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Safety and Efficacy of Praziquantel in Pregnant Women Infected with *Schistosoma haematobium* in Lambaréné, Gabon

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Gerstenberg, Jacob Miguel

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Dekan: Professor Dr. B. Pichler

1. Berichterstatter: Professor Dr. A. A. Adegnika
2. Berichterstatter: Professor Dr. K. O. Kagan

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Dedicated to *Dr. Joseph Knüver-Hopf*, grandfather of my children, who deceased during my research stay due to Covid-19 disease.

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List of abbreviations

AI	Anemia of Inflammation
AIDS	Acquired Immunodeficiency Syndrome
ANC	Antenatal Care
CAA	Circulating Anodic Antigen
CCA	Circulating Cathodic Antigen
CD	Cercarial Dermatitis
CERMEL	Centre de Recherches Médicales de Lambaréné
CR	Cure Rate
DALYs	Disability adjusted life years
EDSG	Enquête Démographique et de Santé du Gabon
ERR	Egg Reduction Rate
FCFA	Franc des colonies françaises d'Afrique
FDA	Food and Drug Administration
FGS	Female Genital Schistosomiasis
GBD	Global Burden of Diseases
HB	Hemoglobin
HIV	Human Immunodeficiency Virus
HSV	Herpes Simplex Virus
IL	Interleukin
KS	Katayama Syndrome
IUGR	Intrauterine Growth Restriction
LBW	Low Birth Weight
LF	Lateral Flow
MDA	Mass Drug Administrations
NTDs	Neglected Tropical Diseases
OR	odds ratio
PZQ	Praziquantel
RCT	Randomized Controlled Study
SCC	Squamous Cell Cancer of the Urinary Bladder
SEA	Soluble Egg Antigen
SD	Standard deviation
SGA	Small for Gestational Age
STDs	Sexually Transmitted Diseases
STH	Soil Transmitted Helminths
TCA	Trichloroacetic Acid
Th-cells	T helper cells
UCP	Up-converting Phosphor Technology
WHO	World Health Organization
YLDs	Years lived with Disabilities
YLLs	Years of life lost

1 Introduction

1.1 Abstract

Schistosomiasis is a human parasitic disease that affects around 220 million people worldwide[1] – mainly in sub-Saharan Africa[2] - and belongs to the neglected tropical diseases[3]. Despite growing efforts, the control and elimination of schistosomiasis is far from being realized,[4] and as many other neglected tropical diseases, human Schistosomiasis affects particularly the poorest sections of the population.[3] In the past decades the efforts of controlling schistosomiasis have been focused on school-aged children, leaving out other important sub-populations.[5] One of these groups are women of reproductive age, who are additionally affected of what is nowadays known as “female genital schistosomiasis”.[6] Since it’s discovery, praziquantel has been the most important drug for treating schistosomiasis,[7] but it’s use has longtime been withhold to pregnant and lactating women due to the fear of adverse effects on their offspring.[8] But as women in sub-Saharan Africa spend up to 25% of their reproductive age pregnant and up to 60% breastfeeding,[9] this omission left significant parts of the population untreated. Pregnant women can therefore be identified as one of the most neglected and at the same time vulnerable sub-populations. Due to the growing knowledge about the morbidity of schistosomiasis in pregnant women,[8,9] the discussion about the use of praziquantel in pregnancy has re-emerged during the past two decades, leading to new recommendations.[10,11] However, there have only been two randomized controlled studies investigating treatment in pregnant women with schistosomiasis and these were carried out with women infected with *S. mansoni*[12] and *S. japonicum*.[13] Safety and efficacy of praziquantel to treat pregnant women infected with *S. haematobium* – the third main infective causative of human schistosomiasis - remains unknown. This thesis therefore aims to provide further data for the use of praziquantel in pregnant women, and to help bringing the treatment to those who are the most affected and the most at risk of living with long-term complications of the underlying parasitic disease.

1.2 The parasite and it's Life Cycle

Human schistosomiasis is a parasitic disease and also known as *Bilharzia,* named coming from its first describer Theodor Bilharz, a German doctor and scientist who first described the causative agent in the year of 1851 in Cairo, Egypt.[14] Schistosomiasis is caused by the infection of blood-dwelling trematode flukes of the genus *Schistosoma,*[15] consisting of 6 human pathogen species: *Schistosoma haematobium, Schistosoma mansoni, Schistosoma japonicum, Schistosoma intercalatum, Schistosoma guineensis* and *Schistosoma mekongi*, from which the first three are the main species responsible for human infections.[7] *S. japonicum* and *S. mekongi* are zoonoses, also infecting a wide range of other mammals (such as dogs, pigs, cattle and rodents), which makes it more difficult to control their reservoir, whereas *S. mansoni* and *S. haematobium* are mainly dependent on humans as definite host.[7,15] Besides, there exists also another dozen of schistosomes, that are infecting animals, but are not primarily responsible for human disease.[15]

Freshwater snails are the intermediate hosts of all schistosomes, but each *Schistosoma* species is dependent on a range of specific snail species, such as snails of the genus *Bulinus* (*Bulinus africanus, B. tropicus, B. camerunensis, B. senegalensis, B. forskalii, B. globosus* and *B. truncatus*) for *S. haematobium, S. intercalatum and S. guineensis*; the genus *Biomphalaria (Biomphalaria sudanica, Bi. alexandrina, Bi. pfeifferi, Bi. Chianomphala)* for *S. mansoni*, the genus *Oncomelania* for *S. japonicum* and the species *Neotricula aperta* for *S. mekongi*.[16,17] Therefore the regional and geographical distribution of each *Schistosoma* species is determined by its intermediate host's habitat range and usually only one species of *Schistosoma* occurs within one region.[7] However, humans can be infected with multiple species at the same time.

S. haematobium and *S. mansoni* are found widespread across Africa and in the Middle East, whereas *S. mansoni* is the only species to be found in the Americas and *S. japonicum* is predominantly found in China and the Philippines. *S. intercalatum* and *S. guineensis* are found in Western and Central Africa, and *S. mekongi* around the Mekong River, but none of the last three is seeming to play

a bigger role in human infections in terms of Epidemiology or Burden of Disease.[7] Europe is not endemic for *Schistosoma*, but some of the recognized intermediate hosts of *Schistosoma* have been found in Spain, Portugal and Corsica (France) and there was a recent case report from 2014 of autochthonous transmission to humans in Corsica (France).[18]

In contrast to other trematodes, schistosomes have two different sexes and are living pairwise, the bigger male forming a trench - the gynaecophoric channel - where the longer and thinner female lives wrapped inside.[15] This living as a couple may also explain the origin of their current name - deriving from the ancient Greek words σχιστος (*schistos)* and σῶμα (*soma)* meaning “split body”. schistosomes live an average of 3-10 year[19] in the veins of their human host where they digest erythrocytes and - because of their blind digestive tract- regurgitate their waste products into the bloodstream.[7,15] The localization of the veins affected, depends on the species - *S. haematobium* lives within the veins around the urinary bladder, leading to urogenital schistosomiasis, whereas *S. mansoni* and *S. japonicum* live in the mesenteric veins leading to abdominal schistosomiasis.[7]

Eggs, which are produced by the female and fertilized by the male, are deposited into the small venules of the perivesical or periportal system - depending on the species - and then migrate towards the lumen of either bladder and ureter or the intestines.[17] The accumulation of eggs in the small venules, causes a blockade and rupture of the small blood vessels, leading to the release of the eggs into either feces or urine, but also causing the characteristic symptom of blood in urine or feces.[17] Regardless of whether they reach the environment or they become trapped in nearby tissues, the fertilized eggs have an average survival time of 1-2 weeks after being released.[7] Once the eggs get in contact with freshwater, they will then hatch and release a free-living, ciliated and motile form - the miracidia - that can subsequently infect a suitable snail as intermediate host.[7,15] Inside the snail the parasite then undergoes asexually replication into multicellular sporocysts and after a period of 4-6 weeks, the infectious cercariae are released,

carrying an embryonic sucker and their distinctive bifurcated tale.[7,15] Release of the cercariae is provoked by light and mainly occurring during daylight[15], human infections therefore mainly occur through contact with freshwater during household activities such as washing, bathing, swimming or fishing. Cercariae can remain infective in freshwater for 1-3 days, and can withstand a temperature range between 15 - 35°C.[20] After being released, they now search for the skin of their definite host and once in contact, they penetrate it through mechanical activity and by the help of proteolytic enzymes.[21]

After the cercariae penetrated the skin of the human host, they migrate into the blood stream and via the lungs to the portal vein where the maturing larvae - the *schistosomula* - need another period of 5-7 weeks to become adult fertile worms before they again start to migrate to their definitive destination - the perivesicular or mesenteric venules - where they start to release eggs.[7,15] During the 5-7 weeks of their maturation - the prepatent period - the infection with *Schistosoma* cannot be detected through standard diagnostics as microscopy based egg detection in excretions (currently the gold standard recommended by the World Health Organization), but the detection of released antigens of the maturing worms may already be possible. Throughout their total life of 3-5 years, schistosomes have a theoretical reproduction capability of shedding 600 billion eggs.[15]

1.3 The Burden of the Disease

Human schistosomiasis belongs to the so called *Neglected Tropical Diseases (NTDs)*, a diverse group of diseases, that are mainly prevalent in the tropical parts of the world, and are estimated to affect at least one billion people worldwide, who mostly live under poor conditions.[3] The World Health Organization (WHO) currently lists 20 different diseases or conditions on their list of NTDs, caused by a variety of different pathogens, including viruses, bacteria, parasites, fungi and toxins.[22] Feasey et al. (2010) stated that the reason why NTDs are predominantly found in tropical areas, is due to the fact that the concentration of poverty is highest among the people living in remote and rural areas nearby the equator.[23] Because of this association with poverty and their origin (at least in part) in lack

of access to clean water, sanitation and adequate housing, the authors state that the diseases should not be defined as tropical diseases but rather as diseases of the "bottom billion".[23] NTDs have been designated as one of the most powerful reinforcements of the poverty trap - a never ending cycle of poverty and illness[24] – but most of them could be prevented, eliminated or even eradicated with improved access to existing and cost-effective measures.[3]

Prevalence of schistosomiasis has been estimated to affect around 238[1] million individuals worldwide, the latter representing about 3.5% of the world population.[1] In terms of mortality, the Global Burden of Diseases Report from 1980-2016 estimates about 10.000 schistosomiasis-related deaths each year.[25] Regarding the regional distribution of schistosomiasis, it was found that about 90% of the disease burden appear to happen in sub-Saharan Africa, followed by eastern Asia and the MENA-Region (Middle East and North Africa),[2] whereas urogenital schistosomiasis, which is caused by *S. haematobium,* is considered to be endemic in 53 countries only in Africa and the Middle East.[26]

Besides the estimation of prevalence, incidence and mortality, today the most common tool to measure and compare the burden of diseases and injuries, is the calculation of "disability-adjusted life years" (DALYs), which is an indicator that was developed as part of the Global Burden of Disease Report 1990, and which can be understood as the sum of "years of life lost" (YLL) and "years lived with disabilities" (YLDs).[27] Hotez et al. (2014) stated that in the year 2010 schistosomiasis was found to be in third place in terms of DALYs caused by NTDS - right behind Leishmaniasis and "other NTDs"- and that it had risen from place 7 to place 3 from the year 1990 to 2010, with an estimated increase of 58% [27 - 67, 95% CI].[2] It is important to mention, that the far more devastating effects of *Schistosoma* infection appear to be caused by chronic infection rather than immediate death. This can be illustrated by taking a closer look at the different proportions of YLLs and YLDs in the calculation of DALYs: In the case of schistosomiasis the YLDs account for more than 90% of the DALYs, compared

to 10% for the YLLs.[2] Therefore schistosomiasis is labeled as “Disabler” rather than “Killer”.

It has been supposed, that approximately 40 million women of reproductive age have been infected with schistosomes in 2014.[8,28] Although there is a lack of epidemiological data on schistosomiasis during pregnancy there have been two recent meta-analyses that estimated prevalence of schistosomiasis in pregnant women. Adam et al. (2021) analyzed the results of 32 studies in 13 sub-Saharan African countries, including a total of 21,024 pregnant women and calculated a prevalence estimate of schistosomiasis of 13.2% (95 % CI 11.0 – 15.4) among pregnant women. Out of these 32 studies, 19 were conducted on *S. mansoni*, 11 on *S. haematobium*, and 2 on both species. The estimated prevalence of *S. haematobium* was higher compared to *S. mansoni*: 21.1% (95% CI 14.1–28.1) vs. 8.7% (95% CI 6.0–11.3), respectively.[28] The prevalence of *S. haematobium* was based on 8,100 pregnant women and ranged from 4.5% in Ghana[29] to 46.8% in Cameroon.[30] In a more recent Review, Cando et al. (2022) calculated a pooled prevalence of *S. haematobium* infection of about 13.44 (95% CI 8.90-19.80) based on the results of 21 studies involving 12,550 women.[31] The prevalence within the analyzed studies ranged from 0.3% in Ghana[32] to 46.8% in Cameroon.[30]

It is notable that there is a substantial difference within the estimated prevalence of *S. haematobium* between these two reviews. Two methodological differences should perhaps be mentioned: Cando et al. (2022) included only studies carried out after 2001 (because it was by then that the WHO recommended praziquantel as a treatment for pregnant women) whereas Adam et al. included also one study from 1992.[29] Another difference was the inclusion of a study from Ahenkorah et al. (2018 and 2020) that reported prevalence of both *S. haematobium* and *S. mansoni* among pregnant women in Ghana[32,33]. Cando et al. (2022) included the reported infections of *S. haematobium* and *S. mansoni* from this study into their analysis of the prevalence of the respective species, whereas Adam et al. (2021) did so only for the analysis of *S. mansoni* but not *S. haematobium*. Because the

reported prevalence of 0.3% was at the very limit of the reported range of the Meta-Analyses, it might have biased the results.

However, both Reviews show that there is a substantial burden of disease among pregnant women in sub-Saharan Africa, and that there is still lack of recent epidemiological data. The authors state, that this lack of epidemiological data on schistosomiasis during pregnancy could be due to the fact, that most of the studies on schistosomiasis have been carried out on school-aged children.

1.4 Therapeutical Approaches

Since its introduction in 1979, pharmaceutical interventions for schistosomiasis treatment and transmission control have largely relied on praziquantel (PZQ) and have become widespread during the past years through preventive-chemotherapy programs, notably for school-aged children. Praziquantel's popularity has been based on its effective use against all *Schistosoma* spp., its relatively good safety profile and its low production cost.[34] While the production costs are estimated around 0.4$ per treatment,[34] and such prices may be considered for cost-effectiveness calculations of MDA programs, this does not account for individual treatment, as prices in local pharmacies can be much higher.

Before the praziquantel era, treatment was based on drugs such as antimony potassium tartrate, first used by Christopherson et al. in 1918,[35] and formerly known only for the treatment of leishmaniasis. Despite its toxicity it remained the treatment of choice for schistosomiasis until the development of praziquantel.[36] Other drugs of use were metrifonate (only active against *S. haematobium* and not commercially available since 2002)[37] and oxamniquine (only active against *S. mansoni).*[34] The current product of praziquantel available is a racemate, consisting of the biological active enantiomer (R-Praziquantel) and the inactive distomer (S-Praziquantel)[38]. After oral administration approximately 80% are absorbed, the drug undergoes a first pass effect in the liver and presents with a maximal serum concentration after 1-3 hours.[39] Approximately 80% are bound to

proteins, nearly exclusive Albumine.[40] Praziquantel is rapidly metabolized by the cytochrome P450 system, its elimination half-life ranges between 0.8-1.5 hours[39] and 2-2-8.9 hours[38] and about 80% are excreted in the kidneys, exclusively in the form of metabolites.[38,39] Interestingly, disease status of patients seems to increase drug exposure (higher serum concentration and longer elimination half-time) compared to healthy volunteers. [41]

The in vivo effect mechanism of praziquantel is still not fully understood, but it has been showed to interrupt Ca^{2+} homeostasis in adult worms provoking a rapid spasmodic muscular contraction leading to immobility of the worm.[42] Additional damage of the worm tegument by vacuolization and disintegration may lead to exposal of worm antigens[38] and facilitate recognition by the host immune system.[43] This in turn demonstrates that effective treatment with PZQ relies in part on intact immune mechanisms capable of killing the adult worm. Data indicate, that the anti-schistosome activity of praziquantel is not related to its peak concentration but rather to the duration of exposure of the drug to the worm.[38] Cochrane Database analyses have shown, that there is a dose-response curve for treatment of *S. mansoni* from 20-40mg/kg but not from 40-60mg/kg, indicating that doses lower than the standard dose of 40mg/kg may be inferior but higher doses do not bring any advantage.[44] In contrast, this dose-response has not been shown in infections with *S. haematobium*.[45]

Measurement of the efficacy of praziquantel is largely dependent on egg detection by microscopy of fecal smears or filtered urine and is quantified in terms of cure rate (CR) or egg reduction rate (ERR), but more recent methods based on detection of circulating anodic antigen (CAA) and/or circulating cathodic antigen (CCA) are increasingly being used. In a meta-analysis of seven trials on 864 individuals, Kramer et al. (2014) quantified the CR of single dose praziquantel (40mg/kg) for the treatment of *S. haematobium* as 60% (treatment failure: RR 0.42; 95% CI 0.29-0.59). A more recent meta-analysis from Ethiopia by Hailegebriel et al. (2021) showed pooled CR of 40mg/kg praziquantel for treatment of *S. mansoni* (12 trials), *S. haematobium* (2 trials) and either of both

species (CR: 89.22[95%CI 85.38-93.07] vs. CR: 93.56 [95%CI 80.63-106.49] vs. CR: 89.83 [95%CI 86.21-93.45], respectively)

Praziquantel has been approved by the United States Food and Drug Administration (FDA) in 1982 for patients one year and older.[39] Dosage recommendations vary between 20mg/kg body weight 3 times per day (FDA)[39] and 40mg/kg body weight in a single dose (WHO mass drug administration recommendations).[11] It is advised to take tablets together with a meal and plenty of water, to increase oral bioavailability.[38,41] Caution is advised in patients with a prior history of epilepsy or involvement of the central nervous system due to schistosomiasis or other pathologies.[39] The use of praziquantel during pregnancy is discussed in the chapter *Praziquantel in Pregnancy.*

Known side effects include general-, nervous-, skin- and gastrointestinal system disorders such as malaise, headache, dizziness, abdominal discomfort, nausea, vomiting and urticaria. An overview of the frequency distribution of symptoms can be found in a recent meta-analysis of 14 observational studies in Ethiopia from Hailegebriel et al. (2021), who found an overall prevalence of side effects in 88.7% of all individuals receiving the drug (n=1217).[46]

In addition to praziquantel, artemether (a derivate of the antimalarial drug artemisinin) has also been studied for the control of schistosomiasis, as its addition to the standard treatment seems to increase CR.[47] It also has the benefit of being effective against the young and immature *schistosomula*, as shown in an animal model of hamsters infected with *S. haematobium* cercariae.[48] Despite this evidence, Artemether has not been introduced on a large scale to treatment regimens in endemic regions, as its use has been prioritized for malaria treatment, and widespread (prophylactic) mass treatments in endemic settings may increase the selection pressure of resistance in *Plasmodium* spp.[21]

1.5 Transmission Control

Transmission control of schistosomiasis will probably be successful if using a multidimensional approach. Effective treatment of infected individuals - reducing the reservoir of the parasite - can only be one pillar apart from the prevention of contamination of freshwater with human excrements and urine and the provision of access to clean water sources.[7]

But also environmental changes due to climate change or human interventions - as the construction of dams or irrigation canals - and subsequent alterations in snail habitats can have an immense impact - positive or negative - on transmission patterns.[7,15] Steinman et al. (2006) described one of the more discouraging examples: The construction of the Diama Dam close to the delta of the Senegal River. The erection of this barrage prevented the penetration of salt water into the river during the dry season and allowed a more extensive irrigation for farming activities. Previously the prevalence of *S haematobium* was (depending on the proximity to the river) somewhat between 0.7% and 29.7%, whereas *S. mansoni* has been absent. The first cases of *S. mansoni* appeared within only 18 months and 10 years later the prevalence of *S. haematobium* and *S. mansoni* had risen up to 51.6% and 71.8%, respectively.[49] Although treatment appears to reduce egg load about 90% in most individuals and the treatment therefore has a very good short-term effect, reinfection following curative therapy is observed frequently, leaving the long-term effectiveness of treatment alone unsatisfactory.[50]

1.6 Diagnostics

While treatment is directed against the adult worms, the diagnostic standard for active schistosomiasis is the microscopic detection of eggs in urine, feces, or tissue biopsies. Urine based microscopy is using either centrifugation or polycarbonate filters to isolate eggs from urine before examining them under a microscope, whereas fecal examination follows the Kato-Katz technique.[51] However, these methods have several disadvantages: The number of eggs excreted is often low, and shows high fluctuation from day to day.[7] Therefore,

examination of specimen needs to be repeated several times, and might still miss some infections. Because of this low sensitivity, exclusion of active infection remains a challenge.[7]

Molecular techniques for detecting schistosomal DNA in urine or feces are more sensitive than standard microscopy.[52] However, they are still limited, as eggs are irregularly distributed in excretions, and therefore infections might still be missed. Additionally, molecular techniques are expensive, require complex laboratory equipment and trained staff and are therefore difficult to realize in many field settings.

Immunological techniques for diagnosis can be subdivided into two categories: (i) serological assays detecting specific host antibodies; and (ii) determination of circulating antigens. Serological tests can be based on enzyme-linked immunosorbent assay (ELISA) or immunofluorescence assay (IFA). There are different antigens with specific epitopes that can be used for the detection of host antibodies including soluble egg antigen (SEA), egg antigen CEF6 or CCA.[53] The use of these techniques has mainly been found to be useful in returning travelers, but in endemic populations active infection cannot be distinguished from previous exposure, and serology therefore remains without further benefit.[53]

Detection of circulating antigens is the most promising approach in current diagnostics. Circulating antigens can be classified into three groups, depending on the development stage of the parasite: (i) cercarial antigens; (ii) adult worm antigens (including tegument and gut-associated antigens) and (iii) egg antigens.[53]

Two of the most researched circulating antigens are *circular anodic antigen* (CAA) and *circular cathodic antigen* (CCA), which belong to the group of gut-associated adult worm antigens.[53] Both of them are genus specific, produced by the gut epithelium of adult worms or primordial gut cells of immature schistosomula or cercariae, and regurgitated by the parasite together with other

undigested waste.[53] They are extremely stable carbohydrate chains, heat resistant, soluble in trichloroacetic acid (TCA) and found in serum and urine of the hosts.[53] The primary function of CAA and CCA is believed to protect the schistosome gut and to participate in the host's immunomodulation.[53] Barsoum et al. (1992) and Van Dam et al. (1996) investigated kinetics of CCA and CAA in mice, infected with *S. mansoni* cercariae. Detectable levels of both antigens appeared within 3 to 5 weeks following infection, with CCA being detectable a few days earlier and higher infection intensity leading to earlier detection.[54,55] Levels of both antigens correlate well with the number of adult worms, and decrease significantly following praziquantel-based treatment in murine models.[56,57]

Several studies have examined post-treatment variability in *S. mansoni* infected individuals and found that serum CCA and CAA and urinary CAA are more stable than fecal egg counts. [58–61] In contrast to this, Van Etten et al. (1997) showed, that in children infected with *S. haematobium*, microscopy fluctuated less than CAA or CCA levels in urine.[62] Due to improving techniques and a consequently decreasing detection limit of circulating antigens, sensitivity has increased and detection of CAA in serum claims to be able to detect single worm infections.[63] Currently, the level of serum-CAA is considered to be the most accurate indicator of actual worm burden.[53]

Detection of CAA and CCA was initially performed using monoclonal antibody dependent sandwich ELISA, which allowed detection in a highly sensitive and specific manner. Detection of both antigens in urine and serum showed specificities above 98%, as reported by Krijger et al. (1994).[64] Sensitivity of the assays ranges between 32% and 100%, depending whether detected in urine or serum, pre-treatment procedures and particularly infection intensity. Significant lower sensitivity in individuals with lower infection intensities, remains a key limitation of this assay.[65] Also, its implementation in routine clinical diagnostics is relatively complex and it has not been developed for single case identification in daily routine.[65]

Due to these limitations, an easy-to-use point of care test strip was developed for detection of CCA in urine, using rapid immunochromatography. This device is currently commercially available (Rapid Medical Diagnostics, Pretoria, South Africa), but has not yet been approved by WHO. Nevertheless, it has been used in various studies and was found to be sufficiently sensitive and specific for determining *S. mansoni* infections. However, in *S. haematobium* infections, the test showed large variations among different studies, and needs further consideration. Another assay that has been described by Corstjens et al. (2008) is a rapid test using up-converting phosphor-technology (UPT) and a lateral flow (LF) test strip for detection of CAA in serum and urine. As we also used this assay in our study, the assay is described in more detail in the chapter 2.4 Diagnostics.

