendations for, amongst others, the prevention of NTDs such as schistosomiasis in endemic areas [26].

Scope and aims of the thesis

The present thesis focuses on the epidemiology of schistosomiasis in Lambaréné and surrounding areas, empirically known to be one of the main areas in the country endemic for schistosomiasis. Although some MDA campaigns with PZQ were implemented in the country and thus in this area for the control of morbidity of the disease as per WHO recommendations, the epidemiological situation of schistosomiasis is not well established. This makes it difficult to estimate the initial burden of the disease in the population, and subsequently to evaluate the impact of the control program. In particular, risk factors for exposure and the level of distribution of the disease in the population are not well known, the real burden of disease in the local population is not well enough documented, and the benefits of the use of PZQ for the treatment of the disease at an individual level or for the control of the disease at the community level have not yet been evaluated, for instance. In addition, not much, or for our area of interest no data at all, are available on the transmission of schistosomiasis. Taking the case of Lambaréné and surrounding areas, the aim of the present work was thus to describe the current epidemiology of schistosomiasis in the area of interest, including investigating the benefit of the use of PZQ in this area. We expect that our findings shall contribute insights into the state of schistosomiasis in the country. As specific aims of our work, we have:

i - Assessed the epidemiology of schistosomiasis in the region of interest. Here the objective was to assess the distribution of the disease within the local population, mapping its geographical distribution and to determine factors associated therewith. The prevalence of the disease is indeed an indicator for the optimal frequency of MDA with PZQ.

ii - Assessed the relationship between the local population and schistosomiasis. Indeed, we strongly consider that the population's knowledge of and their attitudes and practices towards the disease can provide relevant information for understanding the disease in the population, but also for its control.

iii - Provided some basic information on the transmission of the disease in the study area. Knowledge of the intermediate host of schistosomiasis, its geographical distribution and the dynamics of its population throughout the year can help to control the disease. Snail control is indeed one of the recommended axes for the control of schistosomiasis in endemic areas.

iv - Assessed the efficacy of PZQ for the treatment of schistosomiasis and the re-infection in the local population. The treatment of schistosomiasis is one of the main issues with regards to the morbidity of the disease, with PQZ being the only recommended drug. If its efficacy in killing the adult *Schistosoma* worm remains satisfactory in general, it is recommended to continually assess that efficacy over time and to assess any factor which could affect it.

v - Assessed the impact of schistosomiasis on *Plasmodium falciparum* infection. As both infections are endemic and probably the most prevalent parasitic infections in our area of interest, we found it relevant to assess the effect of schistosomiasis, very often diagnosed in the chronic phase, on *Plasmodium* spp. infection, generally known as an acute infection.

vi - Assessed the effect of urogenital schistosomiasis on the haematological parameters widely used for screening in the diagnosis of infectious diseases. Indeed, it has been reported that schistosomiasis can influence different haematological cell counts. We therefore found it relevant to assess the haematological profile of patients with schistosomiasis.

vii - Summarised the state of schistosomiasis research in the region in particular, and in the country in general. Based on online scientific publications, many scientific activities seem to be conducted around schistosomiasis in Gabon. We took advantage of the present work to summarise the research activity on this disease in the country over the two last decades. We also looked at the activities implemented in the country for the control of the disease in the same period of time.

At the end of our work, we expect to considerably contribute to improving knowledge on the epidemiology of schistosomiasis in our area of interest in particular, and in the country in general. The new knowledge we expect to contribute on the state of the disease in the country will help, we hope, to improve the control of the morbidity of the disease in our population.

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CHAPTER 2

Epidemiology of schistosomiasis and soil-transmitted helminth coinfections among schoolchildren living in Lambaréné, Gabon

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ABSTRACT

Schistosomiasis is a parasitic infection highly prevalent in Central Africa where it is co-endemic with many other parasitic infections, including soil-transmitted helminths (STHs). For its optimal control, there is a need of descriptive epidemiological data for each endemic region. The objective of the present study was to determine the epidemiological situation around schistosomiasis in Lambaréné, Gabon. A cross-sectional study was conducted among schoolchildren. One urine sample per day was collected on three consecutive days for the diagnosis of schistosomiasis using a urine filtration technique. One stool sample was collected for the detection of *Schistosoma* spp. and STH spp. eggs using the Kato–Katz technique, and for larvae, using the coproculture technique. A total of 614 schoolchildren were included in the analysis. The overall prevalence of schistosomiasis and STH infections was 26% (159/614) and 15% (70/473), respectively. Human–freshwater contact was the main risk factor for schistosomiasis in the area (relative risk (RR) = 2.96, [2.20–4.00], P < 0.001). Hematuria (RR = 5.53 [4.30–7.10], P < 0.001) and proteinuria (RR = 2.12 [1.63–2.75], P < 0.001) as well as infection with *Trichuris trichiura* (RR = 1.86 [1.33–2.61], P = 0.002) and *Ascaris lumbricoides* (RR = 1.96 [1.19–3.21], P = 0.039) were associated with an increased risk of schistosomiasis. *Trichuris trichiura* was the highest prevalent STH species in the area. Our study reports a moderate prevalence for schistosomiasis with human–water contact as the main risk factor, whereas the prevalence of STH infections appears to be low. Our results stress the need for the implementation of WHO recommendations for schistosomiasis control.

INTRODUCTION

Schistosomiasis is the most devastating parasitic disease worldwide, second only to malaria.[1] According to the WHO, an estimated 207 million people may have schistosomiasis in the world.[2] Of these, more than 90% live in sub-Saharan Africa (sSA),2 which is among the parts of the world where the greatest number of soil-transmitted helminth (STH) infections occurs[3] and where preschool-age and school-age children are particularly exposed to STHs. Indeed, with a just more than 271 million children exposed to schistosomiasis in 2018, the WHO consider Africa second to Southeast Asia as the part of the world which requires most of the billion-preventive chemotherapy for soil-transmitted helminthiases needed globally.[4] The main species of STHs that infect humans are Ascaris lumbricoides, Trichuris trichiura, and hookworm (Necator americanus and Ancylostoma duodenale).

In sSA, two species of schistosomiasis are predominant: *Schistosoma haematobium*, causing urogenital schistosomiasis, and *Schistosoma mansoni*, causing intestinal schistosomiasis. In addition, *Schistosoma intercalatum* and *Strongyloides guineensis*, particularly prevalent in Central Africa, are causes of rectal schistosomiasis. For all types of schistosomiasis, the morbidity depends on population factors. As for STH infections, school-age children and young adults seem to bear the brunt of the disease.[5] In these subpopulations, anemia and malnutrition, among other consequences, are very often reported to be associated with the disease.[6]

Schistosomiasis involves human contact with infested freshwater. The occurrence of the disease is, therefore, highly related to the environment and population behaviors, and the epidemiology will, thus, widely vary from one region to another. Indeed, absence of safe water, lack of sanitation, and culture-associated behavioral patterns can increase the risk of being infected in some subpopulations. For disease prevention, the WHO recommends a universal approach which includes access to safe water and improvement of sanitation and hygiene education (WASH), in addition to mass drug administration (MDA) with praziquantel (PZQ) and snail control.[7] Mass drug administration is recommended for areas with moderate or high schistosomiasis prevalence, and the application frequency depends on the prevalence of infection.[8] This underpins the need to assess the disease prevalence for each particular endemic region.

In Gabon, schistosomiasis cases have been reported already in the 1960s.[9] The disease is present in almost the whole country.[10] The country is also endemic for STH infections. A national baseline mapping for schistosomiasis and STH infections initiated by the government in 2014 showed a low prevalence of schistosomiasis in the northern and eastern health regions of the country.11 For the control of schistosomiasis and STH infections, Gabon has opted for national campaigns of MDA of PZQ and albendazole among schoolchildren. The only known national campaigns of PZQ conducted before the present study which covered Lambaréné town took place in 2016. Lambaréné is the capital of Moyen-Ogooué, one of the nine provinces of Gabon, located centrally in the country and is being known to be endemic for STH infections[12] and schistosomiasis, with *S. haematobium* as the main Schistosoma species encountered.[13,14]

Whereas epidemiological data are available for STH infections,[12] little is known about the descriptive epidemiology of schistosomiasis, particularly on the disease's exact prevalence. Understanding the epidemiological situation of schistosomiasis in this semi-urban area and the transmission dynamics over time are of importance, the prevalence of the infection being an indicator of MDA frequency. The objective of the present study was, therefore, to describe the epidemiology of schistosomiasis in Lambaréné, including its coinfection with STHs.

MATERIALS AND METHODS

Study area. Lambaréné is a semi-urban area with 44,000 inhabitants reported in 2016.[15] The town is 240 km distant by road in a southerly direction from Libreville, capital of Gabon, and situated 100 km south of the equator. The Ogooué River, the main river of the country, is fed by several streams and traverses the town, forming many ponds and lakes, which offer favorable conditions for freshwater snail development. Previous reports have demonstrated the presence of *S. haematobium* in the area.[13,14] There is some evidence of the presence of intestinal schistosomiasis, too.[10,16] Despite a municipal water supply network provided by the National Society of Water and Electricity (Société d'Electricité et d'Eau du Gabon), large parts of the population do not have access to safe piped or pump water because of insufficient capacity of the company. Indeed, small streams, tributaries to the Ogooue River, are used more by a part of the local population for water supply, fishing, and household work, and by children mainly for playing. Lambaréné is also known to be endemic for intestinal helminths.[12]

Study population and sampling. A cross-sectional study was conducted from April to July 2016. The target population was primary schoolchildren from Lambaréné. Among the 26 primary schools across Lambaréné, a total of 17 primary schools were asked to participate in the study. These included all governmental primary schools (n = 8), all confessional primary schools (n = 6), and three randomly selected private primary schools. The schools were distributed across the town. Selection of participants was carried out using a 2-stage cluster sampling procedure. At the first sampling stage, one class batch was selected at random for each academic level (5-year levels) and for each school. For each class selected, all children were invited to participate in the study. The legal representatives were visited at home, and informed consent was requested from them to enable their child to participate in the study. Among those children whose parents granted informed consent, around 10 children were included per class. For the classroom with a size equal to or less than 10 students, all students for whom the parents provided the informed consent were included.

Sample size calculation. A study conducted earlier in Zilé-PK villages, a rural area close to Lambaréné, reported a schistosomiasis prevalence of 45%.[17] For Lambaréné as a semi-urban area, we hypothesize a one-third decrease in schistosomiasis prevalence, compared with that in the rural Zilé-PK villages setting. Thus, to be able to detect a variation in prevalence of at least 15%, with 95% confidence and 0.05 precision using a formula reported elsewhere,[18] we needed to include at least 323 volunteers in the present study.

Data collection. Study nurses collected demographic data (age, gender, and address) and anthropometric parameters (weight, height, and mid-arm brachial circumference) at school before blood sampling. To inquire about human–water contact and potential treatment received (PZQ and anthelminthic drugs) during the past 6 months, trained field workers applied a standardized questionnaire to participants. For very young children for whom the interviewer judged the veracity of the answer as doubtful, the questions were asked to parents or the primary caretaker at home and the answers weighted. In addition, the interviewers collected schools' and participant houses' global positioning system (GPS) coordinates, using handheld GPS monitors. All data collected were transcribed into the case report form (CRF) and then digitalized.

Sample collection and laboratory examinations. The research team provided eligible participants with plastic containers and invited them to provide three urine samples on three consecutive days and one stool sample. Samples were collected at school. For each urine sample, urine filtration was performed for the detection of *S. haematobium* eggs using a micro-filter membrane of 12 µm (Whatman type) as described elsewhere.[19] Furthermore, 11 urine parameters, including erythrocyte and protein, were assessed by urine dipstick (Combur [10] Test). We used the Kato–Katz technique for the detection of eggs of STHs in stool samples as described elswhere.[20] Also, we used the coproculture technique for the detection of hookworm *(N. americanus)* and *S. stercoralis* larvae. In detail, approximately 10 mg of sieved stool was collected using a spatula and transferred onto a slide, which was three-time wound with adsorbent tissue and placed inside a petri dish. Twenty milliliters of tap water weres then added into the petri dish such that the tissue paper was just soaked and the water did not cover the stool preparation. The petri dish was then placed in an incubator for 7 days with the temperature set at 22–28°C. Following this incubation period, 10 mL of liquid obtained from the petri dish by using a syringe was passed through a micro-filter membrane of 10–12 µm. The membrane was then transferred onto a glass slide, mounted on a microscope, and read using a low-power objective (×10) of a light microscope. The slide reading was performed by two independent experienced readers. The result was reported as the number of larvae per 10 mg of stool after calculating the mean larvae count obtained from the pooled results of
both readers. In the case of a quantitative (difference ≥ 20%) or a qualitative discrepancy between both readers, a third independent reader was required, and the mean of the two closest results was considered as the definitive result.

For all examinations performed, the result was recorded in the CRF and then digitalized. Results of laboratory examinations were reported back to the parents or legal representatives of each participant. Participants found infected were treated with 40 mg/kg of PZQ once for schistosomiasis and/or 400 mg of albendazole once a day during three consecutive days for STH infection. For other infections when indicated, participants were referred to an appropriate healthcare center.

Statistics. Data were collected and managed using the REDCap electronic data capture tool[21] hosted at Centre de Recherches Médicales de Lambaréné (CERMEL) and exported into R software (v. 3.2.4, R core team, Vienna, Austria) for statistical analysis. Age was used as the categorical variable. The hemoglobin level threshold to define anemia was set as defined by the WHO for sea level.[22] To determine child nutritional status, the BMI-for-age Z-score for children and adolescents aged from 5 to 19 years was applied, as recommended by the WHO.[23] Intensity of schistosomiasis and STH infections were defined as described elsewhere.[24] Quantitative and normal distributed variables were summarized by mean and SD. Qualitative data were summarized by prevalence and 95% CI. The chi-squared test of independence was performed to assess risk factors and factors associated with schistosomiasis. The level of statistical significance was set as $P < 0.05$.

Ethics. The study protocol was approved by CERMEL's scientific committee (SCR-Number: 2016-01) and by the Institutional Ethic Committee of CERMEL (CEI-CERMEL 002/2016). Agreement to conduct the study in schools was obtained from the administrative authorities. The study waws conducted in line with the Good Clinical Practice principles of the International Conference on Harmonization[25] and the Declaration of Helsinki.[26]

RESULTS

Study population. A total of 670 participants consented in writing to participate in the study. Among them, 629 provided urine, stool, and/or blood samples. In the present analysis, we included only those 614 participants with known schistosomiasis status (Figure 1). As depicted in Figure 2A, the study population distribution was homogeneous across the study area. The mean (SD) age of our study population was 10.9 ± 2.7 years, with a 0.95 female-to-male ratio. Normal values of nutritional status were reported for 81% (95% CI: 77.6–84.0) of the study population, whereas 25% (95% CI: 21–28) had a hemoglobin level less than 11g/dL. Hematuria and proteinuria were present in 21% (95% CI: 18–25) and 20% (95% CI:16–23) of the study population, respectively, whereas 10% (95% CI: 7–12) presented with both proteinuria and hematuria. Nearly half of the study population (44% [95% CI: 40–48]) had declared having had freshwater contact. Ten per cent (95% CI: 8–13) and 22% (95% CI: 19–26) of our study population have declared having taken PZQ and treatment for STH infection (albendazole) in the last six months before the beginning of the study, respectively (Table 1).

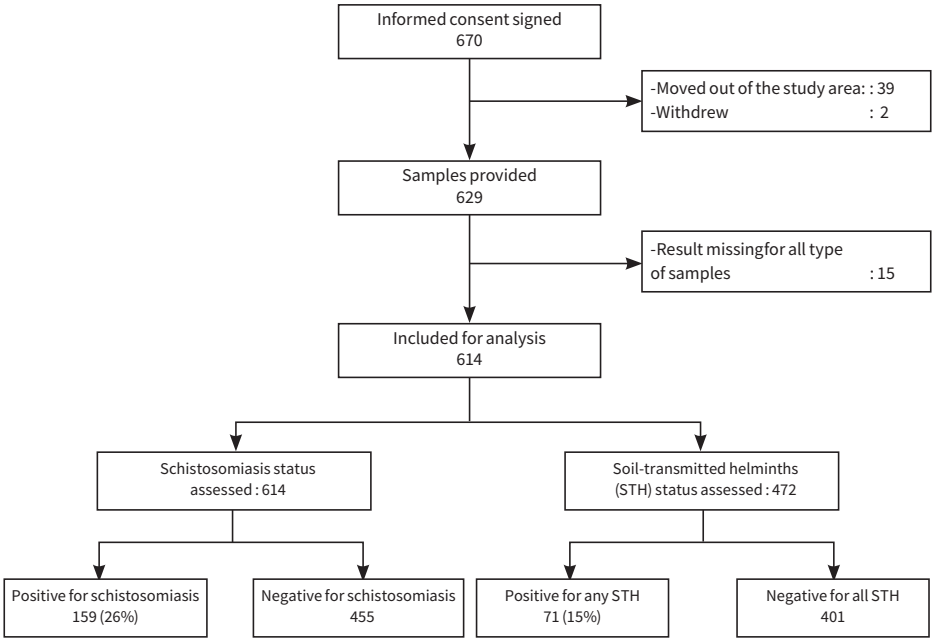

Fig 1: Study flowchart representing the number of participants considered at different study time points.

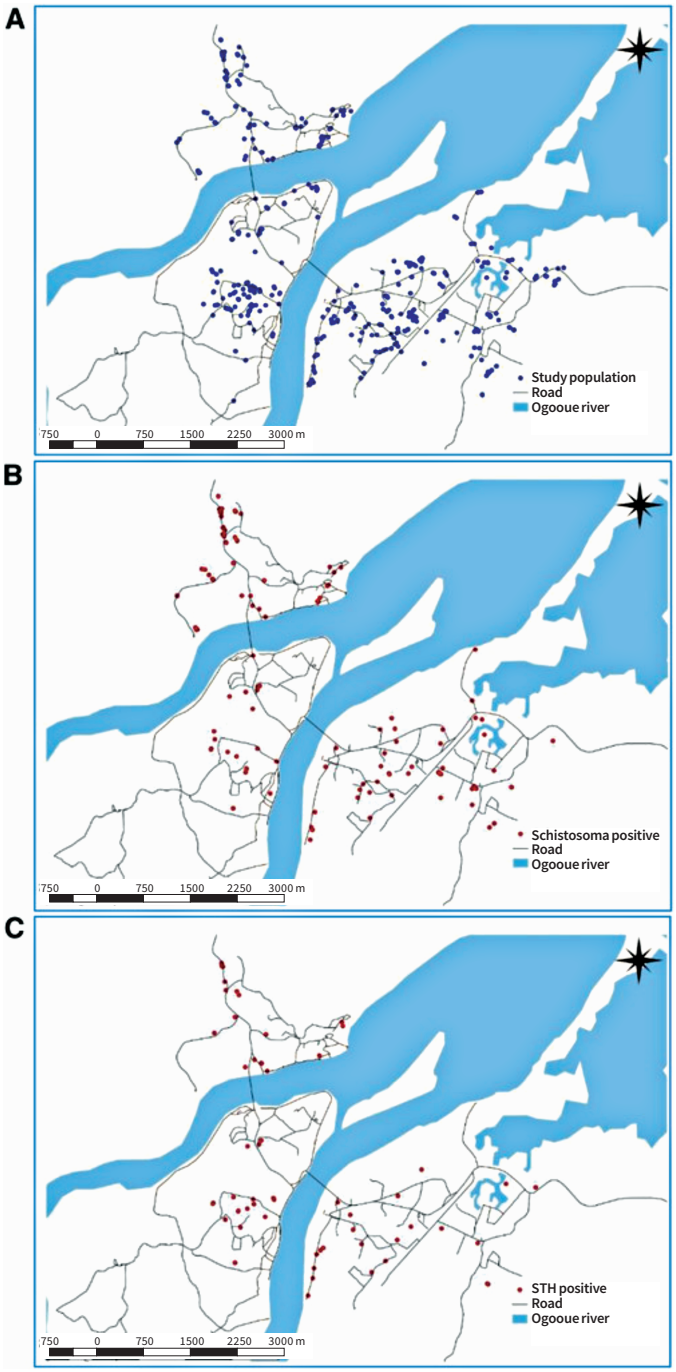

Fig 2: Geographical distribution of study participants using QGIS software version 3.2.2 (Free Software Foundation, Boston, MA); (A) overall study population, (B) participants infected with schistosomiasis, and (C) participants infected with soil-transmitted helminths (STH).

Table 1: Characteristics of the 614 study participants, April to July 2016, Lambaréné, Gabon.

	Study population distribution		
	n	(%)	$_{95\%}$CI (%)
Gender			
Female	300	(48.9)	[44.8 - 52.9]
Male	314	(51.1)	[47.1 - 55.2]
Age			
[5-10[	271	(44.1)	[40.2 - 48.2]
[10-15[	307	(50.0)	[46.0 - 54.0]
[15-19]	36	(05.9)	[04.1 - 08.0]
Nutritional status, BMI-for-Age$			
Thinness	33	(05.4)	[03.8 - 07.5]
Normal	493	(81.0)	[77.6 - 84.0]
Overweight	57	(09.4)	[07.2 - 11.9]
Obese	26	(04.3)	[02.8 - 06.2]
Primary school level			
1st year	127	(20.7)	[17.5 - 24.1]
2nd year	116	(18.9)	[15.9 - 22.2]
3rd year	118	(19.2)	[16.2 - 22.3]
4th year	133	(21.7)	[18.5 - 25.1]
5th year	120	(19.5)	[16.5 - 22.9]
Hemoglobin level (g/dl) $			
≥11	462	(75.5)	[71.9 - 78.8]
<11	150	(24.5)	[21.1 - 27.9]
Hematuria			
Yes	130	(21.3)	[18.1 - 24.8]
Proteinuria			
Yes	120	(19.5)	[16.5 - 22.9]
Stream contact declared			
Yes	266	(43.6)	[39.6 - 47.6]
History of praziquantel treatment*			
Yes	62	(10.1)	[07.9 - 12.9]
History of STH treatment*			
Yes	137	(22.3)	[19.2 – 26.0]

$ missing data: 5 for nutritional status and 2 for haemoglobin level
* taken in the last 6 months

***Schistosoma haematobium* infection prevalence and intensity.** As presented in Table 2, the overall prevalence of schistosomiasis was 26% (95% CI: 23–30). A total of 159 children of 614 who provided urine sample harbored *Schistosoma* spp. eggs, reflecting a prevalence of 26% (95% CI: 22–29) for urogenital schistosomiasis, whereas only three children of 472 who provided a stool sample had *Schistosoma* spp. eggs in the feces, yielding a prevalence of less than 1% (95% CI: 0–2) for potential intestinal or rectal schistosomiasis. Among the infected children, one harbored eggs in urine and stool concomitantly. For *S. haematobium* infection, 72% of 159 infected participants presented with light infection. The geographical distribution of cases of schistosomiasis as shown in Figure 2B reveals that even if schistosomiasis is scattered across the study area, there is some clustering of these cases particularly around the northern entrance of the town which presents a high concentration of cases.

Soil-transmitted helminth infection prevalence and intensity. As shown in Table 2, the overall prevalence of STH infections among the 472 subjects who provided a stool sample was15% (95% CI: 12–19), with all infections classified as light (Figure 3). The most prevalent STH was *T. trichiura*, followed by hookworm and *A. lumbricoides* with 10% (95% CI:8–13), 4% (95% CI: 2–6), and 3% (95% CI: 2–5) prevalence, respectively. *Strongyloides stercoralis* accounted for 1% (95% CI: 0–3). Coproculture technique was performed to improve the diagnosis of hookworm infection in our study population by detecting the presence of larvae in stool. Among the 18 participants classified as positive for hookworm, six (33%) were positive for the presence of eggs only in the stool, four (22%) were positive for the presence of larvae only in the stool, whereas eight (44%) were positive for both.

Table 2: Schistosomiasis and soil-transmitted helminths prevalence of and co-infection between among the study participants, April to July 2016, Lambaréné, Gabon.

	Participants infected with:			Schistosomiasis co-infection with:		
	n	(%)	$_{95\%}$CI (%)	n	(%)	$_{95\%}$CI (%)
Schistosoma spp.						
S. haematobium (N=614)	159	(25.9)	[22.4 - 29.5]	-	-	-
<50 eggs/10mL of urine	115	(72.3)	[65.4 - 79.3]	-	-	-
≥50 eggs/10mL of urine	44	(27.7)	[20.7 - 34.6]	-	-	-
S. intercalatum (N=472)	3	(0.6)	[0.1 - 1.8]	-	-	-
STH infection (N=472)						
T. trichiura	49	(10.4)	[7.8 - 13.5]	23	(4.9)	[3.1 - 7.2]
Hookworm	18	(3.8)	[2.3 - 5.9]	4	(0.8)	[0.2 - 2.1]
A. lumbricoides	15	(3.2)	[1.8 - 5.2]	8	(1.7)	[0.7 - 3.3]
S. stercoralis	6	(1.3)	[0.5 - 2.7]	2	(0.4)	[0.0 - 1.5]
Any STH	**71**	**(15.0)**	**[11.9 - 18.6]**	**28**	**(5.9)**	**[4.0 - 8.5]**

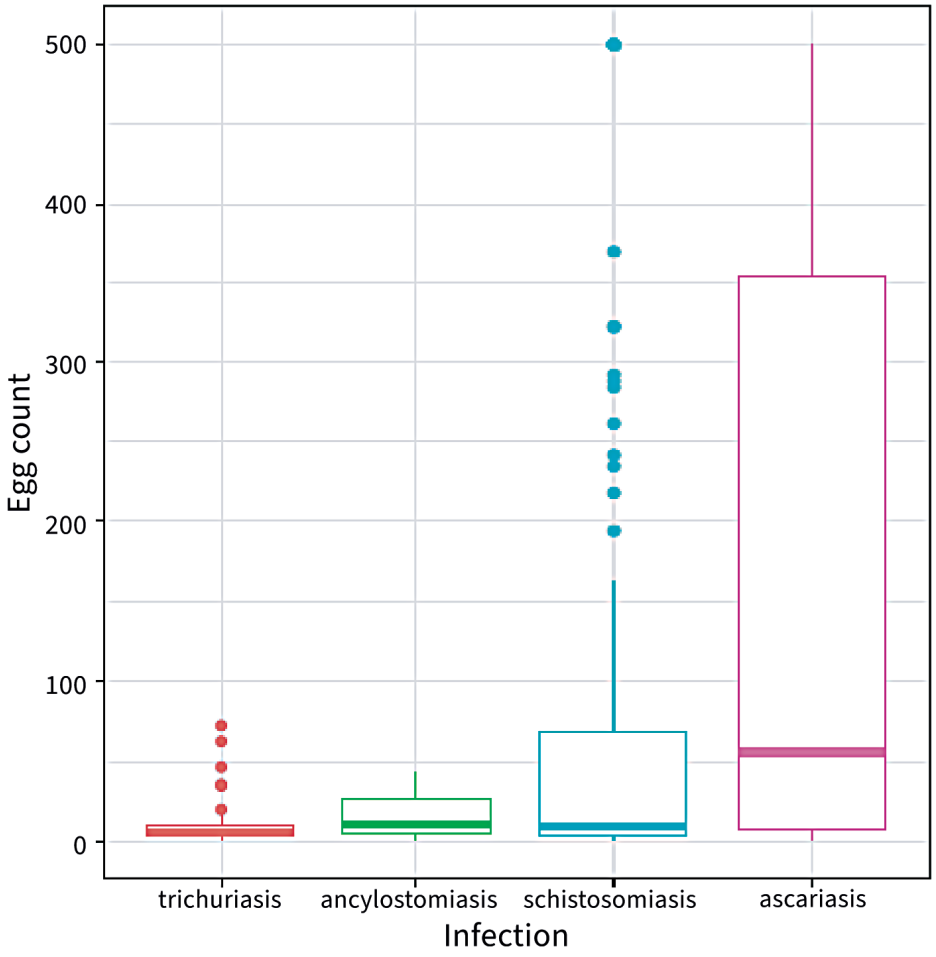

Fig 3: Egg count distribution for soil-transmitted helminth infections and schistosomiasis after Kato–Katz and urine filtration examinations, respectively, showing ascariasis as the infection presenting with the widest distribution in the number of eggs.

Schistosomiasis and STH coinfections. Assessing concomitant infections with other parasites among the 472 participants who provided stool and urine samples, schistosomiasis was found in coinfection with each STH infection investigated in the study, even for those with very low prevalence. As presented in Table 2, the schistosomiasis coinfection rate with any STH infection was 6% (95% CI: 4–8) and the most prevalent coinfection was trichuriasis with 5% (95%CI: 3–7). Regarding coinfections between different species of STHs among the 70 children infected, the prevalence of double infections was 24% (95% CI: 14–34), and the two most prevalent pairings of coinfections were trichuriasis–ascariasis (10% [95% CI: 3–17]) and trichuriasis–ancylostomiasis (9% [95% CI: 2–16]). Some cases of triple infections were found, notably ancylostomiasis–strongyloidiasis–ascariasis (3% [95% CI: 0–7]) and ancylostomiasis–strongyloidiasis–trichuriasis (2% [95% CI: 0–5]).

Factors associated with schistosomiasis. At bivariate analysis as shown in Table 3, age (P = 0.76), gender (P = 0.50), and educational level (P = 0.43) were not statistically associated with schistosomiasis. On the contrary, freshwater contact was highly statistically associated with schistosomiasis. Children who declared freshwater contact had a three-time higher risk (RR = 2.96 [95% CI: 2.2–4.0], P < 0.001) to be infected with *S. haematobium* than those who have not declared a freshwater contact. By contrast, as presented in Table 4, nutritional status (P = 0.61) was not associated with an increased risk of being infected with schistosomiasis (RR = 1.52 [95% CI: 1.09–2.12], P = 0.02).

Table 3: Assessment of risk factors of schistosomiasis infection among the 614 study participants, April to July 2016, Lambaréné, Gabon.

	Bivariate analysis				
	N	n (%)	RR	$_{95\%}$CI (RR)	*P-value*
Age (year old)					**0.76**
[5-10[	271	68 (25.1)	1		
[10-15[	307	83 (27.0)	1.08	[0.82 - 1.42]	
[15-19]	36	8 (23.2)	0.88	[0.46 - 1.69]	
Gender					**0.50**
Female	300	74 (24.6)	1		
Male	314	85 (27.0)	1.06	[0.89 - 1.26]	
Stream contact declared					**<0.001**
No	344	48 (16.9)	1		
Yes	266	110 (48.7)	2.96	[2.20 - 4.00]	
Level of education					**0.43**
1st year	127	40 (36.5)	1		
2nd year	116	31 (26.7)	0.85	[0.57 - 1.26]	
3rd year	118	28 (23.7)	0.75	[0.50 - 1.14]	
4th year	133	35 (26.3)	0.84	[0.57 - 1.22]	
5th year	120	25 (20.3)	0.66	[0.43 - 1.02]	

Table 4: Factors associated with schistosomiasis infection among the 614 study participants, April to July 2016, Lambaréné, Gabon.

	Bivariate analysis				
	N	n (%)	RR	$_{95\%}$CI(RR)	*P-value*
Hematuria					**<0.001**
No	484	64 (13.2)	1		
Yes	130	95 (73.1)	5.53	[4.30 – 7.10]	
Proteinuria					**<0.001**
No	494	105 (21.2)	1		
Yes	120	54 (45.0)	2.12	[1.63 – 2.75]	
Hemoglobin rate*					**0.003**
≥ 11	462	106 (22.9)	1		
< 11	150	53 (35.3)	1.54	[1.17 – 2.02]	
STH#					
T. trichiura	49	23 (46.9)	1.82	[1.30 – 2.56]	**0.003**
Hookworm	18	4 (22.2)	0.79	[0.33 – 1.89]	**0.77**
A. lumbricoides	15	8 (53.3)	1.97	[1.20 – 3.23]	**0.04†**
S. stercoralis	6	2 (33.3)	1.19	[0.38 – 3.74]	**0.67†**
Any STH	71	28 (39.4)	1.52	[1.09 – 2.12]	**0.03**
Nutritional status					**0.61**
Underweight	33	6 (18.2)	0.68	[0.32 – 1.42]	
Normal	493	132 (26.8)	1		
Overweight	57	15 (26.3)	0.98	[0.62 – 1.55]	
Obesity	26	5 (19.2)	0.72	[0.32 – 1.60]	
History of praziquantel treatment&					**0.007**
No	545	132 (24.2)	1		
Yes	62	25 (40.3)	1.66	[1.18 – 2.33]	
History of STH treatment$					**0.15**
No	472	129 (27.3)	1		
Yes	137	29 (21.2)	0.78	[0.54 – 1.10]	
Yes	137	29 (21.2)	0.78	[0.54 – 1.10]	

* 2 missing data
& 7 missing data
$ 5 missing data
# 142 missing data
† Fisher's exact test

Considering each species of STH, there was no statistical association for hookworm infection (RR = 0.79 [95% CI: 0.32–1.89], P = 0.77) and strongyloidiasis (RR = 1.19 [95% CI: 0.38–3.74], P = 0.67) with schistosomiasis, but there was a significant increase in risk of being infected with *Schistosoma* spp. when positive for trichuriasis (RR = 1.82 [95% CI: 1.33–2.56], P = 0.003) or for ascariasis (RR = 1.97 [95% CI: 1.20–3.23], P = 0.04). On the other hand, children with a hemoglobin level less than 11g/dL (RR = 1.54 [95% CI: 1.17–2.02], P = 0.003), presenting with hematuria (RR = 5.53 [95% CI: 4.3–7.1], P < 0.001) or proteinuria (RR = 2.12 [95% CI: 1.63–2.75], P < 0.001), had an increasing risk of being positive for schistosomiasis.

DISCUSSION

The present study confirms urogenital schistosomiasis in Lambaréné as predominating in the area. We found three participants with stool samples positive for the presence of *Schistosoma* eggs identified as *S. intercalatum* eggs, establishing the presence of few cases of intestinal schistosomiasis in the study area. Indeed, if the evidence of *S. haematobium* is widely reported in Lambaréné,[12,27] only scarce data reveal the presence of *S. intercalatum*[10] or S. guineensis16 in the area. Because *S. haematobium* eggs can be found in stool,[28] and confusion between *S. intercalatum* and *S. haematobium* eggs is possible when the differentiation is carried out based only on their shape and size using a microscopic method,[29] as we did, it will be relevant to use a molecular method to reconfirm the presence of *S. intercalatum* and/or *S. guineensis* in the area.

The main objective of the present study was to determine the prevalence of schistosomiasis in Lambaréné. Our results reveal 26% prevalence for urogenital schistosomiasis using the urine filtration technique as a diagnostic tool, classifying schistosomiasis prevalence as moderate for Lambaréné's community[8] and *Schistosoma* spp. as the most prevalent helminths in this town. To the best of our knowledge, this is the first time the level of schistosomiasis prevalence is assessed in Lambaréné, with a detailed mapping effort of schistosomiasis down to the individual level in the county.

The first cases of schistosomiasis were reported in the area in 1966.[9] Only one MDA campaign for schistosomiasis has been conducted in the area for schistosomiasis control before the conduct of this study, and no other specific intervention measures were implemented. We, therefore, expected to find a high prevalence of schistosomiasis, and indeed, a very high prevalence of schistosomiasis is often found in the areas where access to PZQ treatment is scarce or unavailable.[30,31] The moderate prevalence of schistosomiasis we found could suggest a stabilization of the prevalence of the disease in the area. An explanation for this may be the availability of the drug in the community. In addition to the scarce national campaigns of MDA, it should be noted that over the past 20 years, PZQ treatment has been provided free of charge to the infected population during the CERMEL routine or scheduled activities, being very active in the field of schistosomiasis, with significant impact in the community. Indeed,

it has been demonstrated that treatment of the population with even a single dose of PZQ per year can be sufficient to reduce the morbidity of schistosomiasis in an endemic area. [32,33]

Another objective of the present study was to describe risk factors and factors associated with schistosomiasis. As an associated factor, we have assessed the relation of the history of PZQ taken with schistosomiasis, and we found that 10% of the study population declared to have taken PZQ in the past six months, regardless of the source of the drug. Surprisingly, more infected than uninfected children with schistosomiasis declared to have received treatment before, raising the issue of PZQ efficacy or reinfection. Indeed, in our study area, it is common to give 40mg of PZQ per body weight once to treat schistosomiasis as recommended by the WHO,[34] although this protocol is sometimes found to have a limited efficacy.[35] On the other hand, reinfection is common in endemic areas,[35,36] assuming that those found infected despite the history of PZQ taken in the last months are reinfection cases. In both cases, there is a need for a proper investigation.

Assessing contacts of the study population with freshwater bodies considered as potential *Schistosoma* foci, nearly half of the population (44%) declared to have had contact with open freshwater, implying that an important part of the population is at risk of contact with *Schistosoma* parasites. When taking into account schistosomiasis status, the results demonstrate an ambiguous relationship leading to a couple of hypotheses. First, among participants found infected with schistosomiasis, no freshwater contact was declared for one of three of them. Subject to the reliability of their declarations, this fact could raise the possibility of the existence of additional, temporary schistosomiasis foci. In fact, Lambaréné is heavily irrigated by the tributaries of the Ogooué River. The rise of the river's waters during the rainy season is accompanied by flooding of the land, creating a significant number of temporary water pools, which can, thus, become potential foci of schistosomiasis, especially for school-age children. Second, among children not infected with *S. haematobium*, 35% have declared to use stream water for household activities or bathing, suggesting either a low sensitivity of urine filtration technique for the diagnosis of schistosomiasis or a fact that all human–water contact points, particularly streams, are not schistosomiasis foci. Based on what has been described earlier, it appears that schistosomiasis foci are not only streams, and these foci are not homogeneous and geographically distributed in the study area as demonstrated by the geographical distribution map of our participants, which showed clusters of infected children (Figure 2B). Actually, the northwest of the town appears to be a high prevalence area for schistosomiasis, whereas the southeast appears to be a moderate prevalence area. Between the two, the disease is present, but at low prevalence. To better understand the situation of schistosomiasis in the area, the determinants of this picture need to be investigated.

Although freshwater contact is obviously the main risk factor for schistosomiasis, it is known that some factors such as age and gender can affect this risk factor through age or gender-related behaviors. Some studies found that males are more at risk to be infected with schistosomiasis than females,[37,38] whereas others have shown the contrary.[39] For some authors, young

age increases the risk to be infected with schistosomiasis.[38,40] It has been suggested that the impact of culture on the population behavior with regard to contact with open freshwater could explain the difference observed and, therefore, affect the risk level of being infected. In the present study, neither gender nor age was associated with schistosomiasis. We, therefore, assume that the local culture does not lead to significant schistosomiasis exposure differences due to age- and gender-specific behaviors.

Hematuria is the main symptom known to be associated with urogenital schistosomiasis and very often used for indirect diagnosis of the disease. In this study, we found a strong association between any hematuria (microscopic and/or visible) and schistosomiasis. The situation was similar for proteinuria, meaning that these two biomarkers can be of interest for the diagnosis of schistosomiasis in our population. This question will be addressed in a further article. On the contrary to anemia, malnutrition was not associated with schistosomiasis. This corroborates the findings of Abdi et al.[41] who also found no association between intestinal schistosomiasis and malnutrition status among schoolchildren living in northwestern Ethiopia.[41] Even in a highly endemic area, Munisi and collaborators found no association between *S. mansoni* infection malnutrition among Tanzanian schoolchildren.[42] However, our results opposed the findings of some other authors who reported that malnutrition is associated with *S. haematobium* infection in children living in moderate or highly endemic areas for schistosomiasis.[6,43] These conflicting results suggest the existence of other determinants, which could affect the association between the disease and nutritional status. However, this has to be investigated in more detail prospectively in our population. In the case of the present study, we hypothesize a good nutritional status of schoolchildren in Lambaréné.

This study confirms once more that Lambaréné is endemic for STH infections, particularly for *A. lumbricoides*, *T. trichiura*, hookworm, and *S. stercoralis*,12 with *T. trichiura* being the predominant STH species, followed by hookworm and *A. lumbricoides*. On the contrary to the vicinity of Lambaréné where a moderate prevalence of STH infections was recently reported,[17] the low prevalence we report here could be explained by the availability of anthelminthic drug in the area, particularly albendazole, which is very often used for self-medication by the local population. Indeed, about 25% of study participants have declared having received anti-STH treatment in the last six months preceding the study. Moreover, the morbidity of STH infections is associated with the intensity of the disease. Heaviest infection is indeed associated with anemia or malnutrition, among others. In our study population, all cases of STH infections were of light intensity, assuming their non-association with anemia and malnutrition. This result supports our finding on the association of anemia with schistosomiasis in our study population and consolidates the low number of underweight cases we reported.

The geographical distribution of children infected with STHs shows a homogeneous spread of these parasites across the study area (Figure 2C) and, therefore, their local coexistence with schistosomiasis. Schistosomiasis–STH infection co-endemicity is commonly reported in sub-Saharan countries.[44-46] Assessing the coinfection with both trematodes and intestinal

nematodes, we found an increase in risk of having schistosomiasis when infected with STHs, particularly with *T. trichiura* and *A. lumbricoides*. Although some authors found no association between schistosomiasis and STH infections, such as Njaanake et al.[45] in Kenyan schoolchildren from two villages, our results are in line with what was reported by Molvik et al.[44] on South African schoolchildren living in moderate prevalence areas for schistosomiasis and STH infections. Even some immunological mechanism can explain that fact; we hypothesize that this association could be due to the fact that both types of infection share the same environmental risk factors. If the absence of an association with *S. stercoralis* could be due to a lack of statistical power (we found very few cases of strongyloidiasis), the absence of association with hookworm infection, which was more prevalent than ascariasis in our cohort, would support our hypothesis. Indeed, it is known that the life cycle of hookworm requires hot or sandy soils, in contrast to those of *Schistosoma* spp., *Ascaris* spp., and *Trichuris* spp. *Trichuris trichiura* eggs, for instance, must remain in warm moist soil to become infectious. In any case, these results show the necessity to take into account the interaction between schistosomiasis and STH infections, especially with trichuriasis and ascariasis for assessment of the effect of one of these diseases, in particular at the immunological level.

CONCLUSION

Lambaréné represents an area of moderate prevalence for schistosomiasis and low prevalence for STH infections. Our results emphasize the necessity to fully implement the recommendations of the WHO to improve the situation of schistosomiasis in the area where no additional risk factors other than human–water contact could be identified among schoolchildren. To that aim, hematuria and proteinuria strongly associated with the infection could help. Although the picture of schistosomiasis in Lambaréné becomes clearer, the need to establish the transmission dynamic remains. A pilot malacological study conducted in the area few years before already provides some helpful information.

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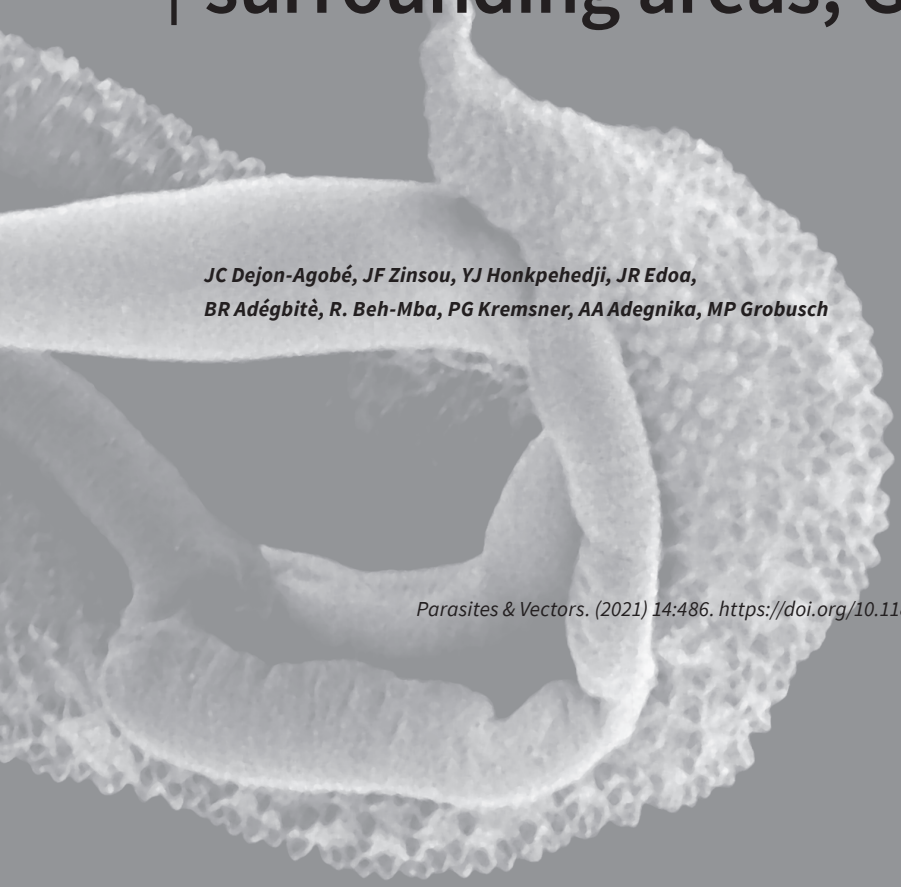
CHAPTER 3

Knowledge, attitudes and practices pertaining to urogenital schistosomiasis in Lambaréné and surrounding areas, Gabon



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ABSTRACT

Background: Control of schistosomiasis remains a priority in endemic areas. Local epidemiological data are necessary for a tailored control programme, including data on population behaviour in relation to the disease. The objective of this study was to assess schistosomiasis-related knowledge, attitudes and practices in the general population of Lambaréné, a small city in Gabon, in order to optimise the design and implementation of a local control programme that is tailored to need.

