Plasmodium falciparum and Necator americanus in Gabon: observation, intervention, and controlled human infection

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CHAPTER 1

General Introduction: Malaria and Hookworms, disproportionate funding despite global burden.

It is the beginning of the 20th century, malaria and hookworms are endemic in North America and Europe.[1] Dr Albert Schweitzer graduates from his medical training in Strasbourg, France, equipped to treat the spectrum of human disease present in Lambaréné, Gabon, even malaria which was at the time also well known in Europe.[2] Not only malaria but hookworm was also part of his medical education. This helminth infection was prevalent in Europe, and the USA where the Rockefeller foundation would pioneer a hookworm control campaign.[3] In 1953 Albert Schweitzer would be awarded a Nobel peace prize for his humanitarian work. Around the same period, efforts to eradicate malaria and hookworm in Europe and America started to bear fruit[4], yet despite this, malaria and hookworm are highly prevalent in many other parts of the world.

Every year, malaria kills ≈600,000 individuals, the majority being children under the age of 5 years (≈75%), concentrated particularly in sub-Saharan Africa (≈94%).[5] High mortality results in the hegemony of malaria in the fields of tropical medicine and parasitology. In recent times, malaria shares some of the limelight with Neglected Tropical Diseases, a diverse group of conditions that includes soil-transmitted helminth infections such as hookworm disease, who's burden of disease is almost as high as for malaria.[6,7] The impact of hookworms are felt in healthy life lost, and indirect mortality. These parasites continue to produce deleterious effects on economic potential in adult workers, and stunted growth and cognition in young children.[8,9] In 2025, Oxford Economics Africa estimated that a 90% decrease in malaria by 2030, from 2015 levels, will add $127 billion to the GDP of African countries.[10] The global economic cost of hookworm disease is >$100 billion a year.[11] Controlling these parasites will result in an exodus from poverty and is eminently achievable.

1 – Faust, E. C. History of Human Parasitic Infections. Public Health Reports (1896-1970)70, 958–965 (1955).

2 – Nobel Peace Prize 1952. NobelPrize.org https://www.nobelprize.org/prizes/peace/1952/schweitzer/biographical/.

3 – Elman, C., McGuire, R. A. & Wittman, B. Extending public health: the Rockefeller Sanitary Commission and hookworm in the American South. Am J Public Health 104, 47–58 (2014).

4 – From malaria control to eradication: The WHO perspective – Mendis – 2009 – Tropical Medicine & International Health – Wiley Online Library. https://onlinelibrary.wiley.com/doi/10.1111/j.1365-3156.2009.02287.x.

5 – World malaria report 2024. https://www.who.int/publications/i/item/9789240104440.

6 – Global report on neglected tropical diseases 2025. https://www.who.int/publications/i/item/9789240114043.

7 – Global burden of 292 causes of death in 204 countries and territories and 660 subnational locations, 1990–2023: a systematic analysis for the Global Burden of Disease Study 2023 - The Lancet. https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(25)01917-8/fulltext.

8 – Hotez, P. J. et al. Hookworm infection. N. Engl. J. Med. 351, 799–807 (2004)

9 – Hookworm infection | Nature Reviews Disease Primers. https://www.nature.com/articles/nrdp201688.

So, one may ask, why are malaria *(Plasmodium falciparum)* and hookworms *(Necator americanus)* still endemic in Gabon, a middle-income country in Central Africa, ≈75 years after Schweitzer, despite the burden of disease and the colossal profit margin of eliminating this burden?

Malaria and Hookworms are in essence diseases of neglected people. They are not only a proxy for poverty, but a proxy for inequitable access to socio-economic and healthcare systems. It is extremely difficult for underprivileged individuals living in remote endemic regions to extricate themselves from these parasites on their own. Living conditions, where vulnerable people live, are a risk factor. Conditions are warm and humid, there is more mud, and no piped chlorinated water. A child running around barefoot outside his/her home is vulnerable to malaria carrying mosquitoes in the air and hookworm larvae in the ground. This child is at risk for infection, reinfection, and superinfection. When repeatedly infected, the child may trade partial immunity for stunted growth and cognitive impairment.[12,13]

The right to health must not be neglected. So how do we eliminate malaria and hookworm infection in endemic areas?