1.7 Pathology of the Disease

Although many cases of schistosomiasis are initially asymptomatic, heavy infections can cause severe morbidity,[7,15] and also light infection might already be associated with chronic morbidities.[66] In endemic regions the initial infection often occurs at the age of 2 years and then infection intensity subsequently increases during the following ten years, having its peak in early adolescence, before usually decreasing throughout adulthood.[7,15] Due to this reason, the focus of research and therapeutical interventions for many decades has been on school-aged children, while overlooking the prevalence and burden of disease of schistosomiasis in pregnant women and infants.[67] However, there are important pathophysiological peculiarities among these formerly neglected populations, as young women can additionally suffer from specific complications as *female genital schistosomiasis* (FGS)[68] and there is growing evidence of the immunological importance of *in utero* sensitization[69] and infections in early childhood.[5]

1.7.1 Immunopathogenesis

In endemic regions and in the absence of treatment, schistosomiasis is primarily a chronic disease lasting for decades, as described above. This lasting infection probably results from both, repeated reinfection[70] but also the longevity of worms.[19] To maintain the infection over such a long period of time without either the parasite nor the host being killed, both must have developed a mutually balanced immunological interaction. The exact mechanisms of interaction between the adult worm, deposited eggs and the host's immune system are complex and still not fully understood.[71] Throughout the period of infection, the host's immune system has to face different life cycle stages of the parasite with many different antigens expressed on their surface.[71] Adult worms dispose different mechanisms to evade from the human immune system, as reviewed by Hambrook et al (2021),[72] including the regeneration of the worm's outer tegument using somatic stem cells,[73] molecular mimicry,[74,75] incorporation of host's antigens[76,77] and manipulation of the host's immune response.[78]

Regarding the pathogenesis, there is wide accordance that pathology due to human schistosomiasis is predominantly caused by inflammation that is based on the host's immune response against eggs that have been trapped in tissue.[7,15,79] Eggs contain and release a variety of proteases and other antigenic and toxic products that can cause severe damage.[80,81] Once they have not reached the environment, but have been trapped in nearby tissue, they are hotspots for the host's immune system, which in response begins to develop granulomas to wall in the eggs and their noxious agents, thereby protecting the surrounding tissues from them.[82] However, uncontrolled granuloma formation would quickly displace large amounts of tissue, constrict venous blood flow and cause extreme consequential damage such as portal hypertension and hepatosplenomegaly with all their complications - including death.[71] The granuloma therefore plays an ambivalent role: on the one side it protects the host's tissue from egg-derived toxins and on the other hand it leads to fibrosis of the tissue.[83] Interestingly, the granuloma seems to play a crucial role for the

parasite itself as it is indispensable for the capability of the excreted eggs to migrate into the lumen of the gut or bladder.[82]

The acute phase of schistosomiasis is known to be characterized by a Th-1 type immune response[71,84] with increased levels of IL-1, IL-6 and TNF-α.[85] Once the first eggs are produced approximately 6 weeks following infection, the immune response shifts towards a Th-2 type, to facilitate the formation of granulomas.[71] Both types can – if unregulated – result in increased tissue damage as excessive production of the Th-2 cytokine IL-13 is strongly associated with liver fibrosis,[84,86] whereas an overwhelming pro-inflammatory Th-1 response also results in non-fibrotic granulomas and higher mortality.[87,88] Regulatory immune mechanisms, including IL-10, regulatory T-cells, B-cells, antibodies and T-cell anergy, which lead to a balanced immune response seems to be the key to limit pathology.[71]

There is also evidence that active schistosomiasis during pregnancy alters the immune status of their offspring.[71,89] The extent of the influence of this in utero sensitization on the offspring has not yet been fully clarified, but it is suggested that it results in an early immunoregulation following the exposition to egg antigens once the newborns are infected themselves.[71] Maternal infection seems to be associated with reduced worm burden and less induced pathology in the offspring[89] at least in murine models.[90] These effects might contribute to the fact that most people from endemic areas establish a stable and chronic infection throughout their life and do not develop overwhelming granulomatous pathology.[71] However, the majority of the burden of disease of schistosomiasis seems to be induced by the chronic inflammation and resulting systemic manifestations (see also chapter *Systemic manifestations*) and not the immediate results of granuloma formation.[91] Other suspected influences on the child's immune system triggered by in utero sensitization concern the immune response in the course of vaccinations and the prevalence of allergic diseases.[69]

1.7.2 Immediate manifestation - Cercarial Dermatitis

The penetration of infectious cercariae can provoke cercarial dermatitis (CD) a temporary maculopapular pruritic rush that sometimes persist for some days as a papular pruritic lesion, mediated by IgE hypersensitivity response.[79] Skin lesions are discrete erythematous and raised with a size of 1-3cm.[21] Cercarial dermatitis - also known as “swimmer’s itch” - has appeared across all continents, but mainly reported from Europe and Northern America, after the erroneously infection of humans with avian trematode cercariae of the genus *Trichobilharzia* - a group of schistosomes that have birds (e.g. ducks) as their definitive host.[92,93] Cercarial dermatitis occurs mainly among travelers, migrants or after primary infection, but has not been commonly reported among endemic populations.[7,15,79]

1.7.3 Acute Schistosomiasis - Katayama Syndrome

Katayama syndrome (KS) - formally known as Katayama fever - is the acute form of schistosomiasis[21] and was long time known in Japan to occur amongst the peasants working in the rice fields around Hiroshima.[94] It had been linked to schistosomiasis in 1904 by two Japanese doctors - Fujiro Katsurada and Akira Fujinami - who identified the causative agents of the disease.[94] KS is thought to be caused by an immune-complex mediated systemic hypersensitivity reaction to the migrating schistosomula and their beginning of egg deposition.[15,21,37,79] Symptoms have been correlated to worm burden but also to eosinophilia and IgE levels.[95] KS can occur between 14-84 days after initial infection.[21,37] Many cases are asymptomatic, but if the disease appears, onset is typically sudden and presents with unspecific symptoms as nocturnal fever, malaise, myalgia, headache, fatigue, nonproductive cough, eosinophilia and abdominal pain.[21] Pulmonary symptoms as dry cough and radiological findings in chest x-ray as beaded and scattered micronodulations as well as thickening of bronchial walls in lower pulmonary fields have been described, following infection.[95] Within chronically exposed populations in endemic regions of *S. haematobium* and *S. mansoni*, KS has rarely been reported.[7,15,21] One potential explanation that has been discussed, is that in-utero priming of B- and T-Lymphocytes of babies born

to infected mothers[96,97] might lead to decreased severity of symptoms.[7,15,21] Ross et al.(2007)[21] have summarized the differing pathology of KS caused by *S. japonicum*, which is not restricted to primary infections, as it does also appear within populations in endemic areas, and also in individuals with a history of previous infection.[98] Manifestation of KS in such individuals can be severe, with persistent fever and hepatosplenomegaly which can evolve to hepatosplenic fibrosis and portal hypertension.[15]

1.7.4 Urinary Schistosomiasis

Schistosomiasis of the genitourinary tract is a specific property of the infection with *S. haematobium*, and characterized by the main symptom of haematuria.[7,15,37] In endemic regions, haematuria can be so widespread in adolescent populations, that it has been mistaken as menses in girls and thought of as a natural sign of puberty in boys.[99,100] First historical mentions of haematuria as a cardinal symptom of the disease are believed to date back around 1900 BC, when Egyptian physicians described this sign and myths of the “menstruating males of Egypt” arouse.[14]

Haematuria is the first symptom of chronic urinary schistosomiasis, and appears around 10-12 weeks after the infection with *S. haematobium*, together with other early symptoms as dysuria and pollakisuria.[7,15,37,99] Proteinuria in turn, seems to appear as a late manifestation, potentially due to renal damage.[37] Chronic lesions can be visible in cystoscopy as areas of roughened bladder mucosa - also called “sandy patches”- which are pathognomonic to urogenital schistosomiasis and are caused by the granulomatous formation in reaction to eggs deposits.[37] Chronic manifestations include calcification of the bladder and the lower ureter, possibly resulting in hydroureter and hydronephrosis, which in turn can lead to bacterial superinfection and renal dysfunction.[7,15,37] Early symptoms as haematuria and dysuria tend to correlate with infection intensity, and to appear more often throughout younger populations[99], whereas chronic manifestations seem to result from the hosts poor regulation of immune reaction towards egg deposition[7], since they correlate with certain cytokine profiles as TNF-α and IL-10.[101]

Infection with *S. haematobium* is a well-known risk factor for the development of squamous cell carcinoma (SCC) of the bladder.[7,15,37,102] In Egypt, where *S. haematobium* is endemic, schistosomiasis-induced squamous cell carcinoma used to be the most common type of bladder cancer in males[103], but its proportion decreased in the past decades (from around 78% in 1980 to 27% in 2005).[104] However, the mechanism of contribution is not yet fully understood. Gryseels et al.(2006) discuss the influence of inflammatory gene damage as a carcinogenic factor as well as the possibility, that chronic lesions of the bladder mucosae could intensify the exposure of epithelial cells to known carcinogenic substrates as Nitrosamines and β-glucuronidase.[15] Martin et al.(2006) propose, that apart from tobacco smoking - which is a risk factor for both SCC and urothelial carcinoma - schistosomiasis, as well as other urinary tract infections and mucosa damage during self-catheterization, creates an environment of chronic inflammation characterized by growth factors and cytokines favoring cell proliferation, migration, angiogenesis and inhibition of apoptosis resulting in squamous dysplasia and leading to cancer proliferation.[102]

1.7.5 Female Genital Schistosomiasis (FGS)

In addition to the above-mentioned pathologies, women infected with *S. haematobium*, may also suffer from female genital schistosomiasis (FGS).[6] For a long time only the urinary tract and nephrological complications were the focus of possible clinical manifestations of an infection with *S. haematobium*, although first reports of schistosomiasis in the female genital tract date back to the 1950's, when Youssef et al. (1957) first reported *Schistosoma* egg deposition in the cervix.[105] Clinicians in endemic countries continue to neglect FGS as a possible diagnosis in women with complaints, as it is not properly described in medical textbooks or nursing curricula in any of these countries, as stated by WHO.[68]

In past decades, international attention on sexual and reproductive health - especially on women - has increased, also visible in the development of the Sustainable Development Goal 3.7 (universal access to sexual and reproductive health) of the United Nations. Today FGS is a well-recognized complication of

infection with *S. haematobium*[68] and affects in between 33%[106] to 75%[107] of infected women. Analogous to the lesions in the bladder found in urinary schistosomiasis, FGS is characterized by the pathognomonic sandy patches in the lower genital tract, which are composed of schistosome eggs with surrounding host inflammatory tissue[6] and increased blood vessel proliferation.[108] It seems that already the presence of eggs in urine is associated with the involvement of the lower genital tract, but symptoms of FGS do not change with the intensity of infection.[109] Organ manifestations of FGS appear within the uterus, ovaries, fallopian tubes, cervix, vagina and vulva, and tend to be almost equally distributed among these, but in clinical practice most manifestations are diagnosed in the cervix in the course of colposcopy.[110] Clinical symptoms of FGS were summarized by Kjetland et al.(2012)[110] and include contact[109,111–113] and postcoital bleeding[112], pelvic pain[106,114], genital itching[109,111,113], vaginal discharge[106,109,111,113] and stress incontinence.[109,111,113] FGS was also linked to secondary infertility[109,111,113,115] and increased risk of abortion[106]. A growing concern is that FGS may also lead to increased transmission of the human immunodeficiency virus (HIV).[6,110,116] Evidence and potential mechanisms how schistosomiasis in general contributes to the burden of HIV are discussed in the following chapter *1.7.6 - Comorbidity with HIV.*

It is well known that urinary egg shedding decreases significantly with age after its peak in adolescence,[7] whereas this phenomenon cannot be observed in the clinical manifestations of FGS. In contrary the prevalence of such lesions tends to be even higher within older age groups.[111] Other reports found, that urinary tract lesions can remain in adults while urinary egg excretion decreases or even totally ceases in these individuals.[117,118] So far, there is only one study from Kjetland et al. (2006)[119] who investigated the development of FGS lesions for a time period of 12 months following treatment. The authors reported no significant change in sandy patches or symptoms whereas egg excretion ceased after therapy. This leads to the conclusion that advanced genital damage might not resolve after causal treatment of the underlying infection, possibly explained by the fact that lesions are also caused by dead or calcified eggs. Up to now, no

other therapeutic options for FGS exists, except for common praziquantel administration for the underlying infection. Regular praziquantel treatment starting during early childhood and conscientiously continued during adolescence remains the only option at present to protect women from progression of FGS.[120] As pregnancy is a very common state in adolescent women in sub-Saharan Africa (the prevalence was estimated to be around 19.3% in sub-Saharan Africa),[121] the importance of proactive treatment guidelines throughout the reproductive age can readily be understood, and will further be discussed in the chapter *1.9 - Praziquantel in Pregnancy.*

1.7.6 Comorbidity with HIV

There has been raising interest on the question, whether and how infection with schistosomiasis contributes to the transmission and acquisition of HIV. Increasing evidence gives reason to fear, that schistosomiasis and its control may play a crucial role in the pandemic of the acquired immunodeficiency syndrome (AIDS). On a global scale, regions with the highest prevalence of schistosomiasis often also have high levels of HIV-infections.[122] Regarding the health of women of reproductive age, AIDS-related illnesses have been the leading cause of death on a global scale.[123] A recent meta-analysis from Patel et al.(2021)[116] including a total of 26 studies, provided new evidence about the co-infection and a possible association of schistosomiasis and HIV.

Regarding the prevalence of schistosomiasis among HIV-positive individuals in this meta-analysis, the authors summarized eleven studies. Seven of these reported prevalence of either *S. haematobium* or *S. mansoni* and were used to generate a pooled estimate of prevalence of 6.86% (95% CI 1.4 - 21.2) among HIV-positive individuals, whereas data from another four studies reporting prevalence of any *Schistosoma* species resulted in a pooled prevalence estimate of 20.7% (95% CI, 2.8 -49.5)[116]. In turn another six studies reported prevalence of HIV-infection among people with schistosomiasis, ranging from 5.8%[124] to 57.3%[125]. Due to different populations among these studies, the authors did not calculate a pooled estimate. Regarding the risks for HIV-acquisition among

women infected with schistosomiasis, Patel et al. combined the results from 6 studies with a total number of 12.925 participants and calculated a pooled odds ratio of 2.31 (95% CI, 1.23 - 4.33) for infection with HIV among women infected with schistosomiasis compared to those uninfected.[116]

Many different possible mechanisms and explanations how schistosomiasis contributes to HIV transmission have been discussed. Especially FGS has been thought to pose affected women at a greater risk of HIV acquisition.[116] The cervix is thought to be the site where most HIV acquisition caused by heterosexual sex is taking place[126] and as cervical schistosomal egg deposition causes lesions in the mucosa, these might be an entry point for HIV. It has been recognized, that breaching the epithelial barrier of genital mucosa, recruiting HIV target cells to the genital tract and generating a proinflammatory local immune milieu, increases the HIV susceptibility.[126] All these conditions might be evoked by urogenital schistosomiasis. Similar evidence was already demonstrated for other sexually transmitted diseases (STDs) causing ulcerative lesions, neovascularization and epithelial disruptions as genital ulcer or HSV-2.[127] FGS has been shown to lead to increased vascularity[108] and contact[109,111–113] and postcoital bleeding[112], facilitating the access of HIV to deeper genital layers and the bloodstream. Schistosome egg deposition induces a cellular and humoral immune response which includes upregulation of Th-2 helper cells, as well as chemokine co-receptors used by HIV to enter the host cells.[128] It has been shown that schistosomiasis increases the number of $CD4^+$ HIV target cells in the vaginal mucosa,[129] as well as the expression of the HIV co-receptor CCR5 on $CD4^+$ cells in peripheral blood,[130] therefore possibly leading to an increased susceptibility of infected women.

Schistosomiasis might also lead to an increased risk of transmitting HIV to the sexual partner, as infection with *S. haematobium* has been discussed to cause increased HIV shedding into the semen of infected men.[131] Additionally, schistosomal lesions might include higher levels of HIV infected host cells than

healthy surrounding tissue[110], as it has already been demonstrated in genital ulcers.[132]

1.8 Systemic manifestations

In addition to the organ damage mentioned, which is caused to different organs depending on the species, schistosomiasis in general is also held responsible for other more systemic pathologies, such as anemia, malnutrition, growth restriction, and impaired physical and cognitive fitness.[7,15,133] These systemic disease outcomes have been long time left unacknowledged, although they seem to be much more prevalent than specific organ pathologies and may represent the greater part of the chronic disease burden of schistosomiasis.[133] One reason for this neglect might be, that it has been difficult to show the particular contribution of schistosomiasis to each of these pathologies, as they are also caused by other parasitic diseases, and many studies are carried out in regions endemic for polyparasitism.[133]

1.8.1 Evidence for the Relationship of Anemia and Human Schistosomiasis

Anemia is a highly prevalent condition, which affects one out of three persons worldwide, and it has been accounted for 8.8% of the total disability from all conditions in 2010.[134] Of the many different causes of anemia, iron deficiency is by far the largest contributor globally, quantified with about 42 million YLDs in 2010.[1] Other important causes for anemia on a global scale include haemoglobinopathies (10.1 million YLDS), malaria (3.3 million YLDs), hookworm disease (1.9 million YLDS) and schistosomiasis (0.6 million YLDs).[1] Considering these aforementioned causes on a regional scale, there exist substantial differences: In sub-Saharan Africa, malaria and schistosomiasis represent two out of the three top reasons for anemia besides iron-deficiency.[134] While most of the causes for anemia have been lowered from 1990 to 2010, schistosomiasis, malaria and chronic kidney disease were the three only causes that had globally increased in prevalence.[134]

Regarding the evidence for the contribution of schistosomiasis to anemia, King et al. (2005) calculated in a meta-analysis of 20 observational studies a pooled standardized mean difference in hemoglobin levels of -0.26 (95% CI, -0.4 to -0.11) between individuals infected with schistosomiasis compared to those uninfected.[91] The total difference in mean hemoglobin was given as 0.4 mg/dl between these groups.[91] The effect on the hemoglobin level was dependent on the infection intensity and improved after treatment: standardized mean differences -0.66 (95% CI, -1.26 to -0.05) and 0.25 (95% CI, 0.15 to 0.36), respectively.[91]

Furthermore, the authors also investigated the impact of schistosomiasis on malnutrition and reduced fitness.[91] Former was measured by weight, height, weight for height or Skin fold thickness, but only studies using weight for height (n=5) resulted in a significant difference of standardized means: -0.36 (95%CI, -0.66 to -0.06).[91] The latter was measured by exercise duration and revealed a standardized mean difference of -1.09 (95% CI, -1.27 to -0.92).[91] Also other more subjective symptoms as diarrhea, pain and exercise intolerance or fatigue were significantly associated with infection status.

1.8.2 Possible Mechanisms of the Contribution to Anemia

The underlying mechanisms how *Schistosoma* spp. contribute to anemia remain unclear. Friedman et al. (2005) discuss four possible mechanisms: iron-deficiency due to extracorporeal blood loss in urine, splenic sequestration, autoimmune hemolysis and anemia of inflammation.[135]

Although haematuria is a well-known condition for urogenital schistosomiasis and both occult and visible blood has been described for intestinal schistosomiasis,[136,137] there is no sufficient evidence for its sole responsibility to the anemic state of infected individuals.[135] For intestinal schistosomiasis there has only been one study that reported a higher prevalence of occult blood among individuals infected with *S. mansoni* compared to an uninfected control group, but blood loss was not associated with lower hemoglobin levels.[138] Another study

conducted on Filipino children and young adults infected with *S. japonicum* found a correlation only with higher infection intensities.[139]

Several studies evaluated the relationship of *S. haematobium* or *S. mansoni* infection with iron deficiency, possibly resulting from previous extracorporeal blood loss.[135] One of these studies conducted on school-aged children in Niger, found a significant correlation of infection with *S. haematobium* to iron deficiency, defined as low serum ferritin combined with a low transferrin saturation and/or high erythrocyte protoporphyrin level.[140] Another study in Tanzania found a significant correlation of urogenital schistosomiasis with anemia and iron deficiency among both, school-aged children and adults.[141] There was also one study that was conducted on pregnant women in Mali, which found a significant correlation of infection with *S. haematobium* and both anemia and low serum iron.[142] Friedman et al.(2005) raised concerns, that the use of erythrocyte protoporphyrin and transferrin as markers for iron deficiency in most of these studies might be confounded by the fact, that both of these markers are also acute-phase reactants and may therefore be falsely elevated in the context of inflammation.[135]

Meanwhile, Mahmoud et al. (1973) showed in a rodent model that infection with sterile cercariae or only male schistosomes that could not produce eggs also provoked the development of anemia, although it was milder and normochromic, compared to more severe and hypochromic anemia induced by the infection with fertile cercariae.[143] This findings further weaken the hypothesis that schistosomiasis related anemia results solely from blood loss.

Intestinal schistosomiasis caused by *S. mansoni* or *S. japonicum* can lead to hepatic fibrosis accompanied by portal hypertension and result in hepatosplenomegaly. As the spleen plays a crucial factor in regulating the lifespan of erythrocytes, Splenomegaly could act as a mediator how schistosomiasis contributes to anemia. Woodruff et al.(1966) used ^{51}Cr marked Erythrocytes to show that splenomegaly due to the infection with *S. mansoni* lead

to increased splenic sequestration, measured by the increased surface radioactivity, and that the life span of erythrocytes reduced approximately about 50%.[144] However, this mechanism does not explain neither the existence of anemia among individuals with urogenital schistosomiasis, nor among people with light infections and anemia, a condition that is far more frequent than hepatosplenomegaly.

Based on animal models that used *S. mansoni* infected mice, Woodruff (1973) and Mahmoud (1972) postulated the hypothesis, that not only splenic sequestration but autoimmune hemolysis would be the leading mechanisms of how schistosomiasis contributes to anemia, as they also found increased erythrophagocytosis in spleenectomized mice infected with sterile cercariae.[145,146] This theory was strengthened by the findings of Kurata (1966) who identified autoantibodies in sera of rabbits infected with *S. japonicum*, that were directed against their red blood cells.[147]

Anemia of inflammation (AI) was recently summarized in a review article by Nemeth et al:[148] AI is characterized by a decrease of erythrocytic lifespan combined with an impaired production of erythrocytes. The clinical presentation often includes a mild normochromic and normocytic anemia. It is defined through the presence of low hemoglobin, low serum iron or transferrin and not depleted iron stores, e.g., normal serum ferritin. The decrease of erythrocyte survival is mainly caused by macrophage activation through inflammatory cytokines as Il-1 and Il-6, leading to enhanced erythrophagocytosis. Whereas the impaired erythropoiesis has two major components: hypoferremia and direct cytokine mediated suppression of erythropoiesis. Hypoferremia is caused by increased iron trapping in macrophages, duodenal enterocytes, and hepatocytes, mediated through Hepcidin. Hepcidin is physiologically responsible for the Homeostasis of serum iron through binding to its molecular target ferroportin, inducing its endocytosis. Ferroportin is then no longer capable of exporting iron to the plasma. Hepcidin production is regulated by plasma and hepatic iron levels as well as by inflammatory cytokines, namely IL-6.[149] This mechanism is believed to be a host

defense mechanism, restricting the availability of iron to invading pathogens.[149] Depression of erythropoiesis is thought to be mediated through TNF-α, Il-1 and Interferon-y, acting on erythroid precursor cells.[148] In the case of primary kidney involvement due to the underlying disease, decreased production of Erythropoietin[148] and increased retention of Hepcidin[150] may additionally reinforce the state of AI.

Several studies have been carried out, investigating cytokine profiles in individuals with *S. mansoni* infection. Two studies found increased levels of both, Interferon-y[151] and TNF-α[151,152] in individuals with hepatosplenomegaly due to *S. mansoni* infection. Other studies found increased levels of TNF-α[153,154] and Intereferon-y[153] in patients infected with *S. mansoni*, with and without hepatosplenic disease. In addition, one of these studies was able to show a trend towards normalization after treatment in individuals with intestinal schistosomiasis, but not in cases with hepatosplenic disease.[153] Another study again found increased levels of Interferon-y in patients with early stages of *S. mansoni* disease compared to patients with hepatosplenic disease.[155] In the case of *S. japonicum*, there have been similar findings. Coutinho et al.(2005) found increased levels of IL-1 and IL-6 in individuals with hepatosplenic disease.[156] Additionally Kurtis et al. (2011) have shown that maternal schistosomiasis due to infection with *S. japonicum* is associated with elevated levels of inflammatory cytokines (as Il-6 and TNF-α) not only in peripheral blood but also in placental and fetal compartments.[157]

All this evidence considered, one can conclude that low hemoglobin levels observed among subjects infected with *Schistosoma spp.,* might be caused by multiple reasons including the influence of pro-inflammatory cytokines, extracorporeal blood loss and dietary iron deficiency, leading to both anemia of inflammation and iron deficiency anemia. It can therefore be assumed that micronutrient and iron supplementation alone is unlikely to eliminate anemia in regions endemic for schistosomiasis.

1.9 Impact on Pregnancy

Pregnant women may experience any of the above-mentioned pathologies but suffer also from potential adverse birth outcomes during pregnancy. Four different mechanisms how schistosomiasis may contribute to poor pregnancy outcomes have been discussed: i) urogenital and placental disease ii) maternal anemia and iron deficiency iii) inflammatory cytokines in maternal and fetal blood and iv) poor maternal nutritional status due to anorexia and subsequent decrease of energy intake.[5,9] Rodent models have provided strong evidence, that maternal schistosomiasis leads to adverse pregnancy outcomes.[9] One study with 60 mice infected with *S. mansoni* cercariae found a significant difference in proportions of abortion, maternal death and infant death, as well as in means of birth weight and longevity of the mothers, compared to a control group.[158] However, it needs to be considered that schistosomiasis tends to have a greater impact in mice than in humans, although similar results have also been found in porcine models.[159] Meanwhile, the exact prevalence of maternal schistosomiasis remains unclear. It has been estimated, that approximately 10 million pregnant women only on the African continent suffer from schistosomiasis,[9] and the prevalence of schistosomiasis in African pregnant women was recently estimated in a meta-analysis from 32 countries to be at 13.2%.[28]

1.9.1 Urogenital Pathologies in Pregnancy

In addition to all reported pathologies in female genital schistosomiasis (FGS), urogenital pathologies during pregnancy may also include inflammation of the placenta[160] and the pregnant cervix.[161] It has also been discussed, whether schistosomiasis could lead to an increased risk of ectopic pregnancies,[9] as several case reports have indicated.[162,163] Also placental involvement of *Schistosoma* infection in pregnancy has repeatedly been reported in multiple case reports, as summarized by Renaud et al. (1972).[164] Another case report describes placental involvement of *S. mansoni* in four cases of pregnant women, of which three (75%) resulted in stillbirths.[160] Renaud et al. (1972) then systematically investigated 322 placentas of mothers in a region endemic for *S. haematobium* and found 72 of them to be infested with eggs of *S. haematobium*,

but birth outcomes were not associated with infection status.[164] Due to the anatomical proximity between the placenta and the blood vessels in which *S. haematobium* settles in urogenital schistosomiasis, the risk of transplacental infection consists particularly in this form of schistosomiasis. It is also possible that the increased blood flow to the vessels in the pelvis during pregnancy leading to the increased circulation of schistosome eggs, facilitates transplacental infection.[9]

In addition, the question was discussed whether maternal schistosomiasis could lead to congenital infection of the unborn child, as described in an animal model.[159] Friedman et al.(2007) discuss the results from one study that found 3 out of 22 examined newborns infected with *S. japonicum*.[9] However, no such occurrence has been reported for other *Schistosoma* spp.

1.9.2 Vertical HIV transmission

As mentioned earlier, there have also been links between schistosomiasis and increased risk of HIV transmission. Pregnant women may additionally be affected by increased risk of mother to child transmission, but knowledge about the impact of schistosomiasis on this risk is scarce. Only one study so far evaluated the relationship of parasitic co-infections and vertical HIV transmission.[165] The authors report a significantly increased risk for mother to child transmission in mothers who were infected with any helminth (OR: 7.3, 95%CI 2.4-33.7, p = 0.008), but for *S. haematobium* only a trend was visible.[165] Another study evaluated the impact of praziquantel based treatment on vertical HIV transmission compared to albendazole or placebo, and found no significant effect.[166] However, this study was conducted in an area prevalent for *S. mansoni* and not *S. haematobium*, the latter being suspected to play a bigger role in both FGS and HIV transmission. Further findings from the same study also indicated no effect of praziquantel on HIV plasma viral load in mothers,[167] leading to the hypothesis that treatment during pregnancy may be too late to impact vertical transmission.