Methods: The study was cross-sectional in nature. Eligible adults and children living in the study area who volunteered (with informed consent) to participate in the study were interviewed using standardised questionnaires, one of which was a simplified version of the primary questionnaire for participants aged 6–13 years. Data on the participants' knowledge, attitudes and practices that enhance the risk for contracting schistosomiasis were collected.

Results: A total of 602 participants were included. The mean (± standard deviation) age was 21.2 (± 15.0) years, the female:male gender ratio was 1.6 and 289 (48%) participants completed the simplified version the questionnaire. Of the 602 participants, 554 (92%) reported past or current contact with freshwater, 218 (36%) reported a history of a diagnosis of schistosomiasis and 193 (32%) reported past intake of praziquantel medication. The overall levels of knowledge and adequate attitudes toward schistosomiasis among young adults and adults were 68 and 73%, respectively. The proportion of participants pursuing risk-enhancing practices (REP) was 60% among the whole study population. Location was significantly associated with differences in knowledge and REP levels. A history of confirmed schistosomiasis and larger family size were significantly associated with an increase in good knowledge and REP levels. However, the indication of freshwater-associated activities was only associated with a significant increase in the REP level.

Conclusions: The results of this survey reveal a high level of population exposure to schistosomiasis, which is in line with known prevalence of schistosomiasis in Lambaréné and its surroundings. The local population has a reasonable level of knowledge of and adequate attitudes toward schistosomiasis but the level of REP is high, particularly in areas where piped water is absent. In terms of interventions, improving hygiene should have the highest priority, but in a context where provision of safe water is difficult to achieve, the effectiveness of praziquantel treatment and the education of at-risk populations on the need for protective behaviours should be a prominent feature of any local control programme.

BACKGROUND

Schistosomiasis ranks only second to malaria amongst the most devastating human parasitic diseases [1] and remains a public health threat in several parts of the world. This is particularly the case in sub-Saharan Africa where schistosomiasis is the most-deadly neglected tropical disease (NTD) after snakebite envenomation, killing an estimated 2,80,000 people each year [1]. Schistosomiasis is especially prevalent in poor communities with limited access to safe water and adequate sanitation [2] and mostly affects communities exposed to these conditions, particularly agricultural and fishing populations, as well as women doing domestic chores involving infested water, such as washing clothes and dishes. Inadequate hygiene and contact with infested water render children in particular vulnerable to infection [2].

Schistosomiasis is a water-borne disease. Human–water contact is therefore an important factor to consider when disease control programmes are being designed. In addition to mass drug administration (MDA) and snail control, the World Health Organization (WHO) strategy for schistosomiasis control is based on the access to safe water (W), improved sanitation (S) and hygiene education (H) of at-risk population groups [2], a strategy known as the WASH programme [3]. However, in a context where achieving access to safe water sources may be difficult, hygiene education becomes the cornerstone of WASH. It has been demonstrated that the implementation of education of at-risk populations can considerably improve the control of schistosomiasis, even in highly endemic areas [4, 5]. In order to propose the appropriate change in behaviour or better adaptative behaviour, and to implement an adequate approach for prevention, it thus becomes necessary to assess the knowledge of the local population in each area where the disease is endemic, and also to identify the attitudes and practices (KAP) of these local populations toward the disease. To this end, the KAP survey appears to be a relevant tool to explore the local situation, as a pre-requisite for targeting group-tailored intervention strategies. A number of previous KAP studies on schistosomiasis that were conducted in rural and semi-urban areas, where sanitation is usually rudimentary and safe water supply is limited, demonstrated that even when the levels of knowledge and attitude in relation to schistosomiasis are moderate or good [6, 7], disease prevention often remains difficult to practise - mainly in terms of personal and sanitation hygiene, in both children [6, 7] and adults [7] with limited access to adequate toilet facilities and safe water, but also possibly in combination with a lack of knowledge of how schistosomiasis is transmitted or could be prevented [8].

Lambaréné is the capital town of Moyen-Ogooué, one of Gabon's nine provinces. At the time of our study, no previous KAP study on schistosomiasis had been conducted in Lambaréné and its surrounding rural areas; thus, there was a lack of important and relevant information. We report here the results of a KAP study that was performed to gain insight into the knowledge, attitudes and practices of local people regarding schistosomiasis, with the goal to facilitate control program optimisation, as the region appears to be one of the areas of the country worst affected by schistosomiasis.

METHODS

Study design

The study was cross-sectional in design, with eligible volunteers invited to participate. Participants who agreed to partake in the study were asked to take part in individual interviews. The data obtained during these interviews were captured on a standardised questionnaire. Because the targeted population was homogenous, no focus group interviews were conducted.

Study site

Lambaréné is a semi-urban area located by road 240 km south of Libreville, the capital of Gabon, and 110 km south of the equator. It is the capital city of the region and hosts the administrative institutions. The city is located on an island in the Ogooué River and its tributaries, and there are many ponds, lakes and streams in the city. The municipal water supply network does not cover all areas of the town. The area is endemic mainly for *S. haematobium* [9–11]. Lambaréné is surrounded by rural areas all endemic for S. haematobium [9, 10] mainly, namely Zilé-PK and Mitonè-PK areas. The Zilé-PK area includes a set of villages located south of Lambaréné along the first national road (RN1) from km 12 to 33 km, while the Mitonè-PK area includes a set of villages extended over 30 km along RN1 to the north of Lambaréné. Both areas are characterised by basic, rudimentary sanitary facilities and the lack of an adequate water supply network. However, each major village in Zilé-PK area has a public pump, although the streams and tributaries of the Ogooué River still constitute the preferable source of water for household activities and bathing. An industrial palm oil plantation is also located in Zilé-PK area, in the proximity of the RN1 (about 500 m) at the PK 15 level; this plantation provides housing to their workers, including electricity and a number of points of piped water in addition to the freshwater used by the inhabitants. The whole locality has four primary schools and two dispensaries. Additional study locations were Bindo and Makouké, two remote rural settlements about 65 km by road north of Lambaréné. Both areas are located on the banks of the Ogooué River and are surrounded by an industrial palm oil plantation that provides accomodation for its workers, including housing, basic facilities for hygiene, water and electricity. In Bindo, water is supplied directly from the Ogooué River to two public taps through a pipeline; in Makouké, water is treated before reaching the public tap or the houses directly. Each locality has one primary school and one dispensary with one nurse from the Bindo site and a medical team led by one medical doctor from the Makouké site. Makouké also has one secondary school, which is attended by children from both Makouké and Bindo. Both localities are linked by a ferry across the Ogooué River.

Only a few MDA campaigns on the control of schistosomiasis have been conducted by the government in these areas. According to the local health authorities, the last praziquantel (PZQ) treatment campaign took place among school children in 2016. No schistosomiasis awareness campaigns were run in the area.

Study population

The Lambaréné population is about 45,000 inhabitants [12], and Bindo-Makouké and the surrounding areas of Lambaréné (Zilé-PK and Mitonè-PK areas) have about 1000 and 2500 inhabitants, respectively [9]. Agriculture and fishing are the main activities of the young population from the surrounding areas, while it is less practiced by residents of Lambaréné and Bindo-Makouké; rather, the populations of Bindo and Makouké are essentially workers on the local palm oil plantation. From the three areas, volunteers, including school-age children and adults, were invited to participate in the study. However, individuals unable to respond to the questionnaire were excluded.

Questionnaire

The main standardised questionnaire (Additional file 1: Text S1) used in this study was developed using Médecins du Monde [13] and Handicap International [14] guidelines and designed to collect data on the knowledge, attitudes (including prevention strategies) and risk-enhancing practices (REP) regarding schistosomiasis through closed questions among participants aged ≥ 14 years (young adults and adults). In order to allow the interviewee to give his/her position on some aspect of the topic, open questions were also asked. For volunteers aged < 14 years, we used a simplified version of the questionnaire that corresponded with the main questionnaire. This simplified version had been developed for children aged 6 to 13 years from the main study questionnaire by removing inappropriate questions for age, and simplifying the language of the remaining questions. In addition, some illustrating images were added to brighten up the questionnaire and facilitate comprehension of the questions (Additional file 2: Text S2). For validation, the simplified version was pretested in the corresponding study population before being used in the survey. The simplified questionnaire thus mainly assessed the REP of children with regard to schistosomiasis. In addition, elementary knowledge and attitude of these children were assessed. Both questionnaires were used in accordance with the participant's age and were adjusted accordingly by removing questions inappropriate for age, or by simplifying the language.

Calculation of sample size and sampling

Awareness of schistosomiasis was considered to be the primary indicator of the population knowledge on schistosomiasis, and we assumed that 75% of the local population was aware of schistosomiasis. With 95% confidence level and a standard error of 5%, we needed at least 288 volunteers to be able to address our main objective, using the formula for cross-sectional study sample size calculation as described elsewhere [15]. Given that two different questionnaires were used, we considered a calculated sample size for each questionnaire, giving a total of 576 volunteers in total to include in the study. These volunteers were selected from the different study areas by applying a three-stage sampling procedure. The first stage included random selection of a neighbourhood or village from each area. The second stage included the selection of households from these neighbourhoods or villages through systematic sampling, with the third inhabited household systematically selected from every three households. At the third stage, a maximum of two respondents was randomly selected from each selected household.

Data collection

The survey was conducted from June to July 2019. Data on knowledge, attitude and practices were gathered through the standardised questionnaire. Socio-demographic data were collected using the same questionnaire, as were clinical data on schistosomiasis and data on history of taking PZQ. The questionnaire was given in the national language, French, to each respondent included in the study during an approximately 15-min face-to-face interview. The simplified version of the questionnaire was used with participants aged 6–13 years. In order to minimise inter-interviewer differences, a (re)training session was organised every three days of data collection. Data were collected using the paper version of the questionnaire and then digitalised with REDCap software [16] hosted at CERMEL (Centre de Recherches Médicales de Lambaréné). The clean database was extracted and imported in R software version 3.4.4 (R core team, Vienna, Austria) for analysis.

Statistical considerations

We were interested in assessing the knowledge, attitudes and practices of the study population regarding schistosomiasis, and these variables were considered to be the variables of interest. Other variables included in the analysis were considered as explanatory variables. In the descriptive analysis, we reported results as a proportion of answers, while for the analysis of association, a score was calculated for each variable of interest, as the total score for each appropriate answer related to that variable. For questions on knowledge and attitudes, a score of one point was given for each correct answer considered as appropriate while for questions on REP, a score of one point was given for each answer indicating a risk of contamination with schistosomiasis. For closed questions with multiple ordinal responses, an additional score of one point was added at each modality level from zero corresponding to the wrong knowledge or attitude, or for absence of risk practices, amounting to potential maximum scores of 13, 4 and 9 points for knowledge, attitudes and REP, respectively (Additional file 3: Table S1). The overall level of appropriate knowledge, attitudes and REP were estimated as the percentages of total scores. The level of this percentage was classified as 'bad' if < 50%; 'acceptable' if < 60%; 'fairly good' if < 70%; 'good' if < 80%; 'very good' if < 90%; and 'excellent' if ≥ 90%. The majority of the questions on knowledge were deemed inappropriate for children aged 6–13 years. In the simplified version of the questionnaire, we thus collected only basic information on the knowledge of children on schistosomiasis; consequently, we could not estimate the level of children's knowledge in the same manner as we did for adults, and we therefore did not include children in the multivariate analysis shown in Table 5. The situation was different for the evaluation of REP in children. Indeed, as for young adults and adults, we were able to estimate the level of REP in children and, therefore, the regression analysis for REP includes the complete study population.

The scores were used as quantitative variables in the comparative analysis. Quantitative variables were described as the mean and standard deviation (SD) while categorical variables were described as proportions with a 95% confidence interval (CI). Linear regression was used to assess the association between the variables of interest, and demographic, socio-economic and clinical variables related to schistosomiasis were considered as explanatory variables.

For multivariable regression, the final model included all variables known to be associated with schistosomiasis and considered in the univariable analysis. The significance of statistical tests was set at < 0.05.

RESULTS

Study population

A total of 304 households were visited across the study area, with a total of 1799 inhabitants aged ≥ 6 years considered to be potential eligible candidates to participate in the study. Among these, 644 volunteers were randomly selected to participate in the study, and 602 (93%) subsequently consented to be included in the survey. The 42 inhabitants who refused to participate mentioned either a lack of time or just not wanting to be involved in the survey. As all data were collected after informed consent, we could not further test differences between those who consented and those who did not. The mean (± SD) age of the overall study population was 21.2 ± 15.0 years, with a 1.6 female:male gender ratio. As shown in Table 1, the majority of the participants came from downtown Lambaréné (*n* = 329, 55%), while almost all of them had obtained either primary (53%) or secondary (43%) educational level. As the main occupation, 84 (27%) participants among the 313 who responded to the main questionnaire were students, 69 (22%) were farmers and/or fishermen, and 75 (24%) reported to have no main occupation. From the whole study population, 313 (52%) responded to the comprehensive study questionnaire, while 289 (48%) participants responded to the simplified version of the questionnaire. Of those who responded to the simplified version of the questionnaire, 280 (97%) were school children.

Table 1. Sociodemographic characteristics of the 602 study participants.

Sociodemographic characteristics	Study population		
	n	%	95%CI(%)
Age (in year)			
6 – 9	117	19.4	16.3 – 22.8
10 – 13	128	21.3	18.2 – 24.9
14 – 17	91	15.1	12.5 – 18.4
18 – 25	102	17.0	14.2 – 20.4
>25	164	27.3	23.9 – 31.2
Gender			
Female	368	61.1	57.1 – 65.0
Male	234	38.9	35.0 – 42.9
Location			
Lambaréné	329	54.7	50.6 – 58.7
Mitonè-PK villages	52	8.6	6.5 – 11.2
Zilé-PK villages	154	25.6	22.1 – 29.3
Bindo-Makouké villages	67	11.1	87.3 – 13.9

Table 1. *(continued)*

Sociodemographic characteristics	Study population		
	n	%	95%CI(%)
School level			
None	13	2.2	1.1 – 3.7
Primary	318	52.8	48.7 – 56.9
Secondary	262	43.5	39.5 – 47.6
University	6	1.0	0.4 – 2.2
Other	3	0.5	0.1 – 1.4
Main occupational status*			
None-working	75	24.0	19.3 – 29.1
Farmer or fisher	69	22.0	17.6 – 27.0
Trader	21	6.7	4.2 – 10.1
Student	84	26.8	22.0 – 32.1
Administrative	4	1.3	0.3 – 3.2
Health care	6	1.9	0.7 – 4.1
Other	54	17.3	13.2 – 21.9
Family size*			
1-3	77	24.6	19.9 – 29.8
4-6	130	41.5	36.0 – 47.2
≥ 7	106	33.9	28.6 – 39.4

*Applicable to young adults and adults only, corresponding to the main version of the questionnaire

Human–water contact profile of entire study population

As shown in Table 2, 311 (52%) of the 602 participants reported having access to piped water at home for their household activities, and 284 (47%) declared using open freshwater sources. A total of 417 (69%) of the study population considered their house close to a water course; among these, 261 (62%) lived near a river, and 51 (12%), 23 (5%), 22 (5%) and 73 (17%) lived near a lake, stream, swamp or the Ogooué River, respectively. A pit latrine was used by 72% of the study population, while 11 and 18% reported using either private toilets or sharing modern and external toilets with the neighbourhood, respectively.

Table 2. Distribution of factors inherent in schistosomiasis among the 602 study participants.

Factors associated with schistosomiasis	Study population		
	n	%	95%CI(%)
History of *Schistosoma* infection			
Yes	217	36.0	32.2 – 40.0
History of visible haematuria			
Yes	241	40.0	36.1 – 44.1
History of PZQ treatment			
Yes	193	32.1	28.3 – 35.9
Avenues of PZQ*			
Local research centre (CERMEL)	95	49.2	42.0 – 56.5
Health centre	42	21.8	16.2 – 28.2
From a parent	23	11.9	7.7 – 17.3
National campaign of MDA of PZQ	20	10.4	6.4 – 15.5
Drugstore	16	8.3	4.8 – 13.1
Other	9	4.7	2.1 – 8.7
Source of water at home			
Tap water	311	51.7	47.6 – 55.7
Stream/River	284	47.2	43.1 – 51.2
Well	100	16.6	13.7 – 19.8
Ogooué River	57	9.3	7.1 – 11.9
Consider their house near a body water			
Yes	417	69.3	65.4 – 72.9
Type of body water considered as near of houses**			
River	261	62.1	57.3 – 66.8
Lack	51	12.2	9.2 – 15.8
Stream	23	5.5	3.5 – 8.2
Swamp	22	5.3	3.3 – 7.9
Ogooué River	73	17.5	14.0 – 21.5
Type of toilets used at home			
Private toilets	69	11.5	9.0 – 14.3
Shared toilets	106	17.6	14.6 – 20.9
Pit latrine	437	72.6	68.8 – 76.1

*Assessed among the 193 participants with history of PZQ taken
**Assessed among the 417 participants who consider their home as near a body water

Exposure of study population to schistosomiasis

Regarding study participants' contact with fresh water in the study area, 554 (92%) individuals reported having been in contact in one way or another with an open body of fresh water, with the majority (473/554; 85%) reporting the main contact was with some of the local small rivers, some of the local small rivers, followed by some of the local lakes (86; 15%) and the Ogooué River itself (75; 13%) (Table 3). When asked about the place closest to the freshwater body, home was reported by 538 (97%) participants, while plantation and school/place of work were reported by 74 (13%) and 21 (4%) participants, respectively. In terms of the frequency of their contact with fresh water, 281 (51%) participants reported daily contact, while 48 (9%) and 220 (40%) reported once weekly or only occasional contact, respectively. The morning was the period of the day when most of the study population was in contact with fresh water (05:00 h to 11:00 h; 51%, 281/554), followed by afternoon (15:00 h to 18:00 h; 23%, 130/554), noon (12:00 h to 14:00 h; 4%, 22/554), and night (< 1%; 19:00 h to 04:00 h; 3/554). Taking a bath (505; 91%) was the most frequently mentioned reason for freshwater contact, followed by household activities (460; 83%), fetching water (413; 74%), fishing (189; 34%), playing (148; 27%) and planting (44; 8%).

Table 3. Distribution of factors in relation with human-freshwater point contact among the 554 study participants who declared past contact.

Factors related to human–freshwater contact	Study population		
	n	%	95%CI(%)
Type of water point			
River	473	85.4	82.2 – 88.2
Lake	86	15.5	12.6 – 18.8
Ogooué River	75	13.5	10.8 – 16.7
Stream	20	3.6	2.2 – 5.5
Others	6	1.1	0.4 – 2.3
Places from water contact			
Home	538	97.1	95.3 – 98.3
School or place of work	21	3.8	2.4 – 5.7
Planting	74	13.4	10.6 – 16.5
Others	4	0.7	0.2 – 1.8
Frequency of contact			
Daily	286	51,6	47.4 – 55.9
Weekly	48	8.7	6.5 – 11.3
Sometimes a monthly	220	39.7	35.6 – 43.9

Table 3. *(continued)*

Factors related to human–freshwater contact	Study population		
	n	%	95%CI(%)
Time of contact during the day			
Morning	281	50.7	46.5 – 55.0
Mid-day: 12H-15H	22	4.0	2.5 – 5.9
Afternoon: 15H-18H	130	23.5	20.0 – 27.2
Night	3	0.5	0.1 – 1.6
No specific time	118	21.3	18.0 – 24.9
Reason declared for water contact			
Bath	505	91.1	88.5 – 93.4
Housework	460	83.0	79.6 – 86.1
Fetch water	413	74.5	70.7 – 78.1
Fishing	189	34.1	30.2 – 38.2
Playing	148	26.7	23.1 – 30.6
Planting	44	7.9	5.8 – 10.5
Others	0	0.0	-

History of schistosomiasis among the study population

Table 2 includes the results on the history of schistosomiasis among the 602 participants included in the study: 241 (40%) declared having already experienced visible haematuria in the past, and 217 (36%) declared a history of schistosomiasis diagnosis. In terms of having taken PZQ as treatment of schistosomiasis, 193 (32%) remembered having received the drug at least once in the past. The Centre de Recherches Médicales de Lambaréné (CERMEL) was found to be the main source of the drug (95; 49%), followed by local health centres (42; 22%), relatives (23; 12%), national mass PZQ administration campaign (20; 10%) and drugstores (16; 8%).

Knowledge of schistosomiasis and associated factors

The only name known to the local population to indicate schistosomiasis is the French word 'bilharzie'. Of the 602 participants included in the study, 475 (79%; 95% CI 75–82) had already heard of schistosomiasis. The knowledge elements presented in Table 4 were assessed among the 313 young adults and adults who responded to the main study questionnaire. Of these, 301 (96%; 95% CI 75–82) had already heard of schistosomiasis. 'Bacteria' (33%; 95% CI 28–38) and 'worms' (27%; 95% CI 23–33) were the most frequently indicated causative agent of the disease, while the river small snails (46%; 95% CI 41–52) were mainly indicated as the 'animal' transmitting the disease, among others listed. Blood in urine was the main disease symptom indicated (91%; 95% CI 87–94), while getting in contact with river water (70%; 95% CI 64–75) was the answer mainly indicated as the way to become infected. A total of 225 (72%: 95% CI 72–81) participants knew that the diagnosis of the disease can be made by urine examination in a laboratory. When asked about the consequences of schistosomiasis, infertility (70%, 95%

CI 65–75) was the main answer selected, followed by smelly vaginal discharge in women (56%, 95% CI 50–61) and girls (53%; 95% CI 48–59), then anaemia (55%, 95% CI 49–60), cancer of the bladder (50%, 95% CI 44–55) and death (48%, 95% CI 42–53).

The mean (±SD) score for knowledge of schistosomiasis was 8.8 ± 2.4 out of a total of possible 13 points, yielding a 68% appropriate knowledge level for the study population. In the multivariable analysis (Additional file 4: Table S2), we found a relationship between knowledge score and history of schistosomiasis ($P = 0.001$), location ($P = 0.005$) and family size ($P = 0.02$). Indeed, a higher score level of appropriate knowledge was found for participants with a history of schistosomiasis (α = 0.90, 95% CI 0.35–1.45) and for those living in a household with a large number of family members (family size: 4–6; α = 0.86, 95% CI 0.20–1.52; family size > 6: α = 0.75, 95% CI 0.04–1.47). Compared to participants living in downtown Lambaréné, we found a lower score of appropriate knowledge only for participants from Zilé-PK (α = − 1.34, 95% CI − 2.11 to − 0.57), while we observed no difference in the score levels for participants from Mitonè-PK (α = − 0.80, 95% CI − 1.89 to 0.30) and Bindo-Makouké (α = − 0.81, 95% CI − 1.75 to 0.14) villages.

Table 4. Distribution of knowledge towards schistosomiasis among the 313 young adults and adults responding the main questionnaire.

Knowledge of schistosomiasis	Study population		
	n	%	95%CI(%)
Ever heard of schistosomiasis			
Yes	301	96.2	93.4 – 98.0
Indicated as causative agent of schistosomiasis			
Worm	86	27.5	22.6 – 32.8
Virus	46	14.7	11.0 – 19.1
Bacteria	103	32.9	27.7 – 38.4
Other	7	2.2	0.9 – 4.5
Do not know	55	17.6	13.5 – 22.2
Indicated as symptom of urogenital schistosomiasis			
Presence of blood in urine	284	90.7	87.0 – 93.7
Fever	15	4.8	2.7 – 7.8
Diarrhoea	9	2.9	1.3 – 5.4
Stomach-ache	21	6.7	4.2 – 10.1
Itching	18	5.8	3.4 – 8.9
Other	1	0.3	0.0 – 1.8
Do not know	14	4.5	2.5 – 7.4
Aware about diagnostic mean			
Yes	225	71.9	66.5 – 76.8

Table 4. (*continued*)

Knowledge of schistosomiasis	Study population		
	n	%	95%CI(%)
Indicated as the way to catch the disease			
Walking barefoot	67	21.4	17.0 – 26.4
Eat without washing hands	38	12.1	8.7 – 16.3
Get in touch with the river	218	69.6	64.2 – 74.7
Drink the river water	144	46.0	40.4 – 51.7
Mosquitoes bite	11	3.5	1.8 – 6.2
During sexual intercourse	19	6.1	3.7 – 9.3
Others	5	1.6	0.5 – 3.7
Do not know	11	3.5	1.8 – 6.2
Indicated as the 'animal' responsible of the disease			
Mosquitoes	10	3.2	1.5 – 5.8
Land snail	3	1.0	0.2 – 2.8
River small snail	145	46.3	40.7 – 52.0
Fly	8	2.6	1.1 – 5.0
Others	5	1.6	0.5 – 3.7
Do not know	137	43.8	38.2 – 49.5
Indicated as consequences of schistosomiasis			
Anaemia	172	54.9	49.3 – 60.5
Smelly vaginal discharge	175	55.9	50.2 – 61.5
Malodorous vaginal discharge in the little girl	167	53.3	47.7 – 59.0
Infertility	220	70.3	64.9 – 75.3
Cancer of the bladder	156	49.8	44.2 – 55.5
Death	149	47.6	42.0 – 53.3
Others	18	5.8	3.4 – 8.9

Attitudes to schistosomiasis

The results on the interviewees' attitudes toward schistosomiasis are shown in Table 5. Among the 289 children who responded to the simplified version of the questionnaire, 282 (98%; 95% CI 95–99) were ready to inform their parents if they experienced haematuria. Of the 602 participants overall, 575 (96%; 95% CI 93–97) were ready to disclose their status if they were to be found positive for schistosomiasis. Asking for the reason for disclosure, a 16-year-old boy stated that: "*if we have bilharzia, we should not be ashamed because it is a disease like any other disease*" while a 17-year-old girl stated that: "*we have to tell others so that they could help us get the drugs*". Of the 313 young adults and adults who responded to the main questionnaire, 295 (94%; 95% CI 91–97) and 61 (19%; 95% CI 15–24) of them indicated hospitals and/or drugstores as the preferable place to seek drug treatment, respectively, while 13 (4%; 95% CI 2–7) of the participants were ready to go to the traditional healer first to seek treatment. If treated for the disease, 136 (46%; 95% CI 40–52) participants were not ready to avoid going back to the water course. In that regard, a 60-year-old woman living in a neighbourhood of Lambaréné town indicated that: "*it is the only place for me to wash and take a bath*". With regard to the severity of the disease, 135 (45%; 95% CI 39–51) and 108 (36%; 95% CI 30–42) of the young adults and adults who responded to the main questionnaire considered schistosomiasis to be a severe or moderate disease, respectively, while 58 (19%: 95% CI 15–24) of them considered the disease as mild.

The mean (± SD) score for appropriate attitudes toward schistosomiasis was 2.9 ± 0.6 points out of a total score of 4 points, giving a 73% of appropriate attitudes to schistosomiasis for the study population.

Table 5. Distribution of attitudes and practices towards schistosomiasis among the study population.

Attitudes and practices to schistosomiasis	Study population		
	n	%	95%CI(%)
Can disclose his/her Schistosoma infectious status			
Yes	575	95.5	93.5 – 97.0
No	27	4.5	3.0 – 6.5
Can share information in case of haematuria with*			
Parents	276	95.5	92.4 – 97.6
Friend	23	8.0	5.1 – 11.7
School teacher	19	6.6	4.0 – 10.1
Consider the gravity of schistosomiasis as:**			
Severe	135	44.8	39.1 – 50.7
Moderate	108	35.9	30.5 – 41.6
Light	58	19.3	15.0 – 24.2
Missing data	12	-	-

Table 5. *(continued)*

Attitudes and practices to schistosomiasis	Study population		
	n	%	95%CI(%)
Preferable source to seek medication**			
Hospital	295	94.2	91.1 – 96.6
Drugstore	61	19.5	15.2 – 24.3
Traditional healer	13	4.1	2.2 – 7.0
Will not seek the drug	0	0.0	-
Used fresh-water at home			
Yes	416	69.1	65.2 – 72.8
No	186	30.9	27.2 – 34.8
Frequency of fresh-water contact reported			
Everyday	286	47.5	43.4 – 51.6
Every week	48	8.0	5.9 – 10.4
Sometime a month	220	36.5	32.7 – 40.5
Never	48	8.0	5.9 – 10.4
Ready to not go to the river if being treated for schistosomiasis**			
Yes	136	46.3	40.4 – 52.1
No	158	53.7	47.9 – 59.5
Missing data	19	-	-
Had reported having already urinating in a watercourse			
Yes	387	64.3	60.3 – 68.1
No	215	35.7	31.9 – 40.0
Had reported having already defecating in a watercourse			
Yes	152	25.2	21.8 – 28.9
No	450	74.7	71.1 – 78.2

* Applicable to children only, corresponding to the simplified version of the questionnaire
**Applicable to young adults and adults only, corresponding to the main version of the questionnaire

Protective practices against schistosomiasis

Among the participants who regularly used a freshwater course for their daily activities, although some of them reported doing nothing as stated by a 56-year-old lady, some techniques are used by others to reduce the risk of being infected when going to the river particularly for a bath. A 16-year-old boy said, for instance, to (carry water from and) "*take his bath aside the river*". In addition, a 25-year-old woman stated to "*heat the water or put it in the sun*" while a 49-year-old man said to "*put the bleach*" in the bucket of water. As another approach, a 68-year-old lady stated to "*keep clean the river all the time*" while a 16-year-old girl said to have "*stop urinating in water and avoid staying in water*". Some inappropriate practices were reported by some participants to protect themselves to schistosomiasis such as a 63-year-old lady who said to "*must not walk without shoes*" or a 29-year old lady who reported "*to drink a lot of water*". A 17-year-old man from his side stated that; "*I must wash myself without making too many waves*".

REPs toward schistosomiasis and exposure-associated risks

Practices putting respondents at risk for schistosomiasis were evaluated among the 602 study participants. Of these, 554 (92%; 95% CI 90–94) declared having been in contact with fresh water in one way or another, while 415 (69%; 95% CI 65–73) reported using freshwater from sources close to their home. As shown in Table 3, 286 (52%; 95% CI 47–56) study participants were in contact with a freshwater body every day while 48 (9%; 95% CI 6–11) and 220 (40%; 95% CI 36–44) participants were in contact with freshwater weekly or several times per month, respectively. A total of 387 (64%; 95% CI 60–68) study participants reported having urinated in a freshwater body and 152 (25%; 95% CI 22–29) reported having defecated regularly/sometimes in a freshwater body (Table 5).

The mean (± SD) score for REP was 5.4 ± 2.3 points out of a total of 9 points, indicating a 60% level of REP toward schistosomiasis in our study population. Examining factors associated with REP (Table 5), we found in multivariate analysis a relationship between the REP score level and history of schistosomiasis ($P < 0.001$), location ($P < 0.001$), the use of freshwater ($P = 0.006$) and family size ($P = 0.007$). Compared to Lambaréné town, we observed a higher score of REP for the populations of Zilé-PK (α = 0.77; 95% CI 0.14–1.41) and Mitonè-PK (α = 2.01; 95% CI 1.11–2.92) but a lower score for that of Bindo-Makouké (α = − 1.36; 95% CI − 2.14 to − 0.57). We found a higher score of REP among participants with history of a diagnosis of schistosomiasis, as compared to their counterparts without such a diagnosis (α = 0.77; 95% CI 0.31–1.22). Similarly, we found a higher score among those who used freshwater, as compared to those who did not (α = 0.79; 95% CI 0.21–1.37) while, compared to families with three members or less, we found a lower score for families with four to six members (α = − 0.70; 95% CI − 1.24 to − 0.15) and 7 to 30 members (α = − 0.87; 95% CI − 1.47 to − 0.28).

DISCUSSION

The prevalence of schistosomiasis is known to be moderate (26%) in Lambaréné itself [17] and high in the surrounding vicinity, particularly in the villages of Zilé-Pk where prevalence can reach 75% in some villages [10]. The present KAP survey, which is the first being conducted in the area, highlights a high level of population exposure to the disease due to their proximity with, and the use of, freshwater for domestic needs. The results of this study will be helpful to explain human factors pertaining to the endemicity of the disease in the region, and particularly to evaluate the role of the population behaviour for a better control of the disease [2, 18].

Treatment, either preventive or curative, and limiting human–parasite contact are key elements in schistosomiasis control. We noted that 32% of the study population had already received PZQ at some point in time. Of these, almost 50% had received the drug from CERMEL during research activities (participation in clinical studies), highlighting the impact of the research centre in the controlling the morbidity of the disease in the community. Surprisingly, only 10% of the population indicated having received PZQ from MDA national campaigns, bringing into question both the coverage and impact of these campaigns on regional disease control. Indeed, as we previously indicated, only one national campaign of MDA of PZQ was conducted among school children during the last decade.

The present survey revealed that almost the entire study population had already heard of schistosomiasis, leading to the assumption that the local population is well aware of the presence of the disease in the community. This situation of high awareness corroborates observations made in other areas endemic for schistosomiasis [19, 20]. In our population, the main avenues of information on the disease was by word of mouth (57%) and at school (30%).

Overall, we found that the local population had a fairly good knowledge level of the disease. However, compared to the other study areas, the level of correct knowledge of schistosomiasis found for the Zilé-Pk villages, known for their high prevalence of schistosomiasis, was low, similar to data reported from some other areas of Africa. Indeed, a low disease awareness level was reported in western Kenya despite the observation of high disease prevalence [21]. Based on our results, although almost the entire population was aware of the presence of the disease, we can assume that some aspects of the disease remain unclear, particularly for the population of Zilé-PK. It has been reported that having heard about the disease is not necessarily associated with correct knowledge on how the disease is acquired, transmitted, or prevented [8, 20, 22]. In northern Senegal, for example, where 86% of the population had heard about schistosomiasis, only 30% had adequate knowledge of its mode of transmission [23]. Up to 91% of our study population indicated haematuria as the main symptom of the disease and up to 30% failed to indicate knowledge of the connection between a freshwater body, and catching the disease. Only 46% indicated river small snails as animals being involved in transmitting the disease in a context where providing safe water is difficult, indicating the necessity of educating people on disease transmission, for example at school or through the media. Indeed, health education for schistosomiasis has been shown to considerably improve

the rate exposure to infested water and the infection rate in a population living in a heavily endemic area with wells as drinking water sources [4].

We found the overall attitude level of our population on schistosomiasis to be good. We noted, for example, that people were open to talking about the disease, which could explain our finding of a positive association between a higher level of knowledge and a higher number of persons living in the same household. Indeed, all children were ready to inform their parents in case of visible blood in urine, and nine out of ten young adults and adults were ready to share their status with others if they were ever found to be infected. It appears that the local population considers schistosomiasis to be like any other disease and therefore think that there is no need to be ashamed when having it; consequently, the disease does not seem to be associated with stigma. By sharing their infectious status, they expect in fact to be advised on how to proceed to find a solution. This situation highlights the predisposition of the population to seek treatment if infected. Interestingly, almost all participants preferred to go either directly to a hospital or drugstore to obtain drugs for treatment, rather than, for example, consult a traditional healer first. We conclude that if PZQ is available and affordable in the area, it will be used by large numbers of the afflicted population. This is in contrast to some observations in the field where some people have been found to be reluctant to participate in an MDA campaign, particularly whilst being asymptomatic; however, this is not specific to the MDA of PZQ.

The population using freshwater for their daily activities, particularly at home, reported some actions taken to protect themselves from the disease. Although these methods need to demonstrate their efficiency, at least it assumes that the population are ready to follow recommended preventive measures, as far as living circumstances permit. We can therefore suppose that education of the population on protective measures to the disease can be successfully implemented in the local population and could further contribute to improving the situation. In addition to door-to-door campaigns, sending messages on protective measures through social networks or billboards at the main human–water contact points could alleviate schistosomiasis transmission in the community.

Two main points were considered in the assessment of the level of population REP to schistosomiasis, namely freshwater contact and urinating/defaecating into a water body. The results of our questionnaire show a rather high level of REP in the population. In an area where around 70% of the population lives close to a water course and where 71% use fresh water at home as the water source for their daily activities, this finding is not surprising, but rather confirms the fact that some parts of the population are living in conditions putting them almost inevitably in contact with the parasite. We can assume that this is particularly true for the areas surrounding the city of Lambaréné, such as the Mitonè-PK area, where no tap water is available, and, to a lesser extent, the villages of Zilé-PK. Compared with the urban setting of Lambaréné, we found that the level of REP is higher in the Zilé-PK area and Mitonè-PK areas, and lower in the villages of Bindo-Makouké where tap water or public water pumps are available for the majority of the

population. We can therefore assume that the high prevalence of schistosomiasis observed for some parts of Lambaréné city [17] is particularly due to the neighbourhoods lacking an adequate water supply network. In order to interrupt the life cycle of the parasite, the most important action to undertake is to provide the population with safe water. Unfortunately, this remains difficult to achieve, at least in the short- or medium-term. In the absence of alternatives to open freshwater use, it is essential to identify the most exposed population groups, as health education measures could then focus on how to adapt their behaviour in order to reduce their level of exposure to the disease.

The absence of safe water cannot solely explain the high schistosomiasis endemicity in the region. One key element in the transmission of the disease is the contamination of a freshwater body by infected individuals. We were therefore interested to know how local population could infest the watercourse. Our study reveals that up to 64% of the population stated having urinated in the watercourses, which makes clear that the habit of urinating in watercourses plays a major role in the presence of the disease. Focusing the education of the population on that simple practice could thus contribute to considerably alleviating the burden of the disease in the locality.

Knowledge as well as REP levels both increased with an individual's past medical history of schistosomiasis. This result supports the notion that the population practices with regard to schistosomiasis are less clearly linked to their knowledge level on the disease, but rather to their living conditions. Indeed, more than half of the population, for well-understood reasons, is not ready to avoid freshwater contact in order not to get infected, or become re-infected, even if requiring treatment for schistosomiasis. Instead of not being aware of the risk to be infected or re-infected, it appears that this attitude is mainly due to the fact that these individuals simply do not have any other water source than stream freshwater for their daily activities. We also noted that the number of household members can influence the practices regarding schistosomiasis. Families with many members perform fewer REP per capita, probably because only some members of the family are assigned to particular household chores. We can imagine that these are the women and/or the children; however, our results did not show any differences in term of REP level according to age and gender. Most likely, people with a high level of REP can be found among those living closest to a watercourse, particularly in areas where pipe water is not available, or scarce. To be efficient, implementation of schistosomiasis control programme should thus focus on this particular population group.

CONCLUSIONS

The level of population exposure to schistosomiasis in Lambaréné and particularly in the surrounding rural areas of the city is high. The present KAP survey reveals a fairly good knowledge level and a good attitude level of the population toward schistosomiasis, but also a high level of REPs, assumed to be due to their living conditions. In the context of this study, carried out in areas where it is difficult to provide safe water for some of the population, the implementation of the WHO schistosomiasis control programme should focus on two mains aspects, namely the effectiveness of PZQ treatment and education of at-risk populations on preventive behaviours as it is in these populations that a lack of knowledge on the disease transmission was observed. Populations with known freshwater contact could, for instance, be encouraged to seek regular treatment at least once per year, in addition to the implementation of measures aimed at reducing their exposure to the disease.

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ADDITIONAL FILE

Additional file 1: Text S1. Questionnaire for adults who participated in the study.

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Numéro de participation à l'étude : SK-|__ |__ |__ | **Initiales** | __ | __ | __ | __ |

Schistosomiase: Connaissance, Attitude et Pratiques des Populations de Lambaréné et ses Environs

Acronyme: SchistoKAP

Version 1.3_16.06.2019

Questionnaire Adulte

SK-|__|__|__|

CONSENTEMENT VOLONTAIRE A PARTICIPER A L'ETUDE

S'il s'agit d'un participant âgé de 18 ans et plus, a-t-il signé le formulaire de consentement éclairé ?

1. ☐ Oui, 2. ☐ Non

S'il s'agit d'un volontaire âgé de moins de 18 ans ;

Le volontaire a-t-il donné son assentiment oral de participer à l'étude ?

1. ☐ Oui, 2. ☐ Non

Le parent ou tuteur responsable légal a-t-il signé le formulaire de consentement éclairé ?

1. ☐ Oui, 2. ☐ Non

Q1. IDENTIFICATION DU PARTICIPANT ET DONNEES SOCIODEMOGRAPHIQUES

Démographie

Q1.1-Quel est votre âge ? |__|__| ans

Q1.2-Quelle est votre date de naissance ?

|__|__|/|__|__|/|__|__|__|__|
Jour Mois Année

Q1.3 Vous êtes ?

1. ☐ Un homme, 2. ☐ Une femme

Q1.4-Dans quel quartier habitez-vous? _____

Q1.5-Avez-vous toujours vécu dans ce quartier/village?

1. ☐ Oui, 2. ☐ Non

Q1.6-Si "Non", quand êtes-vous arrivé ici? |__|__|/|__|__|/|__|__|__|__|
Jour Mois Année

Q1.7-Si "Non", quel quartier/ville habitiez-vous auparavant? _____

1. ☐ En zone rurale, 2 ☐ En zone urbaine, 3 ☐ En zone semi-urbaine

Données socio-économiques

Q1.8-Quel est votre niveau scolaire?

1. ☐ Aucun, 2 ☐ Primaire, 3 ☐ Secondaire, 4 ☐ Université

Q1.9-Quelle est votre occupation principale?

1. ☐ Aucun, 2. ☐ Agriculteur, 3. ☐ Pêcheur,
4. ☐ Employé de bureau, 5. ☐ Commerçant, 6. ☐ Étudiant,
7. ☐ Agent de santé, 8. ☐ Autre, _____

Q1.10-Le revenu mensuel de votre ménage peut-être estimé à :

1. ☐ Non applicable, 2. ☐ <50 000Fcfa, 3. ☐ 50 000-150 000Fcfa,
4. ☐ Non applicable, 5. ☐ <50 000Fcfa, 6. ☐ Ne veut pas répondre

SK-| __ | __ | __ |

Conditions de vie

Q1.11-Quelle est la principale source d'eau pour vos activités quotidiennes à la maison?
1. ☐ SEEG, 2. ☐ Rivière, 3. ☐ Puits,
4. ☐ Autre, précisez: _

Q1.12-Estimez-vous que votre maison est située à proximité d'un point d'eau: lac, rivière, ruisseau, marais?
1. ☐ Oui, 2. ☐ Non

Q1.13-Si "Oui", veuillez spécifier le type de cours d'eau
1. ☐ Lac, 2. ☐ Rivière, 3. ☐ Ruisseau, 4. ☐ Marais

Q1.14–Quelle est la source d'eau pour vos activités quotidiennes à l'école ou au lieu de travail?
1. ☐ SEEG, 2. ☐ Rivière, 3. ☐ Puits,
5. ☐ Autre précisez: _

Q1.15-Estimez-vous que votre école ou lieu de travail est situé à proximité d'un cours d'eau: lac, rivière, ruisseau, marais?
1. ☐ Oui, 2. ☐ Non

Q1.16- Si "Oui", veuillez spécifier le type de cours d'eau
1. ☐ Lac, 2. ☐ Rivière, 3. ☐ Ruisseau, 4. ☐ Marais

Q1.17–Combien de personnes êtes-vous dans votre maison? _ _ _ _ _ _ _ personnes

Environnement sanitaire

Q1.18-Quel type de sanitaire (toilettes, latrine) utilisez-vous chez vous?
1. ☐ Interne (privé), 2. ☐ Latrine commune, 3. ☐ Latrine traditionnelle
4. ☐ Dans la broussaille, 5. ☐ Nous n'avons pas de latrine dans notre maison

Q1.19-Quel type de sanitaire (toilettes, latrine) utilisez-vous à l'école ou à votre lieu de travail?
1. ☐ Interne (privé), 2. ☐ Latrine commune, 3. ☐ Latrine traditionnelle
4. ☐ Dans la broussaille, 5. ☐ Il n'y a pas de latrine dans mon école / lieu de travail

SK-|__|__|__|

Q2. ÉVALUATION DE LA CONNAISSANCE SUR LA BILHARZIOSE

Connaissance sur la définition de la schistosomiase

Q2.1-Avez-vous déjà entendu parler de la bilharziose

1. ☐ Oui, pouvez-vous nous dire en termes simples ce qu'est la bilharziose? _____
_____
2. ☐ Non (=> *Allez à la question Q3.5*)

Q2.2-Si "Oui", où avez-vous entendu parler de la bilharziose pour la première fois?

1. ☐ À l'école
2. ☐ À travers un parent, un ami ou un collègue (bouche à oreille)
3. ☐ À la télévision ou à la radio
4. ☐ À travers un prospectus
5. ☐ Lors d'une campagne de santé
6. ☐ À l'hôpital
7. ☐ À travers le laboratoire de recherche (CERMEL)
8. ☐ Autre: _____

Q2.3-Si "Oui", connaissez-vous un nom local donné à la bilharziose?

1. ☐ Oui, lequel: _____
2. ☐ Non

Q2.4-Si "Oui", pensez-vous que la bilharziose est une maladie?

