UNIVERSITÄTSKLINIKUM HAMBURG-EPPENDORF

Prevalence of *Schistosoma mansoni* among children under one year in the central highlands of Madagascar

Dissertation

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III Abbreviations

ANC	Antenatal Care
BNITM	Bernhard Nocht Institute for Tropical Medicine
CI	Confidence Interval
CICM	Charles Mérieux Center of Infectious Disease
CRCT	Cluster randomized controlled trial
CRF	Case Report Form
CI	Confidence Interval
REDCap®	Research Electronic Data Capture
DALY	Disability-adjusted life years
FAO	Food and Agricultural Organization of the UN
FreeBILy	Fast and reliable easy to use-diagnostics for eliminating bilharzia in young children and mothers
GDP	Gross domestic product
IQR	Interquartile Range
IQR	Interquartile Range
KID	Kit Identification Number
LMIC	Low-and-middle-income countries
LUMC	Leiden University Medical Center
MDA	Mass drug administration
N	Number
NTD	Neglected Tropical Disease
PEPRAMA	Paving the way for pediatric praziquantel accessibility in Madagascar
PC	Preventive chemotherapy
PCR	Polymerase Chain Reaction
PHCC	Primary Health Care Center
PID	Participant Identification Number
POC-CCA	Point-of care circulating cathodic antigen
PSAC	Pre-school-aged children
PZQ	Praziquantel
RDT	Rapid Diagnostic Test
SAC	School-aged children
SAE	Severe adverse events
SDGs	Sustainability Development Goals
SDI	Socio-Demographic Index
SOP	Standard Operating Procedure
TBST	Test-based schistosomiasis treatment
TID	Tube Identification Number
UCP	Up-Converting Phosphor
UN	United Nations
WASH	Water, sanitation and hygiene
WHO	World Health Organization

1 Aim and research questions

The aim of this study is to assess the prevalence of *Schistosoma* infection among children under one year old in an endemic area in the Amoron'i Mania region in the central highlands of Madagascar.

Research questions:

(1) What is the prevalence of *S. mansoni* in children under one year as determined by point-of-care circulating cathodic antigen (POC-CCA) testing of urine?
(2) Does prevalence differ between rural and urban communities?
(3) Are children from infected mothers more likely to be infected than children from non-infected mothers?

2 Introduction and background

Schistosomiasis is one of the Neglected Tropical Diseases defined by the World Health Organization (WHO). Globally, the vector borne parasitic disease is endemic in 78 countries, especially in tropical and subtropical areas. In 2021, no less than 250 million people required preventive treatment. Estimates show that at least 90% of those requiring treatment for schistosomiasis live in Africa where the disease represents one of the major public health issues (WHO, 2022a). The high prevalence in this region puts over 700 million people at risk of infection, predominantly living in poor communities in low-and-middle-income countries (LMIC) without access to adequate sanitation and safe drinking water (WHO, 2022a).

Worldwide, approximately 120 million children are affected by schistosomiasis (Faust et al., 2020). Most data on prevalence are generated from school-aged children (SAC) from African countries. SAC also represent the main target group for disease control programs like mass drug administrations (MDA). Children under the age of 5 are not regularly included in these campaigns, even though it is assumed that around 50 million of the affected children worldwide are pre-school-aged children (PSAC) (Faust et al., 2020).

This thesis aims to put the focus on the population of the PSAC, precisely children under one year in the highlands of central Madagascar, and investigate the prevalence of the disease for this vulnerable age group.

2.1 Neglected Tropical Diseases

Neglected Tropical Diseases (NTDs) are a group of 20 conditions inducing health, social and economic problems to over 1.7 billion people worldwide (WHO, 2023a).

They occur mostly in tropical climates where they are caused by pathogens such as helminth parasites, protozoan parasites, bacteria and fungi and viruses or toxins (Hotez et al., 2020) (**Figure 1**).

Globally, NTDs are the cause for approximately 200.000 deaths per year and account for 19 million disability adjusted life years (DALYs) annually (WHO, 2023a).

NTDs disproportionally often affect populations that are already vulnerable – whether through geographic location, poverty or marginalization because of gender or age. Children and women from impoverished communities are particularly affected (WHO, 2023a).