1.9.3 Maternal Anemia

As discussed above, anemia is a well-known condition of human schistosomiasis, although the underlying mechanisms remain at least partially unclear. This is of particular importance, as pregnancy is a state characterized by increased hematologic demands and Anemia in turn has been known to be a risk factor for adverse birth outcomes[168] as prematurity and low birth weight.[169] Evidence for the prevalence of anemia due to schistosomiasis in pregnant women, was summarized in a recent meta-analysis, including a total of six studies. Adam et al.(2021) quantified the risk for pregnant women infected with *Schistosoma* spp. to develop anemia as 3 times higher, compared to those uninfected (OR = 3.02, 95%CI: 1.25–7.28, p = 0.014).[28] Another study on pregnant women in Tanzania, that was not included in this meta-analysis found anemia to be associated with *S. mansoni* infections only at higher levels of infection (> 400 eggs per gram feces), but not in light infections (OR = 1.87, 95%CI: 1.07 - 3.27 vs OR = 1.04, 95%CI: 0.73 - 1.48, respectively).[170] The risk for anemia particularly applies for urogenital schistosomiasis, as most of these studies have been carried out in individuals with *S. haematobium* infection,[30,33,142,171,172] and increases at higher intensity of infection.[30]

1.9.4 Maternal and fetal Inflammation

Healthy pregnancies are characterized by a placental cytokine milieu related to Th-2 cells (IL-4, IL-5, IL-13).[173,174] Cytokine production at the maternal fetal interface is crucial for many aspects of a healthy pregnancy because it protects the fetus from invading pathogens, maintains fetal tolerance or initiation of labor, and can be disbalanced through maternal infection.[175] Shifts from this physiological state of the placenta towards a more proinflammatory cytokine profile have been found in mothers infected with malaria to be associated with intrauterine growth restriction (IUGR).[176] Kurtis et al.(2011) found an association of maternal schistosomiasis with increased levels of inflammatory cytokines as IL-1, IL-6 and TNF-α in placental blood.[157] In the same study placental IL-1 and TNF-α production were both found to be independently associated with decreased birth weight.[157] However, a direct relationship between

schistosomiasis and low birth weight was not detected. Similar findings have been reported from Abioye et al.(2019), who found that elevated levels of proinflammatory cytokines in the cord blood of infants born to mothers infected with *S. japonicum* were associated with a higher risk of prematurity and being small for gestational age (SGA).[177]

1.9.5 Maternal nutritional status

Pregnancy is a state of increased hematologic and nutritional demands, and low energy intake and subsequently decreased gestational weight gain have been identified as risk factors for a healthy pregnancy.[178] Caloric undernutrition and nutritional deficits have been associated to schistosomiasis in nonpregnant individuals.[179–181] In turn, treatment with praziquantel has been proved to ameliorate nutritional status among nonpregnant subjects[182,183] possibly by increasing appetite.[184] As mentioned above, schistosomiasis is associated with an increase in proinflammatory cytokines which can also cause anorexia[185] and decreased appetite[186] and could therefore represent a mechanistic link to undernutrition and reduced weight gain during pregnancy. The interaction between schistosomiasis and pregnancy might exacerbate nutritional and hematologic deficits and result in a deprived development of the fetus[9].

1.9.6 Low Birth Weight

Low birth weight (LBW) is defined by WHO as a birth weight < 2500g.[187] Birth weight results from two decisive factors: duration of the pregnancy and fetal growth rate. Newborns can therefore present with decreased weight caused by two reasons: preterm birth or intrauterine growth restriction (IUGR).[188] One approaches the latter through determination of small-for gestational age (SGA), which works as a proxy for IUGR. Genital tract infections and poor nutritional status due to low energy intake or maternal malaria infection have been identified as risk factors for prematurity and IUGR.[178] LBW and SGA can both lead to small size at birth, which in turn is the greatest risk factor for more than 80% of all neonatal deaths worldwide, in addition to increasing the risk of impaired growth, post-neonatal mortality and non-communicable diseases in adulthood.[189]

Most evidence for a possible contribution of maternal schistosomiasis to adverse birth outcomes exists for LBW, as it is the parameter that has been most extensively studied also because it is particularly easy to assess. Regarding this evidence, several studies have found contradictory results, as summarized by Freer et al. (2018) in *The Lancet Infectious Diseases*:[5] Siegrist et al. (1974) compared the mean birth weight of children born to mothers infected with *S. haematobium* with an uninfected control group. The authors did not report a significant difference in term deliveries (3012g vs 3103g mean birth weight, respectively) but in preterm deliveries (1768 g vs. 2457 g, respectively, $p<0.005$). However, the sample size of this study was rather small. Quanhua et al.(2000) report a significant lower mean birth weight in a group of 244 women infected with *S. japonicum* compared to a control group (3229g vs 3355g, respectively, $p<0.05$).[190] Although Kurtis et al. (2011) provided further evidence for a potential mechanistic link, and showed a significant relationship of maternal anemia to increased proinflammatory cytokines and in turn a correlation of elevated cytokines to decreased birthweight, in unstratified analyses the birth weight was not related to maternal anemia.[157] Mc Donald et al. (2014) found no significant difference in means of birthweight or proportions of LBW between a group of 56 women infected with *S. japonicum* compared to a control group of 53 women.[191] A more recent study conducted in Lambaréné (Gabon), found a significant association of *S. haematobium* infection to the prevalence of LBW in a group of 90 infected women compared to a control group of 900 women (OR: 1.7, 95% CI: 1.03 - 2.82, $p = 0.04$).[192]

1.9.7 Preterm Birth, Abortion, and Infertility

Regarding other adverse birth outcomes besides birth weight and fetal growth, there is only little evidence for the contribution of schistosomiasis. Concerning preterm birth, Siegrist et al.(1974) reported in their study a higher proportion of preterm deliveries among the group of infected women.[193] McDonald et al.(2014) found a correlation of elevated proinflammatory cytokines to preterm birth in mothers infected with *S. japonicum*, but there was no difference regarding mean birth weight, the prevalence of LBW or mean gestational age between infected

and uninfected women.[191] Two larger studies evaluated the contribution of FGS to adverse birth outcomes, and found contradictory results. Kjetland et al.(2010) found no correlation of genital schistosomiasis with spontaneous abortion, but with infertility.[113] Leutscher et al.(1998) found significantly higher rates of spontaneous abortion in a village with a known high prevalence of schistosomiasis compared to a control village with low endemicity, using an ecological approach.[106] However, in this same study, diagnosis of *S. haematobium* based on urine microscopy was no longer significantly associated to neither spontaneous abortion nor infertility, but a trend was visible. The contribution of urogenital pathology to adverse birth outcomes due to schistosomiasis remains therefore unclear.

1.10 Praziquantel in Pregnancy

Since the availability of praziquantel in 1979, it has been the mainstay of schistosomiasis control over the past decades.[4] Pregnant women have been excluded from this treatment due to the lack of safety information.[8] After its initial approval in 1982, the American Food and Drug Administration (FDA) classified praziquantel as a 'pregnancy class B drug'[194], indicating that *"animal reproduction studies have failed to demonstrate a risk to the fetus and there are no adequate and well-controlled studies in pregnant women".*[195] This classification of praziquantel in class B was based on animal studies on mice and dogs, which did not find any teratogenic effects in offspring.[196,197]

In order to better understand the consequences of this classification, it is worth looking at the definitions of the other classes: Category A drugs have been specifically tested in pregnant women and proved to be safe, category C drugs have reported adverse effects in animal models but not in humans, and category D drugs have known evidence of risk to the human fetus, but potential benefits may still justify the use in pregnant women.[195] Thus for drugs in categories A-C the key question for risk/benefit analysis is disease-induced morbidity, and if there is evidence for any, the recommendation rapidly turns towards treatment.[198] One would expect that the classification of praziquantel as a class B drug would

therefore have led to a treatment recommendation. However, WHO and several national control programs have excluded pregnant and lactating women from regular treatment for several decades, and some have even advocated excluding adolescent girls in general from MDA campaigns.[198]

In 2002, WHO has changed its recommendations for the preventive chemotherapy of helminthiasis, now supporting the treatment of pregnant and lactating women with praziquantel on both, an individual and mass drug administration level.[10] This informal recommendation was based on a risk-benefit analysis that mainly emphasized the risk of additional morbidity that women may experience due to the delay of treatment on one side – although pregnancy-related morbidity was not included[9] - and post market surveillance data indicating no deleterious effects of pregnancy outcomes on the other hand. In addition to this informal recommendation, the WHO pointed out the need for further clinical trials, addressing both safety and efficacy of praziquantel in pregnant women. The recommendation to use praziquantel during pregnancy was then included into the new *Guidelines for preventive chemotherapy for Helminthiasis* in 2006.[11]

After the change of WHO recommendations, two smaller cross-sectional studies evaluated the use of praziquantel during pregnancy.[199,200] One retrospective study interviewed 637 women who lived in a region with annual MDA of praziquantel, of which 88 reported to have received the drug during their pregnancy.[199] The outcome of these pregnancies was compared with a control group of 549 women, and showed no significant difference in terms of abortion or preterm frequencies, as well as no recorded case of congenital anomalies.[199] Another prospective study was conducted on 25 women in Sudan.[200] Again, no stillbirth or congenital anomalies appeared, although there was one case of abortion 3 weeks post-treatment.[200] The authors state, that this frequency of abortion is similar to the one observed in the local community.

Finally, following the updated WHO recommendations in 2002, two large randomized controlled trials were initiated, examining the safety and efficacy of

praziquantel in pregnant women. One included pregnant women infected with *S. mansoni* in Entebbe, Uganda[12] the other one pregnant women infected with *S. japonicum* in Leyte, The Philippines.[13]

The study in Entebbe (Uganda), a region endemic for *S. mansoni*, mimicked a typical mass drug administration program by randomizing 2507 women into four treatment groups: placebo + placebo, albendazole + placebo, praziquantel + placebo and praziquantel + albendazole. Perinatal mortality and congenital anomalies were similar in all four groups, and praziquantel therefore estimated to be safe of use. However, there was no potential benefit in use of praziquantel in terms of maternal hemoglobin level and anemia (differences in mean associated with PZQ: +0.15 g/dl; 95%CI -0.02 - 0.32 and OR: 1.00; 95%CI 0.83-1.21, respectively) or birth weight and LBW (difference in mean associated with PZQ: −0.01 kg; 95%CI, −0.05 - 0.04 kg and OR: 0.96; 95%CI 0.70 - 1.32, respectively).[12] Also subgroup stratifications based on infection status (uninfected vs light vs heavy infected) revealed no effect of praziquantel on anemia or birth weight.[12] However, this study included a large proportion of women who were not affected by schistosomiasis (the prevalence of *S. mansoni* ranged from 18.2% - 19.8% among the groups), and might therefore have missed an effect that would only reveal using a test and treat strategy.

The second RCT[13] was carried out in Leyte (The Philippines) among 360 pregnant women infected with *S. japonicum*, who were randomized to either praziquantel , given as two doses, each of 30mg/kg, with a 4h interval or with a placebo, both given at the beginning of second trimester (week 12-16). Outcomes did not significantly differ between the treatment and placebo group in terms of birthweight and risk of LBW (differences in mean associated with PZQ: -0.002kg; 95%CI −0.088 - 0.083 and OR: 1.319; 95%CI 0.72-2.38) or maternal hemoglobin level (mean [SD] hemoglobin g/dl: 11.04[1.3] vs 11.05[1.18], respectively, p = 0.923). However, iron status of mothers and newborns were significantly improved in the treatment group, possibly indicating a trend towards an improved

hematologic state. In addition, praziquantel was well tolerated with a similar rate of side effects as in other populations.

Both RCTs found that praziquantel is safe to use in pregnancy, in terms of reactogenicity and delivery outcomes. Although neither of them could prove an additional benefit of treatment on the ongoing pregnancy and its outcome, this does not necessarily mean that pregnant women may not benefit from treatment during their pregnancy. Treatment of maternal schistosomiasis during pregnancy may be too late, to improve the hematologic state and subsequently outcomes of this ongoing pregnancy, as peak improvement of hemoglobin has been found to take up to 15 months after treatment with praziquantel.[182] However, in this same study hemoglobin already significantly increased after a time period of 6 months post-treatment, and these findings have been collected among school aged children and not pregnant women. Also, the impact of treatment during pregnancy for women infected with *S. haematobium* has yet to be investigated. Furthermore, there remain other reasons to believe that exclusion of pregnant women from treatment significantly deteriorates their future health status.

1.11 Antenatal Care in our study area

The most recent demographic and health survey of the Gabonese government (*Enquête Démographique et de Santé 2012, EDSG-II*)[201], published in 2012, reports quite a high coverage of antenatal care throughout pregnant women of the Gabonese population. The survey provides information about a total of 3700 women. The authors report that a total of 94.7% of these women have attended at least one antenatal health care visit during their pregnancy, mainly carried out by midwives (68.8%) while only one out of four women visited a doctor (24.1%). However, there appears to be a substantial gap in attendance rates between urban and rural areas (96.1% vs 85.7%, respectively), and between the different regions as well (96.2% in the capital vs. 80.9% in Ogooué-Ivindo, respectively). The Moyen-Ogooué region, in which we conducted our study, was represented at 93.9% in the upper range.

Regarding the number of visits performed during pregnancy, WHO recommends at least four visits (week 8-12, week 24-26, week 32 and week 36-38) as part of the focused antenatal care visit model (FANC-model).[202] In the EDSG-II survey, 77.6% of women, attended all these 4 recommended visits. Again, there was a notable difference between urban and rural areas (80.8% vs. 58.2%). Regarding the time point of the first attended visit during pregnancy, the authors of the EDSG-II found, that only 63.6% of the women attended one visit before completion of the first 4 months gestation, and another 25.3% within the following two months. The reported compliance to prescribed examinations for blood and urine samples was encouragingly high (97.6% and 96.0%, respectively) as well as to prescribed interventions, as the administration of oral iron supplementation and anthelmintic drugs (88.8% and 70.7%, respectively).

A potential restriction of the provided data of the EDSG-II is, that approximately two thirds (n = 2027) of the studied women were situated either in the capital Libreville or in Port Gentil, the second biggest city of the country. Only 105 women who were based in the civil district of Moyen-Ogoouée, the area where we conducted our study, were included in this survey.

In summary we can conclude out of this provided evidence, that antenatal care in Gabon seems to be widely available, well received and the compliance to preventive measures is quite high. The latter is of crucial importance, as the successful introduction of possible new test-based treatment strategies of schistosomiasis in pregnant women would largely depend on their acceptance throughout both the health workers as well as the population.

1.12 Research Question

Despite WHO recommendations and the growing evidence of both, schistosomiasis associated morbidity in mothers as well as praziquantel safety, many nations including Gabon continue to withhold systematic treatment of schistosomiasis during pregnancy, awaiting data from further clinical trials. In fact, no randomized controlled study trial has been carried out on pregnant

women infected with *S. haematobium* so far, which is the major *Schistosoma* species in Gabon, and was found to affect about 10-12% of the pregnant women in recent studies in our study region.[192,203] As a consequence, praziquantel based treatment during pregnancy has recently been reaffirmed as a neglected field of clinical research.[8] If praziquantel could also be proved to positively impact maternal and/or infant health, its use could then be prioritized and included in regular antenatal care in endemic regions. Our study therefore aimed to investigate safety of praziquantel used during second trimester of pregnant women infected with *S. haematobium* and its effect on maternal and newborn health. We hence posed the following research questions: ***Is the use of praziquantel for the treatment of Schistosoma haematobium infection in pregnant women safe for the mother and the unborn child? How efficient is the treatment during pregnancy in terms of parasitic cure? And can we decrease maternal and child morbidity by treating schistosomiasis during pregnancy, compared to the treatment after delivery?***

2 Methods

2.1 Study design

This study is part of the EDCTP funded project entitled *Fast and Reliable Easy-to-Use Diagnostics for Eliminating Bilharzia in Young Children and Mothers (freeBILy)*, which aims to evaluate the use of CCA and CAA based antigen tests for the diagnosis of *Schistosoma* infections in pregnant women and their newborns.[204] Our trial has been embedded as a sub-study of the *freeBILy-Gabon trial,*[205] a set of interlinked prospective observational studies conducted in Lambaréné (Gabon), which aims to investigate the performance of the UCP-LF test for detection of CAA in pregnant women infected with *S. haematobium* and their infants.

This sub-study has an interventional, randomized, controlled and single (investigator) blinded design. Its purpose was to assess the safety, efficacy, and impact of praziquantel for the treatment of *S. haematobium* infection during pregnancy, on maternal and child health outcomes. Participants found positive for *S. haematobium* were randomized by a 3:1 ratio into an intervention group (treatment during pregnancy) and a parallel control group (treatment 6 months after delivery), respectively. After intervention patients of both groups were followed up for the evaluation of treatment success for a period of seven weeks. Immediate reactogenicity was assessed 1h and 48h after the drug administration. During weekly follow up for urine sampling, we also recorded adverse events during the pregnancy. Urine samples collected during follow up were used to measure parasitological efficacy of praziquantel treatment. At delivery we evaluated the outcome of the pregnancy by assessing gestational age, maternal and newborn wellbeing, and congenital abnormalities. Impact on maternal and child morbidity was measured by maternal hemoglobin level at delivery and newborn anthropometric parameters at birth.

2.2 Study area and study population

Our study took place at the *Centre de Recherches Médicales de Lambaréné* (CERMEL)[206] in Lambaréné (Gabon) and the surrounding villages. Gabon is an upper middle-income country in central Africa, with a population of about 2.1 million habitants and a mean gross domestic product (GDP) per capita of 6,830$, although it is estimated that 30% of the population live on less than the minimum guaranteed wage of 150$ per month.[207] Lambaréné, the capital of the central province Moyen-Ogoué, counted about 38.775 habitants in 2013[208] and is located near the equator. The main economic sector in the region is fishery. Lambaréné has a tropical climate with a mean annual temperature of 26.5°C (19.9°C – 32.5°C monthly range), a mean humidity of 82% (81% - 84% range) and a precipitation range from 166mm-393mm in rainy season (October – May) and from 3.2mm-71mm in dry season (June – September).[209] The study area is known, to be endemic for *S. haematobium*, with previous studies showing a prevalence in pregnant women of approximately 10%.[192,203]

Screening of participants was carried out in three local antenatal care centers (ANC) in Lambaréné: The *Albert-Schweitzer Hospital*, the *Hôpital General* and *Hôpital Georges-Rawiri.* Screening and collection of samples at baseline was carried out by a study nurse. Women were eligible for screening if they provided signed informed consent (by the participant or by a legal guardian, if the participant is a minor), were pregnant with a gestational age of 16 - 30 weeks at inclusion (based on the reported date of the last menses), were willing to deliver in one of the two maternities in Lambaréné (*Albert-Schweitzer Hospital* and *Hôpital Georges-Rawiri)* and had no plans to move out of the study area in the upcoming 24 months. Exclusion criteria were a known previous history of complicated pregnancy and reported previous uptake of praziquantel during the current pregnancy. Women with a gestational age less than 16 weeks were scheduled to return later for inclusion.

2.3 Sampling and Data collection

At their ANC visit, we collected general demographic data from the participating women as well as a medical and obstetrical history including information about the current and previous pregnancies. A general physical examination was conducted by one of the study physicians to determine the general health status. Weight, height, and other anthropometric measurements were collected. We conducted an interviewer-assisted paper-based survey to assess socioeconomic status and to determine our participants' knowledge, attitudes, and practices regarding schistosomiasis (the latter results will be reported elsewhere). To evaluate the socioeconomic status, we used a score from 0-15 points based on five items representing education, profession and the personal and household economic situation. Each item could be rated with 0-3 points and the points were summed without additional weighing. The items collected were academic qualification, profession, monthly income, household water source and household toilet facility. Academic qualification was categorized following the national school system into none – primary – secondary and university graduation. The professions were categorized in ascending order into none - farmer or fisher – student and civil servant or another public employee. The monthly income was based on the household income, as most women were not able to differentiate personal income from their household income. Income was categorized into none or unknown - <150.000FCFA – 150-300.000 FCFA and more than 300.00 FCFA. Household water source was categorized in ascending order into river – rain – well and public tab water. The household facility was categorized into none – public/shared – traditional (private) and water closet.

At their first visit, women were asked to provide a blood sample for investigation of hematologic parameters, and concomitant malaria and filaria infection. For determination of *S. haematobium* infection we collected a urine sample of fresh midstream urine (minimum 10ml). On the consecutive days another two urine samples were collected at the homes of the participants by a field worker of the study team. This was done to increase sensitivity of the urine-based microscopy. Within these three days, women were also asked to provide one stool sample, to

control for the impact of geohelminths, notably hookworm infection. All samples were collected in a clean and dry container and transported and tested within a few hours of the same day in the parasitology laboratory of our research center (CERMEL).

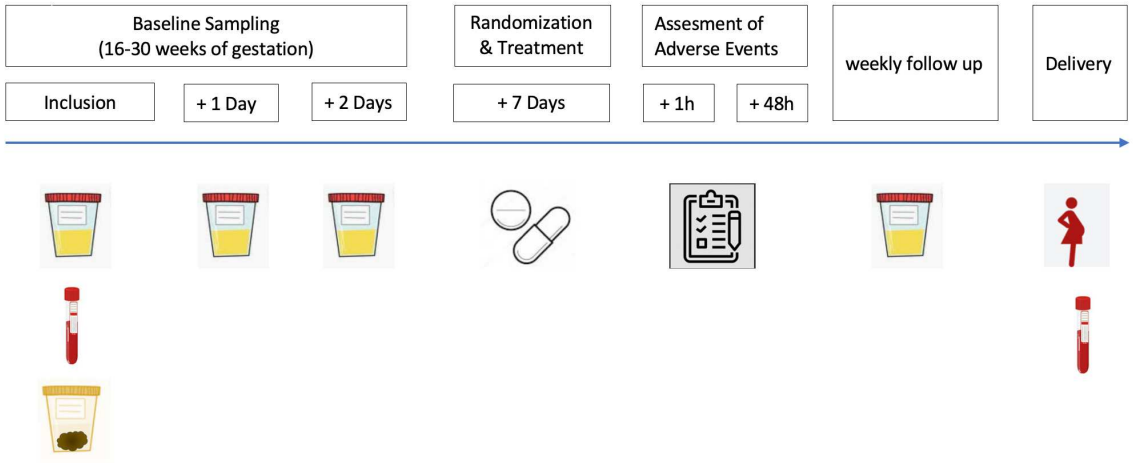


Figure 1: Overview of the sampling during the trial.

(Yellow containers indicate urine sample, tablets indicate the study intervention, clipboard indicates assessment of side effects, brown containers stool sample, red tubes blood sample and pregnant pictogram the delivery.

2.4 Diagnostics

2.4.1 Urine Sample

Diagnosis of *S. haematobium* infection was based on positive results for either *Schistosoma* egg detection by microscopy and/or UCP-LF test for circulating anodic antigen (CAA). Egg detection by microscopy based on urine samples is the current standard of diagnosing urogenital schistosomiasis[7] and allows species identification. However, the number of excreted eggs is often low and shows a high day-to-day fluctuation. We therefore also measured the level of CAA in urine, which is a genus specific antigen (see also chapter *1.6 Diagnostics*) to increase our sensitivity. Because *S. haematobium* and *S. intercalatum* are the only two endemic *Schistosoma* spp. in Gabon[7], and we controlled for *S. intercalatum* infection by examination of stool samples, we could also diagnose

infection with *S. haematobium* based on positive results for CAA detection and negative stool samples.

For *Schistosoma* egg detection the urine sample was thoroughly mixed, to stir up eggs that have possibly settled as sediment, before passing ten (10) ml of urine through a 12 µm filter (Whatman Nuclepore) using a clean syringe. The filter was then carefully placed on a slide and read using an optical microscope at 10x magnification. Reading was executed by two independent competent readers, and the result was based on the mean value of both readings. Results with a reading range greater than 20% were reviewed by a third reader, to ensure quality control. The test was considered positive if at least one *S. haematobium* egg was found. The result was given by number of eggs per 10 ml of urine. Intensity of infection was expressed as negative (0 egg/10 ml urine), light (1-49 eggs/10 ml urine) or heavy (≥ 50 eggs/10 ml urine) following WHO classification.

For analyzing the level of CAA in the urine samples we used the highly sensitive UCP-LF test, developed, and provided by the *Leiden University Medical Center* (LUMC Leiden, the Netherlands). Details of test procedures have been published by Corstjens et al.(2008,2014).[63,65] Briefly, 417µl of fresh or stored (-20°C) urine were concentrated (ultra-centrifugation Amicon 500 filter units, Merck, 10kD cut-off) and pre-treated with 1/6 volume of trichloroacetic acid (TCA). The sample was mixed with an assay buffer that included UCP-particles conjugated with mouse monoclonal CAA-antibodies. After 60min incubation in an orbital shaker, mixture was added in a well of a microtiter plate and a UCP-LF strip was placed in the same well. Strips included a test and a positive control line. After 24h, test-strips were analyzed by a point of care multistrip benchtop reader (Upcon; Labrox Oy, Turku, Finland). Test line signals were normalized to control line signals of each individual strip and results were expressed as a ratio. Threshold of positivity was set at ≥ 2 pg/ml CAA.

2.4.2 Stool Sample

Stool samples were used to investigate the presence of highly prevalent soil transmitted helminths by Kato-Katz[51] technique. Adegnika et al.(2010) reported prevalence of geohelminths in pregnant women in our study area to be as high as 33% for *Ascaris lumbricoides*, 24% for *Trichuris trichuria* and 10% for *Necator americanus* (hookworm),[203] whereas *Schistosoma intercalatum* is only rarely observed in the region.[210] The Kato-Katz method is described in more detail in the WHO bench aids for parasitological diagnosis.[211] Briefly, a small amount of feces is sieved through a metal strainer to sort out fiber and other organic material before placing some of the cleansed material through the hole of a 50mg-standardized template. After removing the template, a pre-soaked cellophane strip is placed over the sample and firmly pressed so that the fecal material will evenly spread. The slide is then read using an optical microscope at 10x magnification. Reading procedures for stool examinations were the same as for urine samples, described above. Because in Kato-Katz technique eggs of hookworm are rapidly cleared and no longer visible after 30-60 minutes, slides were read directly after the preparation.

2.4.3 Blood Sample

Venous blood was collected from the antecubital vein into an Ethylenediaminetetraacetate (EDTA) tube for hematologic and parasitological examination. For diagnostics of concomitant malaria parasite infection (*P. falciparum* and non-falciparum) Giemsa-stained thick- and thin blood smears were performed. For the investigation of filarial parasites (*Loa Loa* and *Mansonella perstans)* we conducted a thin blood smear after lysing the red blood cells with saponin (saponin-leucoconcentration assay). Procedures ensuring quality of microscopic reading were the same for malaria and filaria examinations as described above for urine diagnostics. The EDTA tube was used to perform a full blood count including hemoglobin level and was measured by a Yumizen H500 and Pentra XLR.

2.5 Intervention

Women randomized into the intervention group received a single dose of praziquantel (Biltricide® 600mg, Bayer) within 7 days after detection of *Schistosoma* infection. The dosage followed WHO recommendations of single administration of 40 mg/kg body weight, max 2.400mg, and tended to imitate the situation during a mass drug administration (MDA) campaign. Prescription was carried out by a study doctor and tablets were administered under the supervision of a qualified study nurse. Because bioavailability of praziquantel increases with concomitant food administration,[38] all women were asked to take a meal before or during the treatment visit. Women randomized to the control group did not receive the initial PZQ treatment but six months after delivery, using the same dosage and regimen as used in the intervention group.

2.6 Randomization and blinding

Women who met all eligibility criteria were randomized by a 3:1 ratio into two parallel groups: single-dose treatment during pregnancy and late treatment (same treatment regimen) 6 months after delivery. Randomization was conducted by the study coordinator through a randomization list, based on the block of four. All investigators (study physicians and laboratory staff) were blinded from the treatment allocation. After participants were tested positive, they were invited for the treatment visit. Administration of the tablets for the intervention group was carried out by an unblinded study nurse, who was directly instructed from the coordinator. Participants were not blinded for their treatment. For details of blinding procedure see *Figure 2*.