1. ☐ Oui, 2. ☐ Non, 3. ☐ Je ne sais pas

Q2.5-Si vous pensez que la bilharziose est une maladie, pensez-vous que c'est une maladie grave?

1. ☐ Oui, 2. ☐ Non

Q2.6-Si "Oui", quel est l'agent causal de la bilharziose (le germe responsable de la bilharzie)?

1. ☐ Un virus
2. ☐ Un ver
3. ☐ Une bactérie
4. ☐ Autre : _____
5. ☐ Je ne sais pas

Connaissance sur le diagnostic de la schistosomiase

Q2.7-Pour vous, quels sont les signes qui montrent que quelqu'un est atteint de bilharziose?

1.-_____
2.-_____
3. ☐ Je ne sais pas

Q2.8-Selon vous, comment savez-vous que quelqu'un est atteint de bilharziose?

1. ☐ Quand il a de la fièvre
2. ☐ Quand il fait la diarrhée
3. ☐ Quand il a mal au ventre
4. ☐ Quand il pisse du sang
5. ☐ Quand il a des démangeaisons
6. ☐ Je ne sais pas
7. ☐ Autre: _____
_____

Q2.9-Savez-vous s'il existe un test à l'hôpital pour rechercher la bilharziose chez un individu?

1. ☐ Oui, 2. ☐ Non
Si oui, lequel? _____

SK-|__|__|__|

Connaissance sur la prévention de la schistosomiase
Q2.10-Comment une personne peut-elle se protéger de la bilharziose? 1. ☐ Eviter d'uriner dans la rivière 2. ☐ Éviter de faire des selles dans la rivière 3. ☐ Eviter d'aller à la rivière 4. ☐ Éviter de marcher pieds nus 5. ☐ Dormir sous la moustiquaire 6. ☐ Autre, pouvez-vous donner des détails svp: ________________________ ____
Connaissance sur la transmission de la schistosomiase
Q2.11-Comment peut-on contracter la bilharziose? 1. ☐ En Marchant pieds nus 2. ☐ En mangeant sans se laver les mains 3. ☐ En allant à la rivière 4. ☐ En buvant l'eau de la rivière 5. ☐ Quand on est piqué par les moustiques 6. ☐ Pendant les rapports sexuels avec une personne atteinte de bilharziose 7. ☐ Je ne sais pas 8. ☐ Autre, spécifiez ______________________________________
Q2.12-Pensez-vous que la bilharziose est contagieuse? 1. ☐ Oui, 2. ☐ Non 3. ☐ Voulez-vous commenter votre réponse? __________________________ ____
Q2.13-Connaissez-vous l'animal qui transmet la bilharziose? 1. ☐ Je ne sais pas 2. ☐ Le moustique 3. ☐ Le grand escargot terrestre 4. ☐ Le petit escargot de rivière 5. ☐ La mouche 6. ☐ Autre, précisez ______________________________________
Connaissance sur traitement de la schistosomiase
Q2.14-Pensez-vous que la bilharziose peut guérir sans traitement? 1. ☐ Oui, 2. ☐ Non
Q2.15-Pensez-vous qu'il existe un médicament pour traiter la bilharziose? 1. ☐ Oui, 2. ☐ Non
Q2.16-Connaissez-vous un traitement traditionnel de la bilharziose? 1. ☐ Oui, 2. ☐ Non
Q2.17-Si oui, pensez-vous que ce traitement traditionnel est efficace, que ça traite vraiment? 1. ☐ Oui, 2. ☐ Non
Q2.18-Selon vous, comment pouvez-vous savoir qu'une personne est guérie de la bilharziose? _ ____ ____
Q2.19-Avez-vous des commentaires sur le traitement de la bilharziose? _____________ ____ ____

SK-|__|__|__|

Q3. EVALUATION DE L'ATTITUDE DE LA POPULATION SUR LA BILHARZIE

Perception de la maladie

Q3.1-Pensez-vous que nous devrions avoir honte d'avoir la bilharziose?

1. ☐ Oui,
2. ☐ Non,
3. ☐ Pouvez-vous expliquer votre réponse? _____
_____
_____

Q3.2-Selon vous, la bilharziose est une maladie qui attaque:

1. ☐ Les enfants
2. ☐ Les femmes
3. ☐ Les hommes
4. ☐ Les pauvres
5. ☐ Les gens riches
6. ☐ Les personnes faibles
7. ☐ Avez-vous un commentaire sur cette question? _____
_____

Q3.3-Pensez-vous que la bilharziose peut causer?

1. ☐ L'anémie
2. ☐ La stérilité (infertilité)
3. ☐ Le cancer de la vessie
4. ☐ Les pertes vaginales malodorantes chez les femmes
5. ☐ Les pertes vaginales malodorantes chez la petite fille
6. ☐ La mort
7. ☐ Autre, précisez: _____
_____

Q3.4-A quel niveau de gravité classez-vous la bilharziose? (Encerclez une réponse)

0	1	2	3	4	5
(Pas grave du tout)					(Très grave)

Q3.5-Si vous pensez que vous avez la bilharziose, iriez-vous chercher des soins:

1. ☐ À l'hôpital?
2. ☐ À la pharmacie?
3. ☐ Chez un guérisseur traditionnel?
4. ☐ Personne, je n'irai pas consulter
5. ☐ Avez-vous un autre commentaire sur cette question? _____
_____
_____

Q3.6-Informeriez-vous vos proches si vous avez la bilharziose?

1. ☐ Oui,
2. ☐ Non,
3. ☐ Pouvez-vous argumenter votre réponse? _____
_____
_____

SK-| __ | __ | __ |

Q4. ÉVALUATION DES PRATIQUES PAR RAPPORT À LA BILHARZIE

Pratiques relatives à l'exposition à la bilharziose

Q4.1-Avez-vous été en contact avec un cours d'eau (lac, rivière, ruisseau, marais, etc.)?
1. ☐ Oui, 2. ☐ Non *(=> Allez à la question Q5.1)*

Q4.2-Si "Oui", de quel type de cours d'eau s'est-il agit ?
1. ☐ Lac, 2. ☐ Rivière, 3. ☐ Ruisseau, 4. ☐Autre ……..……..

Q4.3-Si "Oui", où avez-vous été en contact avec le cours d'eau?
1. ☐ À la maison, 2. ☐ À l'école /lieu de travail, 3. ☐ À la plantation
4. ☐ Autre : _

Q4.4-Si "Oui", à quelle fréquence?
1. ☐ Tous les jours, 2. ☐ Toutes les semaines, 3. ☐ Quelques fois par mois

Q4.5-Si "Oui", quand y êtes-vous allé pour la dernière fois? | __ | __ | | __ | __ | __ | 20 __ | __ |
Jour mois Année

Q4.6-Si "Oui", quelles sont les raisons pour lesquelles vous avez été (ou vous êtes) en contact avec le cours d'eau?
1. ☐ Pour les travaux ménagers (vaisselle, lessive…)
2. ☐ Pour puiser de l'eau
3. ☐ Pour prendre un bain
4. ☐ Pour jouer
5. ☐ Pour pêcher
6. ☐ Pour mes activités agricoles
7. ☐ Autre, précisez: _

Q4.7-Si "Oui", à quelle heure de la journée partez-vous habituellement au cours d'eau?
1. ☐ Le matin
2. ☐ Entre 12 heures et 15 heures
3. ☐ Dans l'après-midi, à partir de 15 heures à 18 heures
4. ☐ Le soir ou la nuit
5. ☐ Je n'ai pas d'heures précises pour aller à la rivière

Q4.8- Si "Oui", avez-vous déjà uriné dans ce (ou un autre) cours d'eau?
1. ☐ Oui, 2. ☐ Non

Q4.9- Si "Oui", avez-vous déjà fait les selles dans ce (ou un autre) cours d'eau?
1. ☐ Oui, 2. ☐ Non

Pratiques relatives à la protection contre la schistosomiase

Q4.10-Quelle est votre pratique quotidienne pour éviter d'avoir la bilharziose? _ _ _ _ _ _ _ _
_ _
_ _

Pratiques concernant le traitement de la schistosomiase

Q4.11-Si vous êtes traité pour la bilharziose, pourriez-vous éviter d'aller à la rivière pour ne pas avoir à nouveau la bilharziose?
1. ☐ Oui
2. ☐ Non
3. ☐ Pouvez-vous argumenter votre réponse? _
_ _

SK-|__|__|__|

Q5. EVALUATION CLINIQUE

Q5.1-Avez-vous déjà eu du sang dans les selles?
1. ☐ Oui, 2. ☐ Non

Q5.2-Avez-vous déjà uriné avec du sang?
1. ☐ Oui, 2. ☐ Non (=> *Allez à la question Q5.4*)

Q5.3-Si "Oui", quand avez-vous uriné avec du sang la dernière fois?
1. ☐ Cette semaine
2. ☐ Ce mois-ci
3. ☐ Le mois dernier
4. ☐ Pendant l'année
5. ☐ Plus d'un an
6. ☐ Je ne me rappelle pas

Q5.4-Avez-vous déjà eu la bilharziose?
1. ☐ Oui, pouvez-vous dire quand? _ _ _ _ _ (jour) _ _ _ _ _ _ _ (mois) _ _ _ _ _ _ (années)
2. ☐ Non (=> *Allez à la question Q5.6*)

Q5.5-Si "Oui", comment avez-vous su que vous aviez la bilharziose? _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
_ _

Q5.6-Avez-vous déjà reçu le médicament de la bilharziose?
(Présentez une copie du médicament au volontaire, s'il vous plaît)
1. ☐ Oui, 2. ☐ Non (=> *Allez à la question Q5.9*)

Q5.7-Si "Oui", vous souvenez-vous quand? _ _ _ _ (jour) _ _ _ _ _ _ _ (mois) _ _ _ _ _ _ (années)

Q5.8-Si "Oui", comment êtes-vous entré en possession du médicament?
1. ☐ Je l'ai acheté à la pharmacie
2. ☐ Je l'ai reçu du laboratoire de recherche (CERMEL)
3. ☐ Je l'ai reçu lors de campagnes de traitement de masse (programme national)
4. ☐ Je l'ai reçu dans un centre de santé / à l'hôpital
5. ☐ Je l'ai reçu d'un parent
6. ☐ Autre, précisez: _
_ _

Q5.9-Que pensez-vous du médicament de la bilharziose? _
_ _
_ _

Merci d'avoir pris le temps de répondre à ce questionnaire. Nous espérons que les informations que vous nous avez fournies contribueront à un meilleur contrôle de la bilharziose dans notre localité.

L'équipe de recherche

Date du jour : |__|__| |__|__|__| 2019 Initiales intervieweur : |__|__|__|

Additional file 2: Text S2. Questionnaire for children who participated in the study.

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Numéro de participation à l'Etude: SK-| __ | __ | __ | **Initiales** | __ | __ | __ | __ |

Schistosomiase : Connaissance, Attitude et Pratiques des populations de Lambaréné et de ses Environs

Acronyme : SchistoKAP

Version 1.2_04.06.2019

Questionnaire Enfant

Consentement volontaire à participer à l'étude

Le volontaire a-t-il donné son accord oral de participer à l'étude ?
1. ☐ Oui, 2. ☐ Non (Ne pas procéder au questionnaire svp)

Le parent ou tuteur responsable l'enfant a-t-il signé le formulaire de consentement éclairé ?
1. ☐ Oui, 2. ☐ Non (Ne pas procéder au questionnaire svp)

Q1. Identification et données sociodémographiques

Données démographiques

Q1.1- Quel âge as-tu ? __|__| ans

Q1.2- Quand est-ce que tu fêtes ton anniversaire ? |__|__| |__|__|__| |__|__|__|__|

Q1.3- Es-tu ;

1. ☐ Un garçon ? 2. ☐ Une fille ?

Q1.4- Quel est le nom de ton quartier ? _

Q1.7- Quel quartier habitais-tu avant ? _

1. ☐ Zone rurale 2. ☐ Zone urbaine 3. ☐ Zone semi-urbaine

Données socio-économiques

Q1.8- Où vas-tu à l'école ?
1. ☐ Je ne vais pas à l'école 2. ☐ A l'école primaire 3. ☐ Au lycée

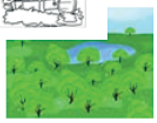
Q1.8a- Si tu vas à l'école, comment s'appelle ton école ? _

Q1.8b- Et quelle classe fais-tu ? _

Conditions de vie

Q1.11- Quelle eau utilisez-vous à la maison ?
1. ☐ L'eau de la SEEG 2. ☐ L'eau de la rivière 3. ☐ L'eau du puit

Q1.12- Penses-tu que votre maison est proche d'un point d'eau ?
1. ☐ Oui 2. ☐ Non



Q1.13- Si oui, s'agit-il :
1. ☐ D'un lac? 2. ☐ D'une rivière ? 3. ☐ D'un marigot ?

Q1.14- Quelle eau utilises-tu à l'école ?
1. ☐ L'eau de la SEEG 2. ☐ L'eau de la rivière 3. ☐ L'eau du puit

Q1.15- Penses-tu que ton école est proche d'un point d'eau ?
1. ☐ Oui 2. ☐ Non

Q1.16- Si oui, s'agit-il :
1. ☐ D'un lac ? 2. ☐ D'une rivière? 3. ☐ D'un marigot ?

SK -| __ | __ | __ |

Environnement sanitaire



Q1.18- Quel type de toilettes utilisez-vous à la maison ?

1. ☐ Moderne 3. ☐ Traditionnelles 5. ☐ Nous n'avons pas de toilette à la maison

Q1.19- Quel type de toilettes utilises-tu à l'école ?

1. ☐ Moderne 3. ☐ Traditionnelles 5. ☐ Nous n'avons pas de toilette à l'école

Q2. Evaluation de la connaissance sur la Bilharzie

Q2.1- As-tu déjà entendu parler de la bilharzie ?

1. ☐ Oui 2. ☐ Non (=> *Allez à la question Q3.6*)

Q2.2- Si oui, où as-tu entendu parler de la bilharzie pour la première fois ?

1. ☐ A l'école
2. ☐ A la maison (Parent, famille)
8. ☐ Autre, précisez _____

Q2.20- Si oui, sais-tu quand quelqu'un est malade de la bilharzie ?

1. ☐ Oui, comment ? _____
2. ☐ Non

Q2.21- Si oui, sais-tu où est ce qu'on attrape la bilharzie ?

1. ☐ Oui, où ? _____
2. ☐ Non

Q2.22- Si oui, que peux-tu faire pour éviter la bilharzie ? _____

_____

Q3. Evaluation de l'attitude vis-à-vis de la bilharzie

Q3.6- S'il t'arrive de pisser du sang, le diras-tu aux autres?

1. ☐ Oui 2. ☐ Non

Q3.6a- S'il t'arrive de pisser du sang, à qui le diras-tu ?

1. ☐ A mes parents
2. ☐ A mon enseignant (e)
3. ☐ A mes amis

SK -| __ | __ | __ |

Q4. Evaluation des pratiques vis-à-vis de la bilharzie

Q4.1- Vas-tu souvent à l'eau ?
1. ☐ Oui 2. ☐ Non (=> *Allez à la question Q5.1*)

Q4.2- Si oui, s'agit-il :
1. ☐ D'un lac ? 2. ☐ D'une rivière ? 3. ☐ D'un marigot ?

Q4.3- Si oui, d'où est-ce que tu pars le plus souvent pour le point d'eau ?
1. ☐ De la maison 2. ☐ A l'école 4. ☐ Autre, _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

Q4.4- Si oui, combien de fois vas-tu au point d'eau ?
1. ☐ Tous les jours 2. ☐ Parfois (De temps en temps)

Q4.6- Si oui, pourquoi vas-tu à ce point d'eau ?
1. ☐ Pour laver le linge ou faire la vaisselle
2. ☐ Pour puiser de l'eau
3. ☐ Pour me baigner
4. ☐ Pour jouer avec mes amis
5. ☐ Pour la pèche
6. ☐ Pendant les travaux champêtres

Q4.7- Si oui, à quel moment de la journée tu y vas le plus souvent ?
1. ☐ En matinée 3. ☐ En après-midi 5. ☐ Je n'ai pas d'heure pour aller à la rivière

Q4.8- Si oui, as-tu déjà uriné dans ce point d'eau quand tu y vas ?
1. ☐ Oui 2. ☐ Non

Q4.9- Si oui, as-tu déjà fait caca dans ce point d'eau quand tu y vas ?
1. ☐ Oui 2. ☐ Non

Q5. Clinique

Q5.1- As-tu déjà cabiné du sang ?
1. ☐ Oui 2. ☐ Non

Q5.2- As-tu déjà pissé du sang ?
1. ☐ Oui 2. ☐ Non

Q5.4- On t'a déjà dit que tu as la bilharzie ?
1. ☐ Oui 2. ☐ Non (=> *Allez à la question Q5.6*)

Q5.5- Si oui, qui te l'a dit ?
1. ☐ Mes parents 2. ☐ A l'hôpital 3. ☐ Mes amis 4. ☐ Autre, _ _ _ _ _

Q5.6- As-tu déjà recu le médicament de la Bilharzie ?
1. ☐ Oui 2. ☐ Non

Q5.8- Si oui, où as-tu recu ce médicament ?
2. ☐ Au CERMEL 3. ☐ A l'école 4. ☐ A l'hôpital 5. ☐ A la maison par mes parents

Nous te remercions pour ta participation à cette étude. Les réponses que tu viens de nous donner vont nous aider à mieux réfléchir sur comment te protéger, toi, ta famille et tes amis contre la bilharzie.

L'équipe de recherche

Date du jour : |__|__| |__|__|__| 2019 Initiales intervieweur |__|__|__|

Additional file 3: Table S1. Description of the scoring of each question and total score for knowledge, attitudes and practices calculated for the participants interviewed during the surveys.

Question reference number[$]	Answer	Mark per answer	Maximum score for	
			Adults	Children
Knowledge				
Q2.1	Oui	+1	1	1
	Non	+0		
Q2.4	Oui	+1	1	NA
	Non	+0		
Q2.6	Un virus	+0	1	NA
	Un ver	+1		
	Une bactérie	+0		
	Autre	+0		
	Je ne sais pas	+0		
Q2.8	Quand il a de la fièvre	+0	1	NA
	Quand il fait la diarrhée	+0		
	Quand il a mal au ventre	+0		
	Quand il pisse du sang	+1		
	Quand il a des démangeaisons	+0		
	Je ne sais pas	+0		
	Autre	+0		
Q2.9	Oui	+1	1	NA
	Non	+0		
Q2.10	Eviter d'uriner dans la rivière	+1	3	NA
	Eviter de faire des selles dans la rivière	+1		
	Eviter d'aller à la rivière	+1		
	Eviter de marcher pieds nus	+0		
	Dormir sous la moustiquaire	+0		
	Autre	+0		
Q2.11	En marchant pieds nus	+0	1	NA
	En mangeant sans se laver les mains	+0		
	En allant à la rivière	+1		
	En buvant l'eau de la rivière	+0		
	Quand on est piqué par les moustiques	+0		
	Pendant les rapports sexuels avec une personne atteinte de la bilharzie	+0		
	Je ne sais pas	+0		
	Autre	+0		
Q2.12	Oui	+0	1	NA
	Non	+1		

Additional file 3: Table S1. *(continued)*

Question reference number[$]	Answer	Mark per answer	Maximum score for	
			Adults	Children
Q2.13	Je ne sais pas	+0	1	NA
	Le moustique	+0		
	Le grand escargot terrestre	+0		
	Le petit escargot de rivière	+1		
	La mouche	+0		
	Autre	+0		
Q2.14	Oui	+0	1	NA
	Non	+1		
Q2.15	Oui	+1	1	NA
	Non	+0		
	Total score for knowledge		**13**	**1**
Attitudes				
Q3.1	Oui	+0	1	NA
	Non	+1		
Q3.5	A l'hôpital ?	+1	2	NA
	A la pharmacie ?	+1		
	Chez un guérisseur traditionnel ?	+0		
	Personne, je n'irai pas consulter	+0		
Q3.6	Oui	+1	1	1
	Non	+0		
	Total score for attitudes		**4**	**1**
Practices				
Q4.1	Oui	+1	1	1
	Non	+0		
Q4.4	Tous les jours	+3	3	3
	Toutes les semaines	+2		
	Quelques fois par mois	+1		

Additional file 3: Table S1. *(continued)*

Question reference number[$]	Answer	Mark per answer	Maximum score for	
			Adults	Children
Q4.7	Le matin	+0	1	1
	Entre 12 heures et 15 heures	+1		
	Dans l'après-midi, à partir de 15 heures à 18 heures	+1		
	Le soir ou la nuit	+0		
	Je n'ai pas d'heures précises pour aller à la rivière	+1		
Q4.8	Oui	+2	2	2
	Non	+0		
Q4.9	Oui	+1	1	1
	Non	+0		
Q4.11	Oui	+1	1	NA
	Non	+0		
Children sub-total score for risk enhanced practices			-	8
Total score for risk enhanced practices			**9**	**9***

[$] Reference number of the question in the adults and children questionnaires

*To harmonise the score between adults and children particularly for multivariate analysis, the total score of children was obtained by multiplying the sub-total score by eight and divided by nine

NA: Not Applicable

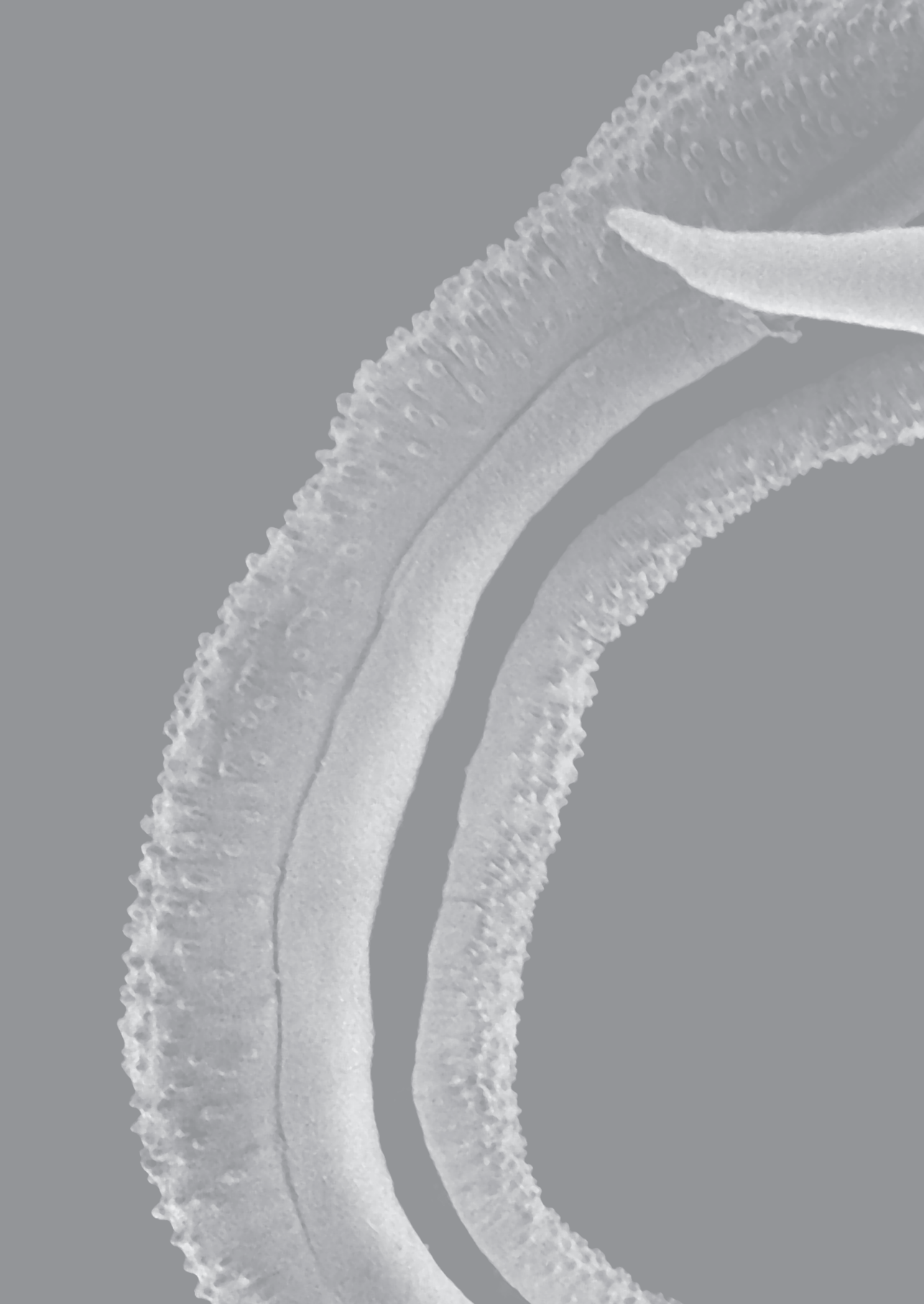
Additional File 4 – Table S2. Factors associated with appropriate knowledge and risk-enhancing practices of the study population towards schistosomiasis.

Variable	Correct knowledge score*							Risk-enhancing practices score						
	Mean	Unadjusted analysis			Adjusted analysis[β]			Mean	Unadjusted analysis			Adjusted analysis		
		α	95%CI(α)	*p*	α	95%CI(α)	*p*		α	95%CI(α)	*p*	α	95%CI(α)	*p*
Age				**0.74**			**0.52**				**0.09**			**0.25**
6 – 9	-	-	-		-	-		5.23	Ref			-	-	
10 – 13	-	-	-		-	-		5.24	0.01	-0.58 – 0.59	0.99	-	-	
14 – 17	8.74	Ref			Ref			6.00	0.77	0.13 – 1.41	0.02	Ref		
18 – 25	8.97	0.23	-0.59 – 1.04	0.59	0.36	-0.47 – 1.19	0.40	5.33	0.10	-0.52 – 0.72	0.76	-0.56	-1.25 – 0.12	0.10
>25	8.75	0.01	-0.76 – 0.77	0.99	0.53	-0.42 – 1.48	0.27	5.24	0.01	-0.55 – 0.56	0.99	-0.43	-1.21 – 0.36	0.29
Gender														
Female	8.83	Ref			Ref			5.28	Ref			Ref		
Male	8.79	-0.04	-0.60 – 0.52	0.88	-0.13	-0.69 – 0.43	0.64	5.49	0.21	-0.18 – 0. 59	**0. 29**	-0.28	-0.74 – 0.19	**0.24**
History of schistosomiasis														
No	8.50	Ref			Ref			4.92	Ref			Ref		
Yes	9.32	0.82	0.29 – 1.35	**0.003**	0.90	0.35 – 1.45	0.001	6.15	1.23	0.85 – 1.60	**<0.001**	0.77	0.31 – 1.22	**<0.001**
Location				**0.004**			**0.005**				**<0.001**			**<0.001**
Lambaréné	9.22	Ref			Ref			4.78	Ref			Ref		
Zilé-PK	8.07	-1.14	-1.78 – -0.51	<0.001	-1.34	-2.11 – -0.57	<0.001	6.41	1.62	1.21 – 2.03	<0.001	0.77	0.14 – 1.41	0.02
Mitonè-PK	8.58	-0.64	-1.59 – 0.32	0.19	-0.80	-1.89 – 0.30	0.15	7.29	2.50	1.88 – 3.13	<0.001	2.01	1.11 – 2.92	<0.001
Bindo-Makouké	8.49	-0.73	-1.57 – 0.11	0.09	-0.81	-1.75 – 0.14	0.09	4.37	-0.42	-0.98 – 0.15	0.15	-1.36	-2.14 – -0.57	<0.001
Educational level				**0.004**			**0.06**				**0.15**			**0.005**
Primary	8.09	Ref			Ref			5.49	Ref			Ref		
Secondary	9.10	1.01	0.41 – 1.61	0.001	0.79	0.11 – 1.47	0.02	5.28	-0.21	-0.59 – 0.17	0.28	-0.41	-0.97 – 0.15	0.15
Other	8.64	0.55	-0.77 – 1.88	0.41	0.52	-0.81 – 1.85	0.44	4.57	-0.92	-1.93 – 0.08	0.07	-1.76	-2.86 – -0.66	0.002

Additional File 4 – Table S2. *(continued)*

Family size				**0.11**			**0.02**				**0.20**			**0.007**
1 – 3	8.36	Ref			Ref			5.61	Ref			Ref		
4 – 6	9.07	0.71	0.04 – 1.37	0.04	0.86	0.20 – 1.52	0.01	5.14	-0.47	-1.08 – 0.13	0.12	-0.70	-1.24 – -0.15	0.01
7 – 25	8.85	0.48	-0.21 – 1.18	0.17	0.75	0.04 – 1.47	0.04	5.55	-0.06	-0.69 – 0.57	0.84	-0.87	-1.47 – -0.28	0.004
Use private toilets at home														
No	8.74	Ref			Ref			5.54	Ref			Ref		
Yes	9.38	0.64	-0.15 – 1.43	**0.11**	0.27	-0.60 – 1.14	0.55	4. 06	-1.48	-2.05 – -0.90	**<0.001**	-0.28	-1.01 – 0.43	**0.42**
Use pit latrine at home														
No	9.11	Ref			Ref			4.79	Ref			Ref		
Yes	8.59	-0.51	-1.04 – 0.01	**0.06**	-0.21	-0.82 – 0.40	0.50	5.58	0.79	0.38 – 1.20	**<0.001**	-0.12	-0.62 – 0.38	**0.63**
Home proximity with water course														
No	8.85	Ref			Ref			4.96	Ref			Ref		
Yes	8.81	-0.03	-0.60 – 0.53	**0.90**	-0.12	-0.73 – 0.49	0.70	5.55	0.59	0.19 – 0.99	**0.004**	0.03	-0.47 – 0.53	**0.89**
Use fresh water														
No	9.13	Ref			Ref			4.43	Ref			Ref		
Yes	8.68	-0.45	-1.02 – 0.11	0.11	0.14	-0.56 – 0.84	0.70	5.79	1.36	0.97 – 1.75	**<0.001**	0.79	0.21 – 1.37	0.008
Main occupation				**0.05**			**0.12**				**0.36**			**0.93**
None	8.45	Ref			Ref			5.16	Ref			Ref		
Student	9.11	0.65	-0.08 – 1.38	0.08	0.69	-0.13 – 1.52	0.10	5.61	0.45	-0.22 – 1.12	0.19	0.20	-0.48 – 0.88	0.56
Farmer/Fisher	8.39	-0.06	-0.83 – 0.70	0.78	0.67	-0.18 – 1.52	0.12	5.68	0.52	-0.18 – 1.22	0.14	-0.12	-0.82 – 0.58	0.73
Trader	8.62	0.17	-0.97 – 1.30	0.77	0.01	-1.12 – 1.14	0.98	5.38	0.22	-0.82 – 1.26	0.68	-0.14	-1.08 – 0.79	0.76
Other	9.41	0.95	0.17 – 1.73	0.02	0.87	0.08 – 1.66	0.03	5.08	-0.08	-0.80 – 0.63	0.82	-0.06	-0.72 – 0.58	0.84

*Assessed among young adults and adults who responded to the main study questionnaire; [α]Coefficient of regression estimating the difference in mark between the associated modality and the modality of reference of the variable; [β]The model of the adjusted analysis included all data considered for crude analysis



CHAPTER 4

Pilot malacology surveys for the intermediate hosts of schistosomiasis in rural and semi-urban areas of the Moyen-Ogooué province, Gabon

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Trop. Med. Infect. Dis. **2022, 7(1), 1; https://doi.org/10.3390/tropicalmed7010001**

ABSTRACT

The objective of this pilot malacological survey was to identify the snail intermediate hosts for *Schistosoma haematobium* in endemic rural and semi-urban areas of Gabon. Snails were collected, morphologically identified, and tested for infection by cercarial shedding. Released cercariae were morphologically identified using low-power light microscopy. A total of six species of snails were collected throughout the study area, with *Bulinus truncatus*, *B. forskalii*, and *Potadoma spp*. being the most predominant species collected. Only the *Bulinus* species were tested for infection by cercarial shedding, of which only *B. truncatus* shed cercariae. Some *B. truncatus* shed mammalian schistosome cercariae, while others shed *Gymnocephalus* cercariae. Our results indicate that *B. truncatus* appears to be a potential intermediate host of schistosomiasis in Gabon, where cases of *S. haematobium*, *S. guineensis*, and *S. intercalatum* infection are reported. However, it will be important to further understand the species diversity and transmission dynamics of schistosomes.

1. INTRODUCTION

Schistosomiasis, a water-borne helminthic disease, is the second most important parasitic infection after malaria in terms of public health and economic impact [1]. Human infections are caused by three main species of flukes, namely, *Schistosoma haematobium* causing urogenital schistosomiasis, and *S. japonicum* and *S. mansoni*, which both cause intestinal schistosomiasis. There are other species that cause intestinal schistosomiasis, although their distribution is restricted to specific foci, including *S. guineensis* and its variant *S. intercalatum* in Central Africa, and *S. mekongi* in South East Asia [2]. The worldwide geographical distribution of the different *Schistosoma* species depends on the presence and distribution of their freshwater snail intermediate hosts; the snail genus is specific to the species of the parasite, with some variations across countries. In Africa, for instance, predominantly snails of the genus *Biomphalaria* serve as intermediate hosts of *S. mansoni*, while snails of the genus Bulinus serve as intermediate hosts of *S. haematobium*, as well as of *S. intercalatum* and *S. guineensis* [3]. *Bulinus* spp. are also known as the intermediate hosts of *S. bovis* [4], a schistosome parasite of ruminants such as cattle, goats, sheep, and pigs. The geographical distribution and density of the snail population and their dynamics over time relate to the epidemiological situation of the disease in a particular human population, rendering schistosomiasis a focal disease.

Freshwater snail control is part of the WHO's recommendation for the control of schistosomiasis [2]. Malacological data is therefore essential for a better understanding of the disease transmission, but also for the implementation of a proper and adequate schistosomiasis control program. Gabon is a central African country located on the equator. Although the region is known to be endemic for schistosomiasis, very few malacological data are available for the country, and most of it is historic. More recent data by Mintsa et al. (2009) reported the presence of *B. globosus* and *B. forskalii* in two different sites in the Estuaire province; Libreville and Ekouk [5]. We conducted a pilot survey in rural and semi-urban areas located central to the country, known to be endemic for urogenital schistosomiasis, with the aim to provide basic information on the snails as intermediate hosts for schistosomiasis and on molluscan diversity in the Moyen-Ogooué, one of the nine provinces of Gabon.

2. MATERIALS AND METHODS

The surveys were carried out at CERMEL [6] and were conducted from 15–19 November 2013 on three different locations: Lambaréné, the provincial capital of the Moyen-Ogooué; the Zilé-PK area, which is a string of villages along the national road (RN1) south of Lambaréné from PK8 to PK33, including Tsouka and Massika I and II villages; and in Mbolani, namely the Bindo-Makouké villages, which is a remote area 65 km from Lambaréné by road (Figure 1). All these locations are either close to the Ogooué river, or are irrigated by its tributaries, with many lakes and swamps. In the region, the vegetation is made up of rainforests, and the weather is characterized by four seasons, long rainy (February to May) and dry (June to September) seasons, followed by short rainy (October to mid-December) and dry (mid-December and Jan-

uary) seasons. These areas are known to be schistosomiasis-endemic, with S. haematobium being the predominant species [7–9]. Indeed, we reported, in 2020, a 26% schistosomiasis prevalence in Lambaréné [9], while a prevalence of around 45% and 15% were reported earlier in 2014 and 2018 for the Zilé-PK area and Bindo village, respectively [7,8].

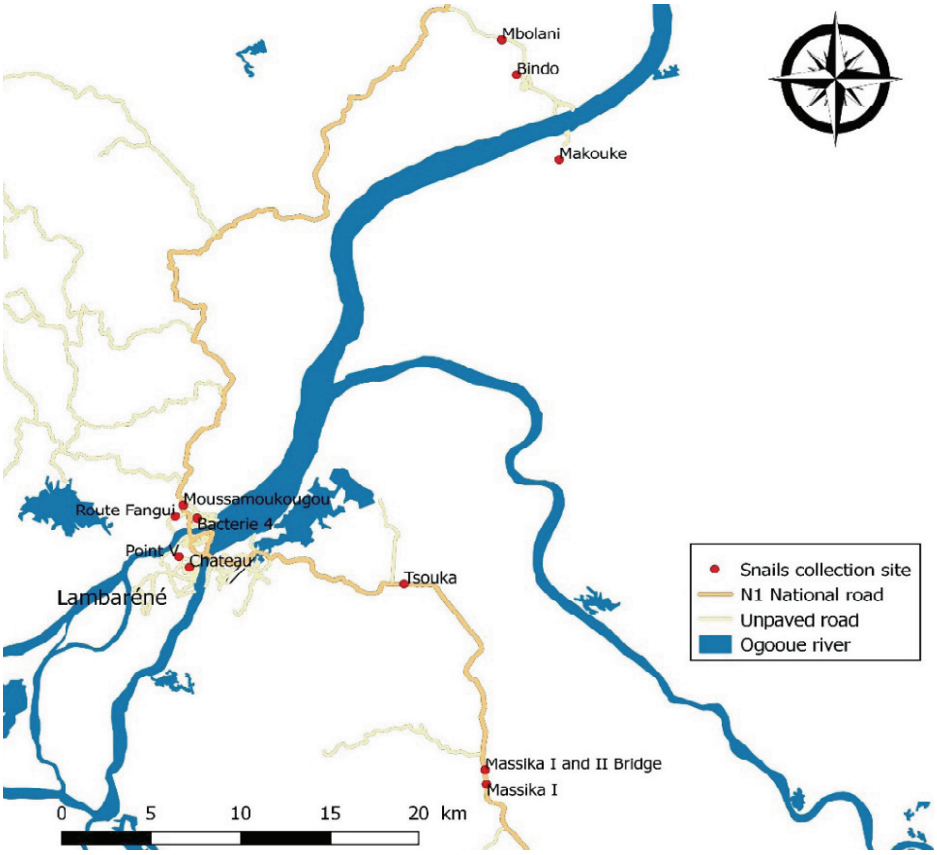

Figure 1. Distribution of the human-freshwater contact points selected for snail collection over the study area.

For each of the three study areas, human-water contact sites, known as potential schistosomiasis foci, were identified. All sites had on average up to 50% vegetation cover, with the watercourse bed being either muddy, sandy, or both. At the selected sites, snails were collected systematically by three collectors for about ten minutes between 8 a.m. and 11 a.m. from aquatic plants and other objects in the habitats. Specifically, vegetation and any materials such as discarded pieces of clothing and tires were thoroughly searched for possibly attached mollusks. During the snail collections, the geographic coordinates of the site were taken using a hand-held GPS, and human-water contact behaviors were observed. All collected snails were placed in a perforated container with wet cotton wool or wet vegetation before being transported back to the CERMEL laboratory.

At the laboratory, snails were separated and identified mostly to the genus level based on the shell morphological characteristics using the standardized taxonomic keys proposed by the WHO identification center [10]. On the day of collection, snails were individually placed in a well plate for cercariae shedding, and dechlorinated clean commercial drinking water was added. The plate was covered to prevent snails from escaping but opened and closed regularly for air circulation. The plate was placed in indirect daylight and left for about three hours from noon to 3 p.m., and then it was examined. The wells were examined under a low-powered microscope for evidence of any emitted cercariae, which were then morphologically differentiated using standardized taxonomic keys [11].

3. RESULTS

3.1. Snail Collection and Species Distribution

A number of snail collection points were selected over the study area (Figure 1). In the Zilé-PK area, the first area visited was Tsouka village, where six water contact sites that appeared as potential transmission hotspots were selected along a tributary of the Ogoouée River, namely, Mikoli River. Other sites that were visited included various sites in Massika I and Massika II villages. Within Lambaréné, snails were collected in small streams of some neighborhoods; Château, Fanguy, and Moussamoukougou, respectively. In Mbolani, Bindo, and Makouké villages, a total of four collection sites were targeted, as these were known as the main human-water contact points.

In total, six snail species were collected from a number of collection points. The overall freshwater snails that were found were: *Potadoma* species (most likely *P. freethi*), *Bulinus truncatus*, *Bulinus forskalii*, *Melanoides* species (most likely *M. tuberculata*), *Lanistes* (most likely *L. nsedweensis*), and *Gabiella* species. Table 1 presents the distribution of snail species collected in each study site. With regard to the *Bulinus* species, a total of 44 snails were collected over the study area, including four *B. forskalii* and 40 *B. truncatus*.

Table 1. Distribution of snail species collected by the study team

Study Area	Snail Genus	Snail Species
Zilé-PK area; Tsouka, Massika I and Massika II villages	*Bulinus*	*B. truncatus*
	Potadoma	*P. freethi*[1]
	Melanoides	*M. tuberculate*[1]
	Lanistes	*L. nsedweensis*[1]
Lambaréné town	*Bulinus*	*B. truncatus*
	Bulinus	*B. forskalii*
Mbolani, Bindo, and Makouké villages	*Bulinus*	*B. forskalii*
	Gabiella	*Gabiella* spp.

[1]Most likely.

3.2. Cercarial Shedding

When testing for cercarial shedding, none of the four *B. forskalii* snails examined were infected, while 12 (30%) of the 40 *B. truncatus* (Figure 2a) examined shed mammalian *Schistosoma* cercariae (Figure 2b), whilst others shed *Gymnocephalus* cercariae. *Bulinus* snails that shed schistosome cercariae were collected only in the Mikoli River of Tsouka village (Zilé-PK area).

(**a**) (**b**)

Figure 2. (a) Some *Bulinus truncatus* snails collected at Tsouka village; (b) Microscopic view of shedding of some mammalian (forked tail) and *Gymnocephalus* (single tail) cercariae.

4. DISCUSSION

The present survey adds malacological information to the scarce data available from the schistosomiasis-endemic region. Our results establish the first evidence of cercarial in the Moyen-Ogooué province. Indeed, we found that *B. truncatus* appears to be an intermediate host of schistosomiasis in the region. It is known that some *Bulinus* snails may act as intermediate hosts of *Schistosoma* bovis [4,12] which cannot be separated from *S. haematobium* by cercariae morphology. However, no domestic animals were observed at the study sites, nor any evidence of bovine game. Moreover, no data are available on the potential presence of *S. bovis* in Gabon, and particularly in the study area. Since the study area is known to be endemic for *S. haematobium* [7–9], we therefore strongly suspect that the mammalian cercariae were actually *S. haematobium* cercariae, shed by *B. truncatus*. However, the use of molecular tools to accurately identify *B. truncatus* as a snail host for *S. haematobium* cercariae in the area remains. As some cases of Schistosome eggs have been reported in the region [9], and cases

of *S. guineensis* have been reported in the country, the role of *B. truncatus* in the transmission of *S. intercalatum* and *S. guineensis* [13] in the country has to be further investigated.

The study was conducted in November, corresponding with the beginning of the rainy season. During the surveys, we observed a low density of snails in the study areas, particularly in the Bindo-Makouké villages. Since seasonal rainfall affects snail density [14], we hypothesize that this reflects the snail population density usually observed during the rainy season. Despite the low density of snails observed, the genus *Bulinus* was present in all three study areas, while *B. truncatus* was found in Lambaréné and in the Zilé-PK areas, known as areas with a moderate or high prevalence of urogenital schistosomiasis [7–9], compared to Bindo-Makouké, where the prevalence of the disease is low [7,8]. This suggests that the distribution of *B. truncatus* could sustain the prevalence of schistosomiasis in the region, and probably in the country.

When exposed to daylight illumination, only *B. truncatus* shed cercariae. Similar to what was reported earlier by Mintsa et al. [5], no *B. forskalii* we collected shed cercariae. However, we found that a high proportion of *B. truncatus* shed cercariae (around 30%), particularly those from the Zilé-PK rural area. This is in contrast to what was reported from southern Mauritania and western Kenya where no to few (1.8%) snails sampled shed cercariae [15,16], respectively. Similarly, the number of cercariae shed by most of the snails was considerably higher than what is usually reported from other naturally infected snails. These results suggest that the *B. truncatus* intermediate hosts we identified are a very efficient vector of schistosomiasis in our study area, which contrasts with the observation of a similar snail species in Kenya, which is refractive to the local *S. haematobium* [17].

In addition to the *Bulinus* snails involved in schistosomiasis transmission, we found other snail intermediate hosts that are capable of transmitting other parasitic diseases. *Potadoma spp*. was one of the snail genera found, particularly in the Zilé-PK area. It has been suspected that *Potadoma* snails may be the intermediate hosts of the lung flukes of the human *Paragonimus* species (most likely *P. africanus* or *P. uterobilateralis*), which are reported to occur in parts of Central (Zaire and Cameroon) and West (Nigeria) Africa, respectively [18]. It would be of interest to clarify the role of this snail in Gabon, and particularly in Lambaréné and its surroundings, where some cases of paragonimiasis have already been reported [19,20].

5. CONCLUSIONS

Bulinus spp., a potential intermediate host of schistosomiasis, appears to be present in Gabon, particularly *B. globosus*, *B forskalii*, and, as we reported, *B. truncatus*, which appears to be an efficient intermediate host of schistosomiasis. However, it remains necessary to properly identify the species in Lambaréné and the surrounding areas using molecular analyses to understand the seasonality of snail transmission and population dynamics to guide an appropriate strategy for schistosomiasis control.