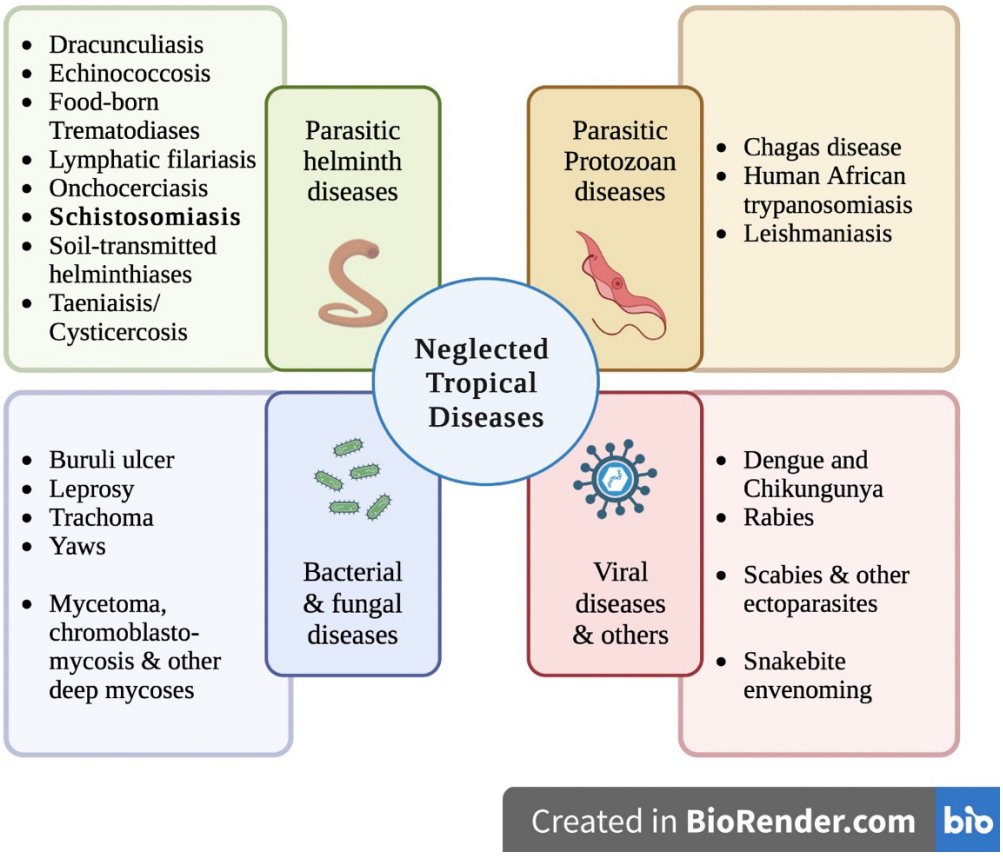


Figure 1 *The 20 Neglected Tropical Diseases*
From (Hotez et al., 2020), own visualization, created with BioRender.com (2023)

Most of these populations are found in countries with a low Socio-Demographic Index (SDI). The Global Burden of Disease Study led by the Institute for Health Metrics and Evaluation (IHME) pointed out the high relevance of NTDs in low SDI countries where NTDs and malaria combined represented the fourth biggest cause of DALYs in 2019, right after maternal and neonatal disorders, respiratory and enteric infections (Institute for Health Metrics and Evaluation (IHME), 2019). In addition to their impact on health, NTDs also contribute to an immense economic and social burden in SDI countries, for example due to loss of work productivity because of physical disabilities, social stigma and exclusion and discrimination (Mitra and Mawson, 2017). It is estimated that NTDs cost the affected countries the equivalent of billions of United States dollars each year (WHO, 2023a).

The fact that this group of diseases has not gotten much attention from traditional profit-oriented pharmaceutical companies and well-funded research groups has contributed to the vicious cycle of poverty and increased health consequences and disabilities (Drugs for Neglected Diseases initiative, 2023).

The movement to fight the NTDs has a long history, dating back to the last decades of the 20th century. A major turning point was the creation of a new department of NTDs by the

WHO in 2005 and a first official list of initially 13 diseases published in *PLOS Medicine* that was expanded the same year (Hotez et al., 2020; Molyneux et al., 2021).

In 2012, a meeting in London, chaired by Bill Gates, brought together the main partners in the support of NTDs, resulting in the London Declaration that significantly increased the global commitment to combat NTDs in the following years (Molyneux et al., 2021). Today, the NTDs are an integral part of the Sustainability Development Goals (SDGs) of the United Nations with the target to end the epidemic of NTDs by 2030 in SDG 3.3 (United Nations, 2023).

In 2020, the WHO published an updated version of the roadmap for NTDs 2021-2030 with strategic approaches aiming to end the neglect to attain the SDGs by 2030 (WHO, 2020). In this roadmap, the WHO defines concrete targets to fight the NTDs and reach overarching global targets like universal health coverage or access to basic water supply, sanitation and hygiene in endemic areas.

Each NTD is listed individually with specific explanations of the actions required to achieve the targets. Concerning schistosomiasis, one of the most ambitious targets is to reduce the proportion of heavy intensity infections to under 1% in all 78 countries where the disease is endemic in order to achieve the elimination of schistosomiasis as a public health problem worldwide (WHO, 2020).