2.7 Outcomes

2.7.1 Safety

Primary outcome for safety was the rate of adverse events (AE) reported by study participants following the treatment. To be able to compare possible adverse events of those taking treatment versus physiological symptoms during pregnancy, both intervention and control group were asked 1 hour and 48 hours

after the intervention visit by a blinded investigator about the appearance of symptoms. Time points reflect the pharmacokinetics of the drug with a relative fast absorption (T_{max} 2-2.6h), and elimination (renal clearance of 80% in 4 days, of which 90% occurs in 24h).[38] This allowed to record most of the possible adverse events within the observation time frame. However, adverse events that were still present after 48h were further observed during follow up.

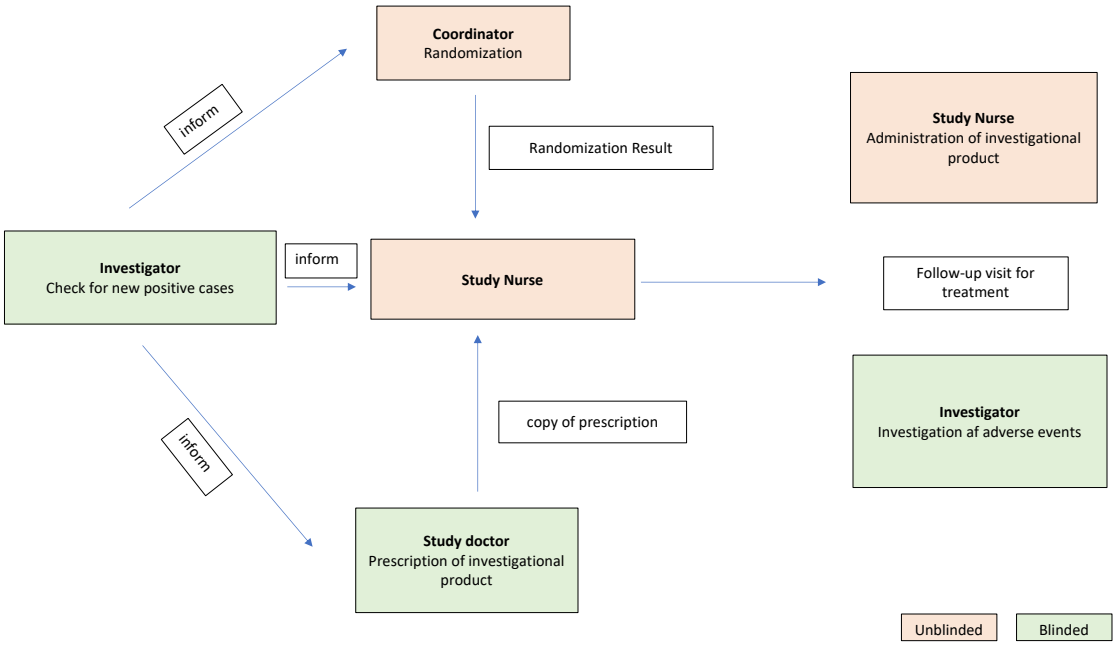


Figure 2: Flowchart for randomization and blinding

(Colors represent study personal who was blinded or not)

At time points 1h and 48h post-treatment we investigated blood pressure, heart frequency and temperature and carried out a standardized survey collection information on side effects that have previously been identified in literature.[7,39,46] These included headaches, stomachache, nausea, vomit, diarrhea, yawning, dizziness, drowsiness, shivering, pruritus, allergic rash, anorexia, myalgia, fatigue, palpitations, other signs of allergy and recent history of fever. Severity of adverse events was classified into mild, moderate or severe on the basis of the observations on both time points: Adverse events were defined as mild if

symptoms occurred only at one of both time points and were presented with low intensity, moderate if symptoms occurred at one of both time points with a moderate intensity or at both time points with a low intensity, and severe if symptoms occurred at both time points with a moderate to high intensity.

Secondary safety outcomes regarding the pregnancy were assessed by the rates of miscarriage, stillbirth, and preterm birth. Miscarriage was defined as fetal death before and stillbirth as fetal death after 20 completed weeks of pregnancy, respectively. Preterm birth was defined as gestational age less than 37 completed weeks of gestation. Additionally, prematurity was classified into categories as extreme preterm (birth before 28 completed weeks of gestation), very preterm (28 - 32 weeks of gestation) and moderate preterm as birth between 32 and 37 completed weeks of gestation, following the definition of WHO.

2.7.2 Efficacy

WHO recommends the use of ERR as appropriate parasitological indicator for evaluating efficacy of praziquantel, using the same diagnostic methodology as for screening.[212] The optimal time interval between therapy and follow-up is given as 14-21 days, since at this time worms that may have re-invaded during a possible reinfection cannot yet shed their own eggs. Since we collected up to 3 urine samples during screening, we also based our calculation of ERR on the mean of 3 samples collected during follow-up. For this purpose, we collected urine samples on days 14, 21 and 28 after the treatment. In contrast to recommendations of WHO, we decided to calculate ERR based on geometric and not arithmetic mean. We therefore used the following equation:

$$Egg\ Reduction\ Rate RR\ (\%) = 100\ x\ \left(1 - \frac{geometric\ mean\ egg\ count\ during\ follow\ up}{geometric\ mean\ egg\ count\ during\ screening}\right)$$

(Equation 1)

Threshold of effective treatment is set at 90% ERR by WHO.[212] Furthermore we calculated CR, which was defined as proportion of infected individuals who were still shedding any eggs in urine at the follow up visits.

2.7.3 Impact on Morbidity

Primary outcome for impact on maternal morbidity was the maternal hemoglobin level at delivery, measured in a venous blood EDTA sample. Additionally, we calculated the rate of maternal anemia (anemia was defined as hemoglobin level < 11 g/dl). Since hemoglobin level is dependent on many factors, we included several predefined confounders in our analysis including maternal malaria and hookworm infection, baseline hemoglobin level, baseline schistosomiasis infection intensity and socioeconomic factor. As loss of blood in urine is a possible mechanistic link between urogenital schistosomiasis and anemia, we also investigated differences in maternal haematuria between the groups, based on a urine sample at day 49 post-treatment. Maternal haematuria was assessed by urine dipstick (Combur test, Roche Diagnostics).

We also performed two subgroup analyses to evaluate the effect of treatment on the hemoglobin level at delivery. First, we stratified participants by the intensity of *Schistosoma* infection at baseline into three groups according to the WHO classification: 1) microscopically negative and only positive by UCAA, 2) mild infection (less than 50 eggs/10 mL urine) and 3) heavy infection (more than 50 eggs/10 mL urine). We then compared the effect of a treatment vs no treatment on maternal hemoglobin levels at birth within the stratified groups, following the hypothesis that the effect of praziquantel might be higher for participants with higher parasitic burden. In a second subgroup analysis we stratified all our followed-up participants into two groups: those with successful parasitic clearance and those with persistent excretion of eggs in their urine, and then compared the mean maternal hemoglobin.

Weight of the newborn, as the primary parameter for impact on newborn morbidity, was measured using a SECA scale with ± 0.2g precision. Anthropometric parameters were assessed by a trained study nurse. We also

calculated rates of LBW, which was defined as a weight < 2500g. Since the birth weight is also dependent on multiple factors, we recorded confounding variables that were defined from previous research[188] and considered them in the analysis. These variables included maternal malaria or hookworm infection, baseline schistosomiasis infection intensity, maternal height and weight at study inclusion, parity, newborn sex, and maternal socioeconomic status. Because LBW is a simplistic parameter for newborn health we also included SGA, as a possible indicator IUGR into our analysis. SGA was calculated using anthropometric parameters at birth and gestational age (based on the last date of menses), and based on gestational age adjusted birthweight, using the multi-racial Williams curve.[213] Additionally, we compared height and cranial perimeter of the newborns.

2.8 Sample Size and Power Calculation

As our trial was embedded into the freeBILy-GAB study, we were limited with a predetermined sample size. The freeBILy-GAB trial was targeted with 1000 participants to be screened for *S. haematobium*, based on previous findings with ~ 10% prevalence. This led to an estimated target size of 100 participants positive for schistosomiasis. For the primary endpoint of safety (rate of adverse events) we did not perform a power calculation.

We performed a power calculation for our primary endpoint of maternal morbidity, the hemoglobin level of mothers at delivery. For the estimation of a treatment impact on hemoglobin level, we searched literature for reported differences in hemoglobin level of pregnant women infected with *S. haematobium* compared to those uninfected. We found three observational studies, that reported differences in mean (±SD) hemoglobin between women infected compared to those uninfected: 9.3 (1.3) vs 9.5 (1.5)[30] ; 9.26 (1.18) vs 10.08 (1.22)[172] and 10.1(1.7) vs 11.3 (1.3).[142] Based on these observations we chose an estimated mean of 9.54 (±1.3) without and 10.29 (±1.3) following treatment. Using a (type-I) α-error of 0.05 and the 3:1 randomization ratio we calculated a power of 80% using the following formula:

$$1-\beta = \Phi\left(\frac{|\mu_A - \mu_B|\sqrt{n_A}}{\sqrt{\sigma_{2A} + \sigma_{2B}/\kappa}} - z_{1-\alpha}\right)$$

with:

$\kappa = n_A/n_B$ is the matching ratio
μA and μB are the means and σA and σB the standard deviations, respectively.
Φ is the standard normal distribution function
α is Type I error
$1-\beta$ is Type II error

2.9 Data management

All data collected data were recorded on paper form case report files (CRF). Laboratory results were collected on standardized result sheets, following quality control guidelines of CERMEL, and used as source documents. All data were entered into a REDCap[214] database software electronic data capture, by a qualified data-entry team, and were double-checked by one of the investigators to ensure quality control. The freeBILy-Gabon trial also included the involvement of an independent Data Safety and Monitoring Board (DSMB).

2.10 Statistical methods

All statistical analyses were performed using the R statistical software[215] (version 4.1.2), and were performed based on a modified per protocol population (participants who truly received the drug vs. no drug uptake). Significance level for all calculations was set at $p < 0.05$ considered to be statistically significant. Continuous data were tested for normal distribution using the Kolmogorov-Smirnov test. Differences between continuous variables were reported in means and significance was calculated using t-test if normally distributed, otherwise Wilcoxon Rank Sum test was used. Categorical variables were compared calculating odds ratio (OR), significance was calculated using fisher's exact test. For comparison of adverse events among study groups we used small sample adjustment to calculate OR, using the "epitools" package. For adverse events we also calculated the relative risk for each symptom. To be able to calculate all ratios we added +0.5 to every field within the four-field table, knowing that this is

not an optimal approach. Effects of treatment on the vital parameters assessed before and after drug administration were compared to the control group using a mixed model with fixed effects and random intercept due to repeated sampling of parameters (baseline, after 1h and after 48h). For the comparison of our primary outcome of newborn morbidity (birth weight) we used multiple linear models. For the comparison of our primary outcome of maternal morbidity (hemoglobin level at delivery), a mixed model was used, again due to repeated sampling during the study (baseline and delivery). Possible confounders for both models were predefined and included in a first model. We then performed stepwise elimination of covariables to find the model with the best fit using the *Akaike information criterion* (AIC). Models with lowest AIC were then selected for final analysis. For calculation of confidence intervals of ERR (*(Equation* 1*)* we used the bootstrap method (based on 1000 repeats) as a possible model-free approach for measuring drug efficacy described by Walker et al. (2016).[216]

2.11 Ethical Approval

The FreeBILy Gabon trial was registered at clinicaltrials.gov (NCT03779347) and was reviewed and approved by the National Ethics Committee of Gabon (PROT N°039/2018/SG/CNE), the Ethics Committee of the University of Tübingen (597/2018BO1), and the Institutional Ethics Committee at CERMEL/Gabon (N° 017/2018). Sponsor for this trial was the *Centre de Recherches Médicales de Lambaréné* (CERMEL), the funding for the FreeBILy Gabon trial was provided by the *European Developing Countries Clinical Trials Partnership* (EDCTP2 RIA2016MC-1626). Additional funding for the work on this thesis was provided by *Deutsche Gesellschaft für Infektiologie / Meta-Alexander Stiftung* (individual research grant) and *Rosa-Luxemburg Stiftung* (individual study scholarship).

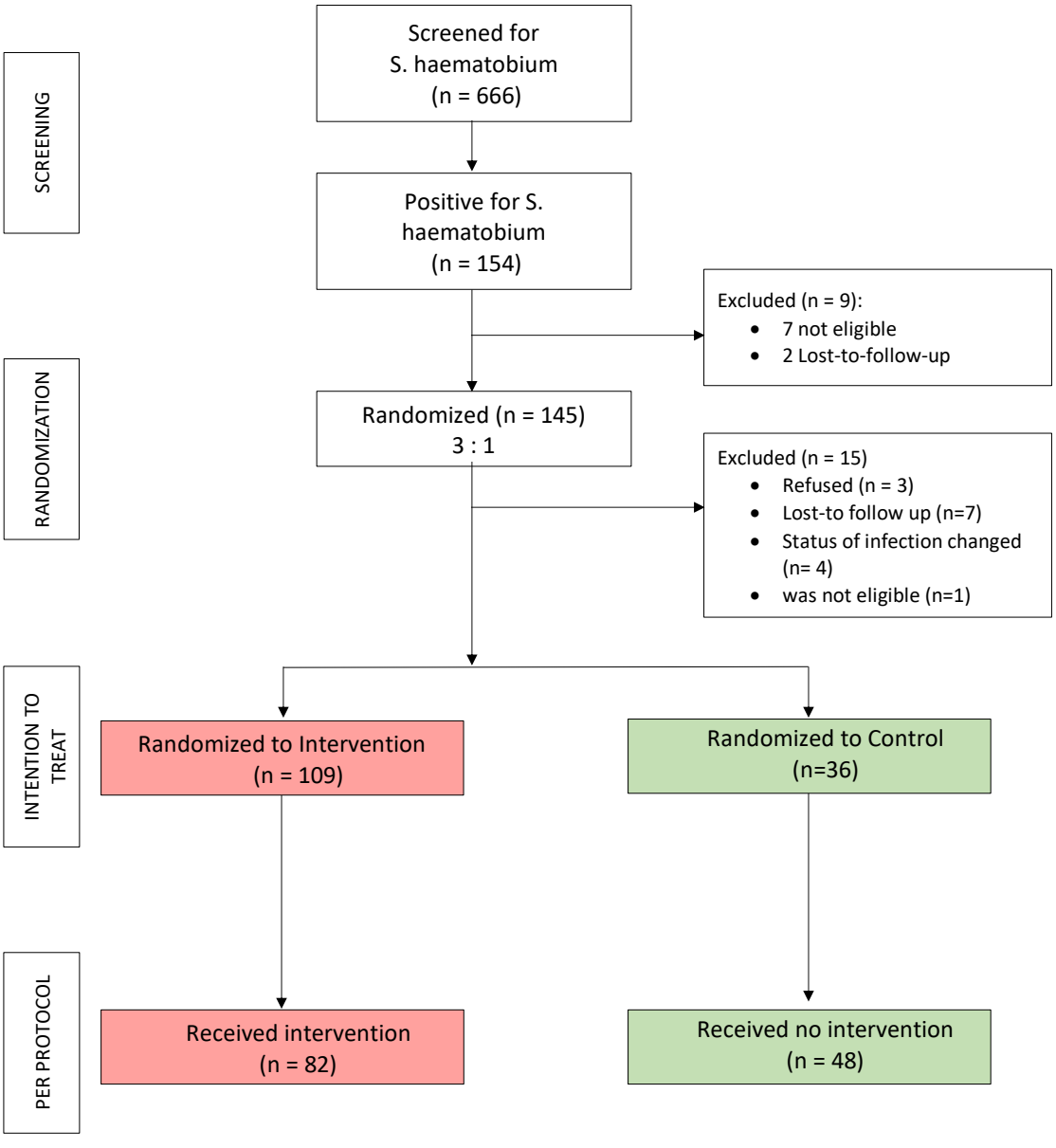
3 Results

3.1 Screening and Follow-Up of Participants

We screened 666 women for *S. haematobium* of whom we found 154 (23.1%) to be positive for *S. haematobium*. From these 58 were only positive by Microscopy, 52 were positive for both Microscopy and UCP-LF CAA and 44 were positive only for the latter. We excluded 9 women who had been screened for infection but were already past 30 weeks of gestation. 145 women were found to be eligible to continue and were randomized into intervention (n=109) and control group (n=36). After randomization there were again 15 cases of exclusion because of refusal (n=3), lost to follow-up (n=7), change in infection status after the laboratory procedures of CAA detection have been reviewed at the early stage of the trial (n=4) and non-eligibility (n=1). On the remaining 130 participants we carried out a modified per-protocol analysis where the group of women that received the intervention was n=82 and women in the control group were n=48. Out of these we were able to analyze a total of 108 participants for safety, 101 participants for efficacy and 103 women for maternal and newborn morbidity. For a detailed overview see also *Figure 3*.

3.2 Study Population characteristics

Most women showed mild or moderate infection intensity of schistosomiasis. Proportion of heavily infected women in the intervention and control group was 16% and 12%, respectively. In both groups, most women were anemic at baseline (71% in the control vs. 74% in the intervention group). Most characteristics at baseline were equally distributed among both groups. Nevertheless, there were some differences, as clinically relevant haematuria differed between the groups (28% the intervention group vs. 17% in the control group) and only women in the intervention group were found to be positive for hookworm infection (n=8, 10%). We controlled for this co-variable in both of our primary endpoints for impact on morbidity. Also mean CAA levels were higher in the intervention group (110 pg/ml ± 548) compared to the control group (72.42 pg/ml ± 168). We discuss this further in chapter 4.1 - *Discussion: Study population*. For detailed information of the baseline characteristics see *Table 1.*



Endpoints	Intervention		Control	Total
Safety	N = 81		N = 27	N = 108
Efficacy	N = 74		N = 27	N = 101
Morbidity	N = 66		N = 37	N = 103

Figure 3: Study Flowchart.

(Colors represent the affiliation to the respective study group.)

Table 1: Baseline characteristics of study participants.

(BMI = body mass index, kg = kilogram, m = meter, g = gram, dl = deciliter, ml = milliliters, pg = picogram)

	Total (N=130)	Control (N=48)	Intervention (N=82)
Age (years)			
<16	7 (5 %)	4 (8 %)	3 (4 %)
[16-18]	16 (12 %)	4 (8 %)	12 (15 %)
]18-30]	68 (52 %)	27 (56 %)	41 (50 %)
>30	39 (30 %)	13 (27 %)	26 (32 %)
BMI (kg/m^2)			
<18.5	6 (5 %)	2 (4 %)	4 (5 %)
]18.5-25]	93 (72 %)	34 (71 %)	59 (72 %)
[25-30]	24 (18 %)	11 (23 %)	13 (16 %)
>30	6 (5 %)	1 (2 %)	5 (6 %)
Missing	1 (0.8%)	0 (0%)	1 (1.2%)
Gestational Age (weeks)			
[16-20[	44 (34 %)	21 (44 %)	23 (28 %)
]20-24]	34 (26 %)	12 (25 %)	22 (27 %)
]24-30]	52 (40 %)	15 (31 %)	37 (45 %)
Number of Gravidity			
Primigravida	38 (29 %)	15 (31 %)	23 (28 %)
Multigravida	90 (69 %)	32 (67 %)	58 (71 %)
Missing	2 (1.5%)	1 (2.1%)	1 (1.2%)
Number of Parity			
Nulliparous	31 (24 %)	12 (25 %)	19 (23 %)
Multiparous	90 (69 %)	32 (67 %)	58 (71 %)
Missing	9 (6.9%)	4 (8.3%)	5 (6.1%)
Mean Hemoglobin (SD) g/dl	10.13 (1.3)	10.02 (1.2)	10.19 (1.3)
Missing	4 (3.1%)	2 (4.2%)	2 (2.4%)
Anemia levels [g/dl]			
Severe <7	1 (1 %)	1 (2 %)	0 (0 %)
Moderate [7-9[	24 (18 %)	9 (19 %)	15 (18 %)
Mild [9-11]	70 (54 %)	24 (50 %)	46 (56 %)
None >11	31 (24 %)	12 (25 %)	19 (23 %)
Missing	4 (3.1%)	2 (4.2%)	2 (2.4%)
Haematuria			
None	61 (47 %)	18 (38 %)	43 (52 %)

	Total (N=130)	Control (N=48)	Intervention (N=82)
Light	35 (27 %)	14 (29 %)	21 (26 %)
Heavy	10 (8 %)	3 (6 %)	7 (9 %)
Missing	24 (18.5%)	13 (27.1%)	11 (13.4%)
***S. haematobium* eggs / 10ml**			
Negative	37 (28 %)	17 (35 %)	20 (24 %)
[1 - 50 eggs / 10ml]	74 (57 %)	25 (52 %)	49 (60 %)
[> 50 eggs / 10ml]	19 (15 %)	6 (12 %)	13 (16 %)
Circulating anodic antigen	95.62 (442.0)	72.42 (168.8)	110.07 (548.4)
Missing	18 (13.8%)	5 (10.4%)	13 (15.9%)
Parasitic Co-Infection			
Negative	94 (72 %)	36 (75 %)	58 (71 %)
Malaria	23 (18 %)	8 (17 %)	15 (18 %)
A. lumbricoides	5 (4 %)	1 (2 %)	4 (5 %)
T. trichuris	10 (8 %)	3 (6 %)	7 (9 %)
Hookworm	8 (6 %)	0 (0 %)	8 (10 %)
Strongyloides	0 (0 %)	0 (0 %)	0 (0 %)
Missing	2 (1.5%)	2 (4.2%)	0 (0%)

3.3 Safety

3.3.1 Drug Side Effects

We found a significant difference in proportions of individuals that reported any symptoms following drug intake in the intervention group compared to the group of none treated schistosome infected pregnant women (76.5% vs. 44.4%; OR: 3.58 [1.61-9.79]; p = 0.004). The most common symptoms following drug uptake were dizziness (58.0%), fatigue (43.2%) and nausea (42.0%) followed by vomiting (27.2%) and headaches (25.9%), but only dizziness, nausea and vomiting were significantly increased (OR: 5.10 [1.96;18.2] p = 0.001; OR: 4.72 [1.49;23.8] p = 0.009; OR: 8.07 [0.99;292] p = 0.003, respectively). All side effects with exact number of frequencies and calculation of odds ratio are found in

Table 2: Frequency and Odds Ratio of Adverse Events. We also calculated the relative risk for side effects in the intervention group, which was significantly higher for having any symptom at all, nausea, and dizziness, whereas vomiting

and fatigue slightly missed the significance level. Details are provided in *Figure 4: Adverse Events with Frequency and Relative Risk.* In the group of none treated schistosome infected pregnant women fatigue (25.0%), yawning (18.2%), dizziness (17.4%) and headaches (17.4%) have been reported most frequently.

Table 2: Frequency and Odds Ratio of Adverse Events.

(OR = odds ratio. OR and 95% CI have been calculated with small sample adjustment using the epitools package.)

	Total (N=108)	**Control (N=27)**	**Intervention (N=81)**	**OR [95% CI]**	**p***
Allergic-Reaction	1 (0.97%)	0 (0.00%)	1 (1.23%)	0.27 [0.03;21.3]	1.000
Anorexia	3 (2.91%)	0 (0.00%)	3 (3.70%)	0.84 [0.10;40.3]	1.000
Shivering	1 (0.97%)	1 (4.55%)	0 (0.00%)	0.00 [0.00;2.24]	0.214
Diarrhea	7 (6.80%)	2 (9.09%)	5 (6.17%)	0.43 [0.12;2.84]	0.640
Dizziness	51 (49.0%)	4 (17.4%)	47 (58.0%)	5.10 [1.96;18.2]	**0.001**
Fatigue	41 (39.0%)	6 (25.0%)	35 (43.2%)	1.91 [0.80;5.88]	0.171
Fever	3 (2.91%)	1 (4.55%)	2 (2.47%)	0.26 [0.06;3.61]	0.518
Headaches	25 (24.0%)	4 (17.4%)	21 (25.9%)	1.31 [0.49;4.80]	0.569
Allergic rash	1 (0.97%)	0 (0.00%)	1 (1.23%)	0.27 [0.03;21.3]	1.000
Myalgia	1 (0.97%)	0 (0.00%)	1 (1.23%)	0.27 [0.03;21.3]	1.000
Nausea	36 (35.0%)	2 (9.09%)	34 (42.0%)	4.72 [1.49;23.8]	**0.009**
Palpitations	7 (6.80%)	0 (0.00%)	7 (8.64%)	2.05 [0.25;82.4]	0.341

	Total (N=108)	Control (N=27)	Intervention (N=81)	OR [95% CI]	p*
Pruritus	2 (1.94%)	0 (0.00%)	2 (2.47%)	0.55 [0.07;30.6]	1.000
Drowsiness	9 (8.74%)	1 (4.55%)	8 (9.88%)	1.14 [0.27;10.0]	0.680
Stomachache	12 (11.7%)	2 (9.09%)	10 (12.3%)	0.93 [0.28;5.21]	1.000
Vomiting	22 (21.4%)	0 (0.00%)	22 (27.2%)	8.07 [0.99;292]	**0.003**
Yawning	18 (17.5%)	4 (18.2%)	14 (17.3%)	0.74 [0.27;2.86]	1.000
Any symptom	74 (68.5%)	12 (44.4%)	62 (76.5%)	3.58 [1.61;9.79]	**0.004**

All side effects have been classified as “mild” or “moderate”. Symptoms most frequently reported to be “moderate” in the intervention group were dizziness (22.2%), nausea (19.8%) and fatigue (13.6%). Only moderate dizziness was significantly increased compared to the control group (OR: 9.83 [1.34;440] p = 0.009). Proportions of adverse events subdivided by severity are depicted in *Figure 5: Proportions of adverse events for each study group.* Most of these symptoms have not only been reported 1h after treatment but appeared also within the 48h time frame following treatment. *Table 3: Temporal occurrence of side effects* gives an overview over the distribution of symptoms following treatment. Time points +1h and +48h reflect the moment of assessing side effects by our study staff. In each case, all side effects were resolved latest after 48 hours. The occurrence of the most common side effects, dizziness, headache, fatigue, and nausea, within one hour and within 48 hours was the same. Vomiting was more frequent within 48h, but there were 8 cases of vomiting within 1h after drug administration. The relevance of vomiting after drug administration is further discussed in the chapter *4.2 - Discussion: Safety.*

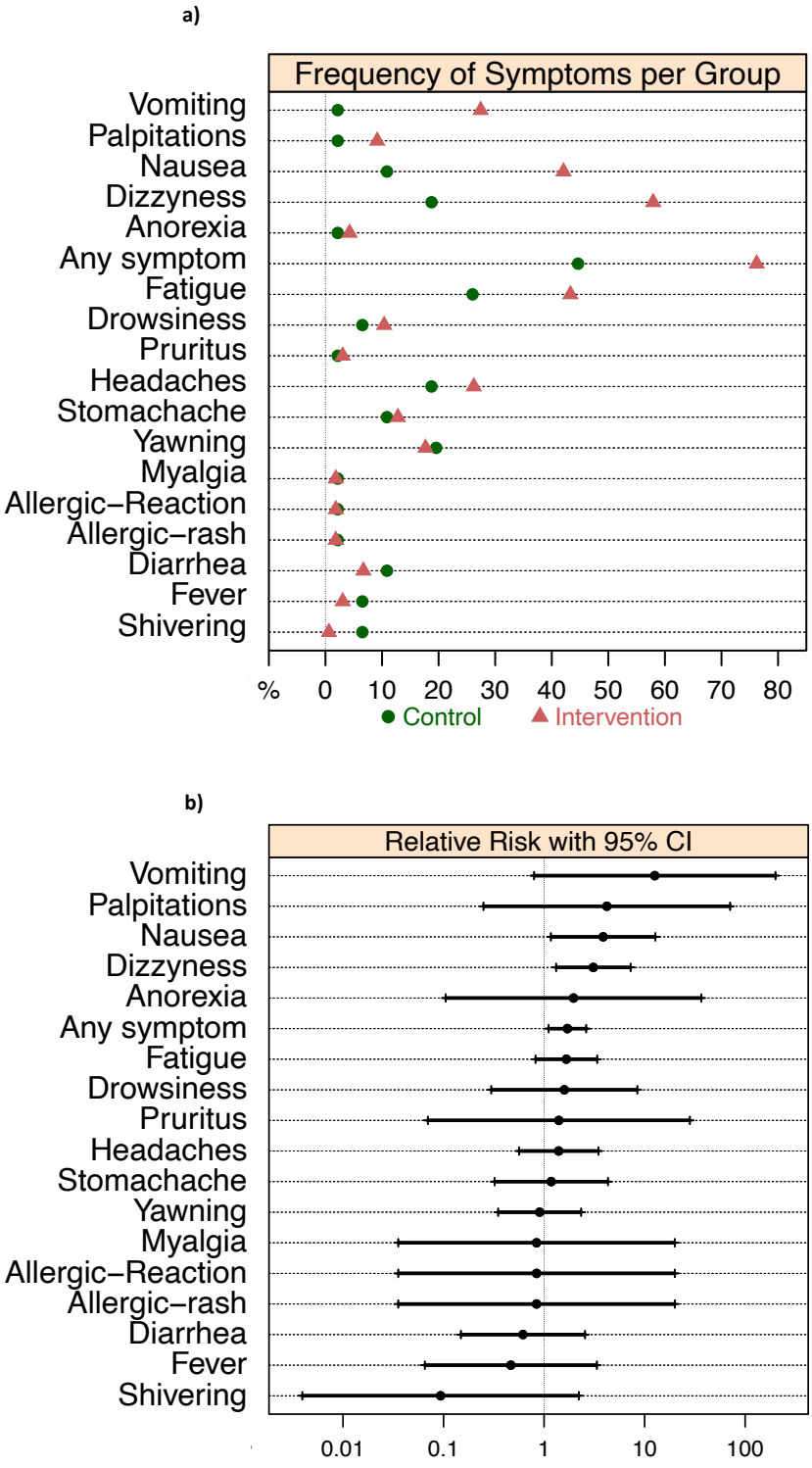


Figure 4: Adverse Events with Frequency and Relative Risk.

a.) Frequency of symptoms by study group in percent. Symptoms are sorted in descending order by Relative Risk. Colors represent study groups. b.) Relative Risk of symptoms in the intervention group compared to the control group on a logarithmic scale. Black lines indicate 95% Confidence intervals (CI) and were calculated using the HH package in R.)