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CHAPTER 5

Schistosoma haematobium infection morbidity, praziquantel effectiveness and reinfection rate among children and young adults in Gabon

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ABSTRACT

Background: Sub-Saharan Africa carries most of the global burden of schistosomiasis. To optimize disease control and reduce morbidity, precise data are needed for control measures adapted to the local epidemiological situation. The objective of this study is to provide baseline information on schistosomiasis dynamics, including praziquantel (PZQ) treatment outcome in children and young adults living in the vicinity of Lambaréné, Gabon.

Methods: Eligible volunteers were included into a prospective longitudinal study. Urine filtration technique was used to detect eggs in urine for schistosomiasis diagnosis. Subjects were treated with 60 mg of PZQ once per month for three consecutive months, and the outcome was assessed by cure rate (CR) and egg reduction rate (ERR).

Results: A total of 328 volunteers were enrolled in the study with a mean (± SD) age of 12.2 ± 4.7 years-old. The female-to-male ratio was 0.99. Out of 258 participants in total, 45% had schistosomiasis during the survey and 43% presented with heavy infections. The incidences of haematuria and schistosomiasis were 0.11 and 0.17 person-years, respectively. After the first and third dose of PZQ, overall ERR of 93% and 95% were found, respectively; while the CR were 78% and 88%, respectively. Both ERR (100 *vs* 88%) and CR (90 *vs* 68%) were higher among females than males after the first dose. The CR increased for both groups after the third dose to 95% and 80%, respectively. After the first PZQ dose, ERR was higher for heavy compared to light infections (94 *vs* 89%), while the CR was higher for light than for heavy infections (87 *vs* 59%). After the third PZQ dose, ERR increased only for light infections to 99%, while CR increased to 98% and 75% for light and for heavy infections, respectively. The reinfection rate assessed at a mean of 44.6 weeks post-treatment was 25%.

Conclusions: The prevalence of schistosomiasis is moderate in communities living in the vicinity of Lambaréné, where a subpopulation with a high risk of reinfection bears most of the burden of the disease. To improve schistosomiasis control in this scenario, we suggest education of these high-risk groups to seek themselves a one-year PZQ treatment.

BACKGROUND

Schistosomiasis is considered the second most important parasitic disease after malaria [1]. It is a neglected tropical disease occurring frequently in sub-Saharan Africa where 85% of the worldwide infected population lives [2]. The disease is poverty-associated, particularly in rural areas where parasite exposure through contact with infested freshwater is frequent. Indeed, parts of the population pursue daily activities such as household chores, bathing, and fishing in potentially infested water. In such areas where reinfection is common [3,4], the WHO recommends implementation of targeted treatment through large-scale treatment to reduce the burden of disease [5], and to prevent morbidity in later life [6]. Administration of treatment at least once a year reduces early (visible haematuria, anemia) and late (portal hypertension, hepatic fibrosis, bladder cancer) schistosomiasis-associated morbidity [5, 7].

Few drugs are available for treatment of schistosomiasis. Metrifonate is an antischistosomal drug indicated for the treatment of schistosomiasis and effective only against *Schistosoma haematobium* [8]; however, the drug is no longer commercially available [9]. Oxamniquine is another antischistosomal drug effective only against *Schistosoma mansoni* [8] but due to its higher price, it is used as an alternative drug when PZQ treatment fails [10]. Praziquantel (PZQ) is currently the WHO-recommended drug of choice, effective against adult worms of all *Schistosoma* species [8] and is used for large-scale treatment. The antimalarial drug artemether also has antischistosomal activity, particularly on juvenile schistosome stages [11] and therefore could play a role in disease prevention as demonstrated by Utzinger et al. [12]. In combination with PZQ, artemether can be used to target all parasite stages during schistosomiasis treatment. Indeed, in comparison to PZQ alone, artemether-PZQ combinations have shown to reduce the prevalence of schistosomiasis in Egyptian children by half, and to reduce disease incidence [13]. However, in Lambaréné and surroundings that are endemic for both malaria and schistosomiasis, the first results were contradictory [14]. Moreover, the regular use of artemisinin, which is the most important antimalarial drug, might contribute to the development of malaria parasite resistance [11] and then jeopardise current malaria control and treatment efforts. Another antimalarial drug, mefloquine, is found to be active on all parasite stages [15] and able to consistently reduce egg excretion [16].

For treatment of *Schistosoma intercalatum*, *S. haematobium* and *S. mansoni*, the main species prevalent in sub-Saharan Africa [5], the recommended dose of PZQ is 40 mg/kg in one or in a split dose, administered 4 hours apart [17]. Due to the confection of the drug (600 mg tablets) and to the usual difficulty to assess patient weight accurately particularly for children during MDA campaigns, PZQ is rarely administered in the most appropriate dosage. Dose scales for praziquantel administration have been developed by the WHO to minimise under-dosage of the drug [7] and to ensure administration of doses between 30 and 60 mg/kg, which is within the dose range that is considered both safe and effective [7, 18].

The WHO recommended diagnostic gold standard for schistosomiasis are urine filtration and Kato-Katz techniques for urogenital and intestinal schistosomiasis, respectively. The objective

is to confirm the diagnosis by detecting *Schistosoma* eggs in fresh urine or stool samples [5]. The continuing presence or absence of *Schistosoma* eggs in urine or stool samples is used to assess PZQ efficacy for schistosomiasis treatment. As such, cure rate (CR) and egg reduction rate (ERR) are the two endpoints commonly used and recommended to evaluate anthelminthic drug efficacy [19]. WHO defines the efficacy of anthelminthic drugs as "the effect of the drug against helminths, in isolation and under ideal conditions" [7]. However, the outcome of these two tests "may vary widely, even in efficacy trials in which the same drug is given at the same dosage under optimal conditions" [7]. Therefore, to enable comparison between studies, the WHO suggested guidelines when assessing anthelmintic drug efficacy [19]. With regards to schistosomiasis, some of these remain difficult to assess, notably the variability in egg output and excretion or preponderance of immature worms less susceptible to PZQ. We therefore think that PZQ efficacy can only be properly estimated from a large number of individual studies across a range of epidemiologically distinct settings.

Lambaréné, a semi-urban town in Gabon, and its surroundings are known to be endemic for schistosomiasis, with *S. haematobium* reported as predominant [20–22] and *S. intercalatum* reported occasionally. An overall prevalence of 30% was recently reported for Lambaréné surroundings [23], rendering the community as having a moderate schistosomiasis prevalence. Whilst the epidemiological picture becomes clearer, there is a lack of information on several epidemiological indicators of schistosomiasis and the impact of PZQ. Therefore, the objective of this analysis is to provide basic information in regard to the parasitological indicators of schistosomiasis in our study population. These indicators include prevalence and incidence of the disease. In addition, the impact of PZQ treatment and reinfection were assessed. This information is relevant for improving schistosomiasis control in the area.

METHODS

Study site

The study was conducted at CERMEL, Centre de Recherches Médicales de Lambaréné, located in Lambaréné, Gabon. Volunteers were recruited from Zilé-PK area and Bindo village, two localities in the vicinity of Lambaréné where schistosomiasis is endemic. Zilé-PK villages is a set of villages located over 20 km (from PK 14 to PK 33) along the national road south of Lambaréné where many human-freshwater body contact points considered as schistosomiasis foci exist (Fig. 1), leading to a considerable level of urogenital schistosomiasis prevalence. Indeed, around 43% prevalence of schistosomiasis was reported, particularly in children [22, 23]. On the contrary, Bindo village, a remote locality located about 50 km north of Lambaréné presents very few human-freshwater body contact points which sustains around 15% schistosomiasis prevalence reported [22, 23].

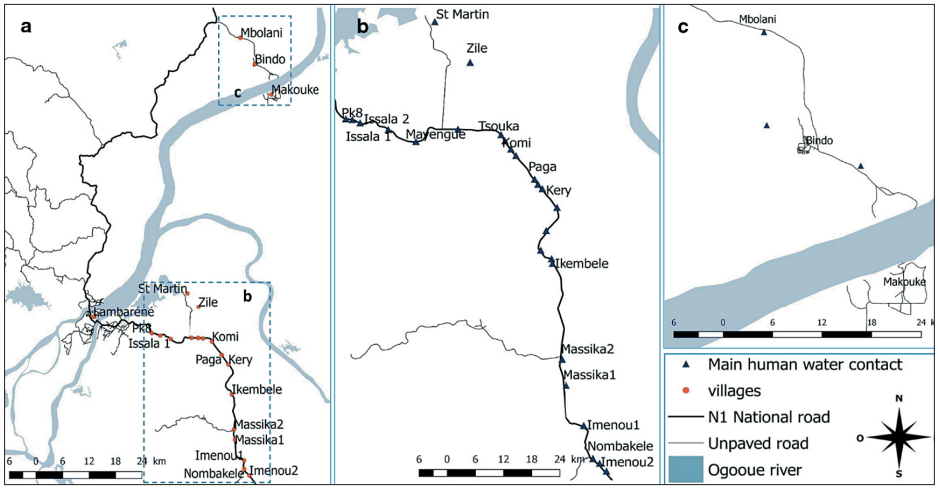

Fig 1: Map of Lambaréné, Gabon, and surrounding localities. a The main human-water contact points in the different study areas. b Zilé-PK villages. c Bindo village

5

Study population

Volunteers aged 6–30 years-old, living in the study area for at least one year and without macroscopic haematuria and no apparent chronic disease during the screening phase were invited to participate in the study. School-age children and young adults are most afflicted by schistosomiasis, and the information from this population reflects best the community's disease burden with the highest incidence. In the present study area, the most common activities that bring young people into contact with open freshwater are fishing and household domestic activities, including water access for daily use.

Study design

The present analysis is a sub-analysis of a longitudinal and prospective study designed to assess the effect of pre- and post-treatment of schistosomiasis with PZQ on malaria transmission. The study was conducted from June 2016 to November 2018. Following the screening phase, eligible participants were followed up for 6 months. At month 6, schistosomiasis status was assessed for the whole study cohort according to the study procedure. Two study groups were therefore considered; study sub-group A, which included participants found to be positive, and study sub-group B, which included all participants found to be negative. Participants of sub-group A were treated during the 3-month treatment phase. From month 9 of the study (end of the study treatment phase), participants were followed for another 6 months (end of the study period), yielding a total follow-up time of 15 months. At the end of the study period, a second schistosomiasis status assessment was performed. In addition to the scheduled visits for schistosomiasis status assessment, participants were invited to actively visit the research centre in case of macroscopic haematuria or other health problems. In case of diagnosis of schistosomiasis and irrespective of the study phase, the participants had to receive a regimen of PZQ of 60 mg/kg body weight once a month during three consecutive months, administered

under supervision of the clinical team. To minimize occurrence of adverse events related to PZQ treatment, participants were asked to eat before taking the drug. To assess treatment success, urine samples were collected four weeks after the first and third PZQ administrations.

Sample size estimation

To address our main objective, the overall sample size to consider was simulated using the sample size calculation formula for cross-sectional studies [24]. Given that an overall prevalence of schistosomiasis of 30% has recently been reported for both study sites [23], and considering 1.96 standard normal variate and 5% precision, we estimated a minimum of 323 volunteers for inclusion in this survey. In addition, the minimum sample size recommended for PZQ efficacy assessment is 50 infected volunteers [19]. A sub-population was therefore analyzed for this secondary purpose.

Laboratory procedures

The urine filtration technique as recommended by the WHO [25] was used to detect the presence of *Schistosoma* eggs in fresh urine samples. On days of sample collection, urine was collected between 10:00 and 15:00 h. For egg detection, the 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 low power objective (10×) of a light microscope. Reading of slides was performed by two independent experienced readers. The final result was reported as the number of eggs per 10 ml of urine after calculating the mean egg count obtained from the pooled results of both readers. In case of a quantitative (difference ≥ 20%) or a qualitative discrepancy between both readers, a third independent reader was required, and the mean of the two closest results was considered as the final result. For the diagnosis of urogenital schistosomiasis, urine samples were collected and processed over 3 consecutive days, unless the participant was found positive with at least 1 parasite egg in any sample before the second, or the third day of sampling. The participant was considered as negative if all 3 urine samples were negative for Schistosoma eggs. In addition, Rapid Dipstick (Combur test, Roche, Rotkreuz, Switzeland) was performed on each urine sample to detect evidence of haematuria.

Statistical analysis

Data were managed using REDCap electronic data capture tool hosted at CERMEL [26]. The final database (Additional File 1) was exported into R version 3.4.4 for statistical analysis. Quantitative variables were summarized as the mean and standard deviation (SD) while qualitative variables were summarized as the proportion and 95% confidence interval (95%CI). Student's t-test was used to compare continuous variables and Chi-square test or Fisher's exact test was used to compare proportions. Significance of the *P-values* was set at < 0.05. With regard to the definition of the variables, a successful cure was defined as the conversion from positive to negative detection of *Schistosoma* eggs in the urine of treated individuals. Reinfection was considered as a new positive case, indicated by the presence of *Schistosoma* eggs in the urine of the participant who had previously been declared cured. In sub-group A, the CR was calcu-

lated as the percentage of volunteers cured among those treated, and the ERR was calculated on the basis of the total arithmetic mean egg counts after vs before treatment and expressed as a percentage as described elsewhere [19]. The intensity of infection was quantified as either light- or heavy-intensity infection using a threshold of 50 eggs per 10 ml of urine. In addition, all cases with visible haematuria were considered as heavy infections [7]. Person-time incidence rates were calculated using the total follow-up period of each participant and expressed in person-years. Incidence of visible haematuria was estimated among the whole cohort during the first study follow-up phase, while incidence of schistosomiasis cases was estimated in sub-group B during the second study follow-up phase.

RESULTS

Study population

We included 351 volunteers in this study. Among them, 328 agreed to join the follow-up phase. The mean (± SD) age was 12.2 ± 4.7 years-old, with 75% of the participants being less than 15 years-old; the female:male ratio was 0.99. From the included volunteers, 79% were from Zilé-PK area (Table 1). Among the participants who joined the follow-up phase, 258 and 188 completed the first and the second study phase, respectively.

Table 1 Study population baseline socio-demographic characteristics and distribution of schistosomiasis cases. The proportion of schistosomiasis cases is distributed at the end of phase1 and at the end of phase2.

	Study population characteristics at baseline			Schistosomiasis cases				*P*-value[a]
				End of phase 1		End of phase 2		
	N	*%*	*95%CI (%)*	*n/N*	*%*	*n/N*	*%*	
Overall	328	–	–	103/258	39.9	33/188	17.5	< 0.0001
Age								
6–8	69	21.0	16.7–25.8	16/61	26.2	10/44	22.7	0.86
9–11	102	31.1	26.1–36.4	34/80	42.5	13/66	19.7	0.006
12–14	81	24.7	19.8–29.4	32/69	46.4	7/50	14.0	0.0004
15–30	76	23.2	18.7–28.1	21/48	43.8	3/28	10.7	0.006
Gender								
Female	163	49.7	44.1–55.2	48/130	36.9	16/97	16.5	0.001
Male	165	50.3	44.7–55.8	55/128	43.0	17/91	18.7	0.0003
Location								
Bindo	69	21.0	16.7–25.8	6/52	11.5	1/39	2.6	–
Zilé-PK	259	79.0	74.1–83.2	97/206	47.1	32/149	21.5	< 0.0001

[a] Chi-square test to compare proportion of schistosomiasis cases between end of phase 1 and end of phase 2
Abbreviations: n, number of schistosomiasis cases; N, number of participants; CI, confidence interval

Schistosoma infection morbidity

As depicted in Fig. 2, among the 328 participants who entered the follow-up phase, 258 (78.7%) were tested for schistosomiasis at the end of the phase 1. During that phase and before the first assessment of schistosomiasis status, 28 (8.5%) participants complained about visible haematuria which was confirmed by Combur test (04510062171). These cases were positive for urine filtration and therefore confirmed as heavy *Schistosoma* infections, and were treated with PZQ. Hence, the haematuria incidence was 0.12 person-years in the cohort. At first assessment, a total of 103 participants (study sub-group A) were found to be infected with *Schistosoma* spp., resulting in 40% (95% CI: 34–46%) of the study population with schistosomiasis. Heavy infection intensity accounted for 45% (46/103). As presented in Table 1, schistosomiasis was more prevalent in Zilé-PK compared to Bindo (47% vs 11%, $\chi 2 = 20.419$, df = 1, $P < 0.0001$). However, there was no evidence of a difference in the percentage of schistosomiasis cases between males and females (43 vs 37%, $\chi 2 = 0.747$, df = 1, P = 0.39).

At the second assessment, a total of 33 participants out of the 188 present at that time point were found to be positive, yielding 17% of the study population with schistosomiasis. Heavy infection intensity accounted for 12% (4/33). Compared to the first round of treatment, no statistically significant improvement was observed among children aged 6–8 years-old (26 vs 23%, $\chi 2 = 0.032$, df = 1, P = 0.86), in contrast to other age groups where a statistically significant decrease in percentage of schistosomiasis cases was observed (Table 2). A similar decrease in percentage of schistosomiasis cases was observed for gender with 37 vs 16% ($\chi 2 = 10.46$, df = 1, P = 0.001) for females and 43 vs 19% ($\chi 2 = 13.14$, df = 1, P = 0.0003) for males. With regard to location, only one (3%) case of schistosomiasis among the 39 participants assessed was found at Bindo, while 21% (32/149) of schistosomiasis cases were found in Zilé-PK area, reflecting a significant decrease in percentage of schistosomiasis cases for both locations ($\chi 2 = 23.42$, df = 1, $P < 0.0001$) compared to the first assessment. The sub-study group B (which included participants negative at first assessment) allowed us to estimate schistosomiasis incidence in our study cohort. Among this sub-population, 12 new schistosomiasis cases out of the 115 participants evaluated at the second assessment were recorded, yielding a 10% cumulative incidence or 0.17 person-year incidence of schistosomiasis.

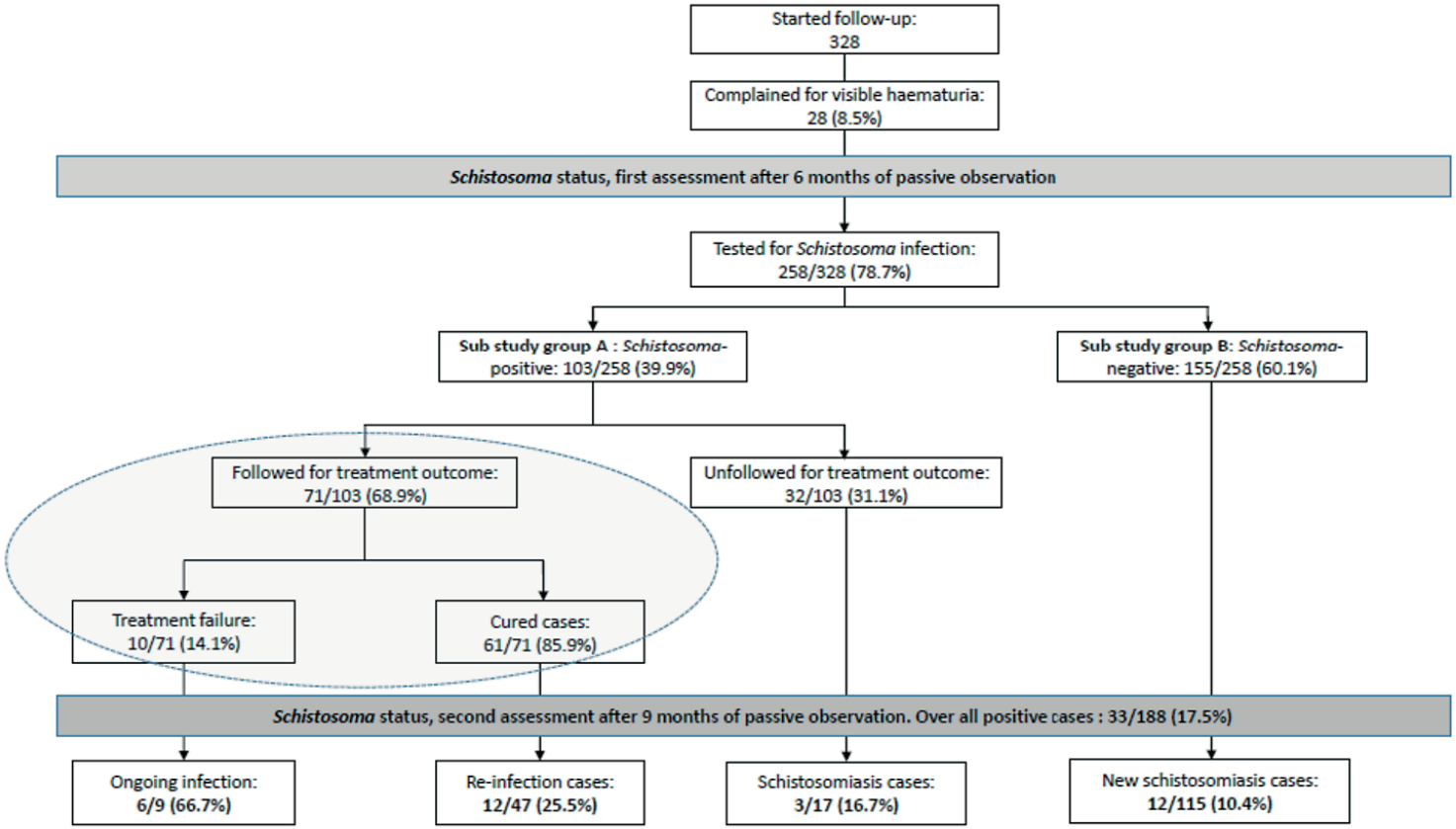

Fig 2: Study participants flowchart. The ellipse indicates participants monitored for praziquantel treatment outcome. For each proportion figure calculated, the denominator represents the number of subjects included (i.e. the numerator from the previous step)

Table 2 Distribution of ERR and CR among the study population and by infection intensity

	Egg reduction rate (ERR)							Cure rate (CR)						
	Post-dose 1			Post-dose 3			*P*-value[c]	Post-dose 1			Post-dose 3			*P*-value[c]
	e/E[a]	%	*P-value*	*e/E*[a]	%	*P-value*		*n/N*[b]	%	*P-value*	*n/N*[b]	%	*P-value*	
Study population	**309/4369**	**92.9**		**408/7953**	**94.9**	-	**0.003**	**52/67**	**77.6**		**72/82**	**87.8**	-	**0.15**
Age			**< 0.0001**			**< 0.0001**	**< 0.0001**			**0.95**[d]			**0.91**	**0.78**
6–8	5/313	98.4		7/2017	99.7			10/12	83.3		13/15	86.7		
9–11	69/1727	96.0		70/2739	97.4			19/25	76.0		24/27	92.0		
12–14	223/1420	84.3		99/1514	93.5			16/21	76.2		21/25	88.9		
15–23	12/909	98.7		232/1683	86.2			7/9	77.8		14/15	93.3		
Gender			**< 0.0001**			**< 0.0001**	**< 0.0001**			**0.058**			**0.09**	0.007
Female	7/1914	99.6		41/4602	99.1			27/30	90.0		39/41	95.1		
Male	302/2455	87.7		367/3351	89.0			25/37	67.6		33/41	80.5		
Location						**0.020**								
Bindo	0/1	100		38/512	92.6			1/1	100		3/4	75.0		
Zilé-PK	309/4368	92.9		370/7441	95.0		0.0001	51/66	77.3		69/78	88.5		0.12
Infection intensity			**< 0.0001**			**< 0.0001**	**< 0.0001**			**0.01**[d]			**0.004**[d]	**< 0.0001**
Light	57/519	89.0		2/566	99.6			40/46	87.0		45/46	97.8		
Heavy	252/3850	93.5		406/7387	94.5			12/21	57.1		27/36	75.0		

a E is the total Schistosoma egg counts at baseline and e is the total Schistosoma egg counts at control
b N is the number of participants treated at baseline and n is the number of participants found negative at control
c P-value to assess the significant difference observed between post-dose 1 and post-dose 3 results
d Fisher's exact test applied
Notes: The ERR and CR was assessed at PZQ post-dose1 (n = 67) and post-dose 3 (n = 82)

PZQ administration

Among the 115 participants that were found to be positive at least once for schistosomiasis, 103 were detected positive at the first assessment and 12 at the end of the follow-up phase. A total of 112 (97%) were treated with PZQ. Of these, 106 (92%) and 100 (89%) completed their second and third doses of treatment, respectively. The PZQ doses administered ranged from 38 mg/kg body weight to 65 mg/kg body weight, with a mean (± SD) of 56.8 ± 6.9 mg/kg body weight. The mean time (± SD) between the first and second dose, and between the second and the third dose was 5.6 ± 1.5 and 4.6 ± 1.7 weeks, respectively. The mean time (± SD) between the first dose, the first control and between the last dose and the last control among those remaining positive was 4.7 ± 0.9 and 3.3 ± 1.3 weeks, respectively. In addition, during the treatment phase we recorded one case of vomiting in the first hour after the first-dose administration.

Outcome of praziquantel treatment

Data for assessment of PZQ treatment outcome were available for 67 and 82 infected participants after the first and third doses of treatment, respectively. Outcomes are presented in Table 2. We found ERR of 93% and 95% after the first and the third dose of PZQ, respectively. The ERR was significantly lower for males compared with females after the first (88 *vs* 100%, $\chi 2 = 231.31$, df = 1, $P < 0.0001$) and the third (89 vs 99%, $\chi 2 = 401.23$, df = 1, $P < 0.0001$) PZQ administration. With regard to the intensity of the disease, the ERR was significantly lower for light than heavy infection intensity after the first PZQ administration (89 vs 93%, $\chi 2 = 13.701$, df = 1, $P = 0.0002$) but was significantly higher after the third PZQ administration (100 vs 94%, $\chi 2 = 28.569$, df = 1, $P < 0.0001$).

In addition to the EER, we found an overall CR of 78% and 88% after the first and the third dose of PZQ, respectively. The CR was somewhat lower for males compared with females after the first (68 *vs* 90%, $\chi 2 = 3.594$, df = 1, $P = 0.058$) and third (80 *vs* 95%, $\chi 2 = 2.847$, df = 1, $P = 0.09$) PZQ administration, respectively, but no statistically significant difference was detected. In contrast to ERR for infection intensity, we found a higher CR among participants with light infection intensity compared to their counterparts with heavy infection intensity after the first (87 *vs* 57%, Fisher's exact test: $P = 0.01$) and the third (98 vs 75%, Fisher's exact test: $P = 0.004$) PZQ administration, respectively. As depicted in Fig. 3, the probability of cure is significantly higher in female than in male patients (Log-rank test: $P = 0.04$), and for individuals with light infection intensity than for those with heavy infection intensity (Log-rank test: $P < 0.001$) during the whole treatment phase.

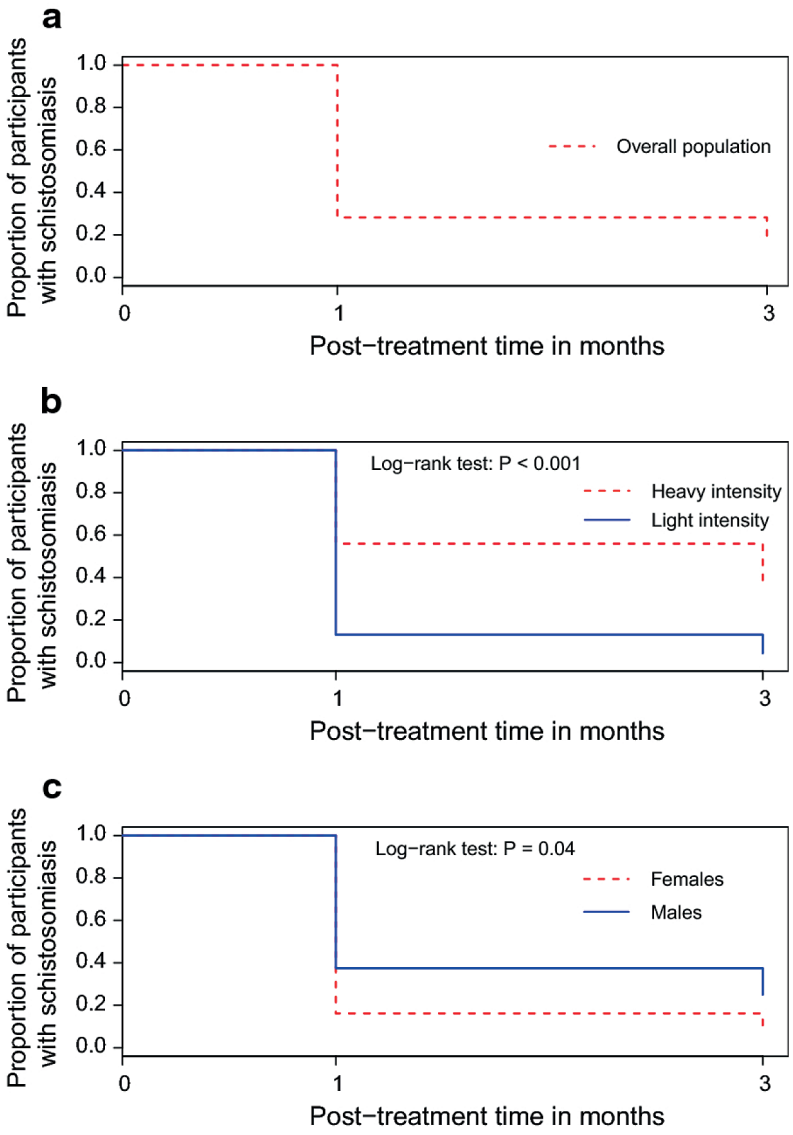

Fig. 3: Kaplan Meier curves showing the probability to cure one month after the first and the third dose of praziquantel, respectively, among the general study population (a), per infection intensity (b) and per gender (c)

Schistosomiasis reinfection

Among the participants who received the full PZQ regimen, post-treatment infection status was assessed for a total of 82 subjects, including 71 during the first follow up phase and 11

at the end of the second follow-up phase. As depicted in Fig. 2, among the 71 participants followed for treatment outcome after the first treatment round, 10 (14%) remained positive for eggs in urine one month after the last dose of PZQ. Out of these 10 participants, three became negative at the end of the second follow up phase. Of the other 61 (86%) participants who became negative (no eggs detected in their urine samples) after the full drug regimen at the first assessment, 12 out of the 47 that were followed-up to the end of the second follow-up period developed schistosomiasis, yielding a reinfection rate of 25%. The mean time to Schistosoma reinfection of these 12 participants was 44.6 weeks.

DISCUSSION

A main objective of this study was to describe the current morbidity of schistosomiasis in our study population. We therefore looked for prevalence, incidence and intensity of the disease as indicators of morbidity, using different diagnostic tools, namely eggs in fresh urine or self-reported visible haematuria. In terms of prevalence, the percentage of schistosomiasis cases we found based on urine filtration reflects the prevalence usually reported from the area. Indeed, although at the time of schistosomiasis assessment some participants who were initially included in the study cohort had withdrawn by the end of the follow-up period, 47% and 11% of schistosomiasis cases that were found in our study cohort for Zilé-PK villages and Bindo, respectively, are comparable to 41% or 43% prevalence for Zilé-PK and 15% or 19% prevalence for Bindo previously found in 2012 [22] and 2014 [23], respectively. These results show that prevalence remains stable and moderate over time in these communities. In addition to the prevalence, to the best of our knowledge the present study also assessed schistosomiasis incidence in the study area for the first time. Eight percent of the study population with visible haematuria were all confirmed as schistosomiasis cases, yielding a 0.12 person-year incidence of self-reported visible haematuria, when taking into account each participant follow up time across all follow-up periods. Based on the urine filtration technique, 10% of the participants who were egg-negative during the first schistosomiasis assessment were found to be egg-positive during the second schistosomiasis assessment, resulting in a 0.17 per person-year schistosomiasis incidence. To our knowledge, the present study also describes an estimation of infection intensity for the first time. When considering only the first cases of schistosomiasis per participant, about half (46%) of the Schistosoma infections were heavy. Heavy schistosomiasis infection is indicative of a high parasite load and is associated with frequent or long-standing Schistosoma exposure [27, 28]. One out of two participants with schistosomiasis, and more males than females, can be assumed to be constantly exposed to a transmission hotspot, most likely due to their daily activities such washing, bathing, swimming or fishing.

The second-most important objective of this study was to report the outcome of schistosomiasis treatment with PZQ. Treatment was intended to be administered with 60 mg/kg body weight. Using 600 mg scored tablets, accurate dosage according to the participants' weight was difficult to reach. In addition, some participants accidentally received a dosage differ-

ent to what had been calculated. Taking this into account, a mean dosage of 57 mg/kg body weight was given, with moderate variation (SD = 7). The drugs were well tolerated; however, as reported above, one participant, a 14-year-old girl weighting 44 kg, vomited less than one hour after having received 4.5 tablets of 600 mg of PZQ. PZQ is indeed commonly reported to be safe [5, 29]. The results of this study show that PZQ efficacy was satisfactory even after the first dose of treatment, as indicated by an ERR of more than 90%. This result is in line with a satisfactory PZQ efficacy reported in several countries in Africa for treatment of *S. haematobium* infection [30–32] as well as for *S. mansoni* [29, 30, 33], although in these studies the regimen was 40 mg/kg. However, in contrast, other studies have reported a doubtful efficacy of PZQ in school children [3]. Factors, such as gender [3], prevalence [4] and intensity of the infection [31] have been found to affect the efficacy of PZQ. In the present study, PZQ efficacy was lower in male than female patients, and for heavy than light infection intensities. This result corroborates the finding of Kabuyaya et al. [3], who in 2017 reported a higher ERR in females compared to male school children aged from 10–15 years-old living in South Africa, even after two doses of PZQ. Interestingly, a higher ERR was found in participants with heavy infections compared to those with light infections. This finding could be explained by the capacity of PZQ to consistently reduce egg excretion through elimination of adult worms, as sustained by the overall ERR we found. However, in both groups a number of participants continued to excrete *Schistosoma* eggs, particularly in those with high-infection intensity, even after three doses of PZQ. Instead of a possible PZQ resistance, we hypothesize that these participants still excrete eggs after treatment, probably because of the schistosomulae present at the time of treatment, or because of the very early reinfection, both scenarios are consecutive to frequent parasite exposure.

Schistosomiasis reinfection is common in areas with moderate or high risk [3, 4]. In the present study, a 25% reinfection rate at 9 months post-treatment was observed. This is higher than what has been reported by Senghor et al. [34] in 2015 among children living in a low transmission area in Senegal two to three months after treatment, and less than what was found at 12 months post-treatment in 1992 by Ofoezie et al. [32] among children living in Nigeria. Although the reinfection pattern varies with location as demonstrated by N'goran et al. [4] among schoolchildren in three neighboring villages in the Ivory Coast, the reinfection rate was reported to increase over time. Indeed, in a study conducted among children in Nigeria, authors reported an increase of reinfection rates over time from 9% at three months posttreatment to 39% at one-year post-treatment, respectively [32]. Our results suggest that reinfection occurs early in the study population. This assumption is supported by the fact that 15% of our participants treated for schistosomiasis remained positive for the presence of eggs in urine even during the three month-treatment phase, and six out of nine of them remained positive up to about one-year post-administration from the first dose of PZQ. Although the hypothesis of PZQ resistance is possible, we assume these cases are frequent reinfection cases; and hypothesize that some people in our study area are continuously exposed to *Schistosoma* spp. due to the proximity of their homes to freshwater bodies, and their daily activities. In that case, the risk of reinfection will be continuous. Our results argue in favour of unequal exposure of the population

to schistosomiasis. A higher proportion of schistosomiasis cases found among males indicates their increased exposure to the parasite than females. Indeed, more engagement in water-contact activities of males was suggested by Onifade et al. [35] to explain the same effect observed among school aged children living in an endemic area of Nigeria. In any case, at the end of study follow-up and as presented in Table 3, three kinds of population groups stand out, which can be discriminated according to the potential level of exposure to schistosomiasis: (i) those who are not exposed to schistosomiasis, meaning that they are not in contact with freshwater bodies, and who can be identified in our study as those who remain negative during the whole survey; (ii) those who are accidentally or occasionally in contact with schistosomiasis foci and can be identified in our survey as those who remained negative during the follow-up after treatment; and (iii) those who are frequently exposed to the parasite, probably due to their daily activities such as bathing or household work known to be associated with a high risk of infection [36]. This last group could be identified in our study population as those who remained positive despite administration of PZQ multiple times, and those found re-infected early after being considered as cured. Therefore, the application of PZQ treatment should be different with regard to the level of exposure. Indeed, if there is no role for untargeted PZQ treatment for the first group cited below, the objective of the treatment for the second group would be to achieve a cure status. In the third group, if the cure is not the main objective due to the high risk of reinfection, repetitive treatment at least once a year during their lifetime exposure will reduce at least the morbidity of the disease, and will be beneficial throughout their adulthood, as reported by WHO [5, 7]. In this scenario, we therefore recommend to complement large scale treatment with education about frequent freshwater contact so that individuals with frequent freshwater contact should then be able to identify themselves and ask for free treatment at least once a year until they leave the endemic area. As mentioned above, artemether is nowadays suggested to be of use to prevent schistosomiasis infection or reinfection [11, 13]; however, it cannot be recommended in our study area where malaria is endemic.

Table 3 Suggestions for recommendation of praziquantel treatment according to the potential exposure level of the population to *Schistosoma* spp.

Population group	Potential level of exposure to *Schistosoma* spp.	Suggestion of the application of praziquantel treatment	Control strategy objective
(i)	No contact with freshwater bodies	No intervention required	-
(ii)	Accidental or occasional contact with freshwater bodies	Provide praziquantel in case of haematuria and one last dose when leaving the area	To cure and prevent morbidity
(iii)	Daily or frequent contact with freshwater bodies	Educate people to seek treatment once a year during their stay in the endemic area and one last dose when leaving the area	To prevent morbidity. Only the last dose will intend to cure

Three participants treated for schistosomiasis who remained positive one month after the third dose of PZQ were found to be negative during the second assessment without any other intervention, raising the issue of *Schistosoma* eggs release following an efficient (adult worm killing) treatment. Indeed, it has been reported that eggs can still be released up to six weeks following PZQ treatment [37]. Therefore, with the outcome of PZQ efficacy assessed four weeks post-treatment as it was done in the present study, the results could possibly be affected by false-positive cases. This differential misclassification bias could result in an underestimation of both ERR and CR. Assessing the viability of eggs released after treatment should allow for controlling this potential bias, but was not done in the present study. However, with regard to the ERR, this should not affect the conclusion drawn on the efficacy of PZQ since the 90% threshold set by WHO to conclude for PZQ satisfactory efficacy [19] was reached. On the other hand, we report a variation of PZQ dosage administered to our study population. We have therefore assessed the outcome of PZQ treatment in an intention-to-treat approach so that instead of efficacy, we report here on the effectiveness of PZQ. Furthermore, the longitudinal study design of the present study enabled us to evoke the dynamics of schistosomiasis in the study cohort, notably the incidence of the infection, which is rarely assessed due to the fact that the accurate starting time point of an infection is difficult to determine.

CONCLUSIONS

The present study confirms a moderate urogenital schistosomiasis prevalence in our community, where part of the population bears the main burden of the disease. Our results highlight different infection patterns which need to be identified and described in order to enable appropriate schistosomiasis control. In a community where snail habitats and human freshwater contact are difficult to control and where PZQ effectiveness is reported, morbidity control should remain a priority particularly for a population with a high risk of exposure. Administration of PZQ in this sub-population should be tailored. Instead of MDA, self-administration of PZQ once a year for people at high risk might be a viable alternative.

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ADDITIONAL FILES

Additional File 1: Table S1. Name and modalities of each variable included in the analysis. Table S2. Database used for the longitudinal analysis. Table S3. Database used for drug efficacy assessment. Table S4. Database used for the survival analysis. (Available on the journal's website)

CHAPTER 6

Haematological changes in *Schistosoma haematobium* infections in school children in Gabon

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ABSTRACT

Background Schistosomiasis is a parasitic disease affecting the blood cell. As a chronic disease, schistosomiasis particularly impacts on the human host's haematological profile. We assessed here the impact of urogenital schistosomiasis on the full blood counts (FBC) as proxy diagnostic tool for schistosomiasis.

Methods A cross-sectional study was conducted among school children living in Lambaréné, Gabon. Schistosomiasis status was determined using urine filtration technique. EDTA blood samples were analysed using a Pentra ABX 60® analyzer.

Results Compared to their infection-free counterparts, school children infected with *Schistosoma haematobium* displayed an altered FBC profile, with changes in all three blood cell lines. Adjusted for praziquantel intake, soil-transmitted helminthic infections and *Plasmodium falciparum* infection status, schistosomiasis was independently associated with a decreasing trend of mean haemoglobin (β = − 0.20 g/dL, *p-value* = 0.08) and hematocrit (β = − 0.61%, *p-value* = 0.06) levels, a lower mean MCV (β = − 1.50µm3, *p-value* = 0.02) and MCH (β = − 0.54 pg, *p-value* = 0.04), and higher platelet (β = 28.2 103/mm3, *p-value* = 0.002) and leukocyte (β = 1.13 103/mm3, *p-value* = 0.0003) counts, respectively.

Conclusions Schistosomiasis is associated with a characteristic FBC profile of schoolchildren living in Lambaréné, indicating the necessity to consider schistosomiasis as a single cause of disease, or a co-morbidity, when interpreting FBC in endemic areas.

INTRODUCTION

Schistosomiasis is one of the two most-common parasitic infections globally, with transmission reported from 78 countries [1]. Globally, 700 million people live in endemic areas [2]. Among the now 240 million people estimated of having schistosomiasis and requiring treatment, more than 90% are estimated to live in sub-Saharan Africa [2, 3].

Schistosomiasis leads both to acute and chronic disease. At both stages, schistosomiasis stimulates the host immune system, being in part reflected in routinely measurable biochemical and, in focus here, haematological alterations. In general, higher mean leukocyte counts and changes in mean of differential leukocyte counts are observed among individuals infected with schistosomiasis compared to those without; both normalise following praziquantel (PZQ) treatment [4]. Eosinophilia is characteristic for schistosomiasis [5]. Thrombocytes play a protective role against schistosomiasis by exerting direct damaging effects on adult worms, with platelet count changes depending on the disease phase [6]. In mice, thrombocytes were shown to adhere to the surfaces of, and to kill mechanically transformed schistosomula, leading to thrombocytopenia in early disease [7]. In the brown rat, a three–fourfold increase of thrombocytes protective against schistosomes was observed 4–6 weeks after initial infection [8]. Few studies were conducted to assess thrombocyte changes in humans [9, 10], and the potential anthelminthic effects thereof. One study reported a thrombocyte reduction by half in patients with intestinal schistosomiasis. However, the authors concluded that this was more likely attributable to portal hypertension rather than directly to the helminths [9]. Another study reported a trend for thrombophilia among Ghanaian children with urogenital schistosomiasis compared to their non-infected counterparts [10]. In addition to leucocytes and platelets, the third blood cell line does not remain unaffected by schistosomiasis either. The main symptom of schistosomiasis is the presence of blood in urine (in urogenital schistosomiasis) [11] or in stool (in intestinal schistosomiasis), due to blood vessel rupture during egg excretion. Chronic schistosomiasis, which particularly affects school-aged children, adolescents and young adults has been reported to be associated with chronic inflammation and iron deficiency anaemia [12].

With schistosomiasis being mainly a chronic infection and very often asymptomatic at that stage, and knowing that schistosomiasis is able to influence the mean FBC parameters as discussed above, we can, thus, assume that in case of co-infections with other diseases such as malaria, schistosomiasis might eventually go unnoticed if the clinician is unaware of the presence of this (co)morbidity. Indeed, in malaria-negative individuals in co-endemic areas, for example, a certain level of microcytic anaemia on its own would probably go unnoticed as it is explained by repeated malaria episodes, with anaemia being one of the malaria diagnostic criteria for disease severity, and chronic anaemia being a well-recognised consequence of repeated malaria episodes. A recent epidemiological assessment demonstrated that Lambaréné is an area of urogenital schistosomiasis with a 26% prevalence, with hematuria and proteinuria being positively associated with the disease [13]. In this follow-up analysis, we examined the

effect of schistosomiasis on FBCs as a surrogate diagnostic parameter, among school children living in Lambaréné, an area endemic for *S. haematobium* and where PZQ is available.

MATERIALS AND METHODS

Study area

Lambaréné is a town in Gabon located 80 km south of the equator known to be endemic for schistosomiasis. The predominant *Schistosoma* species is *S. haematobium* [13–16]. Lambaréné is also known to be endemic for soil-transmitted helminths (STH) [17] and malaria, with highest prevalences in school children and adolescents [18].

Study design and population

The study design was cross-sectional. Volunteers were recruited amongst consenting eligible, apparently healthy school children living in Lambaréné.

Sample size consideration

The current sample size was calculated for a cross-sectional study aiming to determine the prevalence of schistosomiasis in Lambaréné and associated factors, as described elsewhere [13]. In the current analysis, we assessed the difference in haematological parameters between participants with schistosomiasis and those without. Considering a 5% type-I error, and having 161 participants included in the *Schistosoma* positive group and 451 in the control group, we were able, with more than 90% power, to detect a minimum of 10% between both groups for platelet levels, and more than 10% for WBC and RBC levels.