2.2 Schistosomiasis

Human schistosomiasis, also known as bilharzia, named after the physician who first described the disease in Egypt in 1851, is a parasitic infectious disease (LoVerde, 2019). It is transmitted by blood flukes of the genus *Schistosoma*. Worldwide six different species are known as human pathogens: *Schistosoma mansoni*, *Schistosoma japonicum, Schistosoma mekongi*, *Schistosoma guineensis* and *Schistosoma intercalatum* cause the intestinal form of the disease. The sixth species, *Schistosoma haematobium,* leads to the urogenital form (WHO, 2022a).

Clinically most important as well as most studied are *S. mansoni*, *S. japonicum* and *S. haematobium* of which *S. mansoni* and *S. haematobium* are widely endemic in Madagascar (Deka, 2022).

2.3 Transmission and zoonotic life cycle of *S. mansoni*

Schistosomiasis is transmitted to the human body by skin contact with infested open water bodies. *Schistosoma* eggs are excreted into the water through human feces, hatch and release their ciliated free-swimming larvae miracidia. Inside the water, snails of the genus

Biomphalaria spp. serve as intermediate hosts. The miracidia penetrate the snail and develop into sporocysts, which thereupon produce numerous cercaria through asexual reproduction (McManus et al., 2018). The free-swimming cercariae emerge back into the water where they can survive 1-3 days before penetrating the skin of the human host (Colley et al., 2014). Inside the skin, the cercariae transform into schistosomula larvae which are capable of entering the venous blood vessels. The schistosomula are transported to the liver via the lungs and heart reaching the arterial system. Once in the hepatic portal system, they mature into adult worms of both sexes. The male and female worms continuously mate with the male worm entering the gynaecophoral canal of the female. *In copula* they migrate to mesenteric veins of the bowel where the female produces eggs that are fertilized by the male. Eggs are released into the blood vessels, pass the intestinal wall and can thus be excreted in the feces (McManus et al., 2018). If excreted into water the cycle restarts as visualized in **Figure 2**. Schistosomes live inside their human hosts for an average of 3-10 years, in some cases even longer, constantly shedding eggs (Colley et al., 2014).

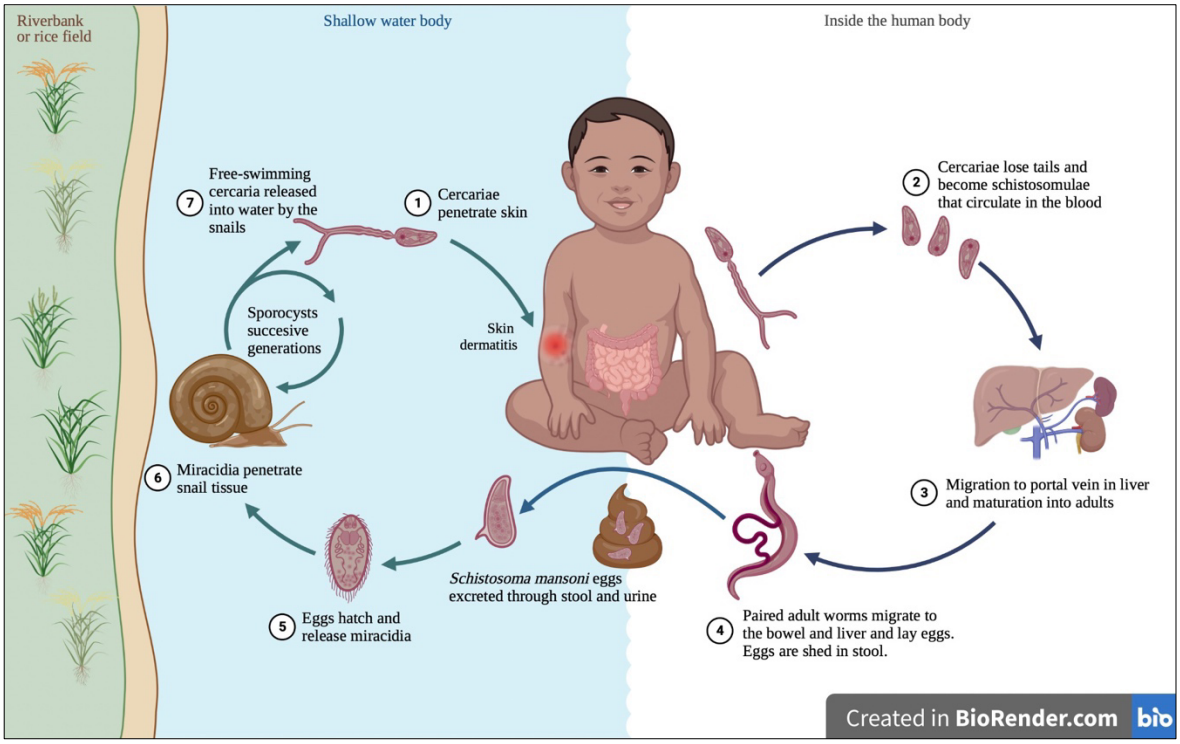


Figure 2 *Visualization of the lifecycle of S. mansoni adapted from "Schistosoma mansoni infection cycle", by BioRender.com (2022). Retrieved from https://app.biorender.com/biorender-template*