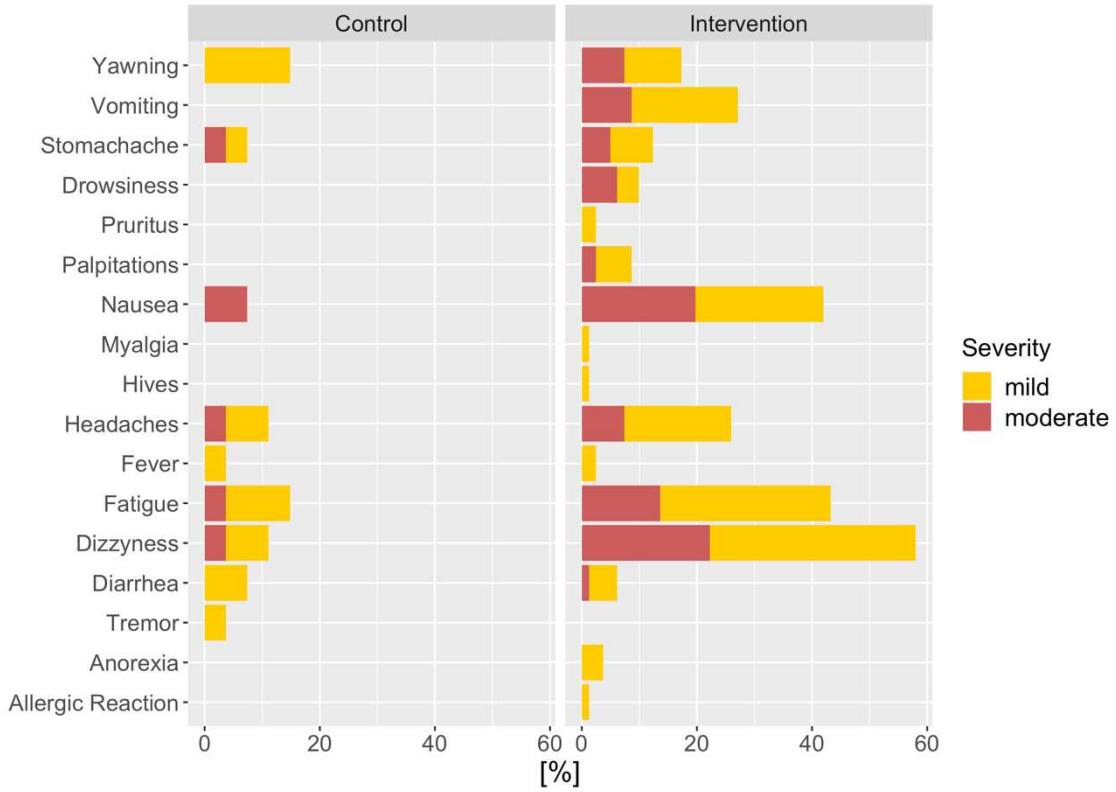


Figure 5: Proportions of adverse events for each study group.

(Colors represent different severity of symptoms.)

Table 3: Temporal occurrence of side effects

(Counts represent number of observations. Each participant underwent two observations. Time points (+1h / +48h) represent time after treatment when individuals have been assessed for side effects. OR = odds ratio. OR and 95% CI have been calculated with small sample adjustment using the epitools package.)

Symptoms	Control (N = 49)	Intervention (N = 162)	OR [95% CI]	p
Headaches				
+1h	3 (6.12%)	14 (8.64%)	1.12 [0.40;4.55]	0.565
+48h	2 (4.08%)	12 (7.41%)	1.28 [0.40;6.61]	0.423
None	44 (89.8%)	136 (84.0%)	Ref.	Ref.
Stomachache				
+1h	0 (0.00%)	8 (4.94%)	2.49 [0.30;94.7]	0.119
+48h	2 (4.08%)	4 (2.47%)	0.42 [0.12;2.76]	0.600
None	47 (95.9%)	150 (92.6%)	Ref.	Ref.
Nausea				
+1h	2 (4.08%)	22 (13.6%)	2.83 [0.92;13.6]	0.032
+48h	1 (2.04%)	22 (13.6%)	4.25 [1.09;31.8]	**0.009**
None	46 (93.9%)	118 (72.8%)	Ref.	Ref.
Vomiting				
+1h	0 (0.00%)	8 (4.94%)	2.80 [0.34;106]	0.095

Symptoms	Control (N = 49)	Intervention (N = 162)	OR [95% CI]	p
+48h	0 (0.00%)	15 (9.26%)	5.25 [0.65;187]	**0.013**
None	49 (100%)	139 (85.8%)	Ref.	Ref.
Diarrhea				
+1h	0 (0.00%)	1 (0.62%)	0.30 [0.04;22.6]	0.771
+48h	2 (4.08%)	4 (2.47%)	0.40 [0.11;2.63]	0.568
None	47 (95.9%)	157 (96.9%)	Ref.	Ref.
Yawning				
+1h	0 (0.00%)	10 (6.17%)	3.06 [0.37;113]	0.074
+48h	4 (8.16%)	6 (3.70%)	0.37 [0.13;1.56]	0.271
None	45 (91.8%)	146 (90.1%)	Ref.	Ref.
Dizziness				
+1h	0 (0.00%)	32 (19.8%)	13.8 [1.71;477]	**<0.001**
+48h	4 (8.16%)	27 (16.7%)	2.34 [0.93;7.72]	0.043
None	45 (91.8%)	103 (63.6%)	Ref.	Ref.
Drowsiness				
+1h	0 (0.00%)	6 (3.70%)	1.87 [0.23;74.2]	0.201
+48h	1 (2.04%)	3 (1.85%)	0.47 [0.11;5.13]	0.910
None	48 (98.0%)	153 (94.4%)	Ref.	Ref.
Convulsion				
+1h	1 (2.04%)	0 (0.00%)	0.00 [0.00;52.6]	1.000
+48h	48 (98.0%)	162 (100%)	0.00 [0.07;171]	1.000
None	0 (0.00%)	0 (0.00%)	Ref.	Ref.
Pruritus				
+1h	0 (0.00%)	2 (1.23%)	0.00 [0.04;712]	1.000
+48h	49 (100%)	160 (98.8%)	0.00 [0.06;166]	1.000
None	0 (0.00%)	0 (0.00%)	Ref.	Ref.
Hives				
+1h	0 (0.00%)	1 (0.62%)	0.00 [0.02;473]	1.000
+48h	49 (100%)	161 (99.4%)	0.00 [0.06;167]	1.000
None	0 (0.00%)	0 (0.00%)	Ref.	Ref.
Anorexia				
+1h	0 (0.00%)	3 (1.85%)	0.00 [0.05;953]	1.000
+48h	49 (100%)	159 (98.1%)	0.00 [0.06;165]	1.000
None	0 (0.00%)	0 (0.00%)	Ref.	Ref.
Myalgia				
+1h	0 (0.00%)	1 (0.62%)	0.00 [0.02;473]	1.000
+48h	49 (100%)	161 (99.4%)	0.00 [0.06;167]	1.000
None	0 (0.00%)	0 (0.00%)	Ref.	Ref.
Fatigue				
+1h	3 (6.12%)	23 (14.2%)	2.05 [0.75;7.88]	0.099
+48h	4 (8.16%)	22 (13.6%)	1.57 [0.62;5.28]	0.236
None	42 (85.7%)	117 (72.2%)	Ref.	Ref.
palpitation				
+1h	0 (0.00%)	4 (2.47%)	1.26 [0.15;54.5]	0.336
+48h	0 (0.00%)	4 (2.47%)	1.26 [0.15;54.5]	0.336

Symptoms	Control (N = 49)	Intervention (N = 162)	OR [95% CI]	p
None	49 (100%)	154 (95.1%)	Ref.	Ref.
Allergic Reaction				
+1h	0 (0.00%)	1 (0.62%)	0.00 [0.02;473]	1.000
+48h	49 (100%)	161 (99.4%)	0.00 [0.06;167]	1.000
None	0 (0.00%)	0 (0.00%)	Ref.	Ref.
History Fever				
+1h	1 (2.04%)	2 (1.23%)	0.00 [0.02;137]	1.000
+48h	48 (98.0%)	160 (98.8%)	0.00 [0.06;169]	1.000
None	0 (0.00%)	0 (0.00%)	Ref.	Ref.

3.3.2 Vital parameters

We also assessed the heart rate, temperature, and blood pressure as safety measurements 1h and 48h post treatment. P-values for testing statistical significance between the means of the two group were calculated for each outcome using a mixed model controlling for baseline values, as measurements were repeated several times within the same individual. The mean temperature 1h after treatment was comparable between the two groups (36.5°C vs. 36.5°C, p = 0.71) as well after 48h (36.5°C vs. 36.6°C, p = 0.58). Heart rate was also comparable between the two groups after 1h (90.3 bpm vs. 91.8 bpm, p = 0.84) and after 48h (94.7 bpm vs. 89.4 bpm, p = 0.2). The same applies to the systolic blood pressure 1h after treatment (107mmHg vs 110mmHg, p = 0.33) and 48h after treatment (114 mmHg vs. 111mmHg, p = 0.87).

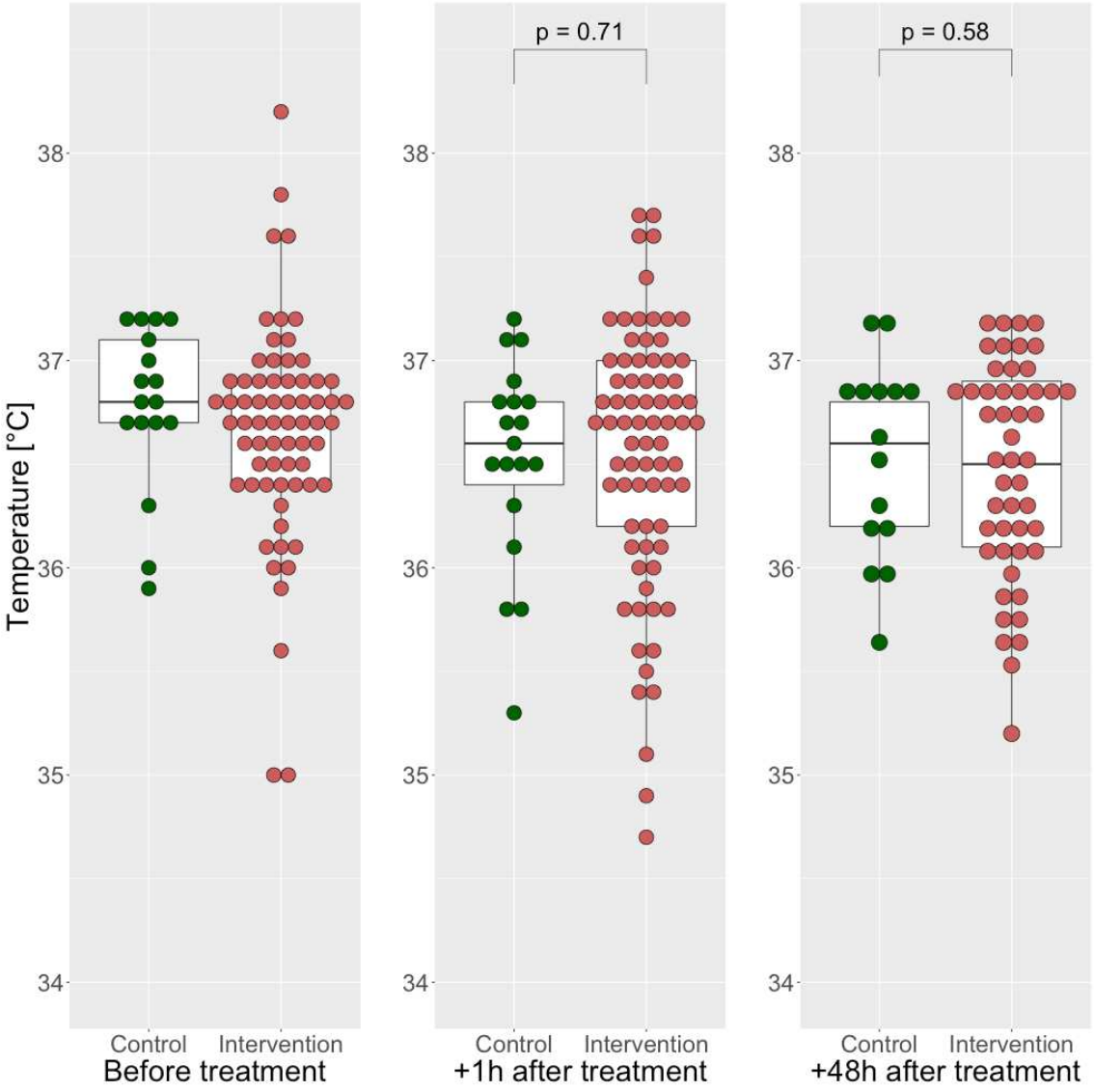


Figure 6: Temperature between study groups before and after treatment

(P-values are obtained by a mixed model controlling for individual temperature before treatment. Colors represent the two different study groups. °C = degree Celsius)

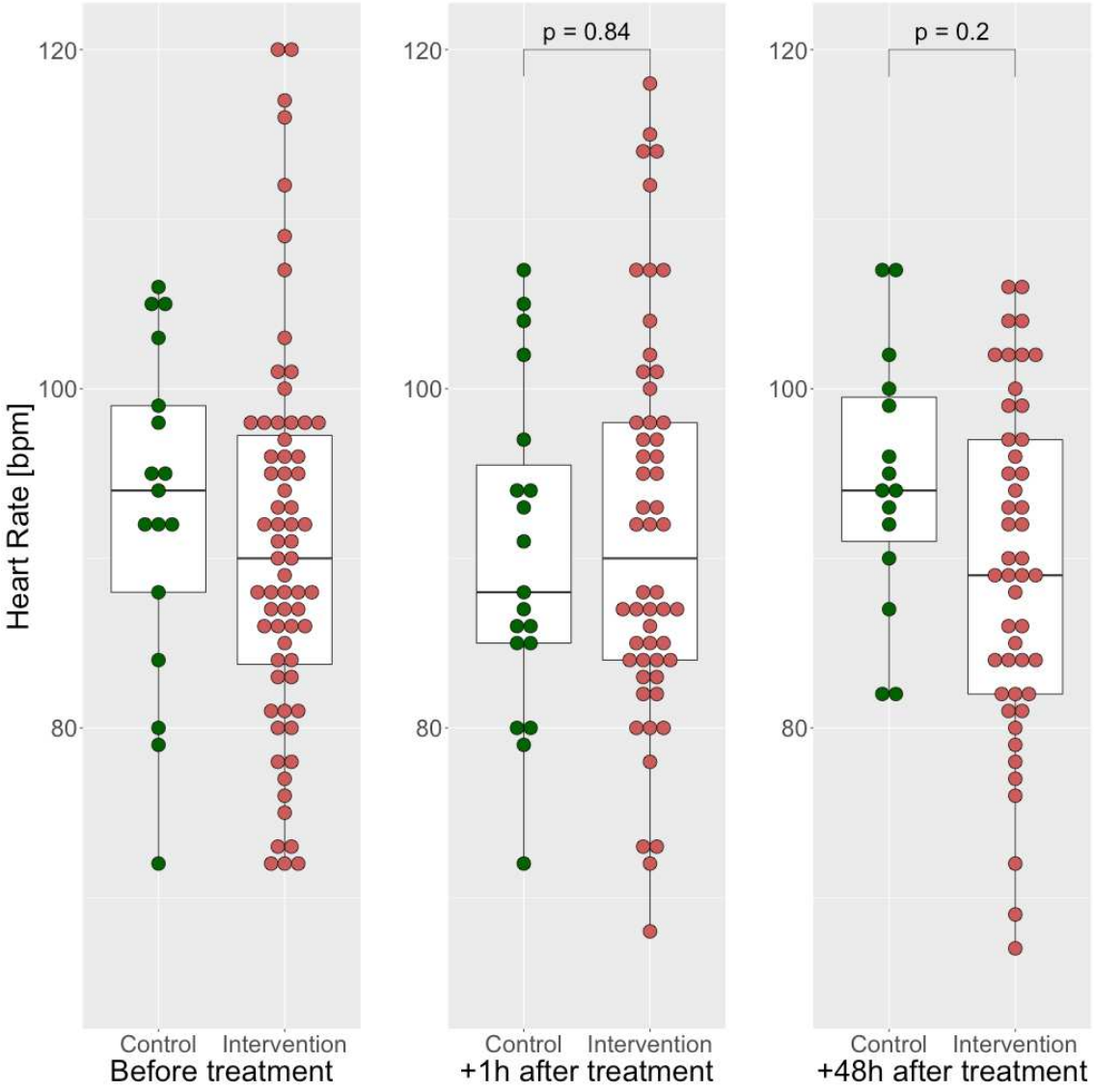


Figure 7: Heart rate between study groups before and after treatment.

(P-values are obtained by a mixed model controlling for individual heart rate before treatment. Colors represent the two different study groups. Bpm = beats per minute)

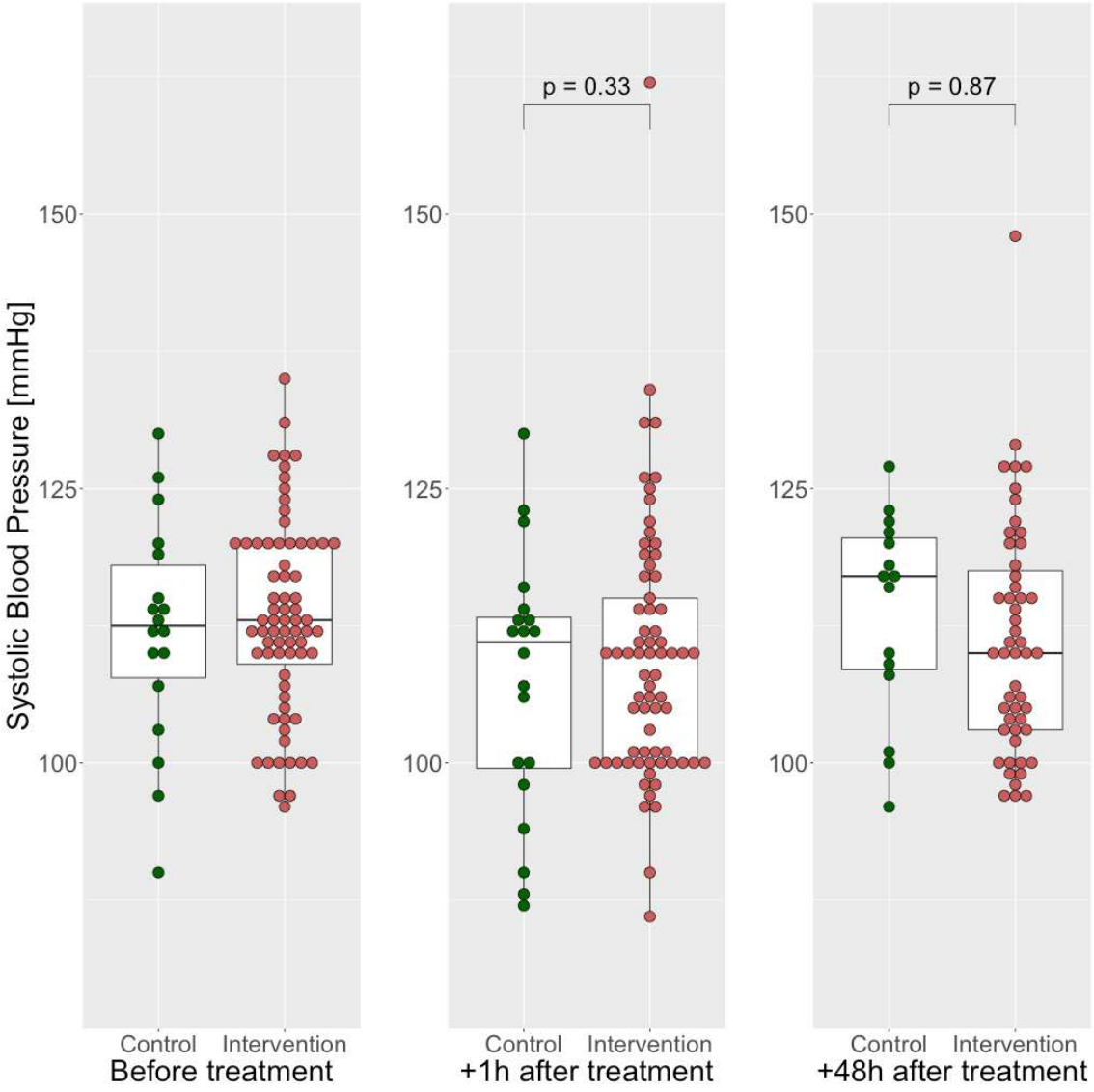


Figure 8: Blood pressure between study groups before and after treatment

(P-values are obtained by a mixed model controlling for individual systolic blood pressure before treatment. Colors represent the two different study groups. mmHg = millimeter mercury)

3.3.3 Outcome of the pregnancy

For safety endpoints concerning the outcome of the pregnancy, we had no case of death of mother, and one case of stillbirth in the intervention as well as in the control group (30th and 37th week of gestation). There was no suspicion of the event being related to prior drug administration, as the time in between was 3 months. There were no cases of abortion or miscarriage in either group reported. We found no relevant difference in rates of preterm births between intervention and control group (0.17 vs 0.13, OR: 1.38 [0.43-4.88], p = 0.57), nor between

different preterm categories (moderate preterm, very preterm, extreme preterm) as seen in the table below. Also, there was no significant difference in means of gestational age at delivery (38.3 vs. 37.2 weeks, p= 0.243).

Table 4: Safety outcome of pregnancies.

*(*p values are generated using Wilcox rank sum test for continuous variables and fisher's exact test for categorical variables. OR = odds ratio)*

	Total N=111	**Control N=44**	**Intervention N=67**	**OR [95% CI]**	**P***
Death of Mother					
No	111(100%)	44 (100%)	67 (100%)		
Yes	0	0	0		
Death of Newborn					1.000
No	109(98.2%)	43 (97.7%)	66 (98.5%)	Ref.	Ref.
Yes	2 (1.8%)	1 (2.3%)	1 (1.5%)	0.63 [0.01;50.2]	0.772
Prematurity [weeks]					0.972
no preterm [>37]	93 (83.8%)	38 (86.4%)	55 (82.1%)	Ref.	Ref.
Preterm [< 37]	18 (16.2%)	6 (13.6%)	12 (17.9%)	1.38 [0.43;4.88]	0.570
Preterm Category [weeks]					0.262
no preterm [>37]	93 (83.8%)	38 (86.4%)	55 (82.1%)	Ref.	Ref.
Moderate preterm [32-37]	10 (9.01%)	3 (6.82%)	7 (10.4%)	1.61 [0.34;10.2]	0.537
very preterm [28-32 weeks]	6 (5.41%)	1 (2.27%)	5 (7.46%)	3.42 [0.36;167]	0.279
extreme preterm [< 28 weeks]	2 (1.80%)	2 (4.55%)	0 (0.00%)	0.00 [0.00;3.85]	0.175
Gestational age [weeks]	37.8 (4.32)	37.2 (5.89)	38.3 (2.83)		0.243

3.4 Efficacy

Concerning the effect of praziquantel on the infection, we found a fivefold chance of being cured after the administration of praziquantel compared to the control group (OR: 5.45 [1.87-16.5], p = 0.001). The CR, defined by the proportion of individuals who were microscopically negative during the three predefined follow-up visits, was significantly higher in the intervention compared to the control group (83.8% vs. 48.1%, respectively p = 0.001). Nevertheless, it is worth mentioning, that almost half of the control group (48.1%, n = 13) were negative during follow up, although they had not received any medication. Six of these cases had initial positive microscopy during screening while another 7 had been only found to be positive by UCAA (range 2.1 – 23.1 pg/ml). None of the latter 7 cases had any eggs been shed out during the extended follow-up (9 visits overall), meaning that they have not been positive for microscopy at any time point. From the 12 cases of the intervention group, that were still positive during follow up, 6 had their last positive urine sample at day 14 and did not shed out any further eggs during the following visits. The other 6 continued with excretion of eggs on a low level (range 2-7 eggs/10ml), but all had very high egg counts before treatment (range 303-948 eggs/10ml).