Study procedures and laboratory examinations

The study was conducted from April to July 2016. Participants were selected at school as described elsewhere [13]. Briefly, legal representatives of volunteers invited at school to partake were visited at home and asked to grant informed consent. Trained field workers used a standardised questionnaire to inquire with parents or other primary caretakers about, among other, history of passing blood in urine and treatment received (PZQ or other anthelminthic drugs) in the previous six months. Nurses collected study subjects' demographic data (age, sex and address) at school. In cases of acute medical concerns, the participant was referred to the clinician for appropriate care.

Eligible participants were provided with plastic containers at school and were invited to provide three urine samples on three consecutive days, and one stool sample at earliest convenience. For each urine sample, urine filtration was performed for the detection of *S. haematobium* eggs using a Whatman microfilter membrane of 10–12 µm as described elsewhere [19]. We used the Kato-Katz technique for the detection of eggs of STH in stool samples as described elsewhere [20] and the copro-culture technique for the detection of hookworm *(Necator americanus)* larvae [13, 20]. One 2.7 mL blood sample was collected into an ethylenediaminetetraacetic

acid (EDTA) tube to perform a FBC, using the automated haematology analyser (ABX Pentra 60®, Horiba Instruments Incorp., Irvine, CA < USA). In addition, 10 µL of fingertip blood was collected for a thick blood smear (TBS) for the detection of *Plasmodium* spp. parasites applying the Lambaréné method [21]. A rapid diagnostic test (RDT) was performed for the rapid detection of circulating *P. falciparum* HRP-2 antigen using the Paracheck Pf® Malaria Test (Orchid Biomedical Systems, Goa, India).

Participants found positive for *Schistosoma* eggs were treated with 40 mg/kg of PZQ once. Those testing STH or *Plasmodium* positive were treated with oral albendazole 400 mg daily for three consecutive days [22], or with artemisinin-based combination therapy (ACT) [23], respectively. For other infections, medical prescription was given by the study clinician, or the participant was referred to an appropriate health centre.

Statistical considerations

We collected data using the patient report form and digitalised it, using the REDCap electronic data capture tool [24] hosted at CERMEL. The original clean database was exported into R software (Version 3.2.4) and a subset database (see Supplementary Database) was obtained for the statistical analyses. Age was used as categorical variable, grouped in 5-year strata. Participants were considered having malaria when testing positive either with TBS or RDT. Qualitative variables were summarised by proportion and 95% confidence interval (CI); while, quantitative variables were summarised by mean and standard deviation (SD) or by median and interquartile (IQ) range where appropriate. The normality of the distribution of continuous variables was assessed by visual inspection and if needed, a log transformation was performed. Chi-square test was used to compare qualitative variables. Student's t-test was used to compare means of continuous variables; while, the Wilcoxon test was used to compare their distribution. Linear Model (LM) regression was used to correct for confounding factors potentially influencing haematological parameters with regard to *Schistosoma* status. The residuals were used to check for assumptions to ensure the usefulness of the model. The level of statistical significance was set at less than 0.05.

Ethical considerations

The original study protocol was approved by the institutional ethic committee of CERMEL (CEI-CERMEL 002/2016). The study was conducted in line with the Good Clinical Practice (GCP) principles of the International Conference on Harmonization (ICH) [25] and the Declaration of Helsinki [26].

RESULTS

Study population characteristics

A total of 614 participants were included in the original study [13], and 612 of them with haematology data available were incorporated in this analysis. The mean age of our study population was 10.1 (SD = 2.7) years, with a 0.95 female:male ratio. The mean haemoglobin level was 11.6 (SD = 1.13) g/dL. Ten per cent [95%CI: 8–13] and 23% [95%CI: 19–26] of our study population, respectively, declared having taken PZQ or albendazole treatment within the past six months prior to study enrolment. The prevalence of *Plasmodium* spp. infection combining TBS and RDT was 20% [95%CI: 17–23]. The prevalence of any STH was 15% [95%CI: 12–18].

Study group characteristics

The 161 (26%) participants found *schistosoma*-egg positive (159 in urine and two in faeces) constituted the *schistosoma*-positive (Sch+) group; while their negative counterparts constituted the *schistosoma*-negative (Sch-) group, leading to a ratio of 2.8 non-infected participants per single infected participant (Table 1). The two groups were comparable for age (*p-value* = 0.33) and sex (*p-value* = 0.39). Of note, the proportion of the participants previously treated with PZQ was significantly higher among the Sch+ group compared to the Sch- group (16% vs 8%, *p-value* = 0.008). The overall prevalence of STH was higher among the Sch+ group compared to the Sch- group (22% vs 12%, *p-value* = 0.005). Trichuriasis was the most prevalent STH infection in both groups, and was mostly prevalent among the Sch+ group compared to the Sch- group (17% vs 8%, *p-value* = 0.001). *Plasmodium* infection was more prevalent in Sch+ group than Sch- group (28% vs 17%, *p-value* = 0.003), particularly when detected using RDT.

Table 1. Characteristics of the 612 study participants with regard to schistosomiasis infection status

	Overall study population		**Schistosoma negative**		**Schistosoma positive**		***p-value***
	N	%, 95%CI	n	(%)	n	(%)	
Sample size	612	-	451	(73.7)	161	(26.3)	
Sex							**0.39**
Female	299	48.9 [40.2-48.2]	225	(49.9)	74	(46.0)	
Male	313	51.1 [40.7 – 55.2]	226	(50.1)	87	(54.0)	
Sex ratio (Female/Male)	**0.95**		**1.00**		**0.85**		
Age (mean, sd)	**10.1**	**2.7**	**10.1**	**2.7**	**10.0**	**2.8**	**0.83**
Age							**0.33**
5-9	352	57.5 [53.5 – 61.5]	262	(58.1)	90	(55.9)	
10-14	239	39.1 [35.2 – 43.0]	171	(37.9)	68	(42.5)	
15-19	21	3.4 [2.1 – 5.2]	18	(4.0)	3	(1.9)	

Table 1. *(continued)*

	Overall study population		Schistosoma negative		Schistosoma positive		*p-value*
	N	%, 95%CI	n	(%)	n	(%)	
Plasmodium* infection							
By microscopy	36	5.9 [4.2 – 8.1]	23	(5.1)	13	(8.2)	0.16
Parasitemia (Geometric mean)		756		574		1229	0.28
By RDT	118	19.3 [16.3 – 22.7]	75	(16.7)	43	(26.7)	0.006
Any method	122	20.1 [17.0 – 23.5]	77	(17.2)	45	(28.3)	0.003
History of praziquantel treatment**							**0.008**
Yes	62	10.1 [7.9 – 12.8]	37	(8.3)	25	(15.7)	
STH***							
Ascariasis	15	3.2 [1.8 – 5.2]	7	(2.1)	8	(6.1)	0.03
Trichuriasis	49	10.4 [7.8 – 13.5]	26	(7.6)	23	(17.4)	0.001
Hookworm	18	3.8 [2.3 – 6.0]	12	(3.5)	6	(4.5)	0.60
Any STH	69	14.6 [11.6 – 18.1]	40	(11.8)	29	(22.0)	0.005
History of STH treatment****							**0.12**
Yes	137	22.6 [19.3 – 26.1]	108	(24.2)	29	(18.1)	

[$] 1 missing data
*5 missing data; 4 for microscopy examination, 2 for rapid diagnosis test including 1 for both
**taken in the last 6 months, 7 missing data
***140 missing data; 111 for Schistosoma negative group and 29 for Schistosoma positive group
****5 missing data

Haematological profile and schistosomiasis status

We assessed the haematological profile of the study participants according to schistosomiasis status (Table 2) in the absence of clinical signs or symptoms hinting at a possible concomitant infectious or non-infectious disease. We found significant lower haemoglobin levels among the *schistosoma*-positive participants compared to their *schistosoma*-negative counterparts (median: 11.5 g/dL vs 11.7 g/dL, *p-value* = 0.001). However, WBC (7.5 103/mm3 vs 6.5 103/mm3, *p-value* < 0.001) and thrombocyte (254 103/mm3 vs 232 103/mm3, *p-value* = 0.002) counts were significantly higher in children with schistosomiasis. Looking specifically at the differential leukocyte count, we found a higher level of lymphocytes (3.57 103/mm3 vs 3.24 103/mm3, *p-value* < 0.0001), neutrophils (2.49 103/mm3 vs 2.29 103/mm3, *p-value* = 0.01), eosinophils (0.52 103/mm3 vs 0.30 103/mm3, *p-value* < 10–4) and basophils (0.07 103/mm3 vs 0.05 103/mm3, *p-value* < 0.001) in Sch+ compared to Sch-.

Table 2. FBC profile of the 612 study participants regarding schistosomiasis status

FBC parameters	Schistosoma status (median, [IQ])		*p-value* (Wilcoxon test)
	Negative	Positive	
Erythrocytes (10^6/mm^3)	**4.56 [4.29-4.83]**	**4.54, [4.30-4.85]**	**0.88**
Hemoglobin (g/dl)	11.7 [11.1-12.3]	11.5, [10.7-12.1]	0.001
Hematocrit (%)	35.9 [34.2-35.8]	35.4, [32.7-36.6]	0.0005
MCV (µm^3)	79.0 [75.0-83.0]	77.0, [73.0-81.0]	<0.0001
MCH (pg)	25.9 [24.4-27.5]	25.1, [23.7-26.5]	<0.0001
MCHC (g/dl)	32.7 [32.0-33.2]	32.5, [31.8-33.2]	0.26
Thrombocytes (10^3/mm^3)	**232 [170-287]**	**254, [195-315]**	**0.003**
Leukocytes (10^3/mm^3)	**6.50 [5.50-8.00]**	**7.5, [6.30-9.10]**	**<0.0001**
Lymphocytes (10^3/mm^3)	3.24 [2.64-4.40]	3.57, [3.11-4.40]	<0.0001
Neutrophils (10^3/mm^3)	2.29 [1.79-3.09]	2.49, [2.04-3.19]	0.01
Eosinophils (10^3/mm^3)	0.30 [0.16-0.50]	0.52, [0.33-0.90]	<0.0001
Basophils (10^3/mm^3)	0.05 [0.04-0.07]	0.07, [0,05-0.09]	<0.0001
Monocytes (10^3/mm^3)	0.39 [0.03-0.61]	0.43, [0.01-0.61]	0.50

A multivariate analysis was carried out to investigate a relationship between haematological constants and schistosomiasis status adjusted for PZQ intake, STH and *P. falciparum* infection status (Table 3), yielding a statistically significant positive correlation between thrombophilia and a diagnosis of schistosomiasis. Similarly, a statistically significant positive correlation between leukocyte count and a diagnosis of schistosomiasis was observed. This applied to all WBC subclasses except for monocytes, too (Table 3). For RBC, a relationship trend was found towards lower haemoglobin and haematocrit levels, respectively (Table 3). With regard to the corpuscular constants of RBCs, a significant relationship was observed for MCV (*p-value* = 0.02) and MCH (*p-value* = 0.04), not for MCHC (*p-value* = 0.83). Indeed, a decrease of 1.50 µm3 and 0.54 pg was observed in MCV and MCH mean level of participants with schistosomiasis, respectively. The adjusted R^2 value for each haematological constant was basically less than 0.01; so, less than 10% of the variation in these constants can be explained by our regression model. The data met the assumptions of homogeneity of variance and linearity and the residuals were approximately normally distributed.

Table 3. Differences in mean of the FBC parameters with regard to schistosomiasis status and adjusted on *Plasmodium* infection, any STH infection status, and history of praziquantel intake among the 472 participants with completed results

FBC parameters	*Schistosoma* positive		
	β	[95%CI(β)]	*p-value*
Erythrocytes (10^6/mm^3)	**0.006**	**[-0.09 - 0.10]**	**0.89**
Hemoglobin (g/dl)	-0.20	[-0.43 - 0.02]	0.08
Hematocrit (%)	-0.61	[-1.25 - 0.02]	0.06
MCV (µm^3)	-1.50	[-2.80 - -0.21]	0.02
MCH (pg)	-0.54	[-1.04 - -0.03]	0.04
MCHC (g/dl)	-0.02	[-0.22 - 0.17]	0.83
Thrombocytes (10^3/mm^3)	**28.2**	**[10.1 - 46.4]**	**0.002**
Leukocytes (10^3/mm^3)	**1.13**	**[1.05 – 1.20]**	**0.0003**
Lymphocytes (10^3/mm^3)	1.11	[1.04 – 1.18]	0.001
Neutrophils (10^3/mm^3)	1.12	[1.03 – 1.22]	0.01
Eosinophils (10^3/mm^3)	1.64	[1.36 – 1.98]	**<**0.001
Basophiles (10^3/mm^3)	1.27	[1.13 – 1.43]	0.0001
Monocytes (10^3/mm^3)	-	-	-

DISCUSSION

The main objective of the present analysis was to assess the effect of urogenital schistosomiasis on FBC parameters among schoolchildren living in Lambaréné, a semi-urban area. Our results reveal that children with schistosomiasis display an altered FBC profile with a change in the cell level count of the three cell types, as compared to those without the disease. However, taking into account some confounding factors which can either affect the haemoglobin and platelet levels [27] or schistosomiasis status such as PZQ treatment which is also known to normalise the leucocytes level in schistosomiasis infected children [4], the relationship between FBC parameters and schistosomiasis status observed was ambiguous for RBC; while, the observed change for thrombocyte and leukocyte counts was independently associated with the disease and was characterised by an increase in cell numbers.

Indeed, our results reveal a significant increase in total leukocyte count levels, as well as for each leukocyte type among children with schistosomiasis, as compared to those uninfected. The high WBC count levels we found among children with schistosomiasis, corroborate the reports of Mohammed et al. [4] and Afrifa et al. [10] among Sudanese and Ghanaian school children, respectively. We hypothesise that the high level of total leukocytes count observed

is to be seen in connection with the host immune response against the presence of adult schistosomes in the bloodstream, and the particular role of eosinophils in the defense against helminthic infections is generally well recognised [28, 29]. Similarly, our results show a significant increase in thrombocyte count levels among *S. haematobium*-infected children. Based on what was demonstrated in brown rats [8], we hypothesise that thrombophilia is related to active defense mechanisms directed against adult worms.

With regard to erythrocytes; although the haemoglobin level was similar in both groups, the FBC yielded significantly lower haemoglobin and haematocrit levels among children infected with schistosomiasis, as compared to those non-infected. Similar erythrocyte profiles were recently reported by Afrifa et al. among Ghanaian children with urogenital schistosomiasis compared to those without [10]. In our study, however, when adjusting for history of PZQ intake, STH and *Plasmodium* spp. infection status, only a trend towards lower haemoglobin and haematocrit levels was observed; while, lower MCV and MCH levels remain significant. Indeed, the haemoglobin levels observed in this population were similar to the levels observed in children without the disease, and were above 11 g/dL, the threshold set by the WHO to define anaemia for populations living at sea level [30]. The absence of a statistically relationship between anaemia and schistosomiasis might appear surprising, since the main symptom of urogenital schistosomiasis is hematuria occurring by blood spilling during egg excretion. However, longer intervals between blood losses, few or no macrohematuria episodes and haematological recovery between episodes might be explanatory. *Plasmodium* spp. infection is usually associated with lower hematocrit and haemoglobin concentrations [31], and could explain the lower level of haemoglobin display by children with schistosomiasis. We indeed found an association between both infections. Interpretation of haemoglobin and platelets levels for one of these two diseases should, therefore, consider the possibility of the presence of both. Similar explanations could apply for cases with STH co-infections.

Some factors such as schistosomiasis chronicity and intensity could influence the relationship between schistosomiasis and FBC parameters we reported here and were not take into account in our analysis, particularly due to the study design. However, the large sample size included in this study should be reassuring with regard to the accuracy of our findings. The study was conducted among schoolchildren knowing to be most affected by the disease and our conclusions are limited to this population. However, further investigations should be conducted particularly among women also known to bear a high burden of urogenital schistosomiasis.

In conclusion, in our setting, schistosomiasis is associated with a characteristic FBC profile, indicating the necessity to consider schistosomiasis as a single cause of disease, or a co-morbidity, when interpreting an FBC in schistosomiasis endemic areas.

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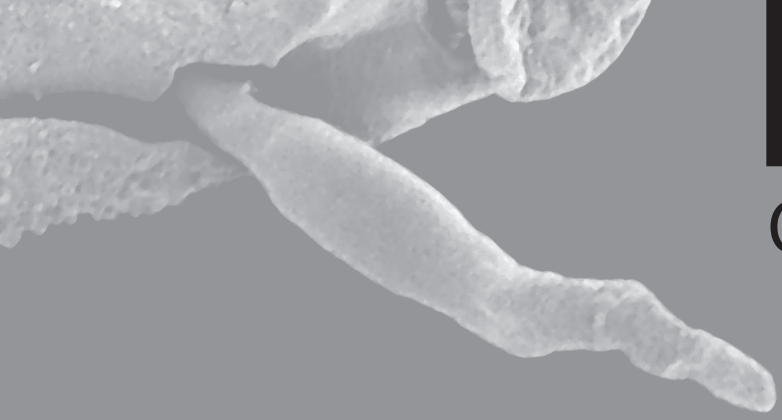

CHAPTER 7

Schistosoma haematobium effects on *Plasmodium falciparum* infection modified by soil-transmitted helminths in school-age children living in rural areas of Gabon

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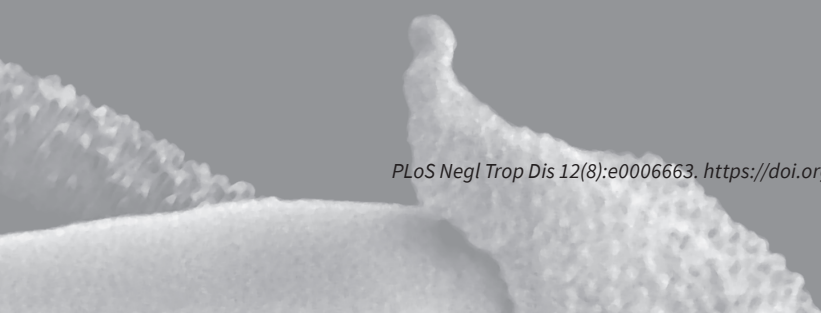

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ABSTRACT

Background: Malaria burden remains high in the sub-Saharan region where helminths are prevalent and where children are often infected with both types of parasites. Although the effect of helminths on malaria infection is evident, the impact of these co-infections is not clearly elucidated yet and the scarce findings are conflicting. In this study, we investigated the effect of schistosomiasis, considering soil-transmitted helminths (STH), on prevalence and incidence of *Plasmodium falciparum* infection.

Methodology: This longitudinal survey was conducted in school-age children living in two rural communities in the vicinity of Lambaréné, Gabon. Thick blood smear light microscopy, urine filtration and the Kato-Katz technique were performed to detect malaria parasites, *S. haematobium* eggs and, STH eggs, respectively. *P. falciparum* carriage was assessed at inclusion, and incidence of malaria and time to the first malaria event were recorded in correlation with *Schistosoma* carriage status. Stratified multivariate analysis using generalized linear model was used to assess the risk of *plasmodium* infection considering interaction with STH, and survival analysis to assess time to malaria.

Main findings: The overall prevalence on subject enrolment was 30%, 23% and 9% for *S. haematobium*, *P. falciparum* infections and co-infection with both parasites, respectively. Our results showed that schistosomiasis in children tends to increase the risk of *plasmodium* infection but a combined effect with *Trichuris trichiura* or hookworm infection clearly increase the risk (aOR = 3.9 [95%CI: 1.7±9.2]). The incidence of malaria over time was 0.51[95%CI: 0.45±0.57] per person-year and was higher in the *Schistosoma*-infected group compared to the noninfected group (0.61 *vs* 0.43, $p = 0.02$), with a significant delay of time-to first-malaria event only in children aged from 6 to 10-years-old infected with *Schistosoma haematobium*.

Conclusions: Our results suggest that STH enhance the risk for *P. falciparum* infection in schistosomiasis-positive children, and when infected, that schistosomiasis enhances susceptibility to developing malaria in young children but not in older children.

INTRODUCTION

Over the past fifteen years, morbidity and mortality due to malaria have globally decreased. However, sub-Saharan Africa, where 90% cases of malaria and 92% of deaths related to malaria have occurred in 2015, still bears the highest burden of the disease [1]. Most of these cases remain confined to rural and semi-urban areas [2-4] where helminths are co-prevalent [5-7]. In these areas where malaria significantly overlaps with helminth infections, several studies have reported interactions between the two parasitic infections at both immunological [8-12] and epidemiological [13-16] levels. Studies have reported an effect of helminths on the cellular and humoral immune responses to the malaria parasites mainly in children [8-12,17]. Some authors have reported that this effect leads to the aggravation of clinical manifestations of malaria. Indeed, it has been shown that *Trichuris trichiura* infection was associated with increased malaria prevalence, while an increased helminth burden was associated with increased *Plasmodium falciparum* or *Plasmodium vivax* parasitemia [18], or enhanced anemia in co-infected children [19]. Another author has reported a positive effect of helminths on malaria outcomes. Indeed, Nacher et al found that helminths, particularly Ascaris, may have a role in the establishment of malaria tolerance in Thai patients [20]. However, HIV co-infection complicated the picture further. Indeed, high prevalences of helminth infections and malaria have been reported in HIV positive people, particularly in pregnant women under anti-retroviral therapy (ART) in Rwanda [21-23] with 10% co-infections with both [23]. These high infection prevalences are found to be associated with a low CD4 counts and moreover, each of these infections is a risk factor for the other [22].

The situation is similarly unclear when it comes to *Schistosoma* spp. infections. It has been reported that the effect of schistosomiasis on malaria may depend on the *Schistosoma* species [24], or may be conflicting even for the same species [15,25-27]. Indeed, some reports have indicated that infection with *S. haematobium* can confer protection against severe malaria in children [25], reducing the risk of progression to symptomatic disease in long-term asymptomatic carriers of *P. falciparum* [15], or can delay the occurrence of a malaria episode in children [26]; whereas others found that *S. haematobium* may increase the prevalence of *P. falciparum* parasites in co-infected children [27]. In contrast, *Schistosoma mansoni* was reported to significantly increase the malaria incidence rate in children [28]. These finding provide evidence of the effect of schistosomiasis on *Plasmodium* infection. Current results on schistosomiasis and plasmodial co-infection are conflicting as reviewed recently by Adegnika et al. [24]. Most studies conducted to address these co-infections are cross-sectional in nature and could be limited in their capacity to precisely examine interactions between schistosomiasis and malaria. In this study, we conducted a longitudinal survey in order to address this issue in an area where *S. haematobium* and *P. falciparum* are the main prevalent species of schistosomiasis and malaria [13,14]. We thus assessed the effect of *S. haematobium* on clinical and parasitological aspects of *P. falciparum* infection in school aged children living in this co-endemic area, including the effect of soil-transmitted helminths (STH) in this association.

METHODS

Ethics statement

The study was approved by the institutional ethics committee of CERMEL, reference number: CEI-MRU 002/2012. Parents or legal representatives of the participant gave a written informed consent. The study was conducted in line with the Good Clinical Practice (GCP) principles of the International Conference on Harmonisation (ICH) [29] and the Declaration of Helsinki [30].

Study site

The study took place at CERMEL (Centre de Recherches Médicales de Lambaréné). Data and samples were collected from May 2012 to December 2014 in Bindo-Makouké villages (BM) and Zilé-PK villages, two settlements in the vicinity of Lambaréné [14] situated approximately at 60 km and 120 km, respectively, to the South of the Equator. The rainfall is perennial except for the long dry season (from June to September) with a mean of 1,216 mm per year [31]. The region is irrigated by the Ogooué River and its tributaries, with many ponds, lakes and streams constituting favourable conditions for fresh-water snail habitation. Recent published data demonstrate that the prevalence in the area for *S. haematobium* range from 15% to 75% [9,13,14]. Water supply, fishing, household work, fetching water and playing are some activities which expose the local population to schistosomiasis. Malaria transmission is perennial and the dominant malaria parasite species is *P. falciparum* [32,33].

Study design

The study was designed as a prospective longitudinal study.

Study population and inclusion criteria

School-age children living in two vicinities of Lambaréné (Nzilé-PK villages and Bindo-Makouké villages) were invited to participate in the study. Volunteers without any known chronic diseases other than possible helminth co-infections, and living in the study area for at least one year before inclusion were eligible to take part to the study. During the survey, participants found with a recurrent or severe disease other than malaria or helminth infection were excluded from the study.

Sample size determination

A previous study conducted in the vicinities of Lambaréné reported a 42% prevalence for *plasmodium* infection among school age children [9]. To be able to detect a minimum of 12.5% prevalence difference of *plasmodium* infection between children with schistosomiasis and those without, with a minimum of 80% power, we needed to include in the study at baseline at least 249 children for each study group, giving a total of 498 volunteers school age children.

Study procedure

Field-workers went to each house and school of both villages to invite through their parents or legal representatives school-age children to participate in the study. Eligible and consenting volunteers were included. At baseline, demographics (age, sex and location) and

anthropological (weight, height) data were collected. Axillary temperature was recorded. *S. haematobium* infection, *P. falciparum* infection and soil-transmitted helminths (STH) status were assessed. Participants were treated if they were found to harbour either of those parasitic infections. The follow-up consisted of two kinds of visit: active visits consisted of monthly home visits for any malaria-like symptoms assessment and recording of any medication intake; and passive visits were ad-hoc presentations of participating children at the research centre for any health issues, including flu-like symptoms.

Malaria status was defined as positive thick blood smear (TBS) associated with fever, or history of fever in the past 48h from the time of visit. Fever was defined as an axillary temperature of 37.5°C or higher. In case malaria was diagnosed, urine filtration was performed to assess evidence of co-infection with urogenital schistosomiasis. Urine filtration was also performed during the follow-up every time the children had visible haematuria.

Study groups were determined based on the schistosomiasis status. This was done differently for baseline analysis and for longitudinal analysis. At baseline and for baseline analysis, participants found infected with *S. haematobium* were assigned to the `*Schistosoma*-positive' (S+) group and the others to the `*Schistosoma*-negative' (S-) group. For longitudinal analysis, study groups were formed at the end of the follow-up period, and we assigned any participants found with infection at baseline and at any time point of the study course to the `*Schistosoma* infected' (SI) group. Those found negative at baseline and who did not experienced schistosomiasis during the study course were assigned to the `*Schistosoma*-uninfected' (SU) group. Time of exposure to malaria infection for incidence calculation did not include the first 28 days after each malaria treatment.

In accordance with the national guidelines, treatment of schistosomiasis consisted of the administration of 40 mg of praziquantel per kilogram body weight once; asymptomatic *P. falciparum* parasitemia and malaria episodes were treated with tablets of 20/120mg of artemether-lumefantrine combination therapy given according to the body weight twice a day, in three consecutive days. Treatment of STH was a once-daily dose of 400 mg of albendazole for three consecutive days [34]. For any other cause of a disease episode, the participant was referred to the appropriate health centre.

Samples collection and laboratory assays

Detection of malaria parasites was done microscopically by TBS using the Lambaréné method as described elsewhere [35-37]. Detection of *S. haematobium* eggs was done by filtration of 10ml of fresh urine using a 12µm pore-size filter as previously described [38-40]. For the diagnosis of urogenital schistosomiasis, urine samples were collected over three consecutive days, unless the participant was found positive with at least one parasite egg in any sample before the second or the third day. The Kato-Katz technique was performed to assess the presence of *A. lumbricoides*, *T. trichiura* and hookworm in fresh stool samples [41]. For each time point

of STH assessment, one stool sample was collected. For each stool sample, two slides were performed and each slide was independently read by two readers.

Statistical analysis

Data were captured on the patient report form (PRF), entered in Access 2013 software and transferred to R software version 3.2.4 for analysis. Univariate and multivariate analysis were performed applying the Generalized Linear Model (GLM). For multivariate analysis, first we considered the interaction between asymptomatic *P. falciparum* infection as the main variable and each explanatory variable. In case of effect measure modification, the analysis was stratified on the variable, and the Breslow test was done to assess the homogeneity of the strata. Otherwise, the variable was evaluated as confounding factor to be include in the final model. Ten per cent (10%) or more difference of estimated measure of association before and after adjustment was used to define confounding factors. The effect of STH infection was assessed separately with respect to the species. Incidence of malaria was estimated in person-year according to each variable. A Kaplan Meier curve was drawn to assess time-to-malaria occurrence. The Log-rank test was used to compare the curves and the Cox model was used for adjusted analysis.

RESULTS

Study population at baseline

Among the participants who were invited to participate in the study, informed consent was granted for 754 children by their parents or their legal representatives. Of those, a total of 739 children with schistosomiasis and *P. falciparum* status available were included at baseline (Fig 1). Among participants enrolled, 68 (9%) children were not able to provide sample stool at baseline and from the others, 31% [95%CI: 27%-35%] were infected with STH. The most prevalent infection was trichuris with 21% [95%CI: 18%-24%] followed by ascaris and hookworm with 19% [95%CI: 16%-22%] and 6% [95%CI: 5%-8%], respectively. Mean age of these study population was 10.4 (SD = 3.1) years, the boy-to-girl sex ratio was 1.1:1 (Table 1). Of participants included, 586 (79%) agreed to be followed-up for malaria incidence.

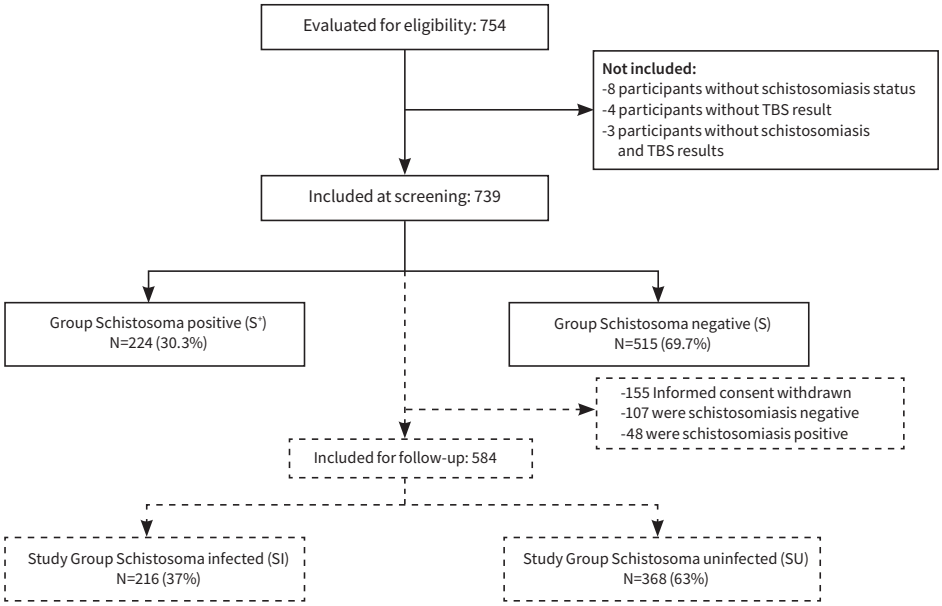

Fig 1: Flow chart of the participants during the study course. The inclusion phase is shown as a solid line and the follow-up phase is shown as a broken line.

Table 1. Characteristic of 739 participants seen at inclusion.

	Study population		
	n	(%)	95%CI(%)
Age			
[6-10]	382	(51.7)	[48.0-55.3]
]10-16]	357	(48.3)	[44.6-52.0]
Age (mean, sd)	(10.4, 3.1)	/	/
Sex			
Female	351	(47.5)	[43.8-51.2]
Male	388	(52.5)	[48.8-56.1]
Sex ratio (M/F)	(1.1)		
Location			
Bindo-Makouké villages	420	(56.8)	[53.2-60.4]
Zilé-PK villages	319	(43.2)	[39.6-46.8]
***S. haematobium* infection**			
Negative	515	(69.7)	[66.2-73.0]
Positive	224	(30.3)	[27.0-33.8]
***P. falciparum* infection**			
Negative	572	(77.4)	[74.2-80.4]
Positive	167	(22.6)	[19.6-25.8]
Soil-transmitted helminths*			
A. lumbricoides	127	(18.9)	[16.0-22.1]
T. trichiura	139	(20.7)	[17.7-24.0]
Hookworm	43	(06.4)	[04.7-08.5]
Any STH	208	(31.0)	[27.5-34.6]

*68 missing data

Distribution of *P. falciparum* and *S. haematobium* infections

The prevalence of *P. falciparum* and *S. haematobium* at baseline was 23% [95%CI: 20%-26%] and 30% [95%CI: 27%-34%], respectively; with 67 (9%, [95%CI: 07%-11%]) participants co-infected with both parasites. Eight per cent of participants infected with *P. falciparum* had fever. As shown in Table 2, both infections were more prevalent in Zilé-PK villages compared to Bindo and Makouké villages with 29% [95%CI: 24%-34%] *vs* 18% [95%CI: 14%-22%], respectively, for *P. falciparum* and 45% [95%CI: 40%-51%] *vs* 19% [95%CI: 15%-23%], respectively, for *S. haematobium*. The prevalence of both infections was similar for age and sex groups.

Table 2. Distribution of *P. falciparum* and *S. haematobium* infections among the 739 participants seen at baseline.

	***P. falciparum* infection**			***S. haematobium* infection**		
	n	% [95%CI(%)]	*p*	n	% [95%CI(%)]	*p*
Total	167	22.6 [19.6-25.6]	/	224	30.3 [27.1-33.7]	/
Sex			0.16			0.74
Female	71	20.2 [16.3-24.8]		104	29.6 [25.1-34.6]	
Male	96	24.7 [20.7-29.3]		120	30.9 [26.5-35.7]	
Age group			0.93			0.17
[6-10]	87	22.8 [18.8-27.0]		107	28.0 [23.7-32.7]	
]10-16]	80	22.7 [18.4-27.0]		117	32.8 [28.1-37.8]	
(Mean, sd)	(10.1, 3.1)			(10.7, 3.0)		
Location			<0.001			<0.001
Bindo-Makouké villages	75	17.9 [14.5-21.8]		80	19.0 [15.6-23.1]	
Zilé-PK villages	92	28.8 [24.1-34.0]		144	45.1 [39.8-50.6]	
STH*						
Ascaris	30	23.6 [16.5-32.0]	0.88	43	33.9 [25.7-42.8]	0.52
Trichuris	41	29.5 [22.1-37.8]	0.05	49	35.2 [27.3-43.8]	0.28
Hookworm	11	25.6 [13.5-41.2]	0.70	21	48.8 [33.3-64.5]	0.13
Any STH	52	25.0 [19.3-31.5]	0.43	72	34.6 [28.2-41.5]	0.24

*68 missing data

Study group characteristics

Among the participants followed-up for malaria incidence assessment, 216 (37%) were found positive for *Schistosoma* infection during the study period, including 176 cases on inclusion and additionally 40 new cases during follow-up and assigned to SI group. The 368 (63%) others participants who remained negative during the whole study course were thus assigned to the SU group (Fig 1). As shown in Table 3, the two study groups were comparable for all parameters except for location and *P. falciparum* parasite carrier status. Indeed, 168 (78%) of SI children

came from Zilé-PK villages while 253 (69%) of SU children came from Bindo and Makouké villages *(p*<0.001). Additionally, prevalence of *P. falciparum* parasite carriage was significantly higher in the SI group compared to the SU group (31% *vs* 20%, *p* = 0.004).

Table 3. Characteristics of study groups considered for longitudinal analysis regarding *Schistosoma* status (N=586).

	***Schistosoma* Infected (n=216)**		***Schistosoma* Uninfected (n=368)**		***P***
	n	% [$_{95\%}$CI (%)]	n	% [$_{95\%}$CI (%)]	
Sex					0.49
Female	111	51.4 [44.5-58.2]	177	48.1 [42.9-53.3]	
Male	105	48.6 [41.8-55.5]	191	51.9 [46.7-57.1]	
Age					0.14
[6-10]	110	50.9 [44.0-57.8]	211	57.3 [52.1-62.4]	
]10-16]	106	49.1 [42.2-55.9]	157	42.7 [37.5-47.9]	
n, (mean, sd)	216	(10.4, 3.1)	368	(10.1, 3.0)	0.19
Location					< 0.001
Bindo-Makouké villages	48	22.2 [16.9-28.4]	253	68.7 [63.7-73.4]	
Zilé-PK villages	168	77.8 [71.6-83.1]	115	31.3 [26.5-36.3]	
***P. falciparum* parasite carriage**					0.004
Positive	66	30.6 [24.5-37.2]	74	20.1 [16.1-24.6]	
STH species					
A. lumbricoïdes	30	13.9 [09.6-19.2]	60	16.3 [12.7-20.5]	0.47
T. trichiura	38	17.6 [12.8-23.3]	58	15.8 [12.2-19.9]	0.56
Hookworm	17	07.9 [04.6-12.3]	14	03.8 [02.1-06.3]	0.05
Any species	58	26.9 [21.1-33.3]	97	26.4 [21.9-31.2]	0.92

Association between *S. haematobium* and *P. falciparum* parasitic infections

At crude analysis as given in Table 4, *Schistosoma* infection (*p* = 0.002) and location (*p*<0.001) were associated with *P. falciparum* infection. Children infected with *S. haematobium* had a 1.8 [95%CI: 1.2-2.5] times odds of being co-infected with *P. falciparum* parasites compared to their non-infected counterpart. After adjustment (Table 4), *P. falciparum* infection remains associated only with location (*p* = 0.02) while a trend of association with schistosomiasis infection was found (*p* = 0.06).

Table 4. Potential risk factors including *S. haematobium* infection associated with *P. falciparum* infection among the 739 participants seen at baseline.

	Crude analysis		Adjusted analysis	
	OR [$_{95\%}$CI(OR)]	*p*	aOR [95%CI(aOR)]	*p*
***S. haematobium* status**		0.002		0.06
Negative	1		1	
Positive	1.8 [1.23-2.53]		1.5 [0.98-2.18]	
Location		<0.001		0.02
Bindo-Makouké villages	1		1	
Zilé-PK villages	1.9 [1.32-2.64]		1.6 [1.09-2.37]	
Sex		0.14		0.38
Female	1		1	
Male	1.3 [0.92-1.84]		1.2 [0.82-1.72]	
Age (years)	1.1 [0.99-1.11]	0.09	0.9 [0.89-1.01]	0.08
T. trichiura		0.06		0.09
Negative	1		1	
Positive	1.5 [0.98-2.28]		1.5 [0.94-2.38]	
A. lumbricoïdes		0.93		0.72
Negative	1		1	
Positive	1.0 [0.64-1.59]		0.9 [0.55-1.49]	
Hookworm		0.72		0.76
Negative	1		1	
Positive	1.1 [0.54-2.25]		0.9 [0.39-1.88]	

In the following analysis, we found effect modification of *Trichuris* or hookworm infections on *P. falciparum* and *S. haematobium* infections association. As presented in Table 5, analysis stratified on those two infections showed that among study participants without *T. trichiura* and hookworm infections, there is no effect of *S. haematobium* on *P. falciparum* parasite carriage; while among those infected with either hookworm or *T. trichiura* or a combination, children co-infected with *S. haematobium* had a 3.1 ([95%CI: 1.5-6.4], $p = 0.002$) time odds of being infected with *P. falciparum*. This finding remained statistically significant when adjusted for age, sex, ascariasis and location (aOR = 3.9 [95%CI: 1.75-9.19], $p < 0.001$). Age, sex and *Ascaris* infection were forced in the final model of the GLM analysis.

Table 5. Association between asymptomatic *Plasmodium falciparum* infection and *Schistosoma haematobium* infection stratified on *Trichuris trichiura* and hookworm infections among the 671 participants with known STH infection status and seen at baseline.

		Crude analysis[+]		Adjusted analysis*	
	N	OR [$_{95\%}$CI(aOR)]	*p*	aOR [$_{95\%}$CI(OR)]	*p*
***T. trichiura* and hookworm negative (N=516)**					
***S. haematobium* status**			0.27		0.84
Negative	360	1		1	
Positive	156	1.3 [0.83-2.01]		1.1 [0.65-1.67]	
***T. trichiura* or hookworm positive (N=155)**					
***S. haematobium* status**			0.002		<0.001
Negative	100	1		1	
Positive	55	3.1. [1.48-6.44]		3.9 [1.75-9.19]	

[+]Breslow-test, p = 0.046
*Adjusted for location, sex, age and *A. lumbricoïdes* infection

Effect of *S. haematobium* infection on *P. falciparum* malaria incidence

During the 19 months follow-up phase for *P. falciparum* malaria incidence assessment, 210 (36%) participants had developed a total of 318 new cases of malaria (Table 6). The overall incidence was 0.51 [95%CI: 0.47-0.55] per person-year. Taking into account the study groups, participants in the SI group had 1.4 [95%CI: 1.1-1.8] times the risk of developing malaria compared to their counterparts in the SU group.

Table 6. Malaria risk and malaria incidence among the 584 participants according to *Schistosoma* status and other risk factors.

Study group	Number of participants exposed	Participants who developed malaria attack		Malaria attack cases				
		n (%)	RR [$_{95\%}$CI(RR)]	Number of cases	Exposure Time [+]	Incidence [$_{95\%}$CI(I)]	RR [$_{95\%}$CI(RR)]	*P-value*
Total	584	210 (36.0)	/	318	627	0.51 [0.47-0.55]	/	
Schistosoma status								0.002
Uninfected	365	109 (29.9)	1	162	373	0.43 [0.38-0.48]	1	
Infected	219	101 (46.1)	1.54 [1.17-2.02]	156	254	0.61 [0.55-0.67]	1.42 [1.14-1.77]	
Location								0.76
Bindo-Makouké villages	301	107 (35.5)	1	163	316	0.51 [0,45-0,56]	1	
Zilé-PK villages	283	103 (36.4)	1.02 [0.78-1.34]	155	311	0.50 [0,44-0,55]	0.98 [0.79-1.22]	
Age								0.73
[6-10]	321	123 (38.3)	1	178	345	0.52 [0.47-0.57]	1	
]10-16]	263	87 (33.1)	0.86 [0.65-1.13]	140	282	0.50 [0.44-0.56]	0.96 [0.77-1.20]	
Sex								0.21
Female	288	97 (33.7)	1	143	304	0.47 [0.41-0.53]	1	
Male	296	113 (38.2)	1.13 [0.86-1.48]	175	323	0.54 [0.48-0.60]	1.15 [0.92-1.43]	

[+]In person-year

The time-to-first malaria episode was assessed for the first twelve months of follow-up of each participant and those who did not develop malaria before the end of that time were censored. Our results show that in the SI group, among the 101 (46%) participants developed malaria, the median time to first malaria episode was 52 weeks. For their counterparts of SU group where 109 (30%) participants developed malaria, the median time to first malaria episode was not reached at the end of 52 weeks of follow-up. As presented in Fig 2, we found a significant delay to malaria in the SU group compared to SI group (Log-Rank test: $p = 0.00037$). Assessing the delay until development of malaria according to schistosomiasis status, we found that SI group participants had a 1.6 (Cox model: $p = 0.0004$) times increased risk of early development of malaria as compared to their counterparts of the SU group. This association remains significant when adjusted for location, age and sex (aRR = 1.9, $p = 0.000034$). Stratifying for age yielded a significant delay in time-to-first-malaria episode in the SU group (median time not reach) compared to the SI group, where the median time was 51 weeks ($p = 0.00003$) for 6±10-year-old children. Children with schistosomiasis had a 2.1 (Mantel-Cox test: $p = 0.00004$) times increased risk of developing malaria compared to children without schistosomiasis. On the contrary, there was no difference in terms of delay in time-to-first malaria episode between the two study groups ($p = 0.41$) in children aged 11±16 years.

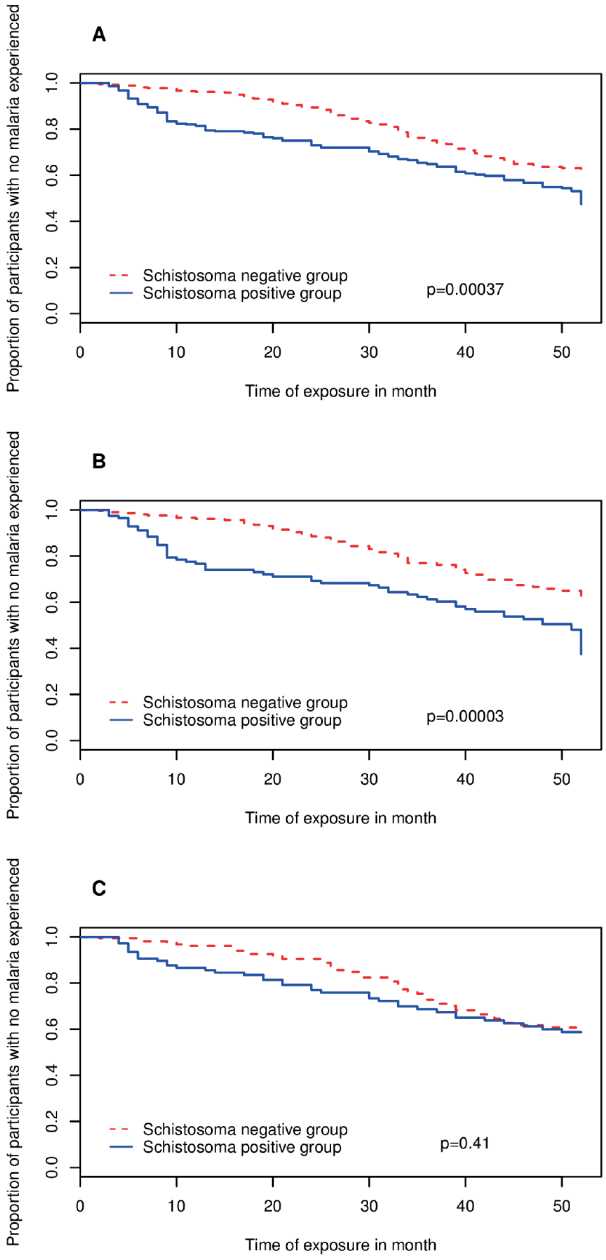

Fig 2. Depicts estimates of time to malaria after 52 weeks of follow-up. Depicted in the vertical axis the proportion of children who did not experience malaria and in the horizontal axis, the follow-up time in months. In red, children in *S. haematobium* non-infected group and in blue children in *S. haematobium* infected group. 2A) Kaplan Meier curve for time-to-first malaria case for overall study population. 2B) Kaplan Meier curve for time-to-first malaria case for children aged from 6 to 10 years old. 2C) Kaplan Meier curve for time-to-first malaria case for children aged from 11 to 16 years old.