Explanation: Starting from (1) free-swimming cercariae entering the body by penetrating the skin, (2) loosing tails and becoming schistosomulae, that circulate in the blood and (3) migrate through the portal venous system into the liver. (4) Maturation, pairing and migration to the bowel; deposition of eggs that are shed in stool. (5) Eggs hatch and release miracidia (6) entering the snail as intermediate host. Asexual production of numerous generations of (7) free-swimming cercariae released into the water.

2.4 Symptoms and long-term effects

Depending on the state and phase of the disease, *Schistosoma* infection can present itself in various clinical pictures.

At first, the cercariae entering the skin can induce cercarial dermatitis. Part of the larvae die inside the skin tissue, causing hypersensitivity reactions. Clinically, this immune response presents itself as temporary maculopapular pruritic lesions on the exposed body parts (McManus et al., 2018). Subsequently, the cercariae that successfully reached the blood system and matured into schistosomula can cause acute schistosomiasis, that, in many cases, is asymptomatic. If symptoms manifest it is called Katayama fever or syndrome. This acute form occurs from 2 weeks to 3 months after exposure and usually affects individuals exposed to schistosomes for the first time, such as travelers to endemic regions (McManus et al., 2018). The pathophysiology behind the Katayama syndrome is a systemic hypersensitivity reaction against the eggs and the schistosomula migrating through the body. Non-specific symptoms such as headache, fever, myalgia, fatigue and eosinophilia as well as the delayed onset of the disease often lead to misdiagnosis. Patients usually recover spontaneously after a few weeks. In some cases, the development of a persistent disease with various symptoms such as diarrhea, abdominal pain, weight loss and dyspnea have been reported (Ross et al., 2007).

In endemic areas, within chronically exposed populations, the Katayama syndrome occurs rarely. When it comes to chronic infection, schistosomiasis presents itself after months to years with systemic effects and various symptoms depending on the *Schistosoma* species.

S. mansoni infection mostly affects the intestinal tract as well as the liver.

Intestinal schistosomiasis is caused by the deposition of eggs in the walls of the bowel. The adult worms living *in copula* in the mesenteric veins depose their eggs that retain in the intestinal tissue and induce the formation of granulomas. The resulting mucosal inflammation leads to microulcerations with superficial bleeding and pseudopolyposis. Hence, typical clinical symptoms are abdominal pain, diarrhea and rectal bleeding (McManus et al., 2018).

In patients with hepato-splenic schistosomiasis, eggs flow from the mesenteric veins into the liver passing the portal venous system where they remain in the periportal tissue. Again, granulomas are formed that can cause a significant enlargement of the liver and spleen (McManus et al., 2018).

Over time, granulomas can develop into fibrotic tissues. Even though patients with periportal liver fibrosis retain their hepatocellular function, the fibrotic remodeling is often irreversible. The fibrosis induces portal hypertension and esophageal varices (Colley et al., 2014). Complications are ascites and esophageal bleeding, clinically presenting with either acute hematemesis that can quickly lead to death or occult or repeated bleeding, eventually leading to anemia, cachexia and hypoalbuminemia (Gryseels et al., 2006).

In rare cases, schistosome eggs can be deposited in or migrate to ectopic locations like the skin, the spleen, the central nervous system or the lungs causing organ-specific symptoms. For example, *S. mansoni* worms sometimes locate in the spinal venous plexus causing neuroschistosomiasis that most often presents with transverse myelitis (McManus et al., 2018).

The intensity of the infection along with the intensity of the organ-specific symptoms is often associated with the amount of eggs in the body, measured by excreted eggs. Hence, recurrent exposure to infested water and thus constant reinfection can lead to intensification of the disease (Colley et al., 2014).

In addition to the organ-specific symptoms, many patients infected with any *Schistosoma* species suffer from non-specific systemic effects. These potentially disabling morbidities include anemia, malnutrition and impaired childhood development (Colley et al., 2014).