We also assessed the ERR and found that it was significantly higher within the intervention group, compared to the control group (ERR: 94.90% [90.95-97.46] vs. ERR: 35.45% [-18.89% - 69.97%], p < 0.001, respectively). We also calculated ERR for each of the (extended) follow-up visits and compared them in between the two groups (see *Figure 9*). It is clearly visible how ERR starts to differ during the follow-up, and how the intervention group is crossing the threshold for successful treatment, settled at 90% ERR, after 14 days and clearly leaving it behind at day 21 post-treatment.

In addition, we also compared the development of the geometric mean of egg counts during follow-up (see *Figure 10*). Here, a similar trend is visible: while the geometric mean for the control group continues to fluctuate with a quite high dispersal, the value in the intervention group is decreasing rapidly within days,

reaching its turning point at approximately 14 days and after 21 days continually remains stable at this very low level.

Table 5: Cure Rate, geometric mean, and Egg Reduction Rate for each study group.

(CR = Cure Rate, GM = Geometric Mean, ERR = Egg Reduction Rate. CR is defined as the proportion of individuals who were microscopically negative at follow up. ERR is based on GM before and after treatment. 95% CI for GM and ERR are calculated by bootstrap method using n=1000 repeats. p value for CR is obtained by fisher's exact test and for ERR by Wilcox rank sum test, OR = odds ratio)

	Control (N=20)	**Intervention (N=73)**	**OR [95% CI]**	**p**
CR				
Negative	13 (48.1%)	62 (83.8%)	5.45 [1.87;16.5]	**0.001**
Positive	14 (51.9%)	12 (16.2%)	Ref.	
Geom. Mean [95% CI]				
Before treatment	4.90 [2.54-8.92]	6.74 [4.14-10.9]		
After treatment	3.53 [1.29-8.76]	0.34 [0.15-0.58]		
ERR [95%CI]	0.35 [-0.19-0.69]	0.95 [0.91-0.97]		**< 0.001**

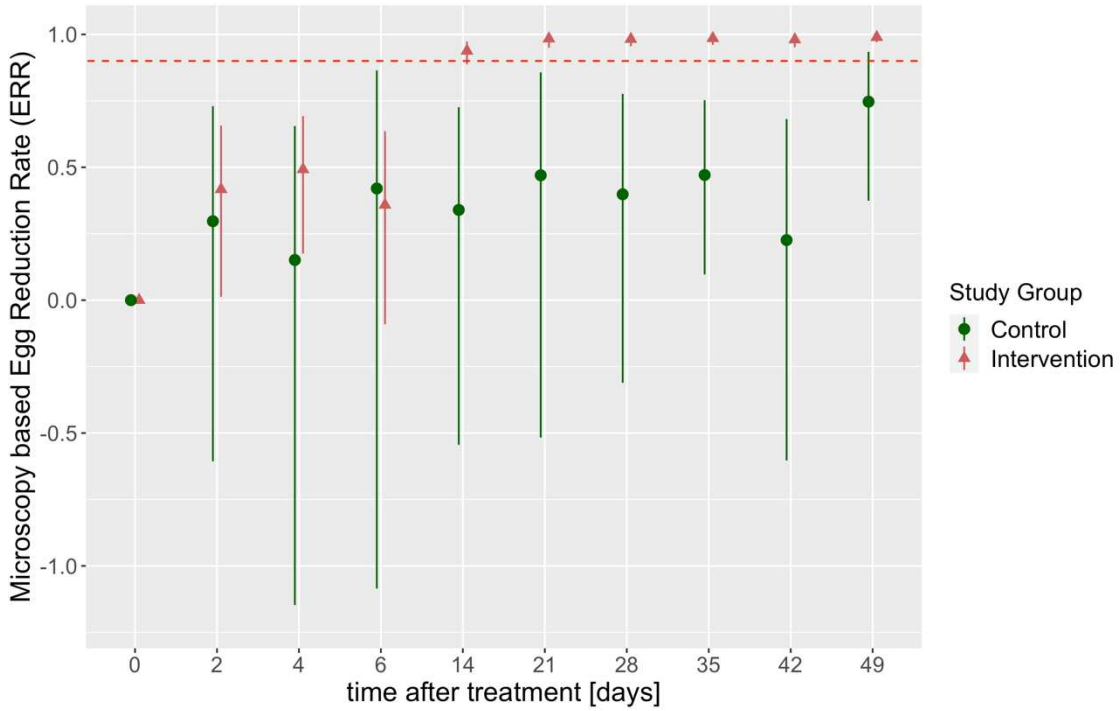


Figure 9: Development of Egg Reduction Rate (ERR) during follow-up.

(ERR is based on geom. mean before and after treatment. Solid lines represent 95% confidence interval (CI) calculated by bootstrap method using n=1000 repeats. The dashed red line indicates the threshold for successful treatment of 90% ERR, as recommended by WHO.)

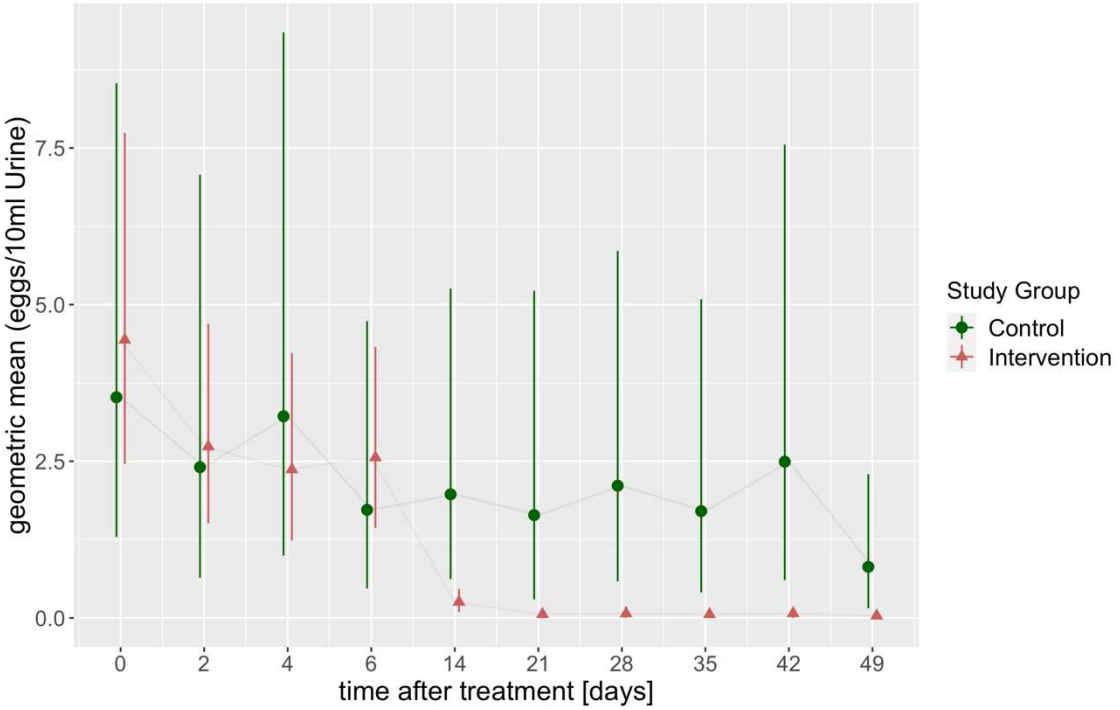


Figure 10: Geometric Mean (GM) during the follow-up.

(Solid lines represent 95% confidence intervals (CI) and are calculated by bootstrap method using n=1000 repeats. ml = milliliter)

3.5 Impact on Morbidity

3.5.1 Maternal Morbidity

Regarding the primary outcome of maternal morbidity, we found no significant difference in means of hemoglobin level between intervention and control group (11.2 ± 1.4 vs. 10.7 ± 1.6, respectively, p= 0.17 unadjusted). Also, after controlling for multiple confounders the difference was still not significant (p = 0.11). Initial preselected covariables included baseline hemoglobin, socioeconomic status and maternal malaria and hookworm co-infection. The linear regression of co-variables and maternal hemoglobin at delivery is depicted in *Figure 11.* The final model only included baseline hemoglobin and co-infections as co-variables, using the model with the lowest complexity and the highest log-likelihood estimate.

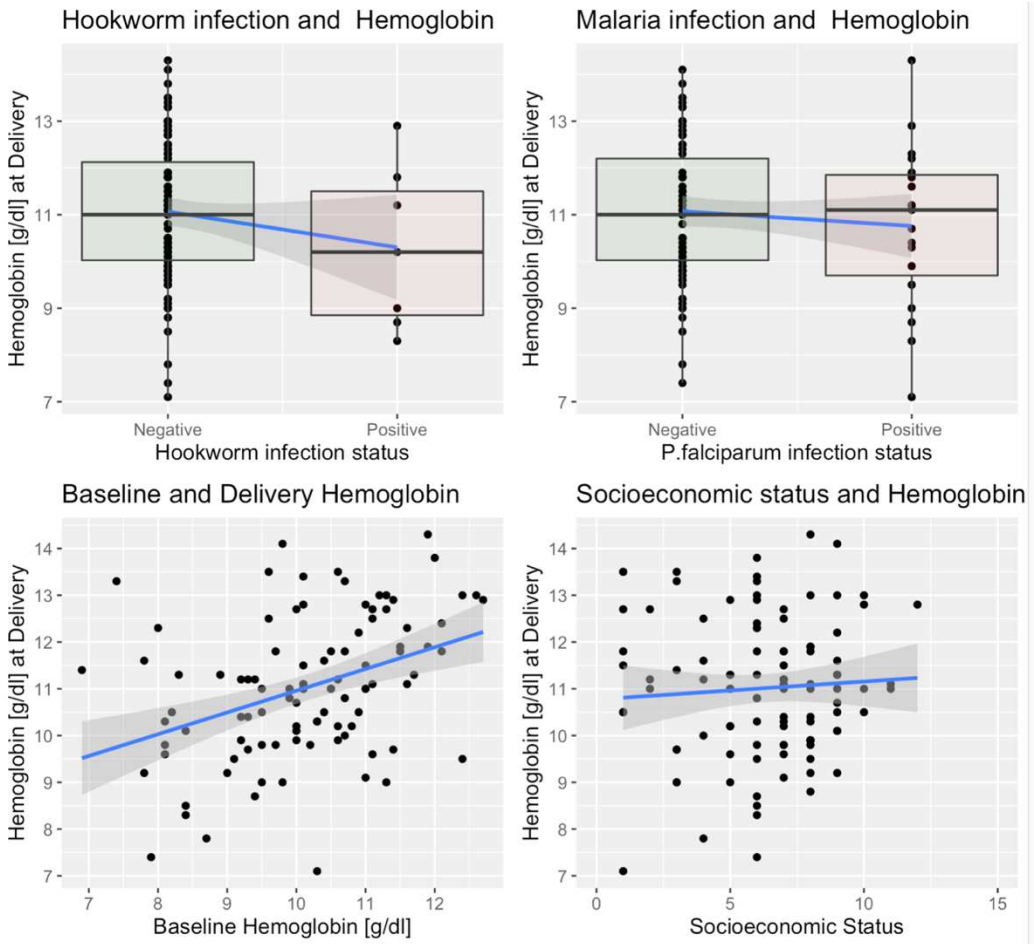


Figure 11: Linear regression of preselected covariables and maternal hemoglobin at Delivery.

(Upper two figures show boxplots with median and interquartile range (IQR), blue lines represent linear models with 95% confidence interval (grey surface), g = grams, dl = deciliters)

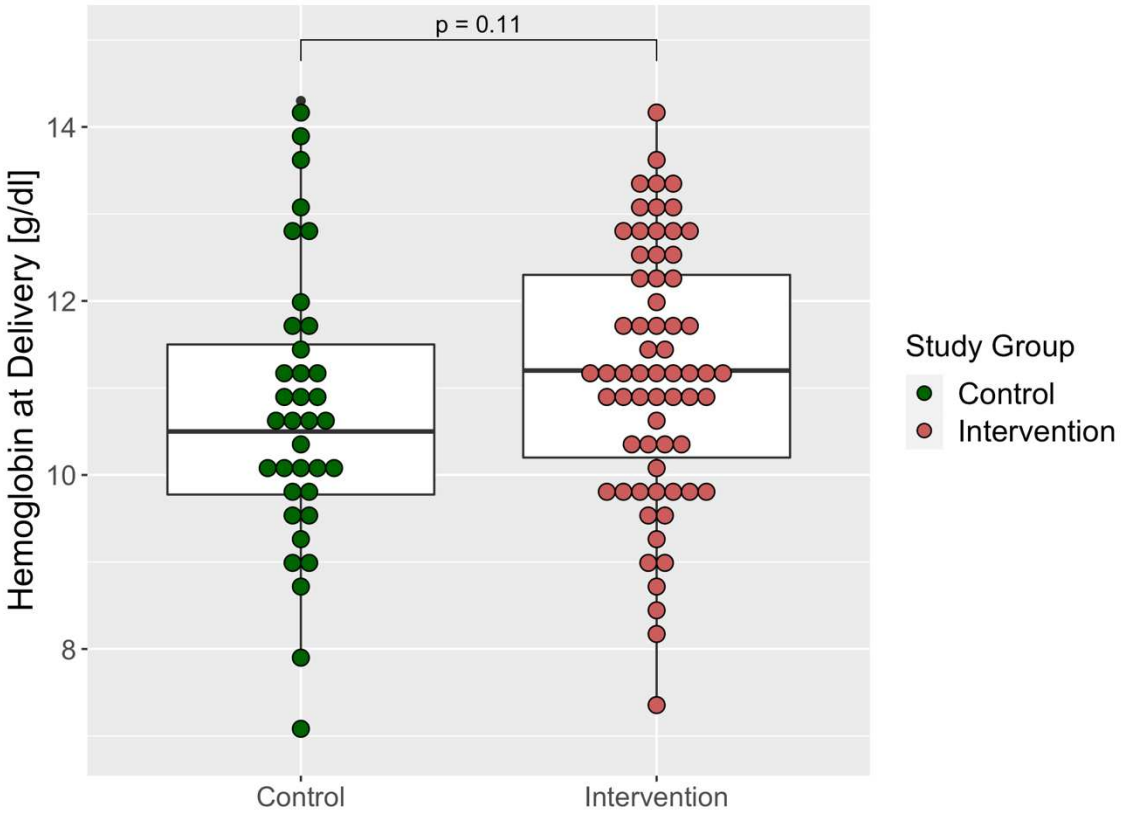


Figure 12: Maternal Hemoglobin at Delivery per study group.

(P-value is obtained from a mixed model using model using baseline hemoglobin, maternal malaria, and hookworm co-infection as co-variables. g = grams, dl = deciliters, Colors represent the affiliation to the respective study group)

Within a subgroup-analysis we compared the impact of treatment on the hemoglobin level at delivery within different subsets of baseline *Schistosoma* infection intensity. In the subsets of low baseline infection intensity and negative baseline microscopy, we found higher levels of hemoglobin at delivery in the intervention group compared to the control group, but neither of the differences was significant. In the subset of heavy baseline infection intensity, we found higher hemoglobin levels within the control group. However, this difference was again not statistically significant, and the case number was very low (n=11). The subgroup analysis is also pictured in *Figure 13: Subgroup analysis stratified by infection intensity.*

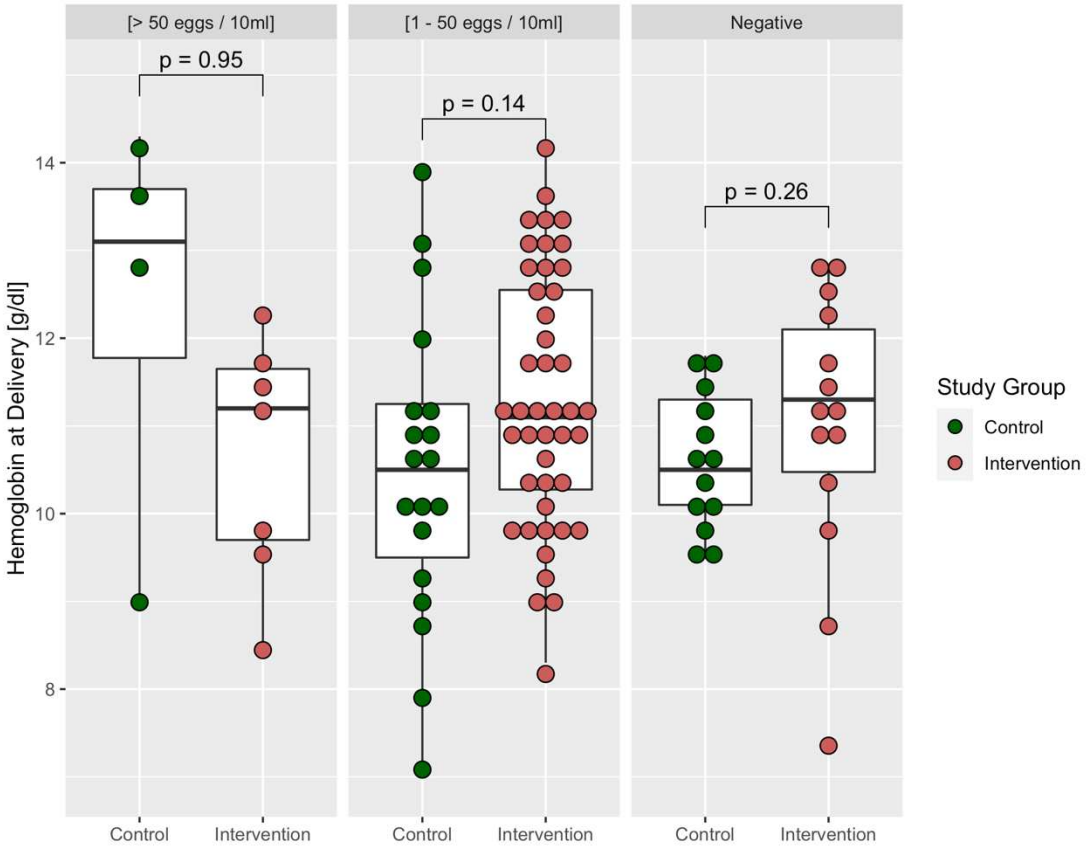


Figure 13: Subgroup analysis stratified by infection intensity.

(Hemoglobin level at delivery for different subsets of baseline Schistosoma infection intensity obtained by urine microscopy. Note that negative participants have only been negative for microscopy but found to be positive by UCAA analysis. P-values are obtained by the same mixed model as used for the main analysis. g = grams, dl = deciliters, ml = milliliters, Colors represent the affiliation to the respective study group)

In a second subgroup analysis we investigated the impact of treatment success on the maternal hemoglobin level at delivery. All participants who were followed up for efficacy of treatment (both intervention and control group) were divided into two subsets: those with successful parasitic clearance and those with persistent shedding of eggs in their urine, regardless of which treatment group they belonged to. We then compared the mean hemoglobin level between these two groups within a mixed model using the same co-variables as for the main analysis. We found that individuals with a successful parasitic clearance had higher hemoglobin values compared to those with persistent shedding of eggs in their urine (11.0 ± 1.4 vs 10.7 ± 1.4, adj. p = 0.02).

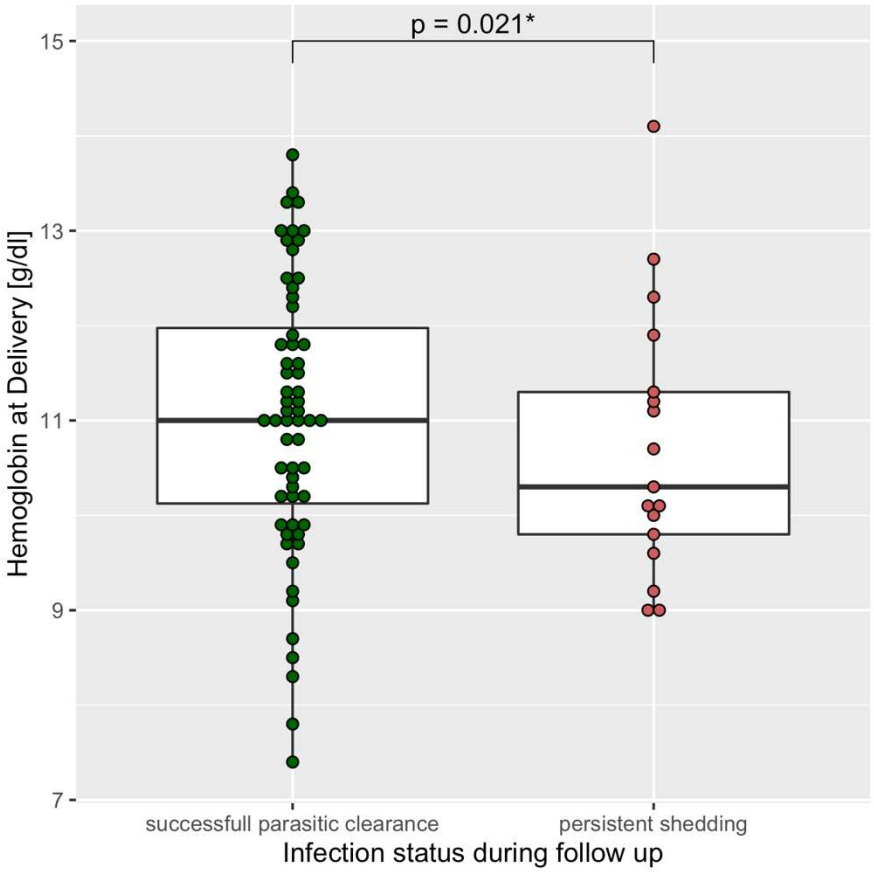


Figure 14: Subgroup analysis stratified by parasitic clearance.

(Hemoglobin level at delivery stratified by infection status during follow up. P-value is obtained by the same mixed model as used for the main analysis. g = grams, dl = deciliters, ml = milliliters, Colors represent the affiliation to the respective study group)

We also compared the proportion of anemic women within the groups. Treatment with praziquantel lowered the odds of being anemic at delivery by half (OR: 0.46 [0.18-1.13], p = 0.06), but this difference was not statistically significant (see *Table 6: Hemoglobin level and proportion of anemia per study group*.). Regarding the effect of praziquantel treatment on clinical parameters as haematuria and leukocyturia, we found that women having received praziquantel had a threefold lower risk (OR: 0.30 [0.08-1.17], p = 0.05) of presenting with blood in their urine at the end of follow-up, 7 weeks post-treatment. The proportion of women with haematuria was significantly lower after treatment compared to controls (18.6% vs. 43.8%, p = 0.05). For leukocyturia there was no such effect, in fact the proportion of women with leucocytes in their urine was similar in both groups (59.3% and 56.2%, OR: 1.13 [0.31-3.97]).

Table 6: Hemoglobin level and proportion of anemia per study group.

*(Unadjusted p-values for hemoglobin is generated with two-sided t.test. *Adjusted p-value for hemoglobin is based on a mixed model using baseline hemoglobin, maternal malaria, and hookworm co-infection as co-variables. p for anemia is obtained by fisher's exact test. g = gram, dl = deciliters, OR = odds ratio)*

	Control	Intervention	OR [95% CI]	p
Hemoglobin (g/dl)	10.7 (1.60)	11.2 (1.44)		0.11*
Anemia				
Not Anemic	13 (36.1%)	36 (55.4%)	Ref.	
Anemic	23 (63.9%)	29 (44.6%)	0.46 [0.18;1.13]	0.068
Anemia categories [g/dl]				
None [>11]	13 (36.1%)	36 (55.4%)	Ref.	
Light [9-11]	18 (50.0%)	24 (36.9%)	0.49 [0.18;1.27]	0.110
Heavy [7-9]	5 (13.9%)	5 (7.69%)	0.37 [0.07;1.88]	0.174

Table 7: Clinical haematuria and leukocyturia per study

(Urine samples were collected group at the end of follow up, 7 weeks post treatment. p values are obtained by fisher's exact test. OR = odds ratio)

	Total N=75	Control N=16	Intervention N=59	OR [95% CI]	p
Haematuria					
Negative	57 (76.0%)	9 (56.2%)	48 (81.4%)	Ref.	Ref.
Positive	18 (24.0%)	7 (43.8%)	11 (18.6%)	0.30 [0.08;1.17]	0.05
Leukocyturia					
Negative	31 (41.3%)	7 (43.8%)	24 (40.7%)	Ref.	Ref.
Positive	44 (58.7%)	9 (56.2%)	35 (59.3%)	1.13 [0.31;3.97]	0.82

3.5.2 Child Morbidity

For child morbidity we found no significant difference in means of birthweight between intervention and control group (2894g ± 452g and 2881g ± 474g, respectively, unadjusted p=0.88). Also, for other newborn parameters such as height and cranial perimeter there was no significant difference, as seen in detail in *Table 8: Outcomes of newborn morbidity per study group*. and *Figure 15: Newborn anthropometric parameters per study group.* We controlled newborn weight for multiple preselected confounders as maternal height, parity, socioeconomic status and maternal malaria and hookworm co-infection. As with calculations for maternal hemoglobin, we chose the model with lowest complexity and highest likelihood to predict our data. The visualizations for linear regression of each co-variable and newborn weight are depicted in *Figure 16: Linear regression of preselected co-variables and newborn weight.* After controlling for these possible confounders, the difference in newborn weight was still not significant (adjusted p = 0.92).

We also investigated the rate of LBW and SGA, the latter being a possible indicator for intrauterine growth restriction. The proportion of LBW was lower in the intervention group compared to the control group (17.9% vs 20.5%, OR: 0.85 [0.29 - 2.54], p= 0.74), but this difference was not statistically significant. The same applied for the proportions of SGA, which was less common in the intervention group (52.4% vs 58.8%, OR: 0.78 [0.17-3.34], p = 0.70), but again the difference was not statistically significant.