DISCUSSION

In area where schistosomiasis is endemic, the question of its effect on malaria outcome is a growing concern. Our study area is endemic for both infections [14], and our study reveals that up to 11% of school-age children could be co-infected with *P. falciparum* and *S. haematobium* parasites, comparing to up to 15% of pregnant women co-infected in Cameroon [42] or 23% of children co-infected with *S. mansoni* in a co-endemic area of North-Western Tanzania [43]. In our study area, poly-parasitism is evident [8,9,13,14,24]. The prevalence of STH species ranged from 32% to 48% among children infected with *S. haematobium*. This finding is not surprising since STH is commonly reported to be prevalent in rural areas [43]. Therefore, the risk to be infected by multiple parasites including *S. haematobium* is high [44]. Since these intestinal parasites are known to be able to modulate the immune system of the host, it would be necessary to assess the effect of these infections on Schistosoma-*P. falciparum* association.

Everything else equal, we found a trend of association between risk of *P. falciparum* carriage and Schistosoma infection. In univariate analysis, Adedoja et al. found that children infected with *S. heamatobium* have equal chances of being infected with *P. falciparum* as children with no worm infection [45], while Ateba and collaborators found a significant increase of plasmodium asexual parasite prevalence among Schistosoma infected children in comparison to the uninfected [9]. Conflicting results found in the literature on schistosomiasis-malaria co-infection issue suggest that there are potential confounding factors not yet established which need to be taken into account. In this study, we found an effect-measure modification of *Trichuris* and Hookworm infections on the association between *S. haematobium* and *P. falciparum*. As well, location was identified as confounding factor. Some authors have previously shown that *T. trichiura* [18] and hookworm [45,46] individually can affect the association between schistosomiasis and malaria co-infection by increasing the risk of being infected with *P. falciparum* parasites. Our analysis was stratified for those two STH infections and adjusted for age and sex which could affect malaria infection [47], and for location found in our analysis as confounding factor. The result shows that when considered only schistosomiasis infection, there is a trend on the risk of being infected with *P. falciparum*. But, in combination with trichuriasis or hookworm infection, schistosomiasis clearly increases the risk of being infected with *P. falciparum*. This result shows that *S. haematobium* alone does not predispose to *P. falciparum* infection in children instead of combined effect. We hypothesize that the cumulative effects of *Schistosoma*, *Trichuris* and Hookworm infections on *P. falciparum* parasite carriage acts at the immunological level. A potential immuno-modulation effect of a poly-parasitism not measured in our study could explain the combined effect of helminths we have observed. Indeed, there is evidence that Schistosoma infection can modulate the immune system in response to *P. falciparum* [9,11]. There is also evidence that *T. trichiura* can exert an influence on the immune response, and for instance negatively affect the antibody response to malaria vaccine candidate in children [17]. However, these potential cumulative effects of helminth infections on *plasmodium* infection need to be properly investigated at immunological level.

The overall incidence of malaria was 0.51 per person-year. This incidence was higher among people infected with *S. haematobium* compared to the uninfected, suggesting that schistosomiasis infection increases the risk of developing malaria. Children infected with *S. haematobium* infection had 1.4 times the risk of developing malaria than uninfected.

Regarding time-to-first malaria infection, we found that malaria occurred earlier in participants infected with *Schistosoma* than those uninfected even after adjustment for age, sex and location. The main symptom we have considered to define malaria was fever, which is one of the results of some endogenous pyrogen molecules activities, notably pro-inflammatory cytokine TNF-α during the infection. Some authors reported that during malaria infection, the production of pro-inflammatory cytokines as well as of anti-inflammatory cytokines can be affected by co-infection with schistosomiasis infection in an age-dependent manner [11,48]. In our study population, the assessment of the delay-to-malaria in relation to age group shows that there is no difference in terms of delay in time-to-first malaria in children aged from 11 to 16 years, while the difference was significant in children from 6 to 10 years. Children aged from 6 to 10 years infected with *S. haematobium* developed malaria earlier than those without *S. haematobium* infection. This finding could support the possible effect of age on the immune responses of malaria in co-infected subjects. However, since the finding is based on statistical significance, biological assessment is suitable for confirmation.

We can retain that exposure to schistosomiasis enhances incidence of, and susceptibility to develop malaria in our study population. This finding corroborates with previous reports like the one by Sokhna and collaborators who reported an increased in susceptibility to developing malaria in co-infected children, even though it was only in children excreting high *S. mansoni* eggs loads [28]; supporting therefore the hypothesis that schistosomiasis negatively affects the outcome of malaria. This stands in opposition to the idea that schistosomiasis possibly improves the outcome of malaria. Indeed, it was reported, for instance, that protection from malaria is conferred by asymptomatic *P. falciparum* infection or co-infection with *S. haematobium* in a Malian study cohort [15]. In the study presented here, we assessed the effect of having been *S. haematobium*-infected on malaria instead of becoming infected at time of malaria, which could affect our conclusion compared to the studies mentioned above. On the other hand, it has been shown that STH can affect susceptibility to malaria infection by acting at the immunological level [49,50]. Not having considered the STH status of participants in our analysis could have affected our results; however, since all participants were assessed and infected ones were treated for STH at inclusion, we assume that the effect of STH was minimized. The prevalence of STH was similar between the both study groups at baseline, and the STH treatment effect was considered as equally distributed between groups.

We have assessed the effect of schistosomiasis on clinical and parasitological aspects of *P. falciparum* infection based on prevalence of *P. falciparum* parasite carriage and malaria inci-

dence. We therefore grouped our study population in relation to the schistosomiasis status. If it was easy at baseline to discriminate children infected or non-infected with *S. haematobium*, the problem we faced during the follow-up phase was to appropriately group our population in accordance with schistosomiasis status. Subjects infected at baseline or during the follow-up phase were treated systematically. However, they were considered as Schistosoma-infected for the whole follow-up phase and those who were not found positive throughout the survey were considered as non-infected. This approach was sustained by the fact that schistosomiasis is known as a chronic infection and, in areas where schistosomiasis is prevalent, the risk factors as playing habits, swimming, taking baths, washing clothes, distance from river are usually constant [51,52] and therefore the probability to be re-infected after treatment is high [53]. Thus, we have assessed the effect of schistosomiasis infection on *P. falciparum* parasite carriage at baseline and on malaria infection during the follow-up study phase.

An earlier conducted study in the same population showed that PCR has a better sensitivity than microscopy for the detection of *P. falciparum* parasites [9]. We have used the light microscopy Lambaréné method for the detection of *P. falciparum* parasites as it is the clinical gold standard. However, this may lead to potential misclassification of the participants regarding *P. falciparum* status at baseline. We think that if prevalence of *P. falciparum* carriage could be underestimated, this potential misclassification of participants would have been equally distributed in both groups and would not therefore affect the trend of our results.

This study confirms that the transmission of schistosomiasis is not evenly distributed in the vicinity of Lambaréné. Schistosomiasis infection is present in many villages but the prevalence varies significantly from one point to another. For example, we found a moderate prevalence for Bindo and Makouké villages where 19% of our study participants were found to be positive when compared to Zilé-PK villages, where 45% of our study participants were found to be positive. This corroborates with a previous pilot study conducted in the same population in 2012. The earlier-indicated prevalences of 15% and 43% for Bindo and Zilé-PK villages, respectively [14], suggest that prevalence of schistosomiasis infection is stable on each location. It was suggested that the difference observed could be explained by the fact that in Zilé-PK villages, streams represent the first source of water compared to Bindo village. The same observation could be applied to Makouké village where piped water is available for the majority of the population. Indeed, the lack of pipe water supply observed in the PK area promotes daily open freshwater contact by the population for household activities, bathing and playing, using the streams well known as schistosomiasis foci. In addition to humans, other ecological factors influence *Schistosoma* host snail density [54], which affect schistosomiasis prevalence. Therefore, we can assume that such factors may also sustain the difference of prevalence for schistosomiasis observed between the both locations, which requires further research. On the other hand, we have observed that areas where *S. haematobium* prevalence is high, a high prevalence of *P. falciparum* carriage was also found. Indeed, the difference in

prevalence observed in favour of the PK area for *S. haematobium* infection was also observed for *P. falciparum*. This observation suggests a correlation of factors affecting both infections as either a consequence of the presence of same environmental risk factors. Another explanation could be indeed the effect of *S. haematobium* infection on *P. falciparum* infection, as demonstrated above. However, this need to be more investigated.

In summary, this study demonstrates that *S. haematobium* infection alone does not increase the risk of being infected with *P. falciparum* parasite but when associated with STH particularly with *T. trichiura* and hookworm, the risk does increase. On the other hand, in people exposed to schistosomiasis infection, risk and susceptibility of developing a malaria event increase in an age-dependent manner. Our results suggest that Schistosoma and probably STH co-infections in general cumulatively impact on malaria outcome in school-age children and therefore need to be accounted for when designing malaria control programs. Thus, in areas of coendemicity and in support of higher efficiency, STH and schistosomiasis control should be considered as an additional tool of malaria control.

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CHAPTER 8

Schistosomiasis in Gabon from 2000 to 2021 - a review

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ABSTRACT

Introduction: Schistosomiasis is a public health issue of concern in Gabon, with the disease being reported from all regions of the country. The topic has been of interest for the local researchers and physicians for over two decades. The objective of this narrative review was to provide an overview of the research activities in the area from 2000 to early 2021.

Methods: We performed a narrative literature review. The search strategy was designed to get a broad overview of the different research topics on schistosomiasis and the national control programme, and included grey literature.

Results: A total of 159 articles was screened, and 42 were included into the review in addition to the grey literature. During the past two decades, the work on schistosomiasis originated from five out of the nine provinces of the country, with diverse aspects of the disease investigated; including immunology, epidemiology, diagnosis and treatment. Several studies investigated various aspects of schistosomiasis-related morbidity in the respective study populations. The body of work demonstrates that much effort was made to understand the details of the host immune response to schistosomiasis, and the immune profile changes induced in patients treated with praziquantel. Although some MDA campaigns were conducted in the country; little, however, is known on the epidemiological situation of the disease, particularly of its distribution within the population, as well as co-infections with other parasitic diseases also endemic in the area.

Conclusion: Progress has been made over the past two decades in the understanding of schistosomiasis in the country, including disease-related morbidity and its interaction with other parasitic infections, and the immunology and epidemiology of the disease. However, for optimising control of the disease, there is a need to finetune these findings with detailed local epidemiological and malacological data. We call for such studies to accomplish the knowledge of schistosomiasis in the country, particularly in areas of moderate or high endemicity, and recommend this approach to comparable schistosomiasis-endemic areas elsewhere.

Key words: schistosomiasis; malacology; control; narrative review; Gabon

1. INTRODUCTION

Schistosomiasis is a parasitic disease caused by the presence of (an) adult pair(s) of a trematode helminth of the genus *Schistosoma* in the venous vasculature of the host. Schistosomiasis is known to be an acute and chronic infection, affecting the immune system of the host at both stages. The immunological interplay between the host immune system and the adult worms and eggs (Angeles et al., 2020) might explain the impact of schistosomiasis on concomitant infectious diseases.

Five main species are responsible for the different manifestations of the disease in humans; *Schistosoma haematobium* being responsible for urogenital schistosomiasis (UGS); *S. mansoni*, *S. japonicum* and *S. mekongi* being responsible for intestinal schistosomiasis; and *S. intercalatum* being responsible for rectal schistosomiasis (Klotz, 1988; WHO, 2021). Some variances are reported; notably *S. guineensis* known as variant of *S. intercalatum* (WHO, 2021). All these species are unevenly distributed across various endemic regions of the world, mainly tropical and subtropical regions (CDC, 2019). Indeed, schistosomiasis is reported from five continents, with Asia and Africa being mostly affected. The African continent in particular bears the highest disease burden, with 93% of the global schistosomiasis cases (Adenowo et al., 2015), rendering schistosomiasis a major public health issue of concern. Mainly *S. haematobium* and *S. mansoni* are present, with some cases of *S. guineensis* and *S. intercalatum* reported particularly from Central Africa (WHO, 2021).

The symptomatology of schistosomiasis is due to the passage of eggs laid by adult worms to the urinary tract and lower gastrointestinal tract lumina. 'Stranded' disintegrating eggs stuck in mainly urogenital and visceral walls lead to granuloma formation, which is responsible for late complications such as bladder cancer. Furthermore, with the distribution pattern being determined by the infecting species, granulomas are reported beyond the urogenital system.

Praziquantel (PZQ) is the current drug of choice recommended for the treatment of all forms of schistosomiasis but active on the adult worms only. A high efficacy of PZQ for the treatment of schistosomiasis is very often reported (Fukushige et al., 2021). However, even in areas where the efficacy of the drug is reported as satisfactory, the re-infection rate could be high, leading to sustained high disease endemicity. Since the elimination or eradication of the disease remains difficult to achieve, the WHO recommends optimising control in order to at least limit the burden of the disease on population level. Depending on the local disease prevalence, one or two rounds of mass drug administration (MDA) of PZQ of at-risk population groups is thus recommended; in addition to striving for access to safe water, improved sanitation, hygiene education, and snail control (WHO, 2021). All taken together, it is clear that for optimising control, the local epidemiological situation of the disease has to be intimately understood.

Gabon is a sub-Saharan country with an estimated 2.1 million inhabitants in 2021. The country is located centrally in Africa, straddling the equator; a region known to be endemic for schis-

tosomiasis. Gabon is divided in nine administrative provinces and the country is irrigated and drained by several large rivers. The Ogooué River is the main river of the country, 1200 km long with an hydrographic basin draining about 80% of the country's territory. Ivindo (500 km long) and Ngounié (300 km long) rivers are its main tributaries. The Ogooué river crosses the entire country in its centre from East to West, cutting through five provinces. These hydrographical conditions render the region favourable for schistosomiasis and could explain the distribution and spacing of the disease across the country. Whereas the whole country is reported to be endemic for schistosomiasis, the bulk of the epidemiological data available seem to be only approximate, and outdated. In the present narrative review, we summarise the literature on schistosomiasis from 2000 to early 2021 to describe the current knowledge on the disease and its management in the country. A wealth of work has been accomplished over the past 20 years to determine the precise epidemiology and clinical manifestations of the disease in Gabon; as well as pathophysiology, diagnosis and treatment aspects have been addressed. Our research question was how all this work best informs the optimisation of the country's schistosomiasis control strategies. Whilst the situation of the disease differs from one country to another and even in the same country, which lessons can be learned from this body of knowledge to possibly identify research gaps and potential for disease control optimisation in other schistosomiasis-endemic regions.

2. METHODS

We conducted a narrative review. However, to ensure the collection of all articles related to schistosomiasis published on the Gabonese population from 2000 to early 2021, a literature search was performed by two independently working researchers (JCDA and JRE) using the following search terms: 'Schistosoma' or 'schistosomiasis', in combination with at least one of the following terms; 'haematobium', 'guineensis', 'mansoni', 'molecular', 'immunology', 'epidemiology', 'serology', 'snail', 'Bulinus', 'biomphalaria', and 'mass drug administration'. Google Scholar and PubMed databases were used to perform the search, which was restricted to articles in English and French. Review articles were excluded from the analysis. In addition, grey literature consisting of the Centre de Recherches Médicales de Lambaréné (CERMEL) website (www.cermel.org), a medical research centre located in Lambaréné and previously known as the Unité de Recherches Médicales (URM) of Lambaréné (Ramharter et al., 2021), and documentation of the 'Programme de Lutte contre les Maladies Parasitaires' from the regional health section of Moyen-Ogooué province (Delegation Regionale de la Santé) of the country's Ministry of Health were consulted for further information on schistosomiasis or on any activities implemented in the country for the control of the disease.

3. LITERATURE REVIEW

Understanding the pathophysiology of schistosomiasis forms the basis for proper disease management and control. To date, it is clear that the disease is due to the presence of adult *Schistosoma* worms in the blood stream while the symptomatology and both early and late disease manifestations are mainly related to the presence of worm eggs, particularly in the wall of the viscera affected. However, to further understand the disease mechanism, its immunological details, its epidemiology and its interaction with other diseases, both basic and applied research are needed to provide the information necessary to optimise control. In Gabon, from 2000 to early in 2021, 42 manuscripts were published pertaining to different aspects of schistosomiasis (Table 1). Of them, one review article was excluded and the remaining 41 manuscripts were mainly on immunology (15), epidemiology (6), disease characteristics (8) and treatment (5) of schistosomiasis. Few articles addressed the issue of schistosomiasis diagnosis (2), and malacology (3). Two articles were study protocols.

Table 1. Schistosomiasis studies conducted in Gabon between 2000 and 2021

Authors and publication year	Study area	Topic	Main findings
Adegnika et al., 2010	Moyen-Ogooué	Epidemiology	The study reports 41 (28%) out of 388 pregnant women living in Lambaréné tested positive for urogenital schistosomiasis using urine filtration technique.
Ateba-Ngoa et al., 2014	Moyen-Ogooué	Study protocol	The study protocol reports a prevalence of 15% and 43% of schistosomiasis in Bindo and Makouké, respectively, obtained from a prior pilot study conducted in schoolchildren.
Ateba-Ngoa et al., 2015	Moyen-Ogooué	Immunology	The study reports that *Schistosoma haematobium* infection was characterised by an increased chemokine profile with at the same time, lower pro-inflammatory markers in malaria co-infected school-aged children from an endemic area of Lambaréné, Gabon.
Ateba-Ngoa et al., 2016	Moyen-Ogooué	Immunology	The study reports a higher prevalence of microscopy detectable *P. falciparum* asexual parasites in *S. haematobium*-infected subjects in comparison to those uninfected with *S. haematobium* (47% *vs* 26%, $p = 0.003$). However, the difference disappeared when the authors considered filarial infection. On the effect of *S. haematobium* infection on the antibody response to *P. falciparum* antigen, the authors report a significant decrease for Pfs48/Pf45 immunoglobulin G titres in Schistosoma-infected children compared to those without schistosomiasis, but no difference for Pfs230 and for antibody against the asexual (MSP1, AMA1, and GLURP) stage of the parasite.

Table 1. *(continued)*

Authors and publication year	Study area	Topic	Main findings
Basra et al., 2013	Moyen-Ogooué, Ngounié	Treatment	The study reports among pregnant women a median reduction of *Schistosoma* eggs of 98% with mefloquine treatment (as a single dose of 15mg/kg of body weight or 2 doses of 7.5mg/kg of body weight on two consecutive days) administered twice in 1-month interval after completion of the first trimester, compared to an increase of 20% in the comparator group, and conclude on the efficacy of mefloquine on *S. haematobium* infection
Bormann et al., 2001	Moyen-Ogooué	Treatment	Assessing different praziquantel (PZQ)-based combination therapies for the treatment of urogenital schistosomiasis in a double-blind, randomised, placebo-controlled study, the author reports a 73%, 27% and 81% cure rate (CR) for PZQ-placebo, artesunate-placebo, and PZQ-artesunate combinations, respectively, while the CR of placebo alone was 20%.
De Jong et al., 2016w	Moyen-Ogooué	Immunology	To know how IgG glycosylation patterns differ with infection pressure among other factors, the authors assessed IgG1 Fc N-glycan galactosylation as a biomarker for immune activation in a multicentric study. The manuscript reports for Gabon cohort a moderate negative correlation between positivity for *S. haematobium* infection and IgG1 galactosylation, while a significant but weak negative correlation was observed for Ghana. Taken together, the authors report a correlation between parasitic infections and lower levels of IgG1 galactosylation in all sites.
Dejon-Agobé et al., 2018	Moyen-Ogooué	Morbidity	As main finding, the manuscript reports that among children with trichuriasis and hookworm infection, those with schistosomiasis had a 3.9 [1.75±9.19] odd to be infected with *P. falciparum* parasites than those without schistosomiasis, while among those negative for the two STH, no risk of *Plasmodium* infection was observed with regard to their schistosomiasis status. In addition, the author found that in infected young children, schistosomiasis enhances susceptibility to developing malaria but not in older children.

Table 1. *(continued)*

Authors and publication year	Study area	Topic	Main findings
Dejon-Agobé et al., 2019	Moyen-Ogooué	Treatment	Assessing in schoolchildren the efficacy of three doses of 60mg of PZQ per body weight given one month apart, the authors report after the first and third dose of PZQ, an overall ERR of 93% and 95%, respectively; while the CR were 78% and 88%, respectively. Both ERR (100 vs 88%) and CR (90 vs 68%) were higher among females than males after the first dose. The CR increased for both groups after the third dose to 95% and 80%, respectively. After the first PZQ dose, ERR was higher for heavy compared to light infections (94 vs 89%), while the CR was higher for light than for heavy infections (87 vs 59%). After the third PZQ dose, ERR increased only for light infections to 99%, while CR increased to 98% and 75% for light and for heavy infections, respectively. The reinfection rate assessed at a mean of 44.6 weeks post-treatment was 25%.
Dejon-Agobé et al., 2020	Moyen-Ogooué	Epidemiology	The study reports a 26% (95%CI: 22–29) prevalence of urogenital schistosomiasis in Lambaréné among schoolchildren living in Lambaréné, with contact with freshwater as the main risk factor. Haematuria and proteinuria were associated with schistosomiasis status, while trichuriasis and ascariasis were associated with a high risk of schistosomiasis.
Dejon-Agobé et al., 2021	Moyen-Ogooué	Morbidity	The study reports haematological changes in *S. haematobium* infections in school children. Adjusted for praziquantel intake, soil-transmitted helminthic infections and *Plasmodium falciparum* infection status, schistosomiasis was independently associated with a decreasing trend of mean haemoglobin (β = − 0.20 g/dL, *p-value* = 0.08) and haematocrit (β = − 0.61%, *p-value* = 0.06) levels, a lower mean MCV (β = − 1.50µm3, *p-value* = 0.02) and MCH (β = − 0.54 pg, *p-value* = 0.04), and higher platelet (β = 28.2 103/mm3, *p-value* = 0.002) and leukocyte (β = 1.13 103/mm3, *p-value* = 0.0003) counts, respectively.

Table 1. *(continued)*

Authors and publication year	Study area	Topic	Main findings
Dejon-Agobé et al., 2021	Moyen-Ogooué	Malacology	In a short communication from a pilot malacological study conducted in Lambaréné, the provincial capital of Moyen-Ogooué, and surrounding rural areas in 2013, authors report the presence of *Bulinus forskalii* and *B. truncatus* as potential intermediate hosts of human-pathogenic schistosome in the area. Exposed to cercariae shedding, only *B. truncatus* shedd mammalian cercariae morphologically identified as schistosome cercariae.
Fitzsimmons et al., 2004	Moyen-Ogooué	Immunology	Assessing the human IgE response to the target *S. haematobium* 22·6 kDa antigen, the manuscript reports a more prevalent IgE response to the antigen in adults than in children, with the highest post-treatment levels seen in people over 14 years.
Flügge et al., 2020	Moyen-Ogooué	Morbidity	Investigating the impact of helminth infections during pregnancy on vaccine immunogenicity in gabonese infants, the manuscript reports no effect of these helminths, including *S. haematobium*, on to the expanded program of immunization (EPI) vaccines; tetanus, diphtheria, pertussis, Haemophilus influenzae type b (HiB), poliomyelitis (polio), hepatitis B, and measles. In addition, *S. haematobium* infection was the most prevalent helminth infection in the study population with 14 (27%) cases out of the 52 tested positive.
Hoekstra et al., 2020	Moyen-Ogooué	Diagnosis	The manuscript introduces a multicentric study (freeBILy project) evaluating the performance of the laboratory-based UCP-LF CAA test for *S. haematobium* detection in pregnant women with the final aim to provide a fast and reliable easy-to-use diagnostic for eliminating bilharzia
Honkpehedji et al., 2020	Moyen-Ogooué	Study protocol	The study aimed to evaluate the accuracy of circulating anodic antigen (CAA) detection for diagnosis of *S. haematobium* infections in pregnant women and to validate CAA as an endpoint measure for anti-Schistosoma drug efficacy.
Honkpehedji et al., 2021	Moyen-Ogooué	Epidemiology	Assessing the association of low birth weight (LBW) and polyparasitic infection during pregnancy in Lambaréné, Gabon, the manuscript reports first a proportion of S. *haematobium* infection of 23% among the 678 pregnant women they included in the study. Secondly, the authors report a decreased of the mean new-born weight with the rising number of maternal parasitic infections from zero to three or more, including schistosomiasis.

Table 1. *(continued)*

Authors and publication year	Study area	Topic	Main findings
Janssen et al., 2015	Moyen-Ogooué	Treatment	The study reports a 5.9% (19/323) prevalence of *S. haematobium* infection in HIV-infected adults who were antiretroviral therapy (ART)-naïve or exposed to ART for at least 3 months. In addition, no association between ART and cotrimoxazole preventive treatment and the risk of having schistosomiasis was found.
Kenguele et al., 2014	Moyen-Ogooué	Diagnosis	The study reports, from non-frozen and frozen urine samples, *Schistosoma*-specific DNA amplification in 61 of 66 samples (92.4%) in which *Schistosoma* eggs were detected. After concentration of the urine by centrifugation, *Schistosoma* DNA was amplified in 64 (97.0%) and 65 (98.5%) of 66 non-frozen and frozen urine pellets from microscopy positive samples, respectively. In addition, *Schistosoma*-specific amplification occurred in some samples in which no *Schistosoma* eggs were found three consecutive times by microscopy.
Labuda et al., 2013	Moyen-Ogooué	Immunology and treatment	Characterising B cell subsets and B cell responses to B cell receptor and TLR 9 stimulation in schoolchildren with *S. haematobium* infection, the manuscript reports as summary of the results that: 'frequencies of memory B cell (MBC) subsets were increased, whereas naive B cell frequencies were reduced in the schistosome-infected group. At the functional level, isolated B cells from schistosome-infected children showed higher expression of the activation marker CD23 upon stimulation, but lower proliferation and TNF-α production'. Six-months after three rounds of a single dose of 40 mg/kg of body weight of PZQ three times every two months, the manuscript reports increase of naive B frequencies cells, MBC frequencies decreased and with the exception of TNF-a production, a restoration of B cell responsiveness to what was seen in uninfected children.
Labuda et al., 2014	Moyen-Ogooué	Immunology	The manuscript reports the pattern-recognition receptors (PRR) responsiveness in children living in the Netherlands compared to those living in semi-urban and rural areas of Gabon and thus exposed to parasitic infections, including schistosomiasis (57% of rural Gabonese children). Comparing inflammatory responses to various TLR and non-TLR ligands, the authors observed a reduced pro-inflammatory response to TLR3 stimulation and enhanced pro-inflammatory response to TLR2/1, TLR2/6 and TLR4 stimulation in children living in Gabon, compared to those living in the Netherlands. No significant differences in cytokine responses to non-TLR ligands.

Table 1. *(continued)*

Authors and publication year	Study area	Topic	Main findings
Labuda et al., 2020	Moyen-Ogooué	Immunology and Treatment	Assessing the effect of PZQ treatment on immune and transcriptome profiles in *S. haematobium*-infected schoolchildren, the study reports that three single-dose of PZQ (40 mg/kg) at 2-month intervals decreases TNF levels in response to TLR stimulation (Pam3) in schoolchildren over 7-months of follow-up, and reversed antigen-specific hypo-responsiveness, which was accompanied by an increase in effector T-Cells. In addition, the author reports an association between CD4+CD25+FOXP3+ T-Cells percentage and innate and adaptive cytokine responses. On the other side, the authors report alteration genes expression in *S. haematobium* infected schoolchildren, even after treatment as compared to non-infected schoolchildren and conclude in an association between schistosomiasis and treatment with distinct transcriptional profiles.
Ludwig et al., 2019	Moyen-Ogooué	Immunology and Morbidity	The manuscript reports a comparison of placental gene expression as well as inflammation markers in maternal and cord blood between Germans and Gabonese women, with 13/54 (23%) of Gabonese women positive for *S. haematobium* infection. The results revealed in Gabonese as compared to Germans women a significantly lower gene expression in maternal placenta side of Vitamin-D-receptor (VDR)1 and Cyp27b1, and a significant lower gene expression of VDR1 and Hsd3b1 in the foetal placenta side. Similarly, the author reports also in maternal and foetal sides a lower expression of Foxp3 and IL10 in placentae from Gabon compared to others from a German cohort. Comparing Gabonese women with and without schistosomiasis, the author reports a lower expression of Hsd3b1 on the foetal side of placenta from infected mothers and lower IFN-γ expression on the maternal side. Assessing the AWA specific IgE and IgG4 antibody levels to schistosomiasis, the authors report a significant higher AWA specific IgG4 levels in maternal and cord blood in the infected group. AWA specific IgE levels were increased in maternal but not foetal plasma from infected Gabonese mothers, and a significantly lower levels of plasma IgG4 in new-borns result in a significantly higher IgE/IgG4 ratio. The author also reports a strong correlation between maternal and cord blood total IgG4 concentrations in all groups however stronger in the German cohort.

Table 1. *(continued)*

Authors and publication year	Study area	Topic	Main findings
Manego et al., 2017	Moyen-Ogooué, Ngounié	Epidemiology	As epidemiology maternal health and of schistosomiasis among other major infectious diseases in the rural department Tsamba-Magotsi, Ngounie Province, in central African Gabon, the manuscript reports a 7% (95%CI: 5–9) prevalence of *S. haematobium* egg excretion in urine at first antenatal care visit assessing in 591 HIV negative pregnant women.
Mebius et al., 2019	Moyen-Ogooué	Morbidity	Studying haemostatic parameters in schoolchildren infected with *S. haematobium*, the manuscript reports that the infection leads to the elevation of the levels of von Willebrand Factor (VWF) antigen, active VWF and osteoprotegerin. Suggesting a direct alteration of the activation status of the endothelium by the parasite, without initiation of coagulation.
Meurs et al., 2011	Moyen-Ogooué	Immunology	Investigating the effect of *S. haematobium* infection on cytokine responses to a number of Toll-like receptor (TLR) ligands (Pam3 ligand of TLR2/1, FSL-1 ligand of TLR2/6, Poly(I:C) ligand of TLR3 and LPS ligand of TLR4), the manuscript reports in infected than uninfected children higher adaptive IL-10 responses against schistosomal antigens (72 h incubation), higher TNF-α responses and significantly higher TNF-α to IL-10 ratios in response to FSL-1 and Pam3. A similar trend was observed for LPS while Poly(I:C) (Mda5/TLR3 ligand) did not induce substantial cytokine responses (24 h incubation).
Mintsa-Nguéma et al., 2014	Estuaire	Parasitology and Malacology	The authors used *Bulinus globosus* snail originating from Benin to evaluate the cercarial emergence pattern of strain of *S. haematobium* originate from Libreville, Gabon, and reports that the 88% of the cercarial emissions occurred between 11 a.m. and 3 p.m., with the average emission peak occurring at 1p.m. and representing 26% of the total daily cercarial production.
Mintsa-Nguéma et al., 2018	Ogooué-Ivindo, Woleu-Ntem	Epidemiology	The manuscript reports a 1.7% prevalence of schistosomiasis across the Northern and Eastern health regions of Gabon, with no significant difference ($p > 0.05$) between the two regions; 1.5% for the Northern and 1.9% for the Eastern regions. Schistosomiasis was mainly caused by *S. heamatobium* with the exception of one respective case of *S. mansoni* and *S. guineensis*, respectively.

Table 1. *(continued)*

Authors and publication year	Study area	Topic	Main findings
Mombo-Ngoma et al., 2017	Moyen-Ogooué, Ngounié	Epidemiology	The study reports a more common low birth weight amongst infants of *S. haematobium*-infected mothers (adjusted Odds Ratio 1.93; 95% confidence interval: 1.08–3.42), compared to those without. The association was unaffected by controlling for demographic characteristics, gestational age and *Plasmodium* infection status.
Nguéma et al., 2010	Estuaire	Parasitology and Malacology	The study reports the presence of *Bulinus forskalii* and *B. globosus* in Melen and Ekouk, and all the snails collected were negative for schistosome infection. On the morphology of the eggs collected from the study population in Melen and Ekouk and from stool and urine, the author reports three morphotypes: *S. haematobium* (predominantly oval and supplied with a well-visible terminal spine), *S. guineensis* (predominantly spindle-shaped with two well-identified extremities) and intermediate morphotypes, with the eggs of the morphotype *S. guineensis* smaller compared to the values found in the literature. Analysing the adult schistosomes, the author reports that all the patterns corresponded to that of *S. haematobium* and concluded on the absence of hybrids from the samples collected.
Nguéma et al., 2018	Estuaire	Parasitology and Malacology	The manuscript reports 4 (0.81%) positive cases of *S. mansoni* out of 495 participants examined and did not identified intermediate snail host of human schistosomes in the whole surveyed water sites of Plaine Orety, an urban area of Libreville, Gabon.
Nouatin et al., 2021	Moyen-Ogooué	Morbidity	The manuscript reports the effect of *S. haematobium* infection among other helminth infection on the immunogenicity and efficacy of the asexual blood-stage malaria vaccine candidate GMZ2. Compared to anti-GMZ2 IgG concentration on D84 in those uninfected, the author reports that participants infected with *S. haematobium* alone presented a higher level of anti-GMZ2 IgG (mean log concentration ±SD: 3.49 ±0.14 vs 3.69 ±0.10, *p-value* = 0.01). On time to malaria, *S. haematobium* (Log-Rank test: *p-value* = 0.008) infections were significantly associated with faster times to the disease.
Remppis et al., 2020	Moyen-Ogooué	Morbidity	Assessing urinary tract morbidity compatible with uro-genital schistosomiasis (UGS) in microscopy positive individuals sonographically, the study reports cases of renal pelvis and/or ureter dilation with some of them presenting bladder wall irregularities. Pathology compatible with UGS was also reported by the authors one and three months after treatment with one dose of 40mg per body weight of praziquantel.

Table 1. *(continued)*

Authors and publication year	Study area	Topic	Main findings
Retra et al., 2008	Moyen-Ogooué	Immunology	The manuscript reports the results of the effect of two Toll-like receptor-2 (TLR2) activating schistosomal lipid fractions (one containing lysophos-phatidylserine (lysoGPSer) plus diacylphosphatidylserine (GPSer) and one containing lysoGPSer and only a trace of GPSer.), in comparison with the known bacterial TLR2 ligands (PAM3CSK4 and MALP-2) on the polarization of the cytokines profiles. The authors found that the TLR2 fraction ligands containing lysoGPSer plus GPSer derived from schistosome parasite generate distinct cytokine profiles than those derived from bacterial (strong TNF-α inducing capacity vs preferential IL-10-activating capacities). In addition, the fraction containing lysoGPSer plus GPSer also showed a strong Th2 response than Th1 when comparing to the PAM3CSK4, MALP-2 and the fraction containing lysoGPSer alone.
Schmiedel et al., 2015	Moyen-Ogooué	Immunology	The study aimed to assess whether *S. haematobium* infection is associated with induction of CD4+CD25hiFOXP3+ regulatory T cells and to evaluate Treg activity during infection. As summary of the results, the author reports that schistosome infection is associated with increased Treg frequency and that Tregs exert a suppressive effect on immune cell function in terms of both proliferation and cytokine production. On the effect of treatment with praziquantel on cytokine responses, the author found a decrease of Treg frequency after anti-schistosome treatment, with their suppressive capacity remains unaltered for cytokine production but their influence on proliferation weakens with treatment.
van den Biggelaar et al., 2000	Moyen-Ogooué	Immunology	The manuscript reports the use of autologous granulocyte-macrophage colony-stimulating factor and dendritic cells (DC) derived from interleukin (IL)–4 as highly efficient antigen-presenting cells (APC) to investigate the hypo-responsiveness to *Schistosoma* parasites in chronically infected persons. Peripheral blood mononuclear cells (PBMC) from persons harbouring *S. haematobium* infection and hyporesponsive to parasite antigen were therefore co-cultured with autologous DC in the presence of adult worm antigen (AWA). The authors found that in contrast to PBMC alone, DC-supplemented cultures responded to AWA by proliferation and by IL-4 and IL-5 production and, in some patients, by production of interferon-γ.

Table 1. *(continued)*

Authors and publication year	Study area	Topic	Main findings
van den Biggelaar et al., 2001	Moyen-Ogooué	Immunology	The manuscript reports "a lower prevalence (odds ratio 0.32 [95% CI 0.16-0.63]) of a positive skin reaction to house-dust mite in children with urinary schistosomiasis than those free of the infection. Schistosome-antigen-specific concentrations of interleukin-10 were significantly higher in infected children, and higher specific concentrations of this anti-inflammatory cytokine were negatively associated with the outcome of skin-test reactivity to mite (0.53 [0.30-0.96])".
van den Biggelaar et al., 2002	Moyen-Ogooué	Treatment and Immunology	At the end of 24-months follow-up of children with schistosomiasis included in three different intervention groups, the study reports 80% (20/25) positive cases of schistosomiasis among those who did not received praziquantel (40mg/kg once), 90% (37/41) among those who received one dose of PZQ at inclusion, and 33% (7/21) among those who received one dose of PZQ every three months for the two years from inclusion with a significantly lower levels of serum CAA and egg output, as compared to the other groups. In response to AWA, multiple treatment induced high level of IL-5 and IL-13 in children at 24-months but only a few children in the group produce INF-γ. High level of SEA-specific IgG1 were found in children treated repeatedly. Children (re) infected at 36 months tended to have higher level of IL-5 and IL-10 to AWA at month 24.
van der Kleij et al., 2004	Moyen-Ogooué	Immunology	The manuscript reports the result of the stimulation of the innate immune system with schistosomal phosphatidylserine (PS), schistosomal glycolipids (GLs), and Escherichia coli lipopolysaccharide (LPS) to study the effect of repeated challenge of the immune system with pathogen-associated molecular patterns. Analysing the cytokine responses (interleukin-6, IL-8, IL-10 and tumor necrosis factor (TNF)- α) to those schistosomal lipids and bacterial LPS, the authors report in infected children living in an area with a high transmission levels of schistosomiasis, as compared to those uninfected and living in an area with moderate transmission level, a significant lower production of mainly IL-8 and TNF– α, but also IL-10, IL-6 following stimulation of schistosomal PS fraction containing the TLR–2 ligand lyso-PS. Responses to the TLR4 ligand, LPS, followed a similar pattern. In contrast, schistosomal adult worm GLs that did not stimulate any of the TLRs tested induced IL-8 and IL-6 responses that were significantly higher in schistosome-infected children than in schistosome-uninfected children.

Table 1. *(continued)*

Authors and publication year	Study area	Topic	Main findings
van der Vlugt et al., 2012	Moyen-Ogooué	Immunology	Investigating in humans (but also in mice) whether schistosome infections can induce functional Breg cells, the manuscript reports significantly higher percentage of CD1dhi B cells in peripheral blood of S. haematobium-infected children compared to uninfected children which were reduced to levels comparable to the uninfected control group six months after treatment with three doses of praziquantel (40 mg/kg) every two months. The authors also found that the CD1dhi B cells from infected children produce more IL-10 after stimulation with anti-IgG/IgM as compared to uninfected children.
van der Vlugt et al., 2014	Moyen-Ogooué	Immunology	Investigating the regulatory characteristics of peripheral B cells from *S. haematobium*–infected individuals and the functional consequences for CD4+ T-cell activation, the manuscript reports as summary of the results that 'an elevated level of B cell IL-10 from *S. haematobium*–infected adults expressed cytoplasmic IL-10 and membrane-bound latency-associated peptide/transforming growth factor β1, compared with uninfected adults. T cells produced less INF-γ, IL-4, and IL-17 upon coculture with B cells from schistosome-infected individuals only, while the conversion to CD25hiFoxP3+ and the percentage of IL-10+ T cells was enhanced. In addition, depletion of the prominent IL-10–producing B-cell subset, CD1dhi cells, resulted in less IL-10+ T cells in the *S. haematobium*–infected group, while levels of FoxP3+ regulatory T cells remained unaffected'.
Zinsou et al., 2020	Moyen-Ogooué	Morbidity	The study reports that individuals overweight/obese positive for urogenital schistosomiasis exhibited lower serum total cholesterol, high-density lipoprotein (HDL)-C and triglyceride levels than their counterpart egg-negative. Similarly, significant negative correlations between the intensity of infection, assessed by serum circulating anodic antigen concentrations, and TC, HDL-C, LDL-C and TG levels were found in overweight/obese individuals but not in lean subjects.

3.1. Epidemiology of schistosomiasis

3.1.1. *Schistosoma* species distribution

A total of four species of *Schistosoma* spp. were reported from the country: *S. haematobium*, *S. mansoni*, *S. guineensis* and its variant *S. intercalatum* (Ateba Ngoa et al., 2014; Borrmann et al., 2001; Dejon-Agobé et al., 2020, 2018; Mintsa Nguema et al., 2018; Nguema et al., 2018). *S. haematobium* was the species reported from all areas where schistosomiasis was reported, while only few cases of *S. mansoni* were reported from Estuaire, Moyen-Ogooué, and Woleu-Ntem provinces, respectively; and few cases of *S. guineensis* from Estuaire and Woleu-Ntem provinces, respectively. Cases of *S. intercalatum* were reported from Lambaréné, the provincial capital of Moyen-Ogooué. The results of these studies confirm that *S. haematobium* is the main *Schistosoma* species endemic in the country, with the presence of some cases of intestinal *Schistosoma* species. Whilst the differentiation between *S. mansoni* eggs from *S. haematobium* or *S. guineensis* eggs can be easily done morphologically by microscope examination, *S. intercalatum* reported from Lambaréné should be speciated at molecular level to be differentiated from *S. haematobium*, since eggs from both species are morphologically similar (Almeda et al., 1996).

3.1.2. Schistosomiasis prevalence

Nine articles specifically addressed the issue of schistosomiasis prevalence over the country, and particularly in four provinces (see Table 2). In the Estuaire province, one study was conducted in 2018 in Plaine Orety, an urban area of Libreville, to investigate the presence of *S. mansoni* in the area. The study reported a prevalence of 0.8% of *S. mansoni* among the general population, but also a 1.2% prevalence for *S. guineensis* (Nguema et al., 2018). No urine samples were collected for the diagnosis of potential *S. haematobium* infection in that population.