The exact pathophysiology behind anemia in patients with schistosomiasis has not been fully understood to date. Since anemia affects approximately one third of the global population, mostly living in LMIC, and often occurs in patients with various anemia-inducing conditions, it is difficult to define the direct correlation with schistosomiasis (Chaparro and Suchdev, 2019).

Potential mechanisms studied so far suggest the following reasons: iron deficiency due to extra-corporeal loss of iron, autoimmune hemolysis, splenic sequestration and anemia of inflammation and chronic disease (Friedman et al., 2005).

2.4.1 Specific effects of schistosomiasis in children

Several studies have shown that children can already be infected at very young age and that schistosome infection can cause impaired childhood development (Odogwu et al., 2006; Colley et al., 2014; Kalinda et al., 2020).

Unfortunately, schistosomiasis in children has again different effects than in adults because it affects them during their childhood development (Osakunor et al., 2018).

Early and chronic exposure to schistosomes leading to high intensity infections are particularly often accompanied by hepato-splenomegaly. Following the chronic infection,

children as young as six years old can already show clinical symptoms of potentially irreversible periportal fibrosis with highly enlarged abdomen and the severe complications described above (Colley et al., 2014).

The damage of the epithelial tissue in the bowel results in malnutrition and anemia and thus eventually, delay of growth (Osakunor et al., 2018).

It has also been shown that infected children can suffer from cognitive impairment due to the anemia of iron deficiency. The chronic inflammation has an impact on the iron status which is important for the neurological development. As a result of the infection, the cognitive function can be reduced (Friedman et al., 2005).

Furthermore, schistosome infection has a direct impact on the gut. To maintain the barrier integrity of the bowel the gut microbiome is essential. During the first years of life in particular, the microbiome is highly variable and any change can have impacts on the child's immune system. However, the exact relationship between schistosomiasis and the microbiome are yet to be understood since we are just starting to appreciate the importance of the microbiome (Osakunor et al., 2018).

Moreover, it has been speculated that chronic exposure can also reduce the efficacy of childhood vaccines due to the constant immune response to schistosomes and their eggs caused by the infection. Hence, children affected by schistosomiasis can be at higher risk of acquiring vaccine-preventable diseases (Osakunor et al., 2018).

2.5 Diagnostic of schistosomiasis

The diagnostic of the disease can be challenging. Clinical symptoms, such as anemia or hepatomegaly, can represent proxy indicators for the diagnosis but are often unspecific. In addition, some patients report blood in stool which can correspond with intestinal schistosomiasis but with a very low specificity (Utzinger et al., 2015).

Various diagnostic tools are available, some of which are explained in the following section with a focus on the test method used in the study: the *point-of care circulating cathodic antigen* (POC-CCA) – test. The main available laboratory test methods for *S. mansoni* detection are displayed in **Table 1**. Some of the listed methods can be applied to diagnose both intestinal (*S. mansoni)*, as well as urogenital (*S. haematobium)* schistosomiasis. Diagnostic tests that are exclusively used to detect urogenital schistosomiasis through *S. haematobium* infection, such as urine filtration and microscopy (Hoekstra et al., 2021), will not further be explained in this thesis.

Table 1 *Diagnostic tools for detection of S. mansoni infection; adapted from McManus et al., 2018*

Test method	Sample	Quantitative Yes/No	Sensitivity	Specificity	Applicability in endemic settings	
					PHCC	Laboratory
Microscopy						
Kato-Katz	10-50 mg of fresh feces	Yes	Low	High	**Yes**	Yes
FECT	10g mg of fresh feces	No	Medium	High	No	Yes
Antigen detection						
ELISA	Serum or urine	Yes	High	High	No	No
POC-CCA	Urine	Semi-quantitative[1]	High	High	**Yes**	Yes
UCP-LF-CCA	Urine	Yes[2]	High	High	No	Yes/no[2]
Antibody detection						
ELISA	Serum	No	High	Low	No	Yes/no[2]
IHA	Serum	No	High	Low	No	Yes/no[2]
DNA detection						
PCR	Fresh or frozen feces, rectal snip, urine or blood	Yes	High	High	No	Yes/no[2]
LAMP		Yes	High	High	No	Yes/no[2]

CAA, circulating anodic antigen; CCA, circulating cathodic antigen; ELISA, enzyme-linked immunosorbent assay; FECT, formalin-ether concentration technique; IHT, indirect haemagglutination assay; LAMP, loop-mediated isothermal amplification; LF, lateral flow; NA, not applicable; PHCC, public health care center; POC, point-of-care; UCP, up-converting phosphor.
[1]The test differentiates between negative, trace, 1+, 2+ and 3+; [2]requires specific equipment, reagents or trained personnel

2.5.1 Laboratory methods

2.5.1.1 Microscopic detection of S. mansoni eggs

Widely used in endemic regions is the microscopic Kato-Katz technique, which is the current WHO reference standard for diagnosing intestinal schistosomiasis (Hoekstra et al., 2021; WHO, 2022a). Through this method, the *S. mansoni* egg count in fecal specimens is measured. The egg count assumably correlates with the worm load of the patient and thus, provides a measure for the intensity of infection. It is relatively inexpensive and simple and can be used in Primary Health Care Centers (PHCCs) if trained staff and a microscope are available. However, it has a low sensitivity and low intensity infections are at risk to be missed (Utzinger et al., 2015).