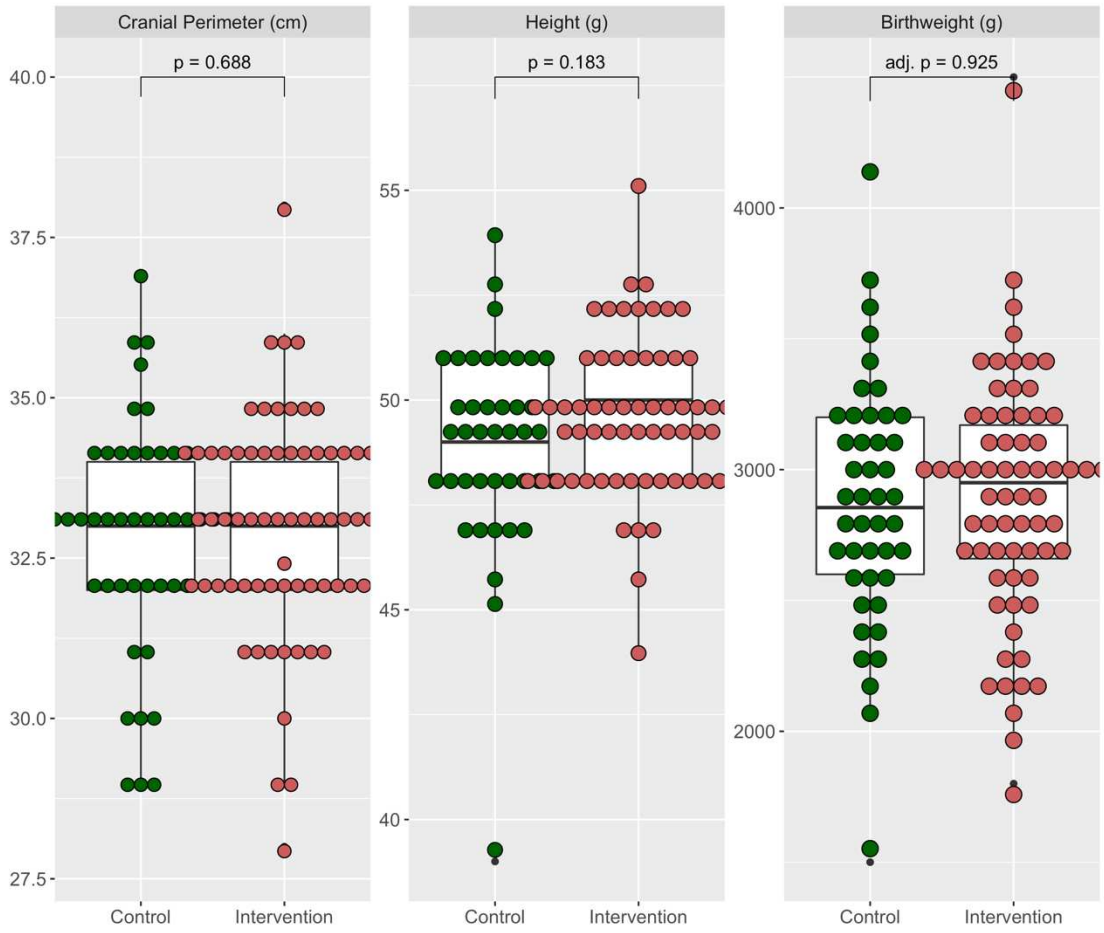


Figure 15: Newborn anthropometric parameters per study group.

(p values are obtained by two-sided t-test. Adjusted p-value for birthweight is obtained from multiple linear regression using maternal height, parity, socioeconomic status and maternal malaria and hookworm co-infection as co-variables. cm = centimeter, g = grams. Colors represent the two different study groups.

Table 8: Outcomes of newborn morbidity per study group.

*(Unadjusted p-values are generated with two-sided t.test for continuous variables and fisher's exact test for categorical variables. *Adjusted p-value for birthweight is obtained from multiple linear regression using maternal height, parity, socioeconomic status, and maternal malaria and hookworm co-infection as co-variables. cm = centimeter, g = gram, OR = odds ratio, LBW = low birth weight, SGA = small for gestational age)*

	Total (N=111)	**Control (N=44)**	**Intervention (N=67)**	**OR [95% CI]**	**p**
Birth-weight (cm)	2889 (459)	2881 (474)	2894 (452)		0.924*
Height (cm)	49.3 (2.12)	49.0 (2.46)	49.6 (1.85)		0.162
Cranial perimeter (cm)	32.9 (1.78)	32.8 (1.84)	32.9 (1.75)		0.682
LBW:					
No [>2500g]	90 (81.1%)	35 (79.5%)	55 (82.1%)	Ref.	Ref.
Yes [<2500g]	21 (18.9%)	9 (20.5%)	12 (17.9%)	0.85 [0.29;2.54]	0.739
SGA:					
No	17 (44.7%)	7 (41.2%)	10 (47.6%)	Ref.	Ref.
Yes	21 (55.3%)	10 (58.8%)	11 (52.4%)	0.78 [0.17;3.34]	0.708

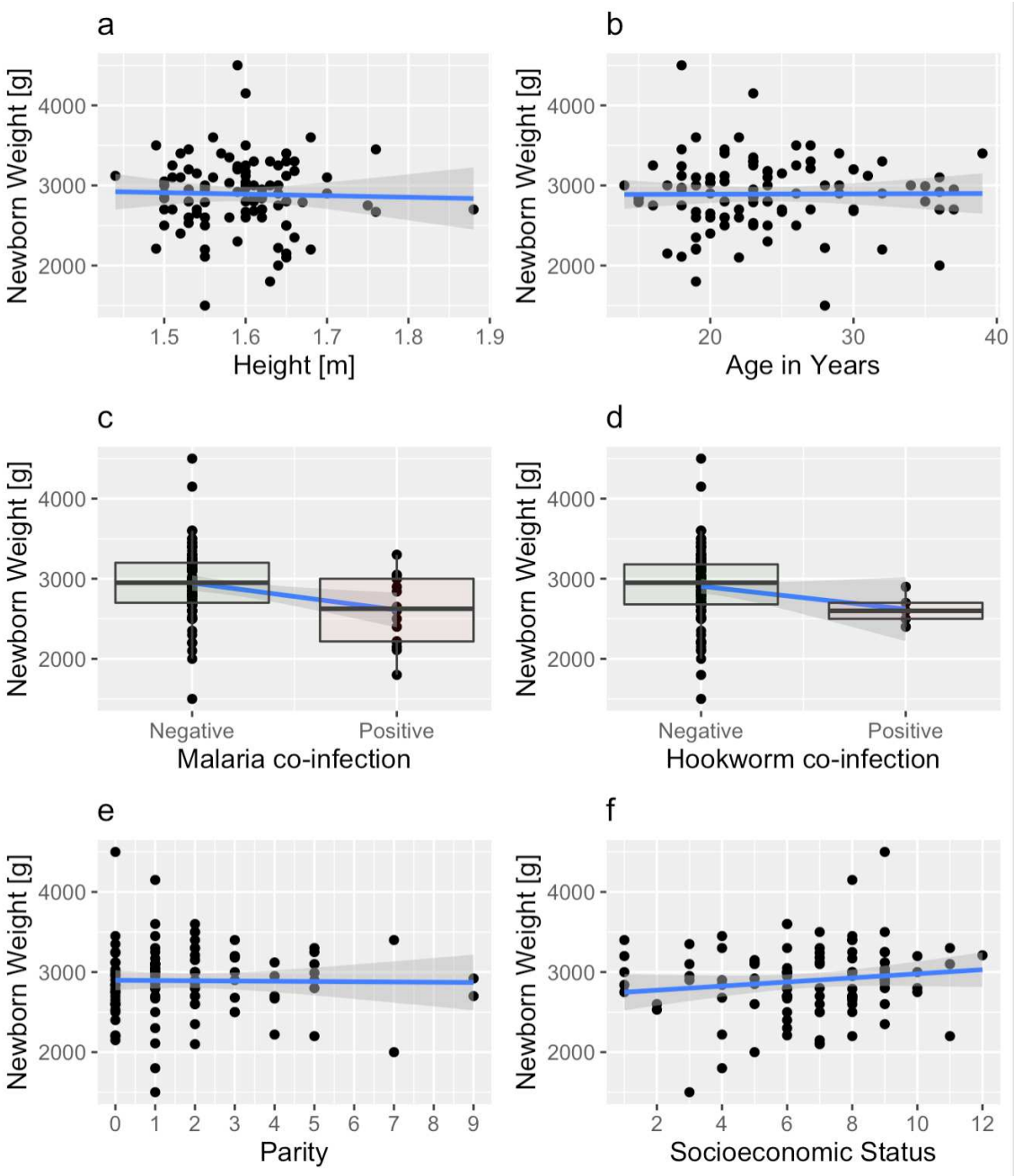


Figure 16: Linear regression of preselected co-variables and newborn weight.

(Boxplots show median and interquartile range (IQR), blue lines represent linear models with 95% confidence interval (grey surface), m = meter, g = grams, ml = milliliters)

4 Discussion

We carried out the first randomized and controlled trial with praziquantel based therapy in pregnant women infected with *Schistosoma haematobium*. Our goal was to weigh up the risks and benefits of a treatment during pregnancy compared to the treatment after delivery. We did this by assessing:

i) the safety of the drug (PZQ) during pregnancy
ii) the efficacy of treating the parasitic disease with PZQ
iii) the impact of PZQ treatment on maternal and newborn morbidity.

We found that praziquantel was safe in use during pregnancy, that it was efficient in reducing the parasitic burden, that it did not increase the birthweight of infants born to infected mothers and that it led towards higher levels of hemoglobin in mothers at delivery. Our findings are in accordance with the current knowledge that praziquantel is safe of use during the pregnancy, even though there is still not enough evidence that treatment alters maternal or newborn health during the ongoing pregnancy. However, our results further strengthen the practice to include pregnant women in treatment and prevention programs of urogenital schistosomiasis to prevent future increase of morbidity.

4.1 Study population

Most characteristics at baseline were equally distributed among both groups, as women were mainly between 18 and 30 years, had already multiple pregnancies before and were negative for malaria co-infection and STH co-infection at baseline. Still, there were some differences in the prevalence of haematuria and hookworm co-infection. As mentioned before, we controlled in the analysis for our primary endpoint for the latter. Another difference was that the mean CAA levels were much higher in the intervention group (110.07 pg/ml $\pm$ 548.4) compared to the control group (72.42 pg/ml $\pm$ 168.8). We suspected one outlier responsible for this effect but decided not to exclude this value. Instead, we also compared geometric means between intervention and control group, and here the difference in means was smaller (7.07 pg/ml in the intervention group vs 11.39 pg/ml in the control group, respectively). We conclude that the difference between the groups, does not alter our further results.

4.2 Safety

Praziquantel is associated with a wide spectrum of side effects including general symptoms, such as malaise and fatigue as well as more specific symptoms of the nervous system, the gastrointestinal tract and the skin, as for example listed by the FDA.[39] However, studies reporting exact frequencies of side effects are limited, especially in urogenital disease and even more in pregnant women. In a Cochrane systematic review on drugs for the treatment of urogenital schistosomiasis, Kramer et al.(2014) reported only two studies, that mentioned exact numbers of side effects comparing praziquantel and placebo.[217] In a meta-analysis of praziquantel used in Ethiopia, Hailegebriel et al.(2021) give a good overview over different side effects and their frequency.[46] However, this analysis again included only two studies on urogenital schistosomiasis.

All side effects reported in our study were known and previously described symptoms[39,46,217] and were only reported at mild to moderate intensity. Regarding the frequency of individual side effects, we noticed slight differences from previous findings. Several meta-analyses reported abdominal pain as the predominant side effect, mainly in school aged children.[46,217] Other frequent reported symptoms are headache[218] and diarrhea.[46] We on the other hand, found symptoms as dizziness, nausea and vomiting to be significantly increased after uptake of praziquantel. One possible explanation to this could be that most of the previous studies looked at cases of intestinal schistosomiasis, whereas our study only included participants with urogenital schistosomiasis. Abdominal pain could appear more frequent after treatment of intestinal schistosomiasis due to an immediate rejection reaction of the dying worms in the intestines. This explanation is further strengthened by the fact, that abdominal pain after administration of praziquantel has also been correlated with the infection intensity of intestinal schistosomiasis.[219] Another important difference is the examined sub-population. School aged children may report very different symptoms compared to pregnant women. It is notable that most of the reported side effects, are symptoms, that often appear physiologically throughout early pregnancy, such as nausea and vomiting. Praziquantel might exacerbate the frequency and

intensity of such symptoms. However, as the number of studies administering praziquantel to pregnant women is scarce, there is not much known about exact frequencies of symptoms in this particular population. One exception is a study from Bustinduy et al.(2020), who reported precise numbers of side effects in pregnant women, even stratified into groups of early and late pregnancy, and found the most common side effects to be nausea and dizziness in early, and nausea and headache in late pregnancy.[220] These findings are confirmed by our results, reporting similar frequencies of these symptoms but without stratification by gestational age.

Although we observed vomiting mostly within 48h after drug administration, we also found 8 cases of vomiting within 1h post treatment. Immediate vomiting could have potentially led to treatment failure due to insufficient absorption of the drug. However, all these 8 cases were free of eggs latest after 3 weeks post intervention, making a treatment failure unlikely.

The fear of adverse pregnancy outcomes caused by praziquantel has been one of the causes, why treatment of pregnant women has been withheld for many decades, even if there has never been a proof of such deleterious impact on the course of the pregnancy. Investigations with praziquantel in animal studies[196,197] have led to the classification of praziquantel as a class B drug by the FDA – indicating that there is no suspected adverse impact on pregnancy in these models but data from human studies are lacking. The growing evidence for the burden of disease in pregnant women and the high burden of morbidity in this neglected population has led to the decision of the WHO in 2002[10] to recommend the use of praziquantel during pregnancy, based on a risk-benefit analysis. This recommendation has opened the possibility for researchers to provide evidence of the effects of praziquantel during pregnancy and close the gap of lacking data. Post-market surveillance data and results from two cross-sectional studies from 2004 and 2005[199,200] have further strengthened this recommendation which has been renewed by WHO in 2006.[11] Following this development, two large RCT studies were conducted in which praziquantel was administered to pregnant

women and which showed no evidence of adverse birth outcomes or an increased prevalence of preterm birth or fetal loss.[12,13]

In our study, we observed one case of stillbirth in each cohort. As the time between drug administration and stillbirth was more than three months, we did not suspect any relation to the administration of the study drug. Apart from that, we found no further cases of birth anomalies, miscarriage or stillbirth, and frequencies of preterm birth did not differ between the groups. In the above mentioned smaller prospective cross-sectional study,[200] the authors also described one case of abortion 3 weeks after treatment, among a total of 25 women treated, but noted that this frequency is normal in the local population. Similar findings have been reported by Olveda et al.(2016), who found no differences in congenital anomalies, fetal death in utero or abortion in pregnant women infected with *S. japonicum* receiving praziquantel treatment compared to those left untreated during pregnancy.[13] We therefore conclude that until today there is no evidence for an increased risk for adverse pregnancy outcomes caused by praziquantel and we therefore support its use for the treatment and prevention of schistosomiasis during pregnancy.

4.3 Efficacy

Besides the question whether it is safe to administer praziquantel to pregnant women, there is also the need to evaluate its usefulness. Overall, praziquantel is a rather easy to use drug for treating schistosomiasis, regarding the simple treatment regimen with the single dose application, which has been very helpful for its use during mass drug administrations. But also the high efficacy of praziquantel makes the drug an indispensable tool in achieving the goals of schistosomiasis control and elimination.[4] Throughout the follow-up of our study, we found that praziquantel was successful in treating almost all of the infections in our study population. Both CR and ERR were significantly higher in our intervention group compared to the control group. We found that women receiving praziquantel had a fivefold chance of being free of eggs in their urine during follow-up compared to the control group (OR: 5.45 [1.87-16.5], p = 0.001).

This result is similar to previously reported findings from the trial in Leyte (The Philippines), that reported an *odds ratio* of 5.815 [3.52-9.61].[13] Also our reported CR of 83.8% is in accordance with previous findings in both non-pregnant individuals (pooled CR: 89.8%)[46] as well as in pregnant women (CR: 83.7%).[13] In addition, we found that CR further increased in the course of follow-up after 7 weeks post treatment up to 95% (data not shown). However, there were 12 individuals who were not completely parasitological negative during the three preselected follow-up visits (days 14,21 and 28 after treatment). 6 of these women had their last positive urine sample at day 14 and were negative during all subsequent follow-up visits. It might be that the time point of 14 days post intervention might be too early to assess treatment efficacy. *Figure 9* shows the time course of ERR and indicates day 21 to be the earliest time point to successfully report treatment success by microscopy. The other 6 participants continued with excretion of eggs on a low level during the extended follow-up. But all 6 of them had very high initial egg counts during screening and therefore benefitted from a substantial reduction of their infection intensity.

4.4 Impact on Morbidity

Besides efficacy in terms of parasitological cure, the question remains whether there is a potential benefit of a treatment during pregnancy for the fetus or for the mother. Anemia was a very frequent condition in our study population, as 73% presented with a lowered hemoglobin level at baseline. We found an increase of 1 mg/dl in mean hemoglobin at delivery compared to baseline for mothers receiving intervention, and the mean hemoglobin level at delivery for those participants was higher compared to the untreated control group, but the difference was not statistically significant. Women who received treatment during their pregnancy had half the risk of being anemic at delivery, but statistical significance was slightly missed. Our results are in accordance with previous findings of the two earlier mentioned RCTs that performed PZQ based treatment in pregnant women, which also found no significant difference in hemoglobin levels or prevalence of anemia. However, both of these studies looked at different species (*S. japonicum* and *S. mansoni*, respectively), while *S. haematobium* has

been discussed as a particular contributor to maternal anemia.[171] Also, it needs to be mentioned, that the trial in Uganda included women into their randomization, who did not have schistosomiasis, as it primarily imitated a mass drug administration without previous testing, diminishing the possible impact of the drug on the target population.

How schistosomiasis exactly contributes to anemia remains unclear, but one of the discussed mechanisms is extracorporeal blood loss in urine.[135] Changes in hemoglobin caused by the administration of praziquantel could be therefore explained by the reduction of haematuria. Most of the participants included in our study showed a light infection intensity (< 50 eggs / 10ml urine), but still one third of the women in both groups had relevant haematuria at baseline. We found that the proportion of persistent haematuria was significantly lower 7 weeks after treatment compared to the control group, which would further strengthen this hypothesis. It remains possible, that the effect of PZQ on maternal anemia may be more distinct in a population with higher intensity of infection. Because of this hypothesis we performed a subgroup-analysis for women only with heavy infection at baseline but did not find any significant difference between hemoglobin levels at delivery in this sub-group (see also *Figure 13).* But there could be a connection between persistent parasite shedding and hemoglobin levels, as we found that hemoglobin levels at delivery were significantly higher among those participants who were free from parasites during the follow up, compared to those with persistent parasituria, regardless of whether they had received intervention or not.

It has been controversially discussed if and how maternal schistosomiasis exactly contributes to LBW of the newborn as summarized in *chapter 1.9.6 Low Birth Weight.* In a previous study carried out in the same study area in Gabon, Mombo-Ngoma et al.(2017) found an increased risk for LBW among pregnant women infected with *S. haematobium,*[192] whereas another more recent study from Zimbabwe, that included about 4.437 pregnant women, did not find any significant evidence for this.[221] Freer et al.(2018) postulated four main

mechanisms how schistosomiasis contributes to adverse birth outcomes: anemia, urogenital disease, maternal inflammation and placental or fetal inflammation.[5] Maternal anemia has already been identified as a risk factor for stillbirth, low-birth weight and preterm birth.[222]

As we showed that maternal anemia at delivery might be affected by treatment during pregnancy, there is a chance of also modifying birth outcomes as birth weight through therapy during the pregnancy. However, causes for maternal anemia are broad, and influenced not only by deworming but notably by the access to iron and folic acid supplementation[222] which has also routinely been applicated to all the pregnant women in our study cohort. Still, any potential action that contributes to increased maternal hemoglobin during pregnancy may decrease the risk of adverse birth outcomes.

Unfortunately we found no impact of PZQ based treatment of the mothers on the birth-weight of their newborns, which is in accordance with earlier findings from the two other RCTs from Uganda and the Philippines.[12,13] It is also possible that maternal schistosomiasis causes LBW by placental inflammation, for example via the transplacental transport and immunogenicity of soluble egg antigens during pregnancy,[9] leading to poor pregnancy outcomes as intrauterine growth restriction.[223] In this case, treatment of maternal schistosomiasis may be too late to have an impact on child morbidity, but it would strengthen the necessity to keep young women in reproductive age free from infection to prevent maternal schistosomiasis in the first place.

4.5 Strengths and Limitations

There were some limitations in our study that need to be addressed. Unfortunately, it was not possible to use a placebo in our control group, since the trial was embedded in a larger project with a more diagnostic focus. This has led to the possibility of reporting bias of the participants, regarding the assessment of adverse side effects of the intervention. However, the randomization and

intervention were investigator-blinded to minimize this impact as much as possible.

Although we found an improved hematologic status of the participants in our intervention group, this effect remained without statistical significance, probably due to the sampling error of our study. We tried to meet this objection by using the substantial diagnostics to identify possible co-infections and thus include potential confounders into our analysis. Also, the time lag between the treatment and delivery was often very short, and not homogeneous due to different pregnancy ages at inclusion and different pregnancy durations.

Based on these experiences we propose that future trials investigating possible benefits of treating urogenital schistosomiasis in pregnant women should include a larger sample size of participants and try to recruit participants consequently at the beginning of the second trimester (around week 16) to allow maximum recovery of the hematological system during the remaining pregnancy.

Despite these limitations, we performed the first randomized controlled study using praziquantel in pregnant women infected with *S. haematobium.* Our study also had some strengths, as it considered important prerequisites, as previously stated by Friedman et al. (2005). For example, our screening was not only based on urine microscopy during multiple subsequent days but was completed by the detection of CAA and therefore allowed the identification of more cases than microscopy on a single urine sample as well as to detect a broader spectrum of infection intensity. In addition to this, our screening included the assessment of potential confounders as the presence of hookworm and *falciparum* malaria infection. We attempted to choose an adequate follow-up period, choosing delivery as the time point to assess maternal morbidity, as this is the earliest point in time when the treatment could be applicated if it was not to be administered during the pregnancy. Also, we based our analysis for the efficacy on multiple urine samples to identify treatment success adequately.

4.6 Conclusion

We found that the use of PZQ for treating urogenital schistosomiasis in pregnant women seems to be safe, effectively cures the parasitic infection and significantly reduces the infection burden. Except for common drug side effects, there is no further indication of risks for pregnancy or adverse birth outcome. However, we were unable to show a significant effect either on the hemoglobin levels of the mothers at the time of birth or on the birth weight of their newborns. The decision regarding the use of praziquantel during pregnancy must therefore be based on a risk-benefit analysis that also considers effects beyond the current pregnancy.

Early adolescence is known to be the time point with highest prevalence of schistosomiasis throughout lifetime,[7] and the rate of teenage pregnancy was recently estimated at 19.3% in sub-Saharan Africa.[121] Moreover it has been estimated that women aged 18-25 years who live in regions endemic for schistosomiasis, spend about 25% and 60% of their reproductive life pregnant and lactating, respectively.[9] This has led to the conclusion that there is little opportunity to treat them outside these periods. It has been shown that praziquantel based treatment before the age of 20 was associated with significantly lower risk for urogenital pathology in women infected with schistosomiasis,[117] and there is evidence that such lesions can persist irreversibly[119] and lead to complications such as incontinence, genital ulcers and infertility.[68]

One can conclude that the exclusion of pregnant women from regular treatment strategies, will most likely result in unnecessarily increased burden of disease and leave them untreated during repeated cycles of pregnancy and lactation. This is further exacerbated by the fact that in countries with limited health care, many young women have closest contact to the health system during their pregnancy. In Gabon for example, up to four routine visits during the pregnancy are free of charge. To take advantage of this offer, the mother must be registered with the state health insurance scheme, which again is free of charge. Most of the women presenting in our screening sites where therefore covered by national health

insurance (one existing barrier often reported was the need for a birth certificate to participate in national health insurance). For many of the women, these visits during pregnancy were the closest contact with the healthcare system that they would have until their next pregnancy. This was also because many women came from more rural areas and had to travel long distances for their prenatal care. Some women even spent the entire last trimester of pregnancy and the period of childbirth in Lambaréné, as there was no or only insufficient gynecological care in the rest of the province. After giving birth, many of these women then returned to their villages, where they again had limited access to the health system.

Prevalence of schistosomiasis among pregnant women was around 23% in our study population and can reach proportions up to 60%[170] in endemic regions. Excluding all these women from schistosomiasis treatment on an individual as well as a mass administration level because of their pregnancy, may therefore pose them at a high risk of delaying treatment for often many years and therefore exposing them and their unborn children at an unnecessary high risk of additional morbidity including FGS. We therefore support the implication of regular test-based treat strategies during routine prenatal examinations and the inclusion of pregnant women into mass drug administration (MDA) programs.

5 Summary (English)

Background

Schistosomiasis is a parasitic disease caused by pathogens of the genus *Schistosoma*, and belongs to the Neglected Tropical Diseases (NTDs).[3] The World Health Organization (WHO) currently lists a heterogeneous group of 20 different diseases as NTDs, which primarily occur in tropical areas and affect around 1 billion people worldwide.[22] NTDs are associated (at least in part) with poverty and poor sanitation,[23] and despite their global prevalence they are – unlike, for example HIV, tuberculosis or malaria - almost absent from the global health and research agenda.[22]

Infections with schistosomes affect around 220 million people,[1] most of whom live in sub-Saharan Africa.[2] A total of six human pathogens are known, of which three are responsible for the predominant burden of disease in humans: *S. mansoni, S. japonicum and S. haematobium*.[7] The latter is the causative agent of urogenital schistosomiasis, which is distinguished from intestinal schistosomiasis by the localization of the pathogen inside the human body and the induced pathologies.[7]

Schistosomiasis can result in local as well as systemic sequelae, such as anemia, malnutrition, growth retardation, and impaired physical and cognitive abilities.[133] In endemic regions, the initial infection often occurs in early childhood, followed by increasing infection intensity, typically peaking in puberty, before both intensity and prevalence decrease in adulthood. [7]

Due to this epidemiological phenomenon, research and interventional measures have focused over decades on school aged children (SAC). However, with the aim of controlling transmission and eliminating the disease, focus has expanded to other populations that had previously been neglected, such as preschool-aged children and pregnant and/or young women.[67] About half of the infected women are also affected by female genital schistosomiasis (FGS),[120] another

complication of urogenital schistosomiasis, which is characterized by genital "*sandy patches*" and symptoms as contact bleeding and vaginal discharge.[68]

In pregnant women with schistosomiasis, there is also the question of consequences for the pregnancy and the newborn. It is estimated that about 13% of pregnant women in sub-Saharan Africa are infected with schistosomiasis. [28,31] Over the last decades increasing evidence for associations between maternal schistosomiasis and adverse pregnancy outcomes has been found.[9] This includes in particular an increased risk of low birth weight (LBW), but also for premature birth or miscarriage, infertility and ectopic pregnancies.[5]

Since its discovery, praziquantel has been the mainstay of individual therapy and disease control of schistosomiasis.[4] Following its approval in 1982, praziquantel was classified by the American Food and Drug Administration (FDA) as a "pregnancy class B drug",[194] indicating that *"animal reproduction studies have failed to demonstrate a risk to the fetus and there are no adequate and well-controlled studies in pregnant women".*[195] Following this classification, recommendations have excluded pregnant and breastfeeding women over decades from individual therapy and mass drug administrations (MDA).[8]

In 2002, WHO changed its recommendations based on an updated risk-benefit analysis, and now recommended for the first time the use of praziquantel in pregnant and lactating women.[10] In the following years, two large randomized controlled trials investigated safety and potential benefits of praziquantel in pregnant women.[12,13]

Both studies found neither an increased risk for the use of praziquantel during pregnancy, nor a positive effect of therapy during pregnancy for the health of the mothers or their newborns.[12,13] However, both studies dealt with pathogens of intestinal schistosomiasis (*S. mansoni* and *S. japonicum*). A corresponding study on the risk and benefit of praziquantel in pregnant women suffering from urogenital schistosomiasis is still pending. We want to close this gap with the

present work and therefore asked the question about safety, efficacy and influence on maternal anemia and newborn weight when using praziquantel in the treatment of infections with *S. haematobium* in pregnant women.