Table 2. Summary of schistosomiasis prevalence over the country reported during the study period, 2000 to 2021

	Province	Department/Locality	Prevalence (%)	95%CI(%)	*Schistosoma* sp	Population	Reference
1	Estuaire	Plaine Orety, Libreville	0.81	[0.22 – 2.06]	S. mansoni	Overall (2 to 94 years)	Nguéma et al., 2018
			1.21	[0.45 – 2.62]	S. guineensis	Overall (2 to 94 years)	Nguéma et al., 2018
		Melen, Libreville	-	-	S. haematobium	NA	Mintsa Nguéma et al., 2014
			16.8	[12.18 – 22.34]	S. haematobium	NA	Nguéma et al., 2010
			3.1	[1.25 – 6.28]	S. guineensis	NA	Nguéma et al., 2010
		Ekouk	26.4	[17.55 – 36.98]	S. haematobium	NA	Nguéma et al., 2010
			8.1	[3.30 – 15.88]	S. guineensis	NA	Nguéma et al., 2010
2	Haut-Ogooué	NDA*	-	-	-	-	-
3	Moyen-Ogooué	Lambaréné	26	[22.47 – 29.55]	S. haematobium	Schoolchildren	Dejon-Agobé et al. 2020
			1	[0.13 – 1.84]	S. intercalatum	Schoolchildren	Dejon-Agobé et al. 2020
		Bindo	15	NA	S. haematobium	Schoolchildren	Ateba-Ngoa et al., 2014
			19.0	[15.6 – 23.1]	S. haematobium	Schoolchildren	Dejon-Agobé et al., 2018
		Zilé village	43	NA	S. haematobium	Schoolchildren	Ateba-Ngoa et al., 2014
			45.1	[39.8 – 50.6]	s. haematobium	Schoolchildren	Dejon-Agobé et al., 2018
		Lambaréné	12	[8.79 – 16.00]	S. haematobium	Pregnant women	Adegnika et al., 2010
		Lambaréné & Fougamou	9	[7.60 – 11.09]	S. haematobium	Pregnant women	Mombo-Ngoma et al., 2017
		Lambaréné & Fougamou	23	[19.89 – 26.36]	S. haematobium	Pregnant women	Honkpéhèdji et al., 2020
4	Ngounié	Tsamba-Magotsi, Fougamou	7	[5.00 – 9.00]	S. haematobium	Pregnant women	Manego-Zoléko et al. 2017
5	Nyanga	NDA	-	-	-	-	-
6	Ogooué-Ivindo	Ivindo	0.8	[0.09 – 2.80]	S. haematobium	Schoolchildren	Mintsa-Nguéma et al., 2018
		Lope	2.4	[0.90 – 5.21]	S. haematobium	Schoolchildren	Mintsa-Nguéma et al., 2018

Table 2. *(continued)*

	Province	Department/Locality	Prevalence (%)	95%CI(%)	*Schistosoma* sp	Population	Reference
		Mvoung	4.4	[2.20 – 7.68]	S. haematobium	Schoolchildren	Mintsa-Nguéma et al., 2018
7	Ogooué-Lolo	NDA	-	-	-	-	-
8	Ogooué-Maritine	NDA	-	-	-	-	-
9	Woleu-Ntem	Woleu	0.8	[0.1 – 2.9]	S. haematobium	Schoolchildren	Mintsa-Nguéma et al., 2018
		Ntem	2.4	[0.9 – 5.2]	S. haematobium	Schoolchildren	Mintsa-Nguéma et al., 2018
			0.4	[0.01 – 2.22]	S. mansoni	Schoolchildren	Mintsa-Nguéma et al., 2018
		Haut Ntem	0.8	[0.09 – 2.75]	S. haematobium	Schoolchildren	Mintsa-Nguéma et al., 2018
		Haut Komo	2.2	[0.71 – 4.98]	S. haematobium	Schoolchildren	Mintsa-Nguéma et al., 2018
			0.4	[0.01 – 2.39]	s. guineensis	Schoolchildren	Mintsa-Nguéma et al., 2018
		Okano	1.2	[0.25 – 3.49]	S. haematobium	Schoolchildren	Mintsa-Nguéma et al., 2018

*NDA: No Data Available

Mapping schistosomiasis in the Northern and Eastern Health Regions of Gabon, Mintsa-Nguéma et al., in 2018, reported a prevalence of schistosomiasis in different departments of Woleu-Ntem and Ogooué-Ivindo provinces. The author reported a range of 0.8% to 2.4% and 0.8% to 4.4% *S. haematobium* prevalence in both provinces, respectively. In addition, 0.4% of *S. mansoni* and *S. guineensis* prevalence were reported by the author from Ntem and Haut Komo, two departments of the Woleu-Ntem province (Mintsa Nguema et al., 2018).

Three articles reported the prevalence of schistosomiasis from Moyen-Ogooué province. The first one published in 2014 was conducted in the surrounding areas of Lambaréné in the framework of a pilot study for immunological studies. The authors reported a 43% prevalence for Zilé village located on the N1 road 15km south of Lambaréné, and 15% prevalence for Bindo village, a remote area located 65km north of the provincial capital of Moyen-Ogooué (Ateba Ngoa et al., 2014). The difference in prevalences between the two areas was explained by the fact that in the Pk15 area, streams represent the population's primary source of water compared to the Bindo village, where public water pumps are available (Ateba Ngoa et al., 2014). Although many manuscripts reported the presence of schistosomiasis for decades in Lambaréné, the first comprehensive prevalence estimation was conducted in 2020, finding a 26% prevalence of UGS among schoolchildren (Dejon-Agobé et al., 2020). Some further articles were not focusing on the epidemiology of schistosomiasis but provided information on the disease distribution in their study population. Indeed, three articles (Adegnika et al., 2010; Honkpéhèdji et al., 2021; Mombo-Ngoma et al., 2017) using similar study methodology reported an increase in schistosomiasis prevalence over time in a specific population subgroup, pregnant women. The first article, in 2010, reported a prevalence of 9% (Adegnika et al., 2010), while the second one reported in 2017 a prevalence of 12% with schistosomiasis associated with a low birth weight delivery (Mombo-Ngoma et al., 2017); an article from 2021 reported a prevalence of 23% (Honkpéhèdji et al., 2021). This observed increase in disease prevalence in pregnant women over time could be considered as an indicator of the absence of, and that way, the need for a schistosomiasis control program in this specific population. From the remaining publications, only the proportion of the participants with schistosomiasis was reported. Having been basically interested in the cohort of schistosomiasis patients, those co-infected with other parasites were compared with those with mono-infections only.

WHO recommends to consider the disease prevalence among children in order to estimate the endemicity level of the disease in a given area (WHO, 2021) and indeed, the prevalence of schistosomiasis reported from some provinces of the country was estimated mainly from children. Therefore, Estuaire, Woleu-Ntem and Ogooué-Ivindo can be considered as provinces with low prevalence for schistosomiasis, while Moyen-Ogooué can be considered as area with moderate prevalence for schistosomiasis. This information is particularly relevant, since the number of MDA rounds estimated to be conducted per year in an endemic area is based on the level of the local prevalence (Crompton and WHO, 2006). It therefore appears that the need for MDA of PZQ for schistosomiasis control is different between the various regions of the country.

3.1.3. Studies areas

Over the past two decades, schistosomiasis has been investigated and reported in five out of the nine provinces of the country; Estuaire, Moyen-Ogooué, Ngounié, Ogooué-Ivindo, and Woleu-Ntem. An effort to map schistosomiasis has been made in 2018 by Mintsa-Nguéma for two provinces of the country; Ogooué-Ivindo and Woleu-Ntem (Mintsa Nguema et al., 2018). In Libreville, the capital of the country and the provincial capital of the Estuaire province, the investigation on schistosomiasis was made in Melen and Plaine Orety, two neighbourhoods of the city known to be endemic for schistosomiasis. In the same province, Ekouk, a rural area located 120km south of Libreville, was also investigated for the presence of schistosomiasis (Nguema et al., 2010). In Moyen-Ogooué province, a wealth of studies on schistosomiasis was conducted in three different areas; namely Lambaréné, the provincial capital; Zilé-PK villages close to Lambaréné, and Bindo village, a remote area located 65 km from Lambaréné (Table 1). The choice of Zilé and Bindo villages was based on the difference in the level of the population exposure to schistosomes, which could indeed differently affect the immune response of the host towards the parasites. Two epidemiological studies were conducted concomitantly in Lambaréné and Fougamou (Honkpéhèdji et al., 2021; Mombo-Ngoma et al., 2017), and another one among HIV negative pregnant women (Manego et al., 2017), providing additional evidence of the presence of schistosomiasis in Ngounié province. A report from 1987 indicated the presence of schistosomiasis in all regions of the country (CNRS-WHO, 1987). However, schistosomiasis was reported only from five provinces over the country during the two last decades, highlighting the lack of a countrywide coordinated registry or the prevalence study. Indeed, it appears that most data come from the capital city of the country, and due to CERMEL location, from Lambaréné and the surrounding areas. As it was recently done for two provinces, efforts should be made at country level to update the situation of schistosomiasis, particularly because many provinces are known to provide favourable conditions for schistosomiasis development, and thus hotspots of schistosomiasis have to be identified.

3.1.4. Studied populations

Children and particularly school-aged children are known to be the population part mostly affected by schistosomiasis. Twenty-six (63%) of the 41 publications on schistosomiasis in the country involving humans were conducted among children; an obvious choice since no data were available on the distribution of the disease in the population, particularly before 2014. However, there are some indications that the highest burden of schistosomiasis is not only amongst school-age children. Indeed, the burden of schistosomiasis in adults and particularly in women are a matter of concern. For example, the estimated prevalence of female genital schistosomiasis (FGS) in endemic areas ranges between 30-50% (Poggensee et al., 1999), or 55-75% in women with *Schistosoma* eggs in urine (Christinet et al., 2016). Among the articles included in our review, eleven focused on an adult population and nine were carried out amongst pregnant women. Of the studies conducted in pregnant women, one assessed the burden of schistosomiasis on the outcome of pregnancy (Mombo-Ngoma et al., 2017), and the two others assessed parasitic co-infections in pregnancy, including schistosomiasis

(Adegnika et al., 2010; Honkpéhèdji et al., 2021). The main risk for schistosomiasis is the contact with infested freshwater. Individuals in contact with freshwater for one reason or another are therefore those exposed to the disease. Since control measures such as MDA of PZQ target the exposed population, it is therefore relevant to identify, for any endemic area, those population parts particularly exposed. For instance, our review documents an increased prevalence of schistosomiasis from 2010 to 2020 in pregnant women, assuming that the situation is at least similar to men, in general. Since schistosomiasis affects populations differently, future studies should report epidemiology of the disease per gender and age. As there is a call to address FGS in endemic areas (UNAIDS, 2019), the female population should be of special interest.

3.2. Diagnosis of schistosomiasis

Urine filtration technique and Kato-Katz using a light microscopy for the evidence of *Schistosoma* eggs in urine and stool are the gold standard for the diagnosis of UGS and intestinal schistosomiasis, respectively. These methods are eventually used in the country. However, if the presence of eggs in urine or stool is considered as a diagnosis of certainty of schistosomiasis, failure to identify eggs in the sample is not a proof that the patient is negative for the disease, given the daily variability of eggs shed by infected individuals. To improve the sensitivity of urine filtration technique for the diagnosis of UGS, practice at CERMEL consists of collecting one urine sample a day usually between 9 am to 3 pm and during three consecutive days. Therefore, a patient is considered as infected if at least one single egg is found in at least one urine sample. Otherwise, the patient is considered as uninfected (Dejon-Agobé et al., 2020). This situation shows the necessity to apply a more sensitive method; ideally a combination of methods, for the diagnosis of schistosomiasis. To improve the sensitivity of the diagnosis of UGS, polymerase chain reaction (PCR) essay detecting *Schistosoma* egg antigen in fresh urine samples is used as a diagnostic method for schistosomiasis. In a cohort of 85 participants living in area highly endemic for urogenital schistosomiasis, Kenguele et al. reported an increase in the number of Schistosoma positive cases by real time PCR when using urine samples concentrated by centrifugation and freezing prior to DNA extraction, as compared to the use of urine samples centrifugated but not frozen. The finding was similar when using urine samples positive (98.5% *vs* 97.0%) or negative (36.8% vs 57.9) for the presence of *Schistosoma* eggs at the microscopy examination. The author therefore suggested that freezing urine before DNA extraction improves the sensitivity of the real-time PCR essay when using urine concentrated by centrifugation (Kenguele et al., 2014).

Haematuria is the main sign of UGS and very often used as diagnostic symptom. In 2020, we reported, in the Lambaréné population, an association between haematuria, but also proteinuria, and schistosomiasis (Dejon-Agobé et al., 2020). However, performance and predictive values for the diagnosis of the disease remain to be further examined since these indicators are affected by the prevalence of these biomarkers in the population, but also by the presence of other causes of haematuria and proteinuria.

Another biomarker of interest for the diagnosis of schistosomiasis are circulating antigens released by schistosomes into the host circulation and eliminated with the urine. The detection of circulating cathodic antigen (CCA) in urine sample by the up-converting phosphorus-based lateral flow (UCP-LF) technology is already used as a point-of-care (POC) diagnostic of *S. mansoni* infection. Similarly, as potentially more sensitive tool for the diagnostic of UGS, an ongoing project conducted in Lambaréné and surroundings aims to assess the accuracy of circulating anodic antigen (CAA) known to be specific for *S. haematobium* detection (Hoekstra et al., 2020; Honkpehedji et al., 2020).

3.3. Schistosomiasis treatment and re-infection

Schistosomiasis treatment remains a matter of concern. Several articles examined the issue of schistosomiasis treatment over the past two decades in the Gabonese population (Basra et al., 2013; Borrmann et al., 2001; Dejon-Agobé et al., 2019; Janssen et al., 2015; Labuda et al., 2020, 2013; Schmiedel et al., 2015; van den Biggelaar et al., 2002). Assessing the effect of mefloquine on schistosomiasis as intermittent preventive treatment against malaria in pregnancy, Basra et al. reported a 98% median reduction of eggs excreted, indicating a satisfactory efficacy of the drug for the treatment of *S. haematobium* infection (Basra et al., 2013). Jansen et al. reported no effect when assessing the efficacy of either anti-retroviral treatment nor cotrimoxazole prophylaxis on *S. haematobium* in HIV-infected patients (Janssen et al., 2015). PZQ is the drug of choice for the treatment of schistosomiasis and is effective against all human-pathogenic *Schistosoma* species, particularly on the adult form of the worm, while artemisinin derivatives are known to be effective on the juvenile form of the parasite. Assessing by cure rate (CR) the use of artesunate for the treatment of *S. haematobium* infection among schoolchildren, Bormann et al. reported a 27% CR of artesunate plus placebo, while the CR for placebo alone was 20%. In the PZQ-artesunate combination both at their usual dosage, the CR increased to 81% while the CR of PZQ alone was 71%. However, the difference between both results was not statistically significant (*p-value*=0.23). These results led the authors to the conclusion that the effect of artemisinin derivatives observed in *S. mansoni* and *S. japonicum* could not be confirmed in *S. haematobium* infections, and that the addition of artesunate to PZQ did not improve the CR (Borrmann et al., 2001). Although the treatment protocol consisted of three doses of 60 mg per body weight once per month for three consecutive months, Dejon-Agobé and colleagues, using the egg reduction rate (ERR), reported more than 90% PZQ efficacy already after the first dose administration, particularly in females (99%) rather than males (88%) (Dejon-Agobé et al., 2019). However, it remains necessary to re-assess the efficacy of a 40mg dose per kilogram of body weight administered once, as this is the routine protocol used.

Four manuscripts investigating the immune response to schistosomiasis treatment with PZQ. Assessing the effect of a single dose of 40mg per body weight of PZQ treatment three times every two months on the host immune response particularly on B cell subsets, Labuda et al. reported reverse B cell frequencies, with their responsiveness restored six months after the last drug dose administration, as compared to uninfected children (Labuda et al., 2013). Van den Biggelaar et al. reported high interleukin (IL)-5 levels and low IFN-γ production after repeated

treatment (van den Biggelaar et al., 2002). To gain insight into which immunologic profiles can predict resistance or susceptibility to schistosome infections, the authors found that high levels of parasite-specific IL-10 were a risk factor for re-infection, and that high levels of IL-5 were associated with haematuria (van den Biggelaar et al., 2002). In the third manuscript, which assessed the association of *S. haematobium* infection with induction of regulatory T cells and evaluated Treg activity during infection, the authors conclude that schistosoma-associated $CD4^{+}CD25^{hi}FOXP3^{+}$Tregs exert a suppressive effect on both proliferation and cytokine production, whilst Treg frequency decreases after praziquantel treatment. However, their suppressive capacity remains unaltered when considering cytokine production, whereas their influence on proliferation weakens with treatment (Schmiedel et al., 2015). In the fourth manuscript, on *S. haematobium*-infected children treated three times with single-dose PZQ (40 mg/kg) at 2-monthly intervals, Labuda et al. concluded that schistosomiasis treatment leads to increased effector T-cell frequencies and decreased levels of $CD4^{+}CD25^{+}FOXP3^{+}$ T-cells, which were associated with decreased TLR-specific tumor necrosis factor (TNF) levels and increased antigen-specific cytokine production (Labuda et al., 2020). The immune profile changes induced by PZQ provide evidence that schistosomiasis treatment has the potential to affect the outcome of other concomitant infectious diseases.

Two articles evaluated the schistosomiasis status of the study participants after treatment (Dejon-Agobé et al., 2019; van den Biggelaar et al., 2002). The first one, in 2002, reported 30% (7/21) positive cases among participants treated with 40 mg/kg of body weight once every three months over two consecutive years (van den Biggelaar et al., 2002), while the other one, in 2019, reported 10 (14%) positive cases out of 71 treated with 60 mg/kg of PZQ once a month during three consecutive months; but also a 25% (12/47) re-infection rate after nine months of follow-up among those found negative after treatment (Dejon-Agobé et al., 2019). The authors considered an early re-infection in individuals frequently exposed to the parasite rather than drug resistance explanatory.

3.4. Schistosomiasis and co-infections

In a cohort of HIV patients from Lambaréné, after loiasis (18%, 56/310), schistosomiasis was the second-most prevalent helminth infection (6%, 19/323) (Janssen et al., 2015). With regard to malaria co-infections, we reported, in 2018, a 10% asymptomatic parasitaemia and schistosomiasis co-infection prevalence in Lambaréné (Dejon-Agobé et al., 2018) and, in 2020, a 6% soil-transmitted helminths-schistosomiasis co-infection prevalence in the same area, with *Trichuris trichiura* being the most-frequently encountered STH species with 5% co-infection prevalence (Dejon-Agobé et al., 2020). These figures highlight the importance of schistosomiasis but also of other helminths in co-infections with other parasitic and viral diseases. We reported, for example, the effect of STH on schistosomiasis-malaria co-infection. Schistosomiasis was associated with a 3.9 odds to be infected with *Plasmodium* parasites in children positive for hookworm and/or trichuriasis, while no effect was observed in children negative for those two STHs. In addition, we reported that schistosomiasis enhances the susceptibility to develop malaria in young children (Dejon-Agobé et al., 2018). Similarly, in an exploratory

analysis of the effect of helminth infections on the efficacy and immunogenicity of a malaria candidate vaccine assessed in Lambaréné's adult population (Dejon-Agobe et al., 2019), Nouatin et al. reported that helminth mono-infections and particularly *S. haematobium* infection were significantly associated with earlier malaria episodes, as compared to those free of helminths (Log-Rank test: *p-value* = 0.008). In addition, the candidate vaccine-specific immunoglobulin (Ig) G concentration was significantly higher (*p-value* = 0.01) in *S. haematobium*-infected study participants (mean log concentration ± SD: 3.69 ± 10), compared to those negative for any helminth investigated (mean log concentration ± SD: 3.49 ± 14); suggesting a potential effect of schistosomiasis on the quality of the antibodies produced in response to the vaccine candidate (Nouatin et al., 2021).

3.5. Immunology of schistosomiasis

Schistosomiasis is known to profoundly affect the host immune system, particularly at the chronic stage. A wealth of studies (Table 1) addressed during the two last decades the issue of immunology in schistosomiasis in the population of Lambaréné and its vicinity, either to understand details of the disease mechanism, or to assess the burden of disease in the immune response to other parasitic diseases, particularly to malaria. Most of these studies focused on the acquired immunity aspect to schistosomiasis, particularly on the cellular immune response to the parasite. Three main aspects were thus of interest; (1) the mechanism of, and the changes in the immunity to schistosomiasis; (2) the effect of PZQ administration for the treatment of schistosomiasis on the immune system; and (3) the effect of schistosomiasis on the malaria immune response of the host.

Several studies investigated details of schistosomiasis host immunity (Fitzsimmons et al., 2004; Labuda et al., 2013; Meurs et al., 2011; Retra et al., 2008; van den Biggelaar et al., 2000; van Den Biggelaar et al., 2000; van der Kleij et al., 2004; van der Vlugt et al., 2014, 2012). Indeed, studying the IgE responses to the *S. haematobium* 22·6 antigen, a *S. haematobium* antigen closely homologous to *S. mansoni* 22·6 and *S. japonicum* 22·6 antigens, for which the level has been correlated with resistance to re-infection after chemotherapy, Fitzsimmons et al. found that like similar molecule in the other species (Sm22 6 and Sj22 6), Sh22·6 antigen is a target in *S. haematobium* infection for the human IgE response. The authors suggested that the changes in the IgE response occur with age or with progressive exposure to key antigens (Fitzsimmons et al., 2004).

Toll-like receptors (TLRs), a class of proteins, are an innate immunity component known to key inflammatory responses and to shape adaptative immunity, thus providing a link between the innate and adaptive immune systems (Akira, 2003; Kawai and Akira, 2005). Four publications examined TLR responses to schistosomal-TLR ligands. Indeed, comparing the innate cytokine responses mediated by various classes of pattern recognition receptors including TLRs, C-type lectin receptors (CLRs) and nucleotide-binding oligomerisation domain-like receptors (NLRs); Labuda et al., in 2014, observed significant differences in cytokine responses to TLR ligands, but not to non-TLR ligands when comparing Dutch (European) and African children

with schistosomiasis. The authors found that children from Lambaréné and Zilé village with schistosomiasis had a lower pro-inflammatory response to TLR3 ligand (polyI:C), but a higher pro-inflammatory response to TLR2/6 (FSL-1), TLR2/1 (Pam3) and TLR4 (LPS) ligands compared to those without (Labuda et al., 2014). Still in Lambaréné and vicinity, Meurs et al. investigated, in a pilot study among children, the effect of *S. haematobium* infection on cytokine responses to a number of TLR-ligands. The authors reported that *Schistosoma*-infected children develop a more pro-inflammatory TLR2-mediated response in the face of a more anti-inflammatory adaptive immune response (Meurs et al., 2011). Previously, in 2007, in the same population, analysing the cytokine responses to schistosomal lipids in order to study the effect of repeated challenges to the innate immune system with pathogen-associated molecular patterns, van der Kleij et al. reported that the schistosomal phosphatidylserine (PS) fraction containing the TLR–2 ligand lyso-PS stimulated the production of IL–8, IL-10, IL-6, and TNF–α lower in children with *S. haematobium* infection, as compared to their uninfected counterparts. This held particularly true for production of IL-8 and TNF- α. The authors also observed a similar pattern with responses to the TLR4 ligand. In contrast, the authors observed that schistosomal adult worm glycolipids that did not stimulate any of the TLRs tested induced IL-8 and IL-6 responses that were significantly higher in schistosome-infected children than in schistosome-uninfected children. The authors concluded that their results indicated that relentless re-exposure to pathogens can lead to altered responses to TLR ligands (van der Kleij et al., 2004). Retra et al. reported a distinct cytokine profiles of two different TLR2-activating schistosomal lipid fractions, and of the known bacterial TLR2 ligands PAM3CSK4 and MALP-2; indicating that not only TLR2 ligands derived from bacteria or from parasites can generate distinct cytokine profiles, but also that the composition of lipid entities reaching the immune system can be important in leading to different immune response outcomes (Retra et al., 2008).

IL-10 is an anti-inflammatory cytokine induced particularly in chronic schistosomiasis. Van den Biggelaar at al. investigated, among Gabonese schoolchildren, the influence of chronic helminth infections including schistosomiasis on the prevalence of atopy, assessed by skin reaction to house-dust mite and other allergens. The authors found that schistosome-antigen-specific concentrations of IL-10 were significantly higher in children infected with schistosomiasis; and that higher specific concentrations of this anti-inflammatory cytokine were negatively associated with the outcome of skin-test reactivity to mites. The authors therefore concluded that the anti-inflammatory cytokine IL-10, induced in chronic schistosomiasis, appears central to suppressing atopy in African children (van den Biggelaar et al., 2000). More specifically, an additional study demonstrated that in mice but also in men, schistosomes have the capacity to drive the development of IL-10-producing regulatory CD1d^{hi} B cells, which are instrumental in reducing experimental allergic inflammation in mice (van der Vlugt et al., 2012).

Chronic helminth infections are found to be associated with down-regulated antigen-specific T cell responses to the parasite. Van den Biggelaar et al. in 2000 were interested to know whether schistosome-specific T cells are present but yet functionally unresponsive, or absent from the peripheral blood of chronically infected young adults living in the vicinity of Lambaréné. As

main finding, the authors reported that in contrast to PBMC alone, DC-supplemented cultures responded to adult worm antigen (AWA) of schistosomiasis by proliferation and by IL-4 and IL-5 production. However, the authors observed production of INF-γ in some patients only. These results therefore enabled the authors to conclude that schistosome-specific T helper cells are indeed present in the peripheral blood during active infection with schistosomes, but are functionally hypo-responsive (van Den Biggelaar et al., 2000).

Immune B cell populations in people with schistosomiasis living in the area of interest were investigated by Van der Vlugt et al. (van der Vlugt et al., 2014). The authors were interested in the influence of regulatory B cells (Bregs) on T-cell cytokines *in vitro* in human schistosomiasis. From blood collected in young adults living in Lambaréné and Bindo, the authors observed reduced levels of effector T-cell cytokines and an increased Treg induction as response to schistosome-induced Bregs. The authors thus concluded that *Schistosoma* spp. can induce functional Bregs in humans that may be instrumental in general T-cell hypo-responsiveness, and may contribute to the increased regulatory milieu found in schistosomiasis.

Antibody responses to *S. haematobium* infection was investigated among local population in two papers. Indeed, de Jong et al. were interested to know whether helminths exposure can lead to a variation in IgG Fc N-glycosylation known to affect antibody-mediated effector functions. In populations with different environmental exposures from different parts of the world, including children from Lambaréné and surrounding area, the authors found that having parasitic infections including schistosomiasis is a significant predictor of reduced IgG1 galactosylation levels (de Jong et al., 2016). Labuda et al. characterised B cell subsets and B cell responses to B cell receptor and TLR-9 stimulation in schoolchildren infected, and concluded that *S. haematobium* infection leads to significant changes in the B cells compartment, both at the phenotypic and functional level (Labuda et al., 2013). Indeed, in response to *S. haematobium* infection, the authors reported an increase in frequencies of memory B cell subsets and a decrease in frequencies of naive B cell. At the functional level of B cells, the authors reported a higher expression of the activation marker CD23 upon stimulation of isolated B cells from schistosome-infected children but a lower proliferation, and TNF-α production (Labuda et al., 2013).

Regarding the immunological effect of schistosomiasis on pregnancy, Ludwig et al. compared placental gene expression and inflammation markers in maternal and cord blood of women living in endemic and non-endemic helminth areas. As specific effect of schistosomiasis on pregnancy, the authors reported on the foetal side of placenta a lower expression of Hsd3b1, a gene playing a vital role in pregnancy. Also, strongly elevated IgE levels known to influence susceptibility or protection against helminth infections was associated with schistosomiasis, as well as an increased AWA specific IgE levels in maternal but not foetal plasma. The authors conclude that exposure *in utero* to different environments alter placental gene expression, with a probable effect in the development/modulation of the offspring immunity (Ludwig et al., 2019).

Two studies conducted by Ateba-Ngoa et al. examined the immunology of schistosomiasis-malaria co-infection (Ateba-Ngoa et al., 2016, 2015), assuming an effect of schistosomiasis on immune response to malaria in the local population. As main finding, the authors reported that *S. haematobium* infection was characterised by increased chemokine production and, at the same time, lower pro-inflammatory markers. In case of co-infection, the authors reported no effect of *S. haematobium* on the immune response of *P. falciparum* infected subjects, neither for the innate nor for the adaptive component of the immune response (Ateba-Ngoa et al., 2015). The same group reported a significant decrease of Pfs48/45 IgG titres in *S. haematobium*–malaria co-infected subjects compared to those infected with malaria only. However, they observed neither any difference for Pfs230 antibody titres, nor for antibodies against asexual (AMA1, MSP1, and GLURP) *P. falciparum* antigens. According to the authors, these findings suggest an effect of *S. haematobium* on antibody responses to some *P. falciparum* gametocyte antigens that may have consequences for transmission-blocking immunity (Ateba-Ngoa et al., 2016). These findings call for a closer examination of schistosomiasis-malaria co-endemicity in areas where both infections are the most-highly prevalent parasitic infections.

3.6. Schistosomiasis morbidity

Haematuria in UGS probably explains anaemia observed in infected children. In two recent publications, a significantly high association of haematuria with schistosomiasis was reported (Dejon-Agobé et al., 2020, 2019), indicating that UGS is the main cause of haematuria in the region. In a more extended investigation, Remppis et al. looked at the burden of schistosomiasis on urinary system, particularly on bladder wall, ureters and kidneys (Remppis et al., 2020). The authors reported some UGS-compatible pathology such as bladder wall irregularities or calcifications, ureter dilation or thickening, and renal dilatation (Remppis et al., 2020). The results of several other studies on the morbidity of schistosomiasis showed that schistosomiasis has the propensity to affect serum cholesterol levels and lipid profile in overweight/obese individuals (Zinsou et al., 2020), induce haemostatic changes by elevating the levels of von Willebrand Factor (VWF) antigen, activate VWF and osteoprotegerin, indicating inflammation-mediated endothelial activation (Mebius et al., 2019), and that it tends to be associated with a low level of erythrocytes and and is significantly associated with a high levels of leukocytes or thrombocytes in children (Dejon-Agobé et al., 2021). One study investigated the morbidity of schistosomiasis in the outcome of pregnancy and reported a low birth weight associated with the disease (Mombo-Ngoma et al., 2017), while another one assessing the effect of helminth infection including schistosomiasis on the immunogenicity vaccines given as part of the WHO Expanded Programme on Immunization (EPI) reported no evidence for a substantial effect on infants' immune responses to vaccines of helminths, including *S. haematobium* (Flügge et al., 2020).

3.7. Malacology

Two recent publications specifically addressed malacological aspects, particularly in Estuaire province (Nguema et al., 2018, 2010). Mintsa-Nguema et al. identified no potential intermediate host species in Plaine-Orety, an urban area of Libreville (Nguema et al., 2018) known to

be endemic for schistosomiasis. This could thus indicate a low abundance of snails in Libreville and consequently, a low transmission level. Testing the presence of hybrids between *S. guineensis* and *S. haematobium* in the province, the same authors reported the presence of *Bulinus globosus* and *B. forskalii* in two waterbodies in Melen, and *B. forskalii* in Ekouk. However, all the collected snails were negative for schistosome infection (Nguema et al., 2010). One pilot malacology study was conducted in 2013 in Moyen-Ogooué Province, particularly in Lambaréné and surrounding areas. As main finding, *B. forskalii* and *B. truncatus* were found in the study area as potential intermediate host of human-pathogenic schistosomes; however, only *B. truncatus* provided evidence of mammalian cercariae shedding, identified as *S. haematobium* cercariae (Dejon-Agobé et al., 2021). *S. haematobium* appears to be the prevalent *Schistosoma* spp. in the whole country. Analysing the chronobiology of the cercarial emergence pattern of *S. haematobium* from Libreville using *B. globosus* from Benin, Mintsa-Nguéma et al. found that *S. haematobium* cercarial emissions begin at 7 a.m., increase gradually from 10 a.m. to its peak at 1 p.m, and decreased gradually before stopping completely at 8 p.m. (Mintsa Nguema et al., 2014). Although some cases of *S. mansoni* are reported in the country, the presence of genus *Biomphalaria* snail known as intermediate host was not documented over the two last decades. Snail control is one of the cornerstones of schistosomiasis control. Therefore, for a tailored control programme, it will be relevant to have a complete depiction of the schistosomiasis transmission situation in the country, notably in the areas known to be endemic for the disease.

3.8. Schistosomiasis control program implementation in Gabon

The WHO recommends control of schistosomiasis in three axes; reduction of the morbidity through MDA campaigns in at-risk-populations using 40mg per body weight of PZQ, the WASH (Water, Sanitation and Hygiene) approach, and snail control (WHO, 2021). According to the Moyen-Ogooué regional branch of the Gabon Ministry of Health's 'Programme de Lutte contre les Maladies Parasitaires', only MDA campaigns are implemented among school children at national level as specific measure for schistosomiasis control, with the last campaign conducted in 2018. For the specific case of Moyen-Ogooué province, some campaigns to administer PZQ to the population are organized at a non-specific frequency targeting certain areas empirically known to be foci of the disease. Considering the WHO recommendation; with the prevalence level of schistosomiasis being different from one region to another, the MDA campaign should be implemented diversely in different parts of the country. The present review noted that schistosomiasis affects more age groups than only children, calling therefore for the extension of the MDA campaigns to particularly population exposed to the disease which remain to be identified.

3.9. Knowledge gaps and future research on schistosomiasis

Whilst the presence of *S. haematobium* and in certain regions *S. guineensis* is well documented, further investigation on the presence of *S. mansoni* is warranted in view of sporadically reported cases.

Schistosomiasis is known to be endemic across the country. However, during the two last decades, data on schistosomiasis were reported only from certain regions, highlighting the necessity to update data as a basis requirement for an optimally tailored control program. Whilst the disease does mainly affect school-age children, the adult population is largely exposed, too. Women in particular bear a high burden of the disease. In regions less-well investigated, the exposure profile of the population to the parasite should be thoroughly assessed, calling for data on disease transmission and population behaviours. Malacological data should thus contribute to identify schistosomiasis foci in these different regions, whilst coordinated, harmonised knowledge, attitudes and practices studies could help to identify highly-at-risk sub-populations and practices.

The real burden of disease at the individual level requires further investigation, particularly for FGS where no data are available so far. Interaction of schistosomiasis with concomitant infections, and the effect of schistosomiasis treatment demand further evaluation. Indeed, aiming to understand the difference of the current covid-19 pandemic across the world, some data show that schistosomiasis endemicity may be associated with negative COVID-19 outcomes. More interestingly, some data seem to suggest that a higher PZQ treatment coverage could reduce COVID-19 active cases and improve the recovery rate (Oyeyemi et al., 2020).

PZQ is the recommended treatment for schistosomiasis at 40 mg per body weight single-dose. Despite a higher repeated dose of PZQ found to be efficacious, we reported an increase risk of reinfection over time for some population in Moyen-Ogooué province (Dejon-Agobé et al., 2019), indicating that efforts should be particularly made on the control of the disease instead of its elimination.

4. CONCLUSION

Progress has been made over the past two decades in the understanding of schistosomiasis in the country, including disease-related morbidity and its interaction with other parasitic infections, and the immunology and epidemiology of the disease. However, for optimising control of the disease, there is a need to fine-tune these findings with detailed local epidemiological and malacological data. In a situation where the control measures of the disease can be improved, the transmission of the disease, its interaction with other disease including non-communicable diseases, its distribution in the population and the role of the population on the endemicity of the disease need to be further investigated. We therefore call for epidemiological, malacological and socio-epidemiological studies at national level to complete the picture of the disease in the country, which should contribute to develop a tailored control program, and recommend this approach to comparable schistosomiasis-endemic areas elsewhere.

ABBREVIATIONS:

AMA: Apical Membrane Antigen
AWA: Adult Worm Antigen
CAA: Circulating Anodic Antigen
CD: Cluster of Differentiation
CERMEL: Centre de Recherches Médicales de Lambaréné
COVID 19: Corona Virus Disease 2019
CLR: C-type Lectin Receptors
CR: Cure Rate
DC: Dendritic Cells
DNA: Deoxyribonucleic acid
EPI: Expanded Program on Immunization
ERR: Egg Reduction Rate
FGS: Female Genital Schistosomiasis
FOXP: Forkhead box Protein
GLURP: Glutamate-Rich Protein
HIV: Human Immunodeficiency Virus
Ig: Immunoglobulin
IL: InterLeukin
INF: Interferon
MALP: Macrophage Activating LipoPeptide
MDA: Mass Drug Administration
MSP: Merozoïtes Surfaces Protein
NLR: Domain Like Receptor
PBMC: Peripheral Blood Mononuclear Cells
PCR: Polymerase chain Reaction
PZQ: Praziquantel
POC: Point of Care
PS: PhosphatidylSerine
STH: Soil-Transmitted helminth
TLR: Toll-Like Receptor
TNF: Tumor Necrosis Factor
UCP-LF: Up-Converting Phosphorus-based Lateral Flow
UGS: Urogenital Schistosomiasis
URM : Unité de Recherches Médicales
VWF: von Willebrand Factor
WASH: Water, Sanitation and Hygiene
WHO: World Health Organization

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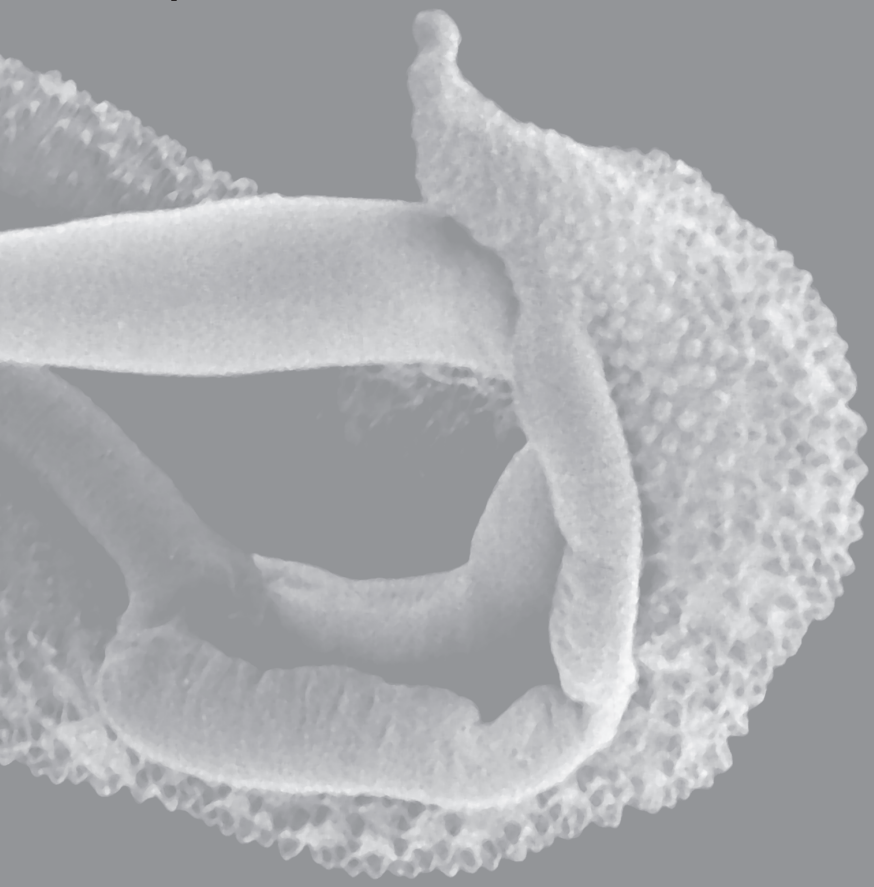
CHAPTER 9

Summary and General discussion



The main objective of the work presented was to provide data on the epidemiology of schistosomiasis in Moyen Ogooué, one of the nine provinces of Gabon; particularly in Lambaréné and surrounding areas. After assessing the role of the population in relation to schistosomiasis by means of a Knowledge, Attitudes and Practice (KAP) study, we reassessed the efficacy of praziquantel (PZQ), the sole licensed drug for the treatment, and used in mass drug administration (MDA) campaigns against schistosomiasis. We were also interested in other aspects of schistosomiasis morbidity with diagnostic and possibly clinical relevance, such as its impact on the full blood count (FBC). Given the importance of the intermediate host of schistosomiasis in transmission of the disease, we took advantage of the present work to report the surveys we conducted on the vectors transmitting schistosomiasis in the study area. As schistosomiasis is frequently reported to potentially affect the outcome of *Plasmodium* spp. infections, we assessed the effects of *S. haematobium* infection on *P. falciparum* infection and disease in our community. As the last step of our work, we summarised the major scientific activities recently conducted on schistosomiasis in Gabon.

The prevalence of a disease and its distribution in the population provides an estimate of its burden. For schistosomiasis, the WHO recommends to use the prevalence among children in order to characterise the disease epidemiology at community level, and to inform appropriately tailored control measures adapted to the specific situation. In **Chapter 2**, we reported a 26% prevalence of schistosomiasis among school children from the urban center of Lambaréné, with a ubiquitous geographical distribution of the disease. For the whole study area including Lambaréné and adjacent villages, and based on our previous publications on the prevalence of schistosomiasis in the surroundings of Lambaréné, we drew the conclusion that the overall risk of schistosomiasis in our community is moderate, but with some parts of the community at high risk of contracting the disease. Indeed, although the disease is present in the whole study area, its prevalence is particularly high in areas where access to piped water is limited or not available, with generally inadequate sanitation. In addition to MDA as recommended by WHO, we suggest specific interventions for the area where schistosomiasis is particularly highly prevalent, such as education on disease prevention and snail control efforts which are essential in any attempt to reduce transmission. If the distribution of the disease needs to be considered for control, human behaviour must also be considered. Indeed, disease-related behaviour can not only explain the epidemiology of the disease in a particular community, but can also form a barrier to the implementation of effective control measures. One of the three main recommendations for the control of schistosomiasis is to provide at-risk populations with safe water, adequate sanitation, and hygiene education (the WASH concept) in order to reduce the disease burden. KAP studies are a relevant tool to explore and to understand the situation of the disease in a community, which in turn is a pre-requisite for targeting group intervention strategies and thus for successful implementation of this recommendation. In **Chapter 3**, we reported the results of the first KAP study conducted on schistosomiasis in the study area. In that study, we first assessed the population's exposure to the disease and found that a large proportion of the population was at risk, mainly due to their living conditions. Obviously, this was particularly the case where piped water is not avail-

able. Specific to the KAP study, at the population level, we reported a lack of knowledge on disease transmission, which is a relevant factor in controlling the disease in a community. Risk-enhancing practices with regards to schistosomiasis remain high in the population, and could explain in part the current state of schistosomiasis in the community. We found that 64% of study participants sometimes, or regularly urinated into a freshwater body. The results of the KAP study we conducted underpin the necessity of thoroughly implementing the WHO WASH recommendations in Lambaréné and surrounding areas. If providing safe water and adequate sanitation remains difficult to achieve for a part of this population, we understand that more effort should be put into hygiene education. Avoiding urination or defecation into a freshwater body, for instance, could considerably reduce the risk of schistosomiasis transmission, and thus reduce the burden of disease at population level. Improving the state of schistosomiasis through population education seems to be feasible in our community, as the population already exhibits some predisposition to this. As we noticed, populations using a freshwater body as the main water source for their household activities already implement some practices to protect themselves from the disease. Population behaviour cannot explain on its own the epidemiology of schistosomiasis in our community. The efficiency of the intermediate hosts of schistosomiasis also plays a significant role in the endemicity of the disease. The pilot malacological survey we conducted aimed to collect basic information on the role of the mollusc intermediate host(s) in the area, and to identify the intermediate host(s) of schistosomiasis. The results of the surveys are reported in **Chapter 4** of this thesis. As the Moyen-Ogooué province is known to be endemic mainly for *S. haematobium*, we were interested in the potential intermediate host for urogenital schistosomiasis reported for central Africa, namely *Bulinus* spp. Our findings confirm that *Bulinus* spp. are present in the whole study area. Amongst two species found, *B. forskalii* and *B. truncatus*, only *B. truncatus* snails shed mammalian cercariae and appear to be a competent and very efficient intermediate host for schistosomiasis in our region. Indeed, some B. truncatus shed a considerable number of cercariae within 24 hours. This finding indicates that the ubiquity of intermediate hosts of schistosomiasis could play a crucial role in schistosomiasis endemicity in the area, and thus their control could considerably impact upon the state the disease in the community. Indeed, snail control is one of the three axes recommended by the WHO for schistosomiasis control but often remains difficult to implement, mainly because of the impact that control may have on the environment. In addition to WASH, MDA using PZQ at regular intervals in areas with moderate or high prevalence of schistosomiasis forms the third WHO recommendation for disease control, as re-infection is common among the exposed population. As it is the case with adherence, PZQ efficacy is key to the implementation of this recommendation. In **Chapter 5**, we addressed the issue of PZQ efficacy and the rate of schistosomiasis re-infection in our community, taking advantage of the TransMal (for Transmission of Malaria) project (clinicaltrials.gov Identifier NCT 02769103) which aimed to assess, amongst other objectives, the effect of schistosomiasis treatment using PZQ on malaria transmission. In that project, participants positive for the presence of Schistosoma eggs in urine by microscopy were treated with 60 mg of PZQ per kg body weight once a month for three consecutive months. The results of our analysis demonstrate a satisfactory efficacy of PZQ (ERR>90%) already following sin-

gle-dose application. Although we used 60 mg instead of 40 mg per kg body weight as recommended by the WHO, this finding seems to confirm that the implementation of MDA campaigns in our population could effectively reduce the morbidity associated with schistosomiasis in our community, as expected. However, the benefit of this treatment on genital schistosomiasis (GS) and particularly on female GS (FGS) remains to be evaluated. In terms of re-infection rates, about 14% of people treated remained positive for the presence of *Schistosoma* eggs in urine four weeks after treatment. We have hypothesised that, instead of indicating drug resistance, this result rather reflects the frequent exposure to the causative agent of the disease in that particular group. Among those found negative after treatment, about one in four were found to be re-infected after approximately nine months of follow-up. In this regard, we assume that the high rate of re-infection we observed indicates that a part of our population is frequently exposed to the disease, and probably would bear more of the burden of the disease in our community. We therefore call for more attention to the morbidity of schistosomiasis in this particular sub-population. Haematuria is the main symptom of urogenital schistosomiasis and suggests that anaemia could be one of the main morbidities related to schistosomiasis. In **Chapter 6**, we reported the results of an analysis aiming to assess the effect of schistosomiasis on haematological parameters. Our findings show that children with schistosomiasis exhibit a different full blood count (FBC) profile as compared to those free of infection. Indeed, schistosomiasis was independently associated with a trend of decreased mean haemoglobin and haematocrit levels, and higher platelet and leukocyte counts. In areas of parasitic co-endemicity, confounding factors could possibly affect the interpretation of FBC results. As this area is in particular co-endemic for schistosomiasis and *Plasmodium* spp. infections, we were also interested in the effect of schistosomiasis on the risk of infection with *Plasmodium* parasites, or of developing a malaria episode. The results of this study are reported in **Chapter 7**. In a longitudinal study, we found on the one hand that schistosomiasis alone does not affect the risk of a *Plasmodium* spp. infection but, in combination with hookworm and *Trichuris trichiura* infections, increases the risk of infection with *Plasmodium* spp. parasites in children. On the other hand, we found an increased propensity to develop a malaria episode, particularly among young children. This finding highlights potential interactions between control programs for different parasitic diseases in co-endemic areas and calls for further investigations. Indeed, the fight against malaria could be impaired by an inefficient control of helminth infections, particularly soil-transmitted helminths and schistosomiasis. In **Chapter 8**, we provide a narrative review on the data reported on schistosomiasis in the country, which revealed that numerous research activities were conducted on the topic during the past two decades, but particularly on the immunology of the disease, and mainly in Lambaréné and its surrounding areas. In addition, the review shows that activities around schistosomiasis control focus mainly on large-scale treatment with PZQ among school children across the country. During those past two decades, only three MDA campaigns with PZQ have been conducted among school children in the country. However, the effectiveness of these interventions still needs to be assessed, probably leading to an array of recommendations to be made for greater efficiency of this control program.