2.5.1.2 DNA detection

In high-resource settings, the PCR-based detection of schistosome DNA in urine, feces or blood is a very sensitive and specific method but it requires high laboratory standards and is often not yet applicable in endemic regions (McManus et al., 2018).

2.5.1.3 Antibody and antigen detection

Enzyme-linked immunosorbent assays (ELISA) and indirect hemagglutination assays (IHA) are sensitive serological methods used for detecting schistosome antibodies (Utzinger et al., 2015). Schistosomes release metabolites, known as circulating anodic antigens (CAAs) and circulating cathodic antigens (CCAs), into the bloodstream while feeding on erythrocytes (McManus et al., 2018). These antigens can be detected in urine or serum using enzyme-linked immunosorbent assay (ELISA) or monoclonal-antibody-based lateral flow assays. Detection of CAAs or CCAs in the body is an indication of an active infection with live worms, and can be detected even before egg production starts (McManus et al., 2018).
However, similar to the PCR method, they require well equipped laboratories. Besides, ELISA cannot differentiate between past and present infections, making it of limited use in endemic regions where individuals may have previously received treatment (Utzinger et al., 2015). Nevertheless, this schistosome-specific antibody detection methods plays a key role in diagnosing travelers (Hoekstra et al., 2021). In those previously naïve returning travelers, the diagnosis is often even more challenging because they usually present a low worm burden (Casacuberta-Partal et al., 2020).

2.5.1.4 The POC-CCA-test

A rapid diagnostic test (RDT) based on the detection of the above mentioned CCAs in urine was developed and became commercially available in 2008: the *point-of care circulating cathodic antigen* (POC-CCA) – test. It has shown a high sensitivity in detecting *S. mansoni* infections, being much more sensitive than the current WHO gold standard Kato-Katz (Colley et al., 2020). However, the specificity is highly variable in different populations ranging from 56% to 94% in a study conducted in 5 different African countries (Colley et al., 2013).
Its major advantage is that it only needs urine which is easy to collect and non-invasive. Also, the handling of the test is uncomplicated and does not require neither highly trained staff nor laboratories (Colley et al., 2017). Therefore, even though its full integration into public health programs is still debated and more data on sensitivity and specificity is needed, it is being increasingly used for field-based surveillance and control programs especially in

endemic regions where it has been repeatedly evaluated over the last years (Colley et al., 2020; Danso-Appiah et al., 2016; Utzinger et al., 2015).

The POC-CCA test consists of a rapid test cassette that shows a positive, negative or invalid result within only 20 minutes after application of urine (ICT INTERNATIONAL, 2018). In addition, it can be used as a semi-quantitative method in research settings, even though a standardization of this methodology is not yet approved by the manufacturer. If the test is valid, a G Score system (**Figure 3**) can be used to determine the intensity of the infection. Several studies have reported a positive correlation between fecal egg counts and increasing test line intensity (Coulibaly et al., 2013; Dawson et al., 2013; Kittur et al., 2016). The G score is a panel of 10 cassettes (G1, G2, G3, G4, G5, G6, G7, G8, G9 and G10) that aims at standardization of the visual reading in order to reduce the reader dependency. This methodology for semi-quantitative interpretation of the POC-CCA tests was developed at the Leiden University Medical Center (LUMC) in the Netherlands (Casacuberta-Partal et al., 2019). Inside each cassette two pre-printed strips show the control line on top and the test line below. The intensity of the test strips is increasing so that it is non-existent in G1 and very intense in G10. Depending on the intensity of the test strip, a score will be assigned to the sample indicating its level of negativity or positivity.