What is required is a combination of interventions. The epidemiologic context, and clinical evaluation of diagnostics and vaccines in Gabon are covered within this thesis. The merits of social medicine are beyond its scope (Figure 1).

10 – The Malaria Dividend Report | RBM Partnership to End Malaria. https://endmalaria.org/related-material/malaria-dividend-report.

11 – The Global Economic and Health Burden of Human Hookworm Infection | PLOS Neglected Tropical Diseases.https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0004922.

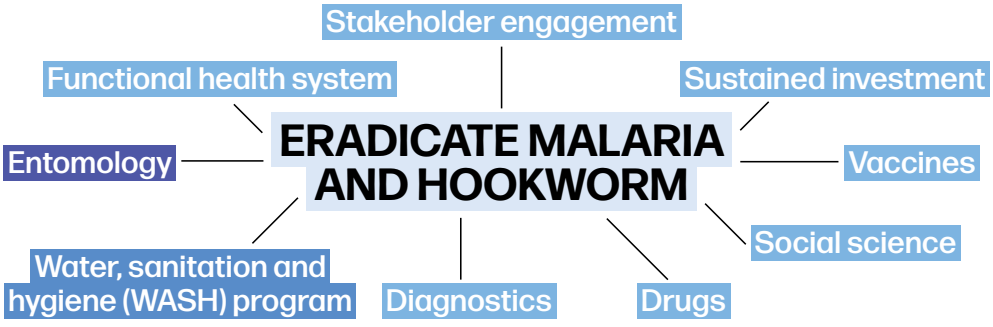
12 – Smillie, W. G. & Spencer, C. R. Mental retardation in school children infested with hookworms. Journal of Educational Psychology 17, 314-321 (1926).

13 – Sakti, H. et al. Evidence for an association between hookworm infection and cognitive function in Indonesian school children. Tropical Medicine & International Health 4, 322-334 (1999).

14 – Cockburn, I. A. & Seder, R. A. Malaria prevention: from immunological concepts to effective vaccines and protective antibodies. Nat Immunol 19, 1199-1211 (2018).

15 – Idem 8.

Figure 1: The key elements in the fight to control and eliminate malaria and hookworm infection. Entomology (in Blue) applies only to malaria. WASH (in Azure) applies only to hookworm. The other elements (in Blue Gray) apply to both malaria and hookworm.



For malaria, we know that humans develop a degree of immunity quite early in life if repeatedly exposed to the parasite at a young age; for those unexposed, it can quickly become a fatal disease.[14] Exposure, however, does not provide lifelong protective immunity. Sterilizing immunity after natural exposure

has not been observed for malaria nor hookworms.[15,16] Besides, these parasites can subvert the human immune system via a plethora of mechanisms, to fulfil their life cycle[17,18] For hookworms, the old friends and hygiene hypotheses postulate that exposure to helminths (and other pathogens and microbes) downregulates the immune response in a manner that protects against autoimmune and allergic disease.[19,20] However, the jury is still out on whether humans develop immunity to hookworm infection following natural exposure, as exposure does not appear to influence clinical manifestations of the disease.[21] Controlled Human Helminth Infection (CHHI), the deliberate inoculation of a consenting volunteer with hookworm larvae, is a polyvalent platform that can be used to not only evaluate cost-effective interventions such as drugs and vaccines for disease control and elimination, but also parasite-host interactions.[22] CHHI is ethically feasible in healthy volunteers, and this is predicated on the careful examination of infective larvae prior to infection, and subsequent abrogation of infection with effective drugs.[23] In this thesis, we will establish CHHI for the first time in an endemic population in Gabon, using it as a platform to investigate a role for prior exposure to hookworm in the profile of clinical disease.

A fundamental aspect of the implementation of CHHI in Gabon is stakeholder engagement. Stakeholders will want to understand the ethics of CHHI and its risk-benefit profile. The socio-cultural context of the region must be considered. Engagement with authorities, regulators, researchers, and the community can purchase valuable social capital, increase local ownership and commitment, and facilitate implementation. Moreover, the risk perception of someone residing in non-endemic areas could be drastically different from a person living in endemic areas. The traveller without previous exposure in most cases experiences severe symptoms of malaria, whereas life-long exposure to malaria is associated with asymptomatic infections.[24] The difference in risk perception can influence how a locally unprecedented scientific intervention is perceived. Paradoxically, the need for such intervention might not be appreciated by those constantly exposed to disease, unless advocated.[25–28] In this thesis, we share the outcomes of a stakeholder meeting apropos CHHI in Gabon.