Methods

We screened pregnant women with a gestational age between 16-30 weeks (based on the date of the last menses) for the presence of *S. haematobium* infection. Participants were recruited in three antenatal care units at local health facilities. Urine samples were collected on three consecutive days and tested for the presence of worm eggs by microscopy and for the presence of the genus-specific *circulating anodic antigen* (CAA) using a lateral-flow assay.[63,65] If one of the two tests was positive, an infection with *S. haematobium* was assumed. In addition, blood and stool samples were collected to determine the maternal hemoglobin level and find concomitant infections with malaria, geohelminths or *S. intercalatum*. The socio-economic status of the participants was determined using a questionnaire. Women who were positive for schistosomiasis were randomized by a 3:1 ratio into an intervention and control group. The intervention consisted of 40 mg praziquantel/kg body weight, administered in a single dose. To improve bioavailability[41] and reduce side effects,[194] we ensured adequate food intake before ingestion.

Side effects were recorded 1 hour and 48 hours after taking the medication using standardized questionnaires. In addition, we collected the vital parameters before, 1 hour and 48 hours after taking the medication. We compared vital signs using a mixed model because of repeated measurements. To investigate the efficacy of the intervention, further urine samples were collected over a period of 7 weeks and used to calculate cure rate (CR) and egg reduction rate (ERR).

For impact on maternal and newborn weight we chose maternal hemoglobin level and birthweight as primary endpoints, since these parameters provide the strongest evidence of a connection to schistosomiasis.[5,9,135] We assessed the prevalence of haematuria in the last urine sample 7 weeks after treatment and

collected another maternal blood sample at birth. Hemoglobin levels were compared using a mixed model, to account for each participant's individual value at baseline, as well as other (potential) confounding factors such as concomitant infections and socioeconomic status. We determined and compared weight, height, and head circumference of the newborns, and used these parameters to calculate the prevalence of LBW and, based on gestational age, whether the newborns were small for gestational age (SGA).

Results

We screened a total of 666 pregnant women for the presence of schistosomiasis and found 154 to be positive. A total of 145 participants were randomized and 130 of them could be included in the two study groups. We performed a modified per-protocol analysis (actual vs. no medication intake) and thus obtained an intervention group with 82 and a control group with 48 participants.

Regarding side effects, we found that fatigue, dizziness, nausea, and vomiting were significantly increased in the intervention group. However, all side effects were of mild to moderate intensity and resolved within 48 hours. The vital parameters after the intervention did not differ between the two groups. We found that participants in the intervention group had a fivefold chance of being cured, and the calculated CR was significantly higher in the intervention group compared the control group (83% vs 48%, $p = 0.001$). Similarly, the ERR was significantly higher in the intervention group compared to the noninterventional control group (95% vs 35%, $p < 0.001$).

Women from the control group had a significantly lower risk of developing haematuria 7 weeks after the intervention. However, we found no significant impact on the maternal hemoglobin level, even after controlling for possible confounding factors. Although the prevalence of anemia was lower in the treatment group, the difference was not statistically significant. Also, after stratification based on the infection intensity, no effect could be detected in the subgroup with severe infections (> 50 eggs/ml urine). A significant difference of

hemoglobin values could only be shown based on stratification by successful parasitic clearance: The hemoglobin value was significantly higher in the subgroup of participants who were free of eggs in their urine after 7 weeks (regardless of an intervention) compared to those who were still excreting eggs.

There were also no differences regarding the effects on child morbidity and the outcome of the pregnancy. The birth weight of the newborns did not differ between the two groups, nor did the other anthropometric parameters. The proportion of children with LBW and those estimated to be SGA did not differ either. We also found no evidence of a higher risk of miscarriage or premature birth after intervention.

Discussion

We conducted the first randomized controlled trial to assess safety and efficacy of praziquantel in pregnant women infected with *S. haematobium*. Our aim was to weigh up the risks and benefits of a treatment during pregnancy versus treatment after childbirth. We found no evidence for any risk to mother or child from the use of praziquantel during pregnancy. We were able to show that praziquantel effectively cures the parasitic infection and significantly reduces the infection burden but has neither a significant effect on the hemoglobin levels of the mothers at the time of birth nor on the birth weight of their newborns. Our results are consistent with the current state of research that praziquantel is safe to use during pregnancy.[8] The side effects we reported are similar to those described in previous publications.[220] Similar to the two previous RCTs in pregnant women,[12,13] we could not find any evidence to prove that therapy during pregnancy has a beneficial effect on the course or outcome of the ongoing pregnancy.

The decision regarding the use of praziquantel during pregnancy must therefore be based on a risk-benefit analysis that also takes effects beyond the current pregnancy into account. The exclusion of pregnant women from therapy programs is also a major problem because many young women in sub-Saharan

Africa spend up to 25% of their reproductive age pregnant and up to 60% breastfeeding.[9] Additionally, in many countries with a limited health care, pregnancy and childbirth represent a period with the closest contact to the health system. Therapy outside of these periods is therefore often much more difficult and could therefore be repeatedly postponed and thus cumulatively delayed over many years.

These delays are believed to contribute to increased morbidity among young women. As mentioned above, the age with the highest burden of schistosomiasis infection is in adolescence. [7] At the same time, the rate of teenage pregnancy in sub-Saharan Africa is around 19%.[121] The exclusion of pregnant women from treatment programs in these countries therefore often means to deprive in particular young women of effective and, according to current evidence, low-risk treatment. However, young women are particularly affected by FGS, and there is evidence that avoiding praziquantel in adolescent women leads to a higher risk of urogenital pathology,[117] which in turn can persist irreversibly[119] and lead to complications such as incontinence, genital ulcers and infertility.[68]

We therefore interpret our results as further encouragement to include pregnant women in treatment and prevention programs for urogenital schistosomiasis to prevent a further increase in their disease burden with unfavorable long-term consequences.

Prof. Ayola Akim ADEGNIKA

6 Zusammenfassung (Deutsch)

Hintergrund

Schistosomiasis ist eine parasitäre Erkrankung, ausgelöst durch Erreger des Genus *Schistosoma*, die zu den *Neglected Tropical Diseases* (NTDs)[3] zählt. Unter diesem Begriff subsumiert die *World Health Organisation* (WHO) aktuell eine heterogene Gruppe von 20 verschiedenen Krankheiten, welche primär in tropischen Gebieten vorkommen und weltweit etwa 1 Milliarde Menschen betreffen.[22] NTDs sind mit Armut und schlechten sanitären Bedingungen assoziiert[23] und sind - anders als etwa HIV, Tuberkulose oder Malaria – im Verhältnis zu ihrer globalen Prävalenz in Forschung und öffentlicher Wahrnehmung stark unterrepresäntiert.[22] Infektionen mit Schistosomen betreffen rund 220 Millionen Menschen,[1] wovon der überwiegende Anteil in Subsahara-Afrika lebt.[2] Es sind insgesamt sechs humanpathogene Erreger bekannt, von denen die folgenden drei für die überwiegende Krankheitslast beim Menschen verantwortlich sind: *S. mansoni*, *S. japonicum* und *S. haematobium*.[7] Der letztgenannte ist Verursacher der urogenitalen Schistosomiasis, welche sich von der intestinalen Schistosomiasis anhand der Lokalisation des Erregers innerhalb des Körpers und der ausgelösten Pathologien unterscheidet.[7]

Schistosomiasis kann zu lokalen sowie systemischen Krankheitsfolgen führen, wie etwa Anämie, Malnutrition, Wachstumsverzögerung und eingeschränkten körperlichen und kognitiven Fähigkeiten.[133] In endemischen Regionen kommt es oftmals zu einer ersten Infektion im Kleinkindalter und dann zu einer zunehmenden Infektionslast, welche typischerweise ihren Höhepunkt in der frühen Pubertät findet, um sich dann wieder schrittweise bis ins Erwachsenenalter zu reduzieren.[7] Aufgrund dieses epidemiologischen Phänomens, haben sich Forschung und interventionelle Maßnahmen über Jahrzehnte vor allem auf Schulkinder fokussiert. Mit dem Ziel der Übertragungskontrolle und Elimination der Erkrankung hat sich jedoch der Blick auf weitere – bis dahin vernachlässigte – Bevölkerungsgruppen ausgeweitet. Hierzu zählen insbesondere Kleinkinder im Vorschulalter sowie schwangere und generell junge Frauen. [67] Etwa die Hälfte[120] der infizierten Frauen sind von einer

weiteren Komplikation der urogenitalen Schistosomiasis betroffen, der sogenannten *Female Genital Schistosomiasis* (FGS), welche sich durch charakteristische „sandige“ Genitalläsionen auszeichnet und mit Symptomen wie Kontaktblutungen und vaginalem Ausfluss einhergeht.[68]

Bei mit Schistosomen infizierten schwangeren Frauen stellt sich auch die Frage nach den Auswirkungen auf die Schwangerschaft und den Gesundheitsfolgen für die neugeborenen Kinder. Schätzungen zufolge sind etwa 13% der schwangeren Frauen in Subsahara-Afrika mit Schistosomiasis infiziert.[28,31] In den letzten Jahrzehnten ist es gelungen, zunehmende Evidenz für die Zusammenhänge zwischen mütterlicher Infektion und nachteiligen Schwangerschaftsfolgen zu generieren.[9] Hierzu zählt insbesondere ein erhöhtes Risiko für ein niedriges Geburtsgewicht, aber auch Hinweise auf eine Risikozunahme für Früh- oder Fehlgeburten, Unfruchtbarkeit und ektope Schwangerschaften.[5]

Praziquantel ist seit seiner Entdeckung der wesentliche Baustein in der individuellen Therapie, sowie in der epidemiologischen Kontrolle der Schistosomiasis.[4] Im Rahmen der Zulassung 1982 wurde Praziquantel durch die *American Food and Drug Administration* (FDA) als „Klasse B Medikament“ eingestuft,[194] da man in Tierversuchen keine Hinweise auf eine Schädigung des Fötus durch Praziquantel feststellen konnte, jedoch keine ausreichenden Studien zu möglichen Folgen beim Menschen vorlagen.[195] Dieser Einstufung folgte eine über Jahrzehnte andauernde Empfehlung, schwangere und stillende Frauen aus individuellen und Gruppen-basierten Therapieprogrammen auszuschließen.[8]

Im Jahr 2002 änderte die WHO ihre Empfehlungen hinsichtlich der Verwendung von Praziquantel bei schwangeren Frauen aufgrund einer aktualisierten Risiko-Nutzen Analyse, und der Einsatz wurde jetzt erstmals empfohlen.[10] In den Folgejahren wurden zwei große randomisierte, kontrollierte Studien durchgeführt, welche Risiko und Nutzen von Praziquantel bei schwangeren Frauen untersuchen sollten.[12,13] Beide Studien fanden kein erhöhtes Risiko für den Einsatz von Praziquantel in der Schwangerschaft, konnten jedoch auch

keinen positiven Effekt einer Therapie während der Schwangerschaft auf den Gesundheitszustand der Mütter oder Neugeborenen nachweisen.[12,13] Beide Studien beschäftigten sich jedoch mit Erregern der intestinalen Schistosomiasis (*S. mansoni* bzw. *S. japonicum*). Eine entsprechende Studie zu Risiko und Nutzen von Praziquantel bei schwangeren Frauen, welche an urogenitaler Schistosomiasis erkrankt sind, steht bis heute aus. Diese Lücke wollen wir mit der vorliegenden Arbeit schließen und stellten daher die Frage nach dem Risiko, der Effektivität und dem Einfluss auf mütterliche Anämie und Neugeborenen Gewicht bei Einsatz von Praziquantel in der Behandlung von Infektionen mit *S. haematobium* bei schwangeren Frauen.

Methodik

Wir untersuchten schwangere Frauen mit einem Gestationsalter von 16-30 Wochen (basierend auf dem Datum der letzten Regelblutung) auf das Vorliegen einer Infektion mit *S. haematobium*. Die Teilnehmerinnen wurden in drei Pränatalsprechstunden in den örtlichen Gesundheitseinrichtungen rekrutiert. Es wurden Urinproben an drei aufeinanderfolgenden Tagen gewonnen und mittels Mikroskopie auf das Vorliegen von Wurmeiern sowie mittels eines lateral-flow assay[63,65] auf das Vorhandensein des Genus spezifischen *circulating anodic antigen* (CAA) getestet. War eines der beiden Testverfahren positiv, wurde eine Infektion mit *S. haematobium* angenommen. Zusätzlich wurden auch Blut- und Stuhlproben erhoben, um den mütterlichen Hämoglobinwert zu ermitteln, sowie Begleitinfektionen mit Malaria, Geohelminthen oder *S. intercalatum* zu finden. Anhand von Fragebögen wurde der sozioökonomische Status der Teilnehmerinnen ermittelt. Frauen, welche positiv für Schistosomiasis waren, wurden in einem 3:1 Verhältnis randomisiert und in eine Interventions- und Kontrollgruppe eingeteilt. Die Intervention bestand aus 40mg Praziquantel / kg Körpergewicht, und wurde in einer einzigen Dosis verabreicht. Zur besseren Bioverfügbarkeit[41] und zur Abmilderung von Nebenwirkungen[194] wurde auf eine ausreichende Nahrungsaufnahme vor der Einnahme geachtet.

Nebenwirkungen wurden 1h und 48h nach Medikamenteneinnahme mittels standardisierter Fragebögen erhoben. Zusätzlich erhoben wir die Vitalparameter vor der Medikamenteneinnahme sowie ebenfalls nach 1h und 48h zwischen den Gruppen. Vitalzeichen wurden aufgrund wiederholter Messungen in einem gemischten Modell miteinander verglichen. Zur Untersuchung der Effektivität der Intervention wurden weitere Urinproben über einen Zeitraum von insgesamt 7 Wochen gesammelt und die Heilungsrate sowie die Reduktionsrate der im Urin ermittelten Wurmeier errechnet.

Als Endpunkte für eine mögliche Auswirkung auf den mütterlichen und kindlichen Gesundheitsstatus wurden der mütterlichen Hämoglobinspiegel bei Geburt sowie das Geburtsgewicht herangezogen, da für diese Parameter die stärkste Evidenz für einen Zusammenhang zur Schistosomiasis besteht.[5,9,135] Wir bestimmten die Prävalenz von Hämaturie in der letzten Urinprobe 7 Wochen nach Behandlung, und nahmen den Teilnehmerinnen bei der Geburt eine erneute Blutprobe ab. Hämoglobinwerte wurden ebenfalls mit einem gemischten Modell verglichen, um den individuellen Ausgangspunkt der jeweiligen Teilnehmer zu Beginn der Studie, sowie weitere (potenzielle) Störfaktoren wie Begleitinfektionen oder den sozioökonomischen Status zu berücksichtigen. Wir bestimmten und verglichen das Gewicht sowie Größe und Kopfumfang der Neugeborenen, und berechneten hieraus auch die Prävalenz von niedrigem Geburtsgewicht und (anhand des Gestationsalters) ob die Neugeborenen als *small for gestational age* (SGA) einzuschätzen waren.

Ergebnisse

Wir konnten insgesamt 666 schwangere Frauen auf das Vorhandensein von Schistosomiasis untersuchen, wovon wir 154 als positiv befanden. Insgesamt konnten 145 Teilnehmerinnen randomisiert und davon 130 in die beiden Gruppen eingeschlossen werden. Wir führten eine modifizierte *per-protocol* Analyse durch (tatsächliche vs. keine Medikamenteneinnahme) und erhielten somit eine Interventionsgruppe mit 82 und eine Kontrollgruppe mit 48 Teilnehmerinnen. Hinsichtlich der Nebenwirkungen fanden wir, dass Fatigue, Schwindel, Übelkeit

und Erbrechen in der Interventionsgruppe signifikant erhöht waren. Alle Nebenwirkungen waren jedoch von milder bis moderater Intensität und innerhalb von 48h wieder abgeklungen. Die Vitalparameter nach Intervention unterschieden sich nicht zwischen den beiden Vergleichsgruppen.

Wir ermittelten, dass die Teilnehmer der Interventionsgruppe eine um das fünffach erhöhte Wahrscheinlichkeit hatten, von Schistosomiasis geheilt zu sein. Die errechnete Heilungsrate war mit 83% in der Interventionsgruppe im Vergleich zu 48% in der Kontrollgruppe signifikant höher. Auch die Reduktionsrate der ermittelten Eier im Urin war in der Interventionsgruppe mit 95% im Vergleich zu 35% in der Kontrollgruppe signifikant höher.

Frauen aus der Kontrollgruppe hatten ein signifikant niedrigeres Risiko für das Auftreten von Hämaturie 7 Wochen nach Intervention. Wir fanden jedoch keinen signifikanten Unterschied zwischen den mütterlichen Hämoglobinwerten der beiden Studiengruppen, auch nicht nach Berücksichtigung möglicher Störfaktoren. Die Prävalenz von Anämie war zwar in der Behandlungsgruppe deutlich niedriger, der Unterschied jedoch nicht statistisch signifikant. Auch nach Stratifizierung anhand der Infektionslast ließ sich in der Subgruppe mit schweren Infektionen (> 50 Eier/ml Urin) kein Effekt nachweisen. Lediglich anhand einer Stratifizierung bezüglich des Therapieerfolgs war ein Unterschied darstellbar: So war der Hämoglobinwert in der Subgruppe der Teilnehmerinnen, welche nach 7 Wochen keine Eier im Urin mehr nachweisbar hatten, unabhängig von der Intervention, signifikant höher im Vergleich zu jenen, die weiter Eier im Urin ausschieden.

Hinsichtlich der Auswirkungen auf die kindliche Morbidität und das Ergebnis der Schwangerschaft fanden sich ebenfalls keine Unterschiede. Das Geburtsgewicht der Neugeborenen unterschied sich nicht zwischen den beiden Gruppen, ebenso wenig die anderen anthropometrischen Parameter. Auch der Anteil von Kindern mit niedrigem Geburtsgewicht oder jenen, die als *small for gestational age*

eingeschätzt wurden, unterschied sich nicht. Ebenso fanden wir keinen Anhalt, für ein höheres Risiko für Fehl- oder Frühgeburten nach Intervention.

Interpretation

Wir führten die erste randomisierte und kontrollierte Studie durch, welche die Risiken und Wirksamkeit von Praziquantel bei mit *Schistosoma haematobium* infizierten schwangeren Frauen ermittelte. Unser Ziel war es, Risiken und Nutzen einer Behandlung während der Schwangerschaft im Vergleich zur Behandlung nach der Geburt abzuwägen. Wir fanden keine Hinweise für eine Gefährdung von Mutter oder Kind durch die Anwendung von Praziquantel während der Schwangerschaft. Ebenso konnten wir zeigen, dass Praziquantel die parasitäre Infektion wirksam heilt beziehungsweise die Infektionslast deutlich reduziert, jedoch weder einen signifikanten Einfluss auf die Hämoglobinwerte der Mütter zum Zeitpunkt der Geburt noch auf das Geburtsgewicht ihrer Neugeborenen hat. Unsere Ergebnisse stimmen mit dem derzeitigen Stand der Forschung überein, dass Praziquantel während der Schwangerschaft sicher angewendet werden kann.[8] Die von uns berichteten Nebenwirkungen entsprechen denen früherer Publikationen.[220] Wie bereits in den zwei zurückliegenden Studien,[12,13] konnten auch wir keine Evidenz dafür finden, dass eine Therapie während einer Schwangerschaft den Verlauf oder das Ergebnis der bestehenden Schwangerschaft günstig beeinflussen könnte.

Die Entscheidung hinsichtlich des Einsatzes von Praziquantel während der Schwangerschaft muss also auf einer Risiko-Nutzen Analyse getroffen werden, die Effekte auch jenseits der derzeitigen Schwangerschaft in Betracht zieht. Der Ausschluss von schwangeren Frauen aus den Therapieprogrammen stellt auch deswegen ein großes Problem dar, da viele junge Frauen in Subsahara-Afrika bis zu 25% ihres reproduktiven Alters schwanger und bis zu 60% stillend verbringen.[9] Zusätzlich hierzu, stellen in vielen Ländern mit einer eingeschränkten Gesundheitsversorgung gerade die Schwangerschaft und Geburt einen Zeitraum mit der engsten Anbindung an das Gesundheitssystem dar. Eine Therapie außerhalb dieser Zeiträume ist daher oft deutlich schwerer

möglich, und wird dementsprechend immer wieder verschoben und kann so kumulativ über viele Jahre verzögert werden.

Diese Verzögerungen sorgen mutmaßlich für eine erhöhte Morbidität unter jungen Frauen. Das Alter mit der höchsten Infektionslast der Schistosomiasis ist, wie eingangs erwähnt, im Jugendalter.[7] Gleichzeitig liegt die Rate für Teenager-Schwangerschaften in Subsahara-Afrika bei etwa 19%.[121] Der Ausschluss von schwangeren Frauen aus den Therapieprogrammen bedeutet in diesen Ländern also oft insbesondere jungen Frauen eine effektive und nach aktueller Evidenz risikoarme Behandlung vorzuenthalten. Diese sind wiederum besonders von *FGS* betroffen. Zudem existiert Evidenz dafür, dass der fehlende Einsatz von Praziquantel bei jugendlichen Frauen zu einem höheren Risiko für spätere Manifestationen von FGS führt,[117] diese wiederum irreversibel persistieren[119] und zu Komplikationen wie Inkontinenz, Genitalulzerationen und Unfruchtbarkeit führen können.[68]

Wir interpretieren unsere Ergebnisse daher als weitere Bestärkung, schwangere Frauen in Behandlungs- und Präventionsprogramme für urogenitale Bilharziose einzubeziehen, um eine weitere Zunahme ihrer Krankheitslast mit ungünstigen Langzeitfolgen zu verhindern.

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8 Own Contribution Statement

The work was carried out at the research center Centre de Recherches Médicales de Lambaréné (CERMEL) in cooperation with the Tropical Institute of the University of Tübingen under the supervision of Prof. Ayola Akim Adegnika.
The superordinated *freeBILy-Gabon* trial was designed by Prof. Ayola Akim Adegnika (Principal Investigator) and Andrea Kreidenweiss (Co-Principal Investigator) in collaboration with Jean-Claude Dejon Agobé (Clinical Investigator) and Yabo Josiane Honkpehedji (Clinical Investigator). The working out of the research question relevant to the present work was carried out by Jacob Gerstenberg (Clinical Investigator) under the supervision of Prof. Ayola Akim Adegnika (Principal Investigator).

After training by Yabo Josiane Honkpehedji [Clinical Investigator], I have independently participated in the recruitment of the participants, the implementation of the intervention, as well as the follow-up and collection of the clinical endpoints together with the other clinical investigators. The delivery and the collection of newborn parameters and maternal blood samples at birth were (usually) performed by study midwives (clinical study nurses) under medical supervision in one of the two local hospitals.

Recruitment of participants included obtaining informed consent for participation, performing a general physical examination, completing questionnaires, and collecting blood, urine, and stool samples. The implementation of the intervention and clinical follow-up included the administration of the study medication, a clinical examination, the completion of standardized questionnaires and the collection of urine samples.

During my research stays at CERMEL, I have participated independently in the recruitment of 224 participants and carried out the intervention and clinical follow-up independently under medical supervision for 50 of the randomized participants.

The microscopic diagnosis of the urine and stool samples for the participants I recruited and followed up was carried out by me in collaboration with the laboratory staff [Mirabeau Nzamba Maloum, Roméo Laclong Lontchi and Alvyn Nguema Moure]. All urine samples from the participants recruited by the other Clinical Investigators were analyzed by the laboratory staff. All *circulating anodic antigen* (CAA) measurements were performed by Mirabeau Nzamba Maloum [laboratory staff] and Roméo Laclong Lontchi [laboratory staff].

The statistical evaluation of the present work was carried out independently by me. A final consultation was carried out by Dr. You-Shan Feng from the Institute for Clinical Epidemiology and Applied Biometrics (IKEAB).
I certify that I wrote the manuscript independently and that I did not use any sources other than those I have indicated.

Hannover, den

[Jacob Gerstenberg]

9 Erklärung zum Eigenanteil

Die Arbeit wurde am Forschungszentrum *Centre de Recherches Médicales de Lambaréné* (CERMEL) in Kooperation mit dem Tropeninstitut der Universität Tübingen unter der Betreuung von Prof. Ayola Akim Adegnika durchgeführt.
Die Konzeption der übergeordneten *freeBILy-Gabon* Studie erfolgte durch Prof. Ayola Akim Adegnika (Principal Investigator) und Andrea Kreidenweiss (Co-Principal Investigator) in Zusammenarbeit mit Jean-Claude Dejon Agobé (Clinical Investigator) und Yabo Josiane Honkpehedji (Clinical Investigator). Die Ausarbeitung der für die vorliegende Arbeit relevanten Fragestellung erfolgte durch Jacob Gerstenberg (Clinical Investigator) unter der Supervision von Prof. Ayola Akim Adegnika (Principal Investigator).

Die Rekrutierung der Teilnehmerinnen, die Durchführung der Intervention, sowie die Nachbeobachtung und Erhebung der klinischen Endpunkte wurden von mir eigenständig, nach Einarbeitung durch Yabo Josiane Honkpehedji [Clinical Investigator], sowie durch die anderen Clinical Investigators durchgeführt. Die Entbindung sowie die Erhebung der Neugeborenen Parameter und mütterlichen Blutproben bei Geburt erfolgten (in der Regel) durch Studien-Hebammen (clinical study nurses) unter ärztlicher Supervision in einem der beiden örtlichen Krankenhäusern.

Die Rekrutierung der Teilnehmerinnen umfasste die Erhebung des Einverständnisses zur Teilnahme, die Durchführung einer allgemeinen körperlichen Untersuchung, das Ausfüllen von Fragebögen sowie das Abnehmen von Blut-, Urin und Stuhlproben. Die Durchführung der Intervention und klinischen Nachbeobachtung umfasste das Verabreichen der Studienmedikation, die klinische Untersuchung, das Ausfüllen standardisierter Fragebögen sowie die Abnahme von Urinproben.

Während meiner Forschungsaufenthalte am CERMEL habe ich eigenständig an der Rekrutierung von 224 Teilnehmerinnen teilgenommen und bei 50 der randomisierten Teilnehmerinnen eigenständig und unter ärztlicher Supervision die Intervention sowie die klinische Nachbeobachtung durchgeführt.

Die mikroskopische Diagnostik der Urin- und Stuhlproben für die von mir rekrutierten und nachbeobachteten Teilnehmerinnen wurde von mir unter Supervision durch das Laborpersonal [Mirabeau Nzamba Maloum, Roméo Laclong Lontchi und Alvyn Nguema Moure] durchgeführt. Alle Urinproben der durch die anderen Clinical Investigators rekrutierten Teilnehmerinnen wurden durch das Laborpersonal untersucht. Alle Messungen des *circulating anodic antigen* (CAA) wurde durch Mirabeau Nzamba Maloum [Laborpersonal] und Roméo Laclong Lontchi [Laborpersonal] durchgeführt.

Die statistische Auswertung erfolgte eigenständig durch mich. Es erfolgte eine abschließende Beratung durch Dr. You-Shan Feng vom Institut für Klinische Epidemiologie und angewandte Biometrie (IKEAB).

Ich versichere, das Manuskript selbstständig verfasst zu haben und keine weiteren als die von mir angegebenen Quellen verwendet zu haben.

Hannover, den

[Jacob Gerstenberg]

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