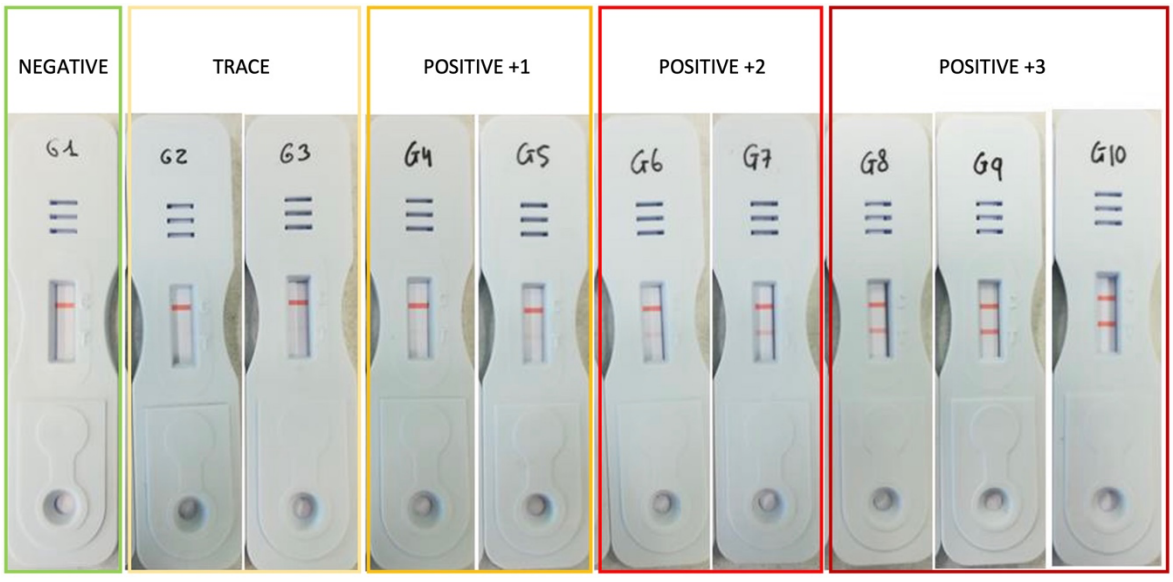


Figure 3 *POC-CCA G Score; cassettes used for intensity interpretation (SOP-LAB-07-FRA, 2020)*

2.6 Treatment of schistosomiasis

The treatment of schistosomiasis involves the use of the oral anthelmintic drug Praziquantel, which is the drug of choice since over 30 years (McManus et al., 2018). The exact mechanism of the drug has not yet been fully understood. It is assumed that Praziquantel

induces tetanic contractions of the adult worms causing surface damage that leads to detachment of the parasite from venous walls and death (Ross et al., 2002).

The drug is effective against all species of *Schistosoma*. Nevertheless, its effectiveness has shown high variability in different studies. A recently published meta-analysis evaluating the efficacy of Praziquantel in Ethiopia showed a cure rate of 89.2 % for *S. mansoni* and an egg reduction rate of 90.2% when administered at the appropriate single dose of 40mg/kg recommended by the WHO (Hailegebriel et al., 2021). But, previous studies only revealed cure rates variating from 36.0 % to 76.7%, using the same dosage (Doenhoff et al., 2008; Zwang and Olliaro, 2014).

Furthermore, Praziquantel is only effective against adult worms and has no effect on immature schistosomes. Thus, it does not alter the schistosome life cycle and re-infections are possible. This is important in regard of the management of treatment because it explains why multiple intakes of praziquantel might be required in order to eliminate all schistosomes that emanate from one infection (McManus et al., 2018).

Generally, Praziquantel is a safe drug. Adverse events are transient and rarely severe. They include headache, dizziness, abdominal pain, nausea and pruritus (McManus et al., 2018).

Praziquantel is registered for use in children older than 4 years (Zwang and Olliaro, 2017). Several studies have investigated the efficacy and safety of Praziquantel in PSAC and SAC. The recommended dose of 40mg/kg has been proven to be efficient and safe, with few reports of adverse events (Coulibaly et al., 2017a; Osakunor et al., 2018; Zwang and Olliaro, 2017). Nonetheless, there is no official recommendation on dosage for children under 4 years. Unfortunately, the Praziquantel tablets are of large size and have a bitter taste which complicates the treatment of PSAC. Currently, Praziquantel is administered crushed and mixed with sweet liquids, but there is few information on the pharmacokinetics in PSAC when using this method (Osakunor et al., 2018). A new potential pediatric formulation for children aged 3 months to 6 years is under development. It is a smaller, orodispersible tablet with an acceptable taste and thus, more suitable for treatment of PSAC (The Pediatric Praziquantel Consortium, 2023). A phase 3 trial conducted in Ivory Coast and Kenya showed promising first results with cure rates similar to those of standard Praziquantel and no safety concerns (N'Goran et al., 2023).