Schistosomiasis is prevalent mostly in tropical and sub-tropical areas and particularly where rivers, streams, dams or swamps are present. In Lambaréné and the surrounding areas, where a part of the population does not have access to piped water, the presence of the Ogooué River, the main river of the country, creates favorable conditions for the development of schistosomiasis. The area is known to be endemic for urogenital schistosomiasis, which is the most prevalent parasitic infection in the local community, but with the local epidemiology of the disease not having been well established previously. Determining the distribution of a disease in a community, assessing factors associated with it and the actions implemented to control it, are key elements to understanding the epidemiology of the disease in the community, and thus to adequately addressing issues that underlie the disease. Although the presence of schistosomiasis in our community was evident, and some interventions were implemented for its control, such as large-scale administration of PZQ, various aspect of its epidemiology remained to be established. With the work presented in this thesis attempting to close that gap for Moyen-Ogooué province in particular, we assume that our findings can be extrapolated to a certain extent to the populations of many other similar endemic areas in the country, or to other endemic countries.

Our findings presented in this thesis confirm that the study area is endemic mainly for *S. haematobium*, with a moderate overall prevalence. However, the living conditions of some sub-populations and the high presence of the intermediate hosts in some parts of the study area could explain in part the higher prevalence observed in some parts of the study area, considered to be the main foci of the disease. Moreover, we have described, for the first time, the intermediate host of schistosomiasis in the area, namely *B. truncatus,* which we found to be very efficient in the transmission of the disease and which could play a role in the unequal distribution of the disease across the overall study area. With regard to preventive measures, we noticed that the population is aware of the presence of the disease, and that those persons with frequent freshwater contact implement individual actions to protect themselves; while at the country level, some national MDA campaigns have been conducted for the control of schistosomiasis-related morbidity. Whereas these measures are appropriate, they seemed to be insufficient to control the disease in the area. Indeed, we highlighted for instance an increase in schistosomiasis prevalence in pregnant women over the past decade in Moyen-Ogooué province.

At the end of this thesis, we understand that our work contributes considerably to completing the picture of schistosomiasis epidemiology in Moyen-Ogooué province, and that it provides an impression of the state of schistosomiasis in the country over the past two decades. However, we think that additional work still needs to be accomplished in order to gain a full picture of the epidemiology of schistosomiasis particularly at country level. Indeed, although a wealth of research work was conducted on schistosomiasis in the country, this was done mainly at the immunological level to understand the mechanism of the disease. The transmission of schistosomiasis is not yet definitively established in our area of interest, and insufficiently at the country level. We morphologically identified snails and cercariae during this project

and we intend, in a future project, to identify the intermediate host of schistosomiasis in the region at the molecular level, as well as to formally identify the *Schistosoma* spp. involved in intestinal schistosomiasis in our community, if any. We recommend similar investigations on schistosomiasis transmission in other parts of the country, particularly where the presence of schistosomiasis is already documented. In addition, the dynamics of snail density over time also needs to be established, which could help to better plan any governmental intervention for the control of schistosomiasis, such as MDA campaigns which could be more efficient if conducted after periods of snail abundance, which are assumed to be associated with increased disease transmission. Interventions after a period of intense transmission could be relatively more beneficial, as they would prevent more morbidity in the community as many persons will be treated just after becoming infected. The other main future work to be carried out will be on genital schistosomiasis, and in particular on female genital schistosomiasis (FGS), which is increasingly of concern. The state of genital schistosomiasis in Gabon remains largely unknown. No data are available yet on either the prevalence of FGS in the country, nor on its burden. This can only be estimated based on the presence of urinary schistosomiasis in women. In addition to the morbidity due to genital schistosomiasis, we intend to investigate further the burden of schistosomiasis in the population in general, as some studies already provided relevant data. Moreover, it will be of interest to repeat epidemiological studies every two to four years at least at the level of Moyen-Ogooué province, particularly for the determination of disease prevalence in the population, and thus to keep measuring the impact of different implemented interventions. Indeed, large-scale treatment with PZQ has been implemented in the country with the aim of reducing the burden of schistosomiasis in exposed populations in particular, although the frequency of those campaigns is lower than that recommended by the WHO for a moderate-risk community: only three national MDA campaigns were conducted during the past two decades. However, this burden is not yet well enough documented in our community, and therefore the benefit of those control programs is difficult to evaluate. To insure the efficiency of MDA campaigns conducted in the area, the efficacy of PZQ should be constantly re-assessed and if found to be insufficient, other treatment protocols should be evaluated. It was suggested that treatment of schistosomiasis could prevent reinfection. As the area is co-endemic for schistosomiasis and other parasitic infections, it will also be relevant to investigate, at epidemiological level, the interaction between schistosomiasis and other parasitic diseases such as *Plasmodium* infection, as well as viral or non-communicable diseases, in addition to the interaction between control programs for different parasitic infections, particularly schistosomiasis and *Plasmodium* spp. infections. Looking at the further investigations to be conducted on schistosomiasis, it is evident that there is a need to further expand our knowledge of the epidemiology of schistosomiasis in our area and in any endemic area for better control of the disease. Indeed, if understanding the epidemiology of schistosomiasis and its interaction with other diseases is relevant for the management of the disease in the population and its impact on the burden of other diseases, then control of the disease with the objective of its elimination, and ultimately its eradication, should be a leitmotiv for health policy-makers.

CHAPTER 10

Nederlandse samenvatting

Het hoofddoel van het gepresenteerde werk was om gegevens te verstrekken over de epidemiologie van schistosomiasis in Moyen Ogooué, een van de negen provincies van Gabon; met name in Lambaréné en omstreken. Na beoordeling van de rol van de bevolking in relatie tot schistosomiasis door middel van kennis, attitudes en praktijk (KAP)-onderzoek, hebben wij de effectiviteit van praziquantel (PZQ) opnieuw geëvalueerd en gebruikt in campagnes voor massale toediening van geneesmiddelen (MTG). PZQ is momenteel het enige toegelaten geneesmiddel voor de behandeling tegen schistosomiasis. Daarnaast waren wij geïnteresseerd in andere aspecten van de morbiditeit van schistosomiasis met diagnostische en mogelijk klinische relevantie, zoals de impact op het volledige bloedbeeld. Gezien het belang van de tussengastheer van schistosomiasis bij de overdracht van de ziekte, hebben wij verslag uitgebracht over de onderzoeken die zijn uitgevoerd naar de vectoren die schistosomiasis in het studiegebied overbrengen. Aangezien vaak wordt vermeld dat schistosomiasis de uitkomst van Plasmodium spp. infecties kan beïnvloeden, hebben we de effecten van S. haematobium infectie op P. falciparum infectie en ziekte in onze gemeenschap geëvalueerd. Als laatste stap in ons werk hebben wij de belangrijkste wetenschappelijke activiteiten samengevat die recentelijk zijn uitgevoerd bij schistosomiasis in Gabon.

De prevalentie van een ziekte en de verspreiding ervan over de bevolking geven een schatting van de ziektelast. De WHO beveelt voor schistosomiasis aan om de prevalentie onder kinderen te gebruiken om de epidemiologie van de ziekte op gemeenschapsniveau te karakteriseren en op de specifieke situatie toegesneden controlemaatregelen te melden. In **hoofdstuk 2** rapporteerden we een prevalentie van 26% van schistosomiasis onder schoolkinderen uit het stedelijk centrum van Lambaréné, met een alomtegenwoordige geografische spreiding van de ziekte. Voor het hele studiegebied, inclusief Lambaréné en de aangrenzende dorpen, en op basis van onze eerdere publicaties over de prevalentie van schistosomiasis in de omgeving van Lambaréné, kwamen we tot de conclusie dat het algehele risico op schistosomiasis in onze gemeenschap matig is. Hierbij lopen sommige delen van de gemeenschap een hoog risico op de ziekte. Hoewel de ziekte in het hele studiegebied voorkomt is de prevalentie vooral hoog in gebieden waar de toegang tot leidingwater beperkt of niet beschikbaar is, met over het algemeen ontoereikende sanitaire voorzieningen. Naast MTG, zoals aanbevolen door de WHO, stellen wij specifieke interventies voor in het gebied waar schistosomiasis bijzonder veel voorkomt. Denk hierbij aan voorlichting over ziektepreventie en slakkenbestrijding, die essentieel zijn bij elke poging om de overdracht te verminderen. Als de verspreiding van de ziekte in afweging moet worden genomen bij de bestrijding, moet ook rekening worden gehouden met het menselijk gedrag. Ziektegerelateerd gedrag kan namelijk niet alleen de epidemiologie van de ziekte in een bepaalde gemeenschap verklaren, het kan ook een barrière vormen voor de implementatie van effectieve controlemaatregelen. Een van de belangrijkste aanbevelingen voor de bestrijding van schistosomiasis is om risicogroepen te voorzien van veilig water, adequate sanitaire voorzieningen en voorlichting over hygiëne (het WASH-concept) om zo de ziektelast te verminderen. KAP-onderzoeken zijn een relevant hulpmiddel om de situatie van de ziekte in een gemeenschap te onderzoeken en te begrijpen. Dit is op zijn beurt een voorwaarde voor het richten van groepsinterventiestrategieën en dus voor een

succesvolle implementatie van deze aanbeveling. In **hoofdstuk 3** rapporteerden we resultaten van de eerste KAP-studie die is uitgevoerd naar schistosomiasis in het studiegebied. In deze studie hebben we eerst de blootstelling van de bevolking aan de ziekte beoordeeld en vastgesteld dat een groot deel van de bevolking risico liep, voornamelijk als gevolg van hun leefomstandigheden. Dit was met name het geval in gebieden waar geen leidingwater beschikbaar is. Specifiek voor de KAP-studie rapporteerden wij op populatieniveau een gebrek aan kennis over de ziekteoverdracht. Dit is een relevante factor bij de bestrijding van de ziekte in een gemeenschap. Risicoverhogende praktijken met betrekking tot schistosomiasis blijven hoog in de bevolking en zouden gedeeltelijk de huidige toestand van schistosomiasis in de gemeenschap kunnen verklaren. We stelden vast dat 64% van de deelnemers aan de studie soms of regelmatig in een zoetwatermeertje urineerden. De resultaten van de door ons uitgevoerde KAP-studie benadrukken de noodzaak om de WASH-aanbevelingen van de WHO in Lambaréné en omstreken te implementeren. Als het voor een deel van deze bevolking moeilijk blijft om veilig water en adequate sanitaire voorzieningen te bieden, begrijpen we dat er meer aandacht moet worden besteed aan de voorlichting over hygiëne. Zo zou het vermijden van urineren of defeceren in een zoetwatermeertje het risico op overdracht van schistosomiasis aanzienlijk kunnen verminderen en daarmee de ziektelast op bevolkingsniveau kunnen verlagen. Verbetering van de toestand van schistosomiasis door voorlichting van de bevolking lijkt haalbaar in onze gemeenschap, aangezien de bevolking hier al enige aanleg voor vertoont. We hebben gemerkt dat bevolkingsgroepen die een zoetwatermeertje als voornaamste waterbron voor hun huishoudelijke activiteiten gebruiken al bepaalde praktijken toepassen om zich tegen de ziekte te beschermen. Het gedrag van de bevolking kan op zichzelf de epidemiologie van schistosomiasis in onze gemeenschap niet verklaren. De efficiëntie van de tussengastheren van schistosomiasis speelt ook een belangrijke rol in het voorkomen van de ziekte. Het malacologische proefonderzoek dat wij hebben uitgevoerd was bedoeld om basisinformatie te verzamelen over de rol van de tussengastheer, of -heren, van weekdieren in het gebied. Daarnaast wilden wij de tussengastheer(en) van schistosomiasis identificeren. De resultaten van de onderzoeken worden gerapporteerd in **hoofdstuk 4** van dit proefschrift. Aangezien bekend is dat de provincie Moyen-Ogooué voornamelijk endemisch is voor S. haematobium, waren wij geïnteresseerd in de potentiële tussengastheer voor urogenitale schistosomiasis gerapporteerd voor Centraal-Afrika, namelijk Bulinus spp. Onze bevindingen bevestigen dat Bulinus spp. in het gehele studiegebied aanwezig is. Van de twee gevonden soorten, B. forskalii en B. truncatus, werpen alleen B. truncatus-slakken cercariae van zoogdieren uit en lijken een competente en zeer efficiënte tussengastheer te zijn voor schistosomiasis in onze regio. Sommige B. truncatus-slakken werpen zelfs binnen 24 uur een aanzienlijk aantal cercariae uit. Deze bevinding geeft aan dat de alomtegenwoordigheid van tussengastheren van schistosomiasis een cruciale rol zouden kunnen spelen in het voorkomen van schistosomiasis in het gebied. Hierdoor zou controle op tussengastheren dus een aanzienlijke impact kunnen hebben op de toestand van de ziekte in de gemeenschap. Slakkenbestrijding is een van de drie door de WHO aanbevolen aspecten voor de bestrijding van schistosomiasis, maar dit blijft vaak moeilijk uitvoerbaar. Vooral vanwege de impact die bestrijding op het milieu kan hebben. MTG met PZQ met regelmatige intervallen als toevoeging

op WASH, vormt de derde aanbeveling van de WHO. Deze aanbeveling geldt in gebieden met matige of hoge prevalentie van schistosomiasis, aangezien herinfectie veel voorkomt onder de blootgestelde bevolking. Zoals het geval is met therapietrouwheid, is de effectiviteit van PZQ de sleutel tot de implementatie van deze aanbeveling. In **hoofdstuk 5** hebben we de effectiviteit van PZQ en het aantal herinfecties van schistosomiasis in onze gemeenschap onderzocht. Hierbij is gebruik gemaakt van het TransMal (for Transmission of Malaria) project (clinicaltrials.gov Identifier NCT 02769103) dat onder andere gericht was op de beoordeling van het effect van schistosomiasis behandeling met PZQ op de malariatransmissie. In dit project werden deelnemers die bij microscopie positief waren voor de aanwezigheid van Schistosoma-eieren in de urine, gedurende drie opeenvolgende maanden eenmaal per maand behandeld met 60 mg PZQ per kg lichaamsgewicht. De resultaten van onze analyse tonen voldoende effectiviteit van PZQ aan (ERR>90%) na eenmalige toediening. Hoewel 60 mg gebruikt is in plaats van 40 mg per kg lichaamsgewicht, zoals aanbevolen door de WHO, lijkt deze bevinding te bevestigen dat de implementatie van MTG-campagnes in onze populatie de morbiditeit geassocieerd met schistosomiasis in onze gemeenschap effectief kan verminderen. Het voordeel van deze behandeling op genitale schistosomiasis (GS) en in het bijzonder op vrouwelijke GS (VGS) moet echter nog worden geëvalueerd. Wat het percentage herinfecties betreft bleef ongeveer 14% van de behandelde personen vier weken na de behandeling positief voor de aanwezigheid van schistosoma-eieren in de urine. We hebben verondersteld dat dit resultaat niet wijst op resistentie tegen het geneesmiddel, maar op de frequente blootstelling aan de verwekker van de ziekte in die specifieke groep. Van degenen die na behandeling negatief werden bevonden, bleek ongeveer één op de vier na negen maanden follow-up opnieuw besmet te zijn. Hierdoor veronderstellen we dat het hoge percentage vastgestelde herinfecties erop wijst dat een deel van onze bevolking frequent aan de ziekte wordt blootgesteld en waarschijnlijk een groter aandeel van de ziektelast in onze gemeenschap draagt. Om deze reden vragen wij om meer aandacht voor de morbiditeit van schistosomiasis bij deze specifieke subpopulatie. Hematurie is het belangrijkste symptoom van urogenitale schistosomiasis en suggereert dat anemie een van de belangrijkste morbiditeiten kan zijn die verband houden met schistosomiasis. In **hoofdstuk 6** hebben wij de resultaten beschreven van een analyse die gericht was op het beoordelen van het effect van schistosomiasis op hematologische parameters. Onze bevindingen laten zien dat kinderen met schistosomiasis een ander bloedbeeld profiel vertonen dan kinderen zonder infectie. Schistosomiasis was onafhankelijk geassocieerd met een trend van verlaagde gemiddelde hemoglobine- en hematocrietwaarden, en hogere aantallen bloedplaatjes en leukocyten. In gebieden met een co-endemie van parasieten kunnen verstorende factoren de interpretatie van het bloedbeeld beïnvloeden. Aangezien dit gebied in het bijzonder co-endemisch is voor schistosomiasis en Plasmodium spp. infecties, waren we ook geïnteresseerd in het effect van schistosomiasis op het infectierisico met Plasmodium parasieten of de ontwikkeling van een malaria-episode. De resultaten van deze studie worden gerapporteerd in **hoofdstuk 7**. In een longitudinale studie vonden we enerzijds dat enkel schistosomiasis geen invloed heeft op het risico van een Plasmodium spp. infectie. In combinatie met haakworm- en Trichuris trichiura-infecties was het risico op infectie met Plasmodium spp. parasieten bij kinderen echter verhoogd. Anderzijds vonden we een

verhoogde neiging tot het ontwikkelen van een malaria-episode, vooral bij jonge kinderen. Deze bevinding wijst op mogelijke interacties tussen bestrijdingsprogramma's voor verschillende parasitaire ziektes in co-endemische gebieden en vereist verder onderzoek. De strijd tegen malaria zou namelijk belemmerd kunnen worden door een inefficiënte bestrijding van worminfecties, met name bodem overdraagbare wormen en schistosomiasis. In **hoofdstuk 8** geven we een beschrijvend overzicht van de gerapporteerde gegevens over schistosomiasis in het land. Hieruit blijkt dat er de afgelopen twee decennia tal van onderzoeksactiviteiten over het onderwerp zijn uitgevoerd, met name over de immunologie van de ziekte, en vooral in Lambaréné en de omliggende gebieden. Bovendien blijkt uit het overzicht dat de activiteiten rond de bestrijding van schistosomiasis vooral gericht zijn op grootschalige behandeling met PZQ onder schoolkinderen in het hele land. In de afgelopen twee decennia zijn in het land slechts drie MTG-campagnes met PZQ onder schoolkinderen uitgevoerd. De doeltreffendheid van deze interventies moet echter nog worden geëvalueerd. Dit zal waarschijnlijk leiden tot een reeks aanbevelingen voor een grotere doeltreffendheid van dit controleprogramma.

Schistosomiasis komt vooral voor in tropische en subtropische gebieden, met name op plekken waar rivieren, beken, dammen of moerassen aanwezig zijn. In Lambaréné en omliggende gebieden, waar een deel van de bevolking geen toegang heeft tot leidingwater, geeft de aanwezigheid van de Ogooué rivier, de belangrijkste rivier van het land, gunstige omstandigheden voor de ontwikkeling van schistosomiasis. Het is bekend dat het gebied endemisch is voor urogenitale schistosomiasis, de meest voorkomende parasitaire infectie in de lokale gemeenschap. De plaatselijke epidemiologie van de ziekte is echter nog niet eerder goed vastgesteld. Het vaststellen van de verspreiding van een ziekte in een gemeenschap, het beoordelen van de factoren die met de ziekte in verband worden gebracht en de acties die worden ondernomen om de ziekte onder controle te krijgen, zijn essentiële elementen om de epidemiologie van de ziekte in de gemeenschap te begrijpen en de problemen die hieraan ten grondslag liggen adequaat aan te pakken. Hoewel de aanwezigheid van schistosomiasis in onze gemeenschap duidelijk was en er enkele interventies werden uitgevoerd om de ziekte onder controle te krijgen, bijvoorbeeld grootschalige toediening van PZQ, moesten verschillende aspecten van de epidemiologie nog worden vastgesteld. Met het werk dat in dit proefschrift wordt gepresenteerd om de kloof voor de provincie Moyen-Ogooué te dichten, gaan we ervan uit dat onze bevindingen tot op zekere hoogte kunnen worden geëxtrapoleerd naar de populaties van vergelijkbare endemische gebieden in het land of in andere endemische landen.

De bevindingen gepresenteerd in dit proefschrift bevestigen dat het studiegebied voornamelijk endemisch is voor S. haematobium, met een matige algemene prevalentie. De leefomstandigheden van sommige subpopulaties en de hoge aanwezigheid van de tussengastheren in sommige delen van het studiegebied kunnen echter gedeeltelijk de hogere prevalentie verklaren die is waargenomen. Deze worden beschouwd als de belangrijkste brandhaarden van de ziekte. Bovendien hebben wij voor het eerst de tussengastheer van schistosomiasis in het gebied beschreven, namelijk B. truncatus. Volgens ons is B. truncatus zeer efficiënt bij de overdracht van de ziekte en kan deze tussengastheer een rol spelen bij de ongelijke

verspreiding van de ziekte over het gehele studiegebied. Wat de preventieve maatregelen betreft merken we dat de bevolking zich bewust is van de aanwezigheid van de ziekte en dat personen die frequent in contact komen met zoet water individuele maatregelen nemen om zich te beschermen. Op nationaal niveau zijn enkele nationale MTG-campagnes gevoerd ter bestrijding van aan schistosomiasis gerelateerde morbiditeit. Hoewel deze maatregelen adequaat zijn, leken ze onvoldoende om de ziekte in het gebied onder controle te krijgen. Zo is bijvoorbeeld gebleken dat de prevalentie van schistosomiasis bij zwangere vrouwen in de provincie Moyen-Ogooué het afgelopen decennium is toegenomen.

Aan het eind van dit proefschrift begrijpen we dat ons werk aanzienlijk bijdraagt aan het completeren van het beeld van de epidemiologie van schistosomiasis in de provincie Moyen-Ogooué en dat het een indruk geeft van de stand van zaken van schistosomiasis in het land in de afgelopen twee decennia. Wij zijn echter van mening dat er aanvullend onderzoek moet worden verricht om een volledig beeld te krijgen van de epidemiologie van schistosomiasis, met name op nationaal niveau. Hoewel er veel onderzoek is gedaan naar schistosomiasis in het land, is dit voornamelijk gedaan op immunologisch niveau om het mechanisme van de ziekte te begrijpen. De overdracht van schistosomiasis is in ons interessegebied nog niet definitief vastgesteld en is nog onvoldoende op landelijk niveau. Tijdens dit project zijn er slakken en cercariae morfologisch geïdentificeerd. We zijn van plan om in een toekomstig project de tussengastheer van schistosomiasis in de regio op moleculair niveau te identificeren. Daarnaast willen we de Schistosoma spp., die betrokken is bij intestinale schistosomiasis in onze gemeenschap, indien aanwezig, formeel identificeren. We raden soortgelijke onderzoeken aan naar de overdracht van schistosomiasis in andere delen van het land, vooral waar de aanwezigheid van schistosomiasis al is gedocumenteerd. Bovendien moet de dynamiek van de slakkendichtheid in de loop van de tijd worden vastgesteld. Dit kan helpen om eventuele overheidsinterventies voor de bestrijding van schistosomiasis beter te plannen, zoals MTG-campagnes. Deze zouden efficiënter kunnen zijn als ze worden uitgevoerd na periodes van overvloed aan slakken, geassocieerd met een verhoogde ziekteoverdracht. Interventies na een periode van intense overdracht zouden relatief gunstiger kunnen zijn, omdat hierdoor meer ziektegevallen in de gemeenschap kunnen worden voorkomen. Dit aangezien veel personen behandeld zullen worden net na besmetting. De andere belangrijke toekomstige werkzaamheden zullen betrekking hebben op GS (in het bijzonder op VGS) die in toenemende mate zorgwekkend is. De stand van zaken met betrekking tot GS in Gabon blijft grotendeels onbekend. Tot op heden zijn er geen gegevens beschikbaar over de prevalentie van VGS in het land, noch over de ziektelast.

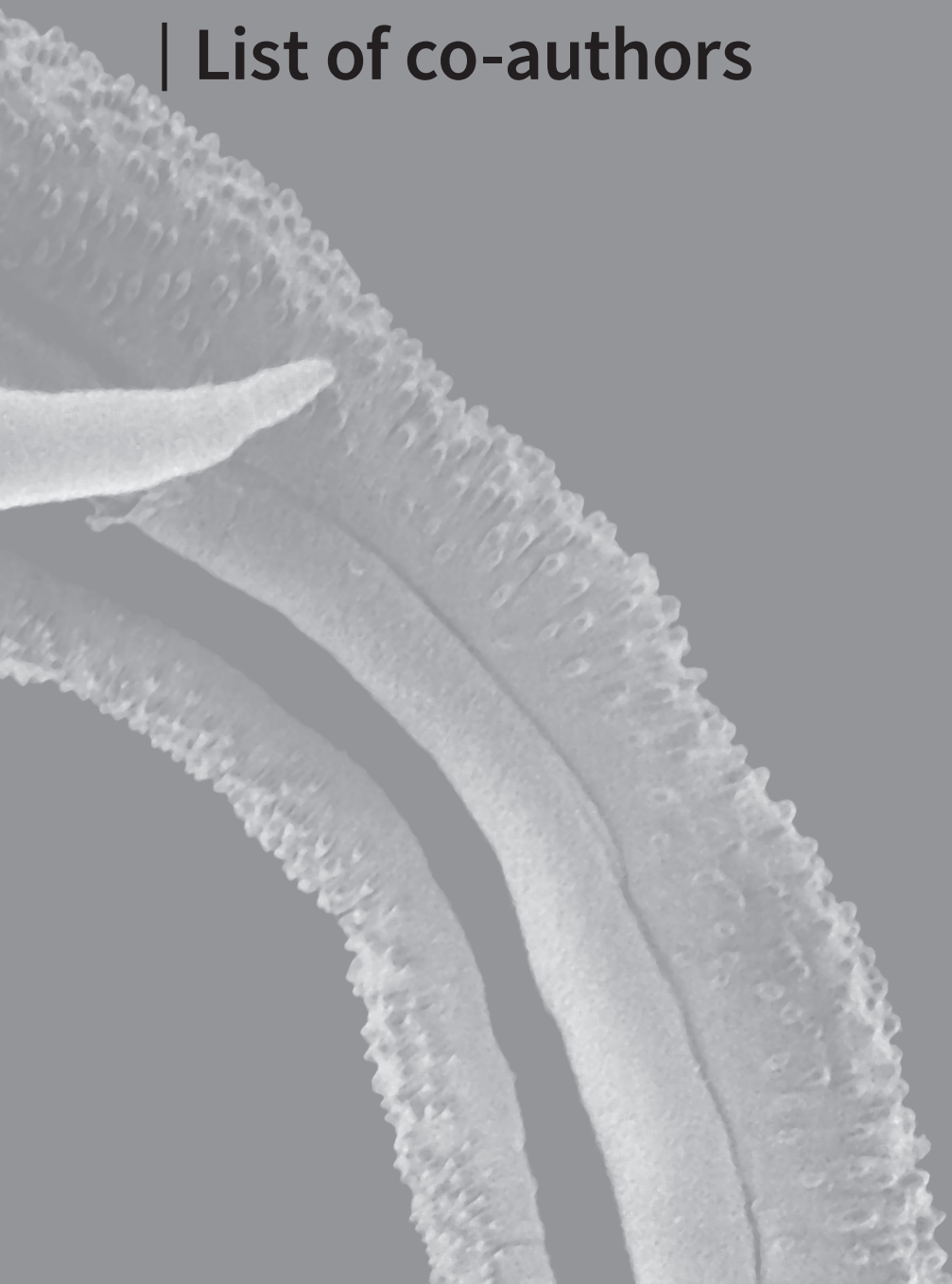
Dit kan alleen worden geschat op basis van de aanwezigheid van urinaire schistosomiasis bij vrouwen. Naast de morbiditeit ten gevolge van GS zijn we van plan de last van schistosomiasis in de bevolking in het algemeen verder te onderzoeken, aangezien sommige studies al relevante gegevens hebben opgeleverd. Bovendien zal het van belang zijn om epidemiologische studies om de twee tot vier jaar te herhalen op het niveau van de provincie Moyen-Ogooué. Dit is met name van belang om de ziekteprevalentie onder de bevolking te bepalen en zo het effect

van verschillende geïmplementeerde interventies te blijven meten. In het land is wel degelijk grootschalige behandeling met PZQ ingevoerd om de last van schistosomiasis, met name bij blootgestelde bevolkingsgroepen, te verminderen. De frequentie van deze campagnes is echter lager dan door de WHO voor een gemeenschap met matig risico wordt aanbevolen. In de afgelopen twee decennia werden slechts drie nationale MTG-campagnes uitgevoerd. Deze belasting is in onze gemeenschap echter nog niet goed genoeg gedocumenteerd waardoor het nut van de bestrijdingsprogramma's moeilijk te beoordelen is. Om de efficiëntie van de MTG-campagnes die in het gebied worden uitgevoerd te verzekeren, moet de doeltreffendheid van PZQ voortdurend opnieuw geëvalueerd worden. Als deze onvoldoende blijkt, dan moeten andere behandelingsprotocollen worden geëvalueerd. Er werd gesuggereerd dat behandeling van schistosomiasis herinfectie zou kunnen voorkomen. Het gebied is co-endemisch voor schistosomiasis en andere parasitaire infecties zoals Plasmodiuminfectie, alsmede virale of niet-overdraagbare ziekten. Hierdoor zal het ook relevant zijn om op epidemiologisch niveau de interactie te onderzoeken tussen schistosomiasis en andere parasitaire ziekten, met name tussen schistosomiasis en Plasmodium spp. infecties.

Het is duidelijk dat onze kennis over de epidemiologie van schistosomiasis in ons gebied en in elk endemisch gebied verder moet worden uitgebreid om de ziekte beter onder controle te krijgen. Als inzicht in de epidemiologie van schistosomiasis en de interactie met andere ziekten relevant is voor de behandeling van de ziekte en de impact ervan op de last van andere ziekten, dan moet de ziektecontrole met het oog op eliminatie en uiteindelijke uitroeiing, de rode draad zijn voor de beleidsmakers van de volksgezondheid.

APPENDIX

List of co-authors



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APPENDIX

Curriculum vitae

CURRICULUM VITAE

Jean Claude Dejon Agobé

Author name: JC Dejon-Agobé
Date and place of birth: June 20, 1977. Cameroun
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Lambaréné/Gabon
ORCID N° : 0000-0003-3816-998X

Academic Background:

2018-2022: **PhD,** Academic Medical Center, University of Amsterdam, Amsterdam/the Netherlands

2014-2015: **Master 2, Clinical Epidemiology**, ISPED, University of Bordeaux Segalen, Bordeaux/France.

2012-2013: **University Degree, Statistic Methods applied in Health,** online, ISPED, University of Bordeaux Segalen, Bordeaux/France.

2011-2012: **University Degree, Methods and Practices in Epidemiology,** online, ISPED, University of Bordeaux Segalen, Bordeaux/France.

2003-2010: **Medical Doctorate**, Institut des Sciences de la Santé, Université des Montagnes, Bangangté/Cameroun.

1996-1997: **Bachelor of Secondary Education**: Mathematics and Natural Sciences. Tibati/ Cameroun

Professional Experience:

As Clinical Investigator: (August 2010-present)
At Centre de Recherches Médicales de Lambaréné (CERMEL), Lambaréné, Gabon:

1. 2019-Present, DFG: Multi-Drug Combination-Therapies to Prevent the Development of Drug Resistance: Phase II Controlled Clinical Trial Assessing Candidate Regimens of Multiple-Antimalarial Combinations for the Treatment of Uncomplicated Malaria in Africa. **MultiMal, Co-Principal Investigator (Co-PI)**

2. 2018-Present, EDCTP: Prospective, observational study to assess the performance of CAA measurement as a diagnostic tool for the detection of Schistosoma haematobium infections in pregnant women and their new-born and child in Lambaréné, Gabon. **freeBILy, Investigator**

3. 2016-2018, DFG: Assessing the effect of neglected tropical diseases on Plasmodium falciparum transmission in an area of co-endemicity. **TransMal, Investigator**

4 2015-2016, CERMEL: A randomized, controlled, double blind, single-center phase 1clinical trial to evaluate safety, tolerability, immunogenicity and efficacy of CAF01 an aluminium hydroxide as adjuvant s for the malaria vaccine candidate GMZ2 in healthy adult African volunteers. **GMZ2CAF01, Investigator**

5 2013-2019, EU: Developing and testing a novel, low cost, effective HOOKworm VACcine to Control Human Hookworm Infection in endemic countries. HOOKVAC FP7-HEALTH-2013-INNOVATION. **HookVac**, **Investigator**

6. 2014-2015, SANARIA: "Effect of *Plasmodium falciparum* exposure and sickle cell trait on infection rates and kinetics after intravenous administration of PfSPZ Challenge". **LaCHMI**, **Investigator**

7. 2012-2014, EDTCP: "Impact of *Schistosomiasis haematobium* infection on immunological and clinical aspects of *P. falciparum* malaria in children". Senior Fellowship applicant. **SF**, **Investigator**

8. 2010-2013, EDCTP: A Phase II, randomized, controlled, double-blind, multi-centre study to evaluate the efficacy, safety, and immunogenicity of GMZ2 candidate malaria vaccine in Gabonese, Ghanaian, Burkinabe and Ugandan children aged 12 – 60 Months". **GMZ2**, **Investigator**

<u>As Clinician</u>, (August 2009 - August 2010)
At Clinique de l'Université, Douala/Cameroun

Workshops:

<u>GCP training</u>

- GCPs training: Global Health Trial (February 2018, March 2016, August 2013), AMANET (2010).
- March 2012: GCLPs (Good Clinical and Laboratory Practice) training. Lambaréné/ Gabon

<u>Oral Presentations</u>

· Monthly scientific meeting held at Centre de Recherches Médicales de Lambaréné (CERMEL).

· June 6-7, 2019. Vième Journée Médicales du Gabon (JMG): ***[Epidemiology of schistosomiasis and soil-transmitted helminth co-infections among school-age children living in Lambaréné, Gabon]***, Libreville, Gabon

· October 28 – November 1, 2018. 67th ASTMH Annual Meeting: "***Increase in the number of malaria attacks among children infected with Schistosoma haematobium living in rural areas around Lambaréné, Gabon".*** New-Orleans, USA

· May 02-04, 2018. Kickoff Meeting of freeBILy study: "*Fast and reliable easy-to-use-diagnostics for eliminating Bilharzia in young children and mothers*". Tübingen, Germany

·March 22-23, 2017. TES Conference; A paradigm shift. "***Schistosoma haematobium infection associated with Plasmodium falciparum infection burden in school aged children living in the vicinities of Lambaréné, Gabon"***. Yaoundé, Cameroun.

· June 1-4, 2016. DFG meeting; Conference of African-German Cooperation Projects on Infectious Diseases. "***Assessing the effect of neglected tropical diseases on Plasmodium falciparum transmission in an area of co-endemicity***". Yaoundé, Cameroon.

· July 2014. Network Meeting under the Controlled Human Malaria Infection Platform, SANARIA/CREATES. Bagamoyo, Tanzania.

Poster Presentations

· December 4-6, 2019. First International Conference on NTDs in Africa in conjunction with the 13th Kenya MoH and KEMRI Annual NTD Conference. ***Complete blood count changes and haematuria associated with Schistosoma haematobium infection among school children living in Lambaréné, Gabon.*** Nairobi, Kenya

· June 6-7, 2019. Vième Journée Médicales du Gabon (JMG): ***[Schistosoma haematobium infection; morbidity, praziquantel efficacy and re-infection rate among children and young adults living in Lambaréné and vicinity, Gabon]***. Libreville, Gabon

· September 17-20, 2018. Ninth EDCTP Forum: ***Schistosoma haematobium infection increases the number of malaria episodes in children living in rural areas around Lambaréné, Gabon***. Lisbon, Portugal

· November 28-29, 2016. UNESCO-MERCK African Research Summit. Empowering Women in Research, south-south collaboration: ***Effect of Schistosoma Haematobium Infection on Plasmodium Falciparum Malaria Burden in Lambaréné, Gabon***. Adis-Abeba, Ethiopia.

· November 6-9, 2016. Eighth EDTCP Forum: ***Effect of Schistosoma Haematobium Infection on Plasmodium Falciparum Malaria Burden in Lambaréné, Gabon***. Lusaka, Zambia.

Training

- April 2022: QWArS (Qualifying the Workforce for AMR Surveillance in Africa and Asia) Professional Qualification
- January 2020, Master class: Basic Immunology and helminth immunology short course, Lambaréné, Gabon
- October 2017: EDCTP Financial and Project Management Training. Dakar, Sénégal
- November 2015: Introduction to statistic with R. On line training. University of Paris-Sud
- January 2011: Formation avancée sur la Réanimation Pédiatrique (PALS). Lambaréné/Gabon
- June 2010: Formation Power to Cure of Sanofi-Aventis. Douala/Cameroun

Scientific Production:

Publications:

Please, see portfolio for list of publication

Reviewer for:

1. Infectious Disease of Poverty Journal (BMC)
2. Transactions of The Royal Society of Tropical Medicine and Hygiene

Langues:

French: Excellent
English: Intermediate

APPENDIX

PhD portfolio

PHD PORTFOLIO

Name PhD student:	Jean Claude Dejon Agobé
PhD period:	February 2018 to November 2022
PhD supervisors:	Prof. Dr. Martin Peter Grobusch
	Prof. Dr. Ayôla Akim Adegnika

		Year	Workload (Hours/ECTS)
1.	**PhD training**		
General courses			
-	Systematic Review	2019	0.7
-	Research Data Management	2019	0.9
-	Practical Biostatistics	2019	1.1
-	Oral presentation	2019	0.8
-	Scientific Writing in English for Publication	2019	1.5
-	Randomized Controlled Trials	2019	0.6
Seminars, workshops and master classes			
-	QWArS (Qualifying the Workforce for AMR Surveillance in Africa and Asia) Professional Qualification; Libreville, Gabon	2022	
-	Master class: Basic Immunology and helminth immunology short course, 13 to 18 January, 2020, Lambaréné, Gabon	2020	
-	Workshops: Challenges to women's health in sub-Saharan Africa, Lambaréné, Gabon	2020	
Presentations			
-	Protective effect of ascaris lumbricoides infection on malaria recurrence among children and young adults living in rural areas of Gabon, Central Africa. **DGI-DZIF-Joint Annual Meeting**.	2022	0.5
-	[Protective effect of ascaris lumbricoides infection on malaria recurrence among children and young adults living in rural areas of Gabon, Central Africa]. **15th Word Malaria Day meeting**	2019	0.5
-	Complete blood count changes and haematuria associated with Schistosoma haematobium infection among school children living in Lambaréné, Gabon. **1st IncoNTD meeting**	2019	0.5
-	[Épidémiologie de la schistosomose et co-infections des géo-helminthes chez des enfants en âge scolaire vivant à Lambaréné, Gabon] **V^{èmes} JMG**	2019	0.5
-	[Infection à Schistosoma haematobium ; morbidité, efficacité du praziquantel et taux de réinfection chez les enfants et les jeunes adultes vivant dans les environs de Lambaréné, Gabon] Vèmes **V^{èmes} JMG**	2018	0.5

		Year	Workload (Hours/ECTS)
-	Schistosoma haematobium infection increases the number of malaria episodes in children living in rural areas around Lambaréné, Gabon. Eight EDCTP meeting	2018	0.5
-	Increase in the number of malaria attacks among children infected with Schistosoma haematobium living in rural areas around Lambaréné, Gabon. Sixty-seventh ASTMH conference	2018	0.5
-	Evidence-based on Circulating Anodic Antigen (CAA) of the Schistosoma haematobium infection early in the first two years of the life of infants living in Lambaréné, a semi-urban endemic area of Gabon. Ten EDCTP forum	2018	0.5
-	High rate of malaria recurrence among children and young adults living in endemic rural areas of Gabon. Ten EDCTP forum	2018	0.5
(Inter)national conferences			
-	10th EDCTP Forum, October 17 – 21, 2021. Maputo, Mozambique, Virtual	2021	0.25
-	[Fifteenth edition of World Malaria Day], April 29, 2022. Libreville, Gabon	2019	0.25
-	1st International Conference on NTDs in Africa in conjunction with the 13th Kenya MoH and KEMRI Annual NTD Conference, December 4-6, 2019. Nairobi, Kenya	2019	0.25
-	Les Vèmes journées médicales du Gabon, June 6 and 7, 2018. Libreville, Gabon	2018	0.25
-	Eight EDCTP meeting, September 17 – 21, 2018. Lisbon, Portugal	2018	0.25
-	Sixty-seventh ASTMH, October 28 - November 1, 2018. New-Orleans, USA	2018	0.25
-	Congrès Africain Des Essais Cliniques, 1Ier édition, November 8 – 11, 2018. Lambaréné, Gabon	2018	0.25

2. Teaching

	Year	Workload (Hours/ECTS)
3. Parameters of Esteem		
Grants		
- Supervisor for the RSTMH Small Grants Programme to Jeannot F. Zinsou	2021	
- Supervisor for the RSTMH Small Grants Programme to Yabo Josiane Honkpehedji	2021	
Awards and Prizes		
- Travel award for the Eighth European and Developing Countries Trials Partnership Forum. Lisbon, Portugal	2018	

		Year	Workload (Hours/ECTS)
4.	**Publications**		
Peer reviewed			
-	Dejon-Agobé et al., **Schistosomiasis in Gabon from 2000 to 2021 - a review**. Acta Tropica. 228 (2022) 106317. https://doi.org/10.1016/j.actatropica.2022.106317	2022	
-	Dejon-Agobé et al., **Pilot malacology surveys for the intermediate hosts of schistosomiasis in rural and semi-urban areas of the Moyen-Ogooué province, Gabon**. Trop. Med. Infect. Dis. 2021 December 22.	2021	
-	Dejon-Agobé et al., **Knowledge, attitudes and practices pertaining to urogenital schistosomiasis in Lambaréné and surrounding areas, Gabon**. Parasites & Vectors. 2019. DOI:	2021	
-	Dejon-Agobé et al., **Haematological changes in Schistosoma haematobium infections in school children in Gabon**. Infection (2021). doi: 10.1007/s15010-020-01575-5	2021	
-	Dejon-Agobé et al., **Epidemiology of Schistosomiasis and Soil-Transmitted Helminth Coinfections among Schoolchildren Living in Lambaréné, Gabon**. Am J Trop Med Hyg. 2020 May 18. doi:10.4269/ajtmh.19-0835	2020	
-	Dejon-Agobé et al., **Schistosoma haematobium infection morbidity, praziquantel effectiveness and reinfection rate among children and young adults in Gabo**n. Parasites & Vectors. 2019. DOI: 10.1186/s13071-019-3836-6	2019	
-	Dejon-Agobé et al., **Controlled human malaria infection of healthy lifelong malaria-exposed adults to assess safety, immunogenicity and efficacy of the asexual blood stage malaria vaccine candidate GMZ2**. Clin Infect Dis 2018 Dec 18. Epub 2018 Dec 18.	2018	
-	Dejon-Agobé et al., **Schistosoma haematobium effects on Plasmodium falciparum infection modified by soil-transmitted helminths in school-age children living in rural areas of Gabon**. PLoS Negl Trop Dis. 2018 August 6. DOI: 10.1371/journal.pntd.0006663	2018	

APPENDIX

Acknowledgements

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My gratitude also goes to **Prof. Peter G. Kremsner** who make all this possible. Indeed, the tree you have planted decades ago continues and will continue to bear fruits. We are immensely grateful to you. You allow me to know the relevance of the clinical research, which is a need for Africa. We have an obligation to do our best to perpetuate the work.

I would also like to express here my gratitude go to **Dr. Ulysse Ateba Ngoa** who first proposed me years ago to join the world of clinical research; and to **Profs Akim Adegnika** and **Bertrand Lell** for giving me the benefit of the doubt regarding my abilities in this area that was totally foreign to me. I will always remember that day in Douala. I could not forget the late **Dr. Saadou Issifou**, the former Director of the URM (CERMEL today) who accepted me in 2010 in his research group at the URM. All this was my gateway to clinical researcher in particular, and in general to medical research.

A special thanks to all peer colleagues and all collaborators at CERMEL, particularly those from my research group led by **Prof. Akim**. To **Josiane**, **Ronald**, **Roméo**, and **Jeannot**, thank you for your contribution to the realisation of this project. Studies conducted under the present PhD project benefited from your support, in their conception as well as in their implementation.

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I would like to take advantage of this page to thank CERMEL and its co-directors (**Profs Akim Adegnika, Maxime Agnandji**, and **Bertrand Lell**) for their commitment to maintaining an environment conducive to the development of researchers, particularly young African researchers. I believe that this is worthwhile work that Africa needs. Future researcher generations will be grateful to you.

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To all, THANK YOU.