Vaccines are helpful in combating pathogens to which the immune system develops robust acquired immunity; this is not the case for malaria nor hookworms. So, an effective vaccine will have to surpass any existing natural immunity. Thorough biological understanding is therefore necessary for rational vaccine development. These parasites also have

16 – Doolan, D. L., Dobaño, C. & Baird, J. K. Acquired Immunity to Malaria. Clinical Microbiology Reviews 22, 13–36 (2009).

17 – Poespoprodjo, J. R., Douglas, N. M., Ansong, D., Kho, S. & Anstey, N. M. Malaria. The Lancet 402, 2328–2345 (2023).

18 – The immunology of human hookworm infections - McSORLEY - 2010 - Parasite Immunology - Wiley Online Library. https://onlinelibrary.wiley.com/doi/10.1111/j.1365-3024.2010.01224.x.

19 – Yazdanbakhsh, M., Kremsner, P. G. & van Ree, R. Allergy, Parasites, and the Hygiene Hypothesis. Science 296, 490–494 (2002).

20 – Rook, G. A. W. Review series on helminths, immune modulation and the hygiene hypothesis: the broader implications of the hygiene hypothesis. Immunology 126, 3–11 (2009).

21 – Gaze, S., Bethony, J. M. & Periago, M. V. Immunology of experimental and natural human hookworm infection. Parasite Immunol 36, 358–366 (2014).

22 – Experimental human hookworm infection: a narrative historical review | PLOS Neglected Tropical Diseases. https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0009908.

23 – Erwin, G. et al. Manufacture of Necator americanus as an infectious challenge agent: Accelerating human hookworm vaccine development. Microb Pathog 204, 107592 (2025).

24 – Idem 16.

25 – Pistone, T. et al. Malaria risk perception, knowledge and prophylaxis practices among travellers of African ethnicity living in Paris and visiting their country of origin in sub-Saharan Africa. Trans R Soc Trop Med Hyg 101, 990–995 (2007).

26 – Peeters Grietens, K. et al. Low perception of malaria risk among the Ra-glai ethnic minority in south-central Vietnam: implications for forest malaria control. Malar J 9, 30 (2010).

27 – Afolabi, M. O. et al. Caregivers' perception of risk for malaria, helminth infection and malaria-helminth co-infection among children living in urban and rural settings of Senegal: A qualitative study. PLOS Glob Public Health 2, e0000525 (2022).

28 – Shah, S. The Fever: How Malaria Has Ruled Humankind for 500,000 Years. (Picador, New York, 2010).

complex life cycles and exist as a series of complicated stages during the infectious process, each stage being physiologically different.[29,30] Nevertheless, the use of attenuated infective stages, *Plasmodium falciparum (Pf)* sporozoites and *Necator americanus* L3 larvae (*Na*L3), as inoculate, are viable approaches for vaccine development.[31–33] This thesis will (1) investigate the efficacy of a whole irradiated sporozoite vaccine in preventing *Pf* in young Gabonese children, and (2) establish the CHHI as a platform for future assessment of hookworm drug and vaccine candidates.

29 – Idem 9.

30 – Idem 17.

31 – Mwakingwe-Omari, A. et al. Two chemoattenuated *Pf* SPZ malaria vaccines induce sterile hepatic immunity. Nature 595, 289–294 (2021).

Diagnostics in the tropics

Besides parasitic infections; respiratory infections, enteric infections, sexually transmitted infections, and other infectious diseases represent a high burden of infection globally, especially in the tropics.[34] When differentially diagnosing and treating these diseases, a more comprehensive approach is required, where more than one pathogen could be present that leads to a similar clinical presentation. The cost of poor management is the emergence of resistance. For example, mass drug administration (MDA) of antimalarials and anthelminthics are an important component of control programs, but Darwinian pressure stipulates that these parasites will evolve to counteract elimination efforts. The dilemma here is that we cannot be sure as to if the genes of drug resistance are already present in the parasite population prior to infecting an individual or if resistance develops through mutation after infection. If the former, then MDA selects for the gene of resistance already present in the parasite population, and this selective advantage drives resistance into the population. Darwinian pressure applies to any intervention. The introduction of *Pf* histidine-rich protein 2 (*Pf* HRP2) based rapid diagnostic tests (RDTs) resulted in increasing detection of *Pf* lacking the *Pf* HRP-2 gene (these have now been detected in 57 countries as of writing, including Gabon).[35] A complementary solution to this dilemma, besides a vaccine, is the implementation of easy-to-use diagnostic tools that can accurately diagnose disease and guide antimicrobial stewardship by healthcare professionals. The World Health Organisation (WHO) recommends the use of RDTs for the diagnosis of malaria.[36] The WHO also recommends a switch to non-*Pf* HRP2 based RDTs if 5% or more of *Pf* cases in an area are missed by the standard RDTs.[37] However, is there a role for diagnostics even when malaria is not diagnosed?
Can a biomarker guide antimicrobial prescription in the tropics? We will aim to address these questions in this thesis.

32 – Chapman, P. R. et al. Vaccination of human participants with attenuated Necator americanus hookworm larvae and human challenge in Australia: a dose-finding study and randomised, placebo-controlled, phase 1 trial. Lancet Infect Dis 21, 1725–1736 (2021).

33 – Hoogerwerf, M.-A. et al. Protective efficacy of short-term infection with Necator americanus hookworm larvae in healthy volunteers in the Netherlands: a single-centre, placebo-controlled, randomised, controlled, phase 1 trial. Lancet Microbe 4, e1024–e1034 (2023).

34 – Idem 7.

35 – Malaria Threat Map. https://apps.who.int/malaria/maps/threats/.

36 – Global Malaria Programme. https://www.who.int/teams/global-malaria-programme/case-management/diagnosis/rapid-diagnostic-tests.

37 – Idem 36.

A brief overview of this thesis

The chapters in this thesis address Malaria **(chapters 2, 3, and 4)** and hookworm infections **(chapters 5 and 6)**. Much of the work is done in Lambaréné, Gabon with contributions from Brazil and Malawi in **chapter 2** , which help contextualise disease epidemiology in Gabon.

In chapter 2, a standardized, multi-step diagnostic approach, using a variety of diagnostic tools, identifies the causes of acute febrile illness (AFI) in Gabon, Malawi, and Brazil. Our results show the continued prominence of malaria in SSA and the need for a reliable point-of-care biomarker that distinguishes bacterial from non-bacterial AFI, and cases of co-infection. However, this approach is too costly and not implementable in low resource settings. Based on this conclusion, **chapter 3** evaluates a dual RDT that detects both *Pf*HRP2 and Plasmodium lactate dehydrogenase (biomarkers for malaria), and C-reactive protein (CRP) (a biomarker of inflammation) in Lambaréné, Gabon with the aim of optimizing antibiotic use. Although, we show good performance of the malaria biomarkers, the measurement of CRP in parallel, could still not improve the antimicrobial stewardship. This indicates that there is a strong need for either optimization of the CRP cut off values or new inflammation biomarkers.

Chapter 4 turns our focus to vaccination. We show that an irradiated whole *Pf* sporozoite (*Pf*SPZ) vaccine is safe but did not provide significant protection against *Pf* in 1–12-year-old Gabonese children. Therefore, alternative strategies to improve vaccine efficacy in children are needed.

Chapter 5 presents a major step toward establishing CHHI in Gabon. Engaged local stakeholders, proposed developing a study protocol, tailored ethical guidelines, and developed a regulatory pathway for approval. The first CHHI in hookworm endemic areas of the world (in Lambaréné, Gabon) is then conducted in **chapter 6,** with locally produced NaL3. The proof-of-concept shows safety and infectivity and starts to shed light on possible factors affecting egg output and symptoms.

Chapter 7 discusses the implications of the findings in this thesis on the control and elimination of malaria and hookworms in Gabon and extrapolate to SSA. The work emphasizes that the clinical management of malaria in Gabon requires a holistic approach and innovation at the point-of-care, including a possible role for artificial intelligence. It is important to consider the consequences of pre-exposure on vaccination and controlled human infections. Finally, I explain how meeting discussions with stakeholders evolved into the final implementation of CHHI in Gabon.