2.7 Control strategies and elimination attempts

To achieve schistosomiasis control and elimination as a public health problem, several strategic prevention interventions need to be implemented. In absence of a vaccine against schistosomiasis, alternative preventive measures must be adopted to control the disease. The

main control measure worldwide is the use of Praziquantel as preventive chemotherapy (PC) in MDA campaigns aiming to reduce the prevalence of high intensity infections (WHO, 2022a). In MDA campaigns targeted populations should be treated regularly with a single dose of Praziquantel without prior testing. The main target group for this secondary prevention is SAC because they are easy and cost-effectively accessible through campaigns at school. In the last decades it has always been the goal to reach a national coverage higher than 75% of SAC in targeted endemic intervention areas receiving PC for schistosomiasis. The new NTD Roadmap 2021-2030 showed that a worldwide coverage for targeted SAC of 67% was reached in 2020 (WHO, 2020). Unfortunately, during the COVID-19 pandemic, the provision of NTD interventions, such as MDA programs for schistosomiasis, decreased drastically. In 2021, only 43.3% of targeted SAC globally received PC for schistosomiasis (WHO, 2022b).

Several studies have shown that PSAC as well as adults, especially those with occupational or domestic infested water contact are at high risk of infection, too, and should be included in MDA programs (Faust et al., 2020; Gruninger et al., 2023).

Consequently, the WHO issued a new guideline for the control and elimination of human schistosomiasis in 2022. They recommend the extension of PC to all people at risk aged 2 years and older in areas with a prevalence higher than 10% (WHO, 2022c).

Despite all the effort for implementing MDA programs, PC alone is not sufficient to achieve elimination as a public health problem. Another core strategic intervention addressed in the NTD roadmap is WASH (Water, sanitation and hygiene) (WHO, 2020). The lack of toilets, management of infested feces and access to safe water across most of the endemic countries make it very difficult for the communities to avoid infection. In many communities, contact with water is an essential part of daily activities, whether through occupational exposure while fishing or farming, or domestic work like laundry or dish washing and bathing in contaminated water bodies. Moreover, many children are at risk through recreational swimming, playing in shallow water or when being bathed by their parents (Grimes et al., 2015; McManus et al., 2018). Hence, the national implementation of better WASH infrastructures including individual hygiene education is crucial, not only to achieve schistosomiasis control but also to prevent many other diseases, create healthy environments and improve the overall quality of life (WHO, 2023b).

Furthermore, health education to raise awareness of the disease and attain behavioral changes and community participation is needed (WHO, 2020). There are several studies conducted on awareness levels in different populations. A study from Western Ethiopia

showed that only 13% of the surveyed participants living in an endemic area had heard about schistosomiasis (Assefa et al., 2021). A study conducted in a province in Mozambique with high prevalence of up to 90% revealed an awareness of 91% (Rassi et al., 2016). Unfortunately, in both populations, misconceptions about the transmission, treatment and prevention of the disease were common, and even the local health workers had insufficient knowledge (Assefa et al., 2021; Rassi et al., 2016). Of course, these studies do not represent the awareness in all endemic countries, but they show the importance of integrating awareness and educational programs on a community level. Nevertheless, even in presence of a potential good knowledge about the disease, awareness alone is often not sufficient to prevent the infection. Many, particularly poor, communities simply do not have a choice but have to adopt behaviors that put themselves at risk because of lacking infrastructures that could avoid contact with water (Klohe et al., 2021).

Another NTD Roadmap approach to eliminate schistosomiasis is through vector control (WHO, 2020). In order to perpetuate the *Schistosoma* transmission life cycle, an intermediate host is required: the fresh water snails, which best survive in shallow still water. By eradicating these snails, the transmission is interrupted and consequentially, the risk of infection reduced. Snail control can be done by killing them through the use of chemicals released into the water regularly. This method is very effective but time-consuming and expensive, especially when large geographic areas are involved. Other concerns include the toxicity to other animals such as fish and the general pollution. More ecological strategies of snail control involve the use of natural snail predators such as fish and crustaceans or the introduction of schistosome-resistant snail strains into the wild snail population, and environmental methods, such as digging drainage tunnels or ditches to bury or flood the snail habitats (McManus et al., 2018).

2.8 Consequences and long-term chronic burden

If left untreated, schistosomiasis has a huge impact on the individual health and well-being of the infected person. In 2016, the disease was accountable for approximately 24.000 deaths and about 2.5 million DALYs (WHO, 2020). In addition to that, it also creates a high burden for the society. The disabilities caused by the disease lead to impaired school performances and subsequently, reduced work performances which prevents upward socioeconomic mobility for the affected people. A weak national work force as well as high expenses for health care and long-term treatment put an additional financial burden on the endemic countries. Hence, poverty is not only the source of schistosome infections, but also the effect (King, 2010).

The following **Figure 4** visualizes the vicious cycle of poverty from birth to adulthood perpetuated by schistosomiasis if no control interventions are carried out and no treatment is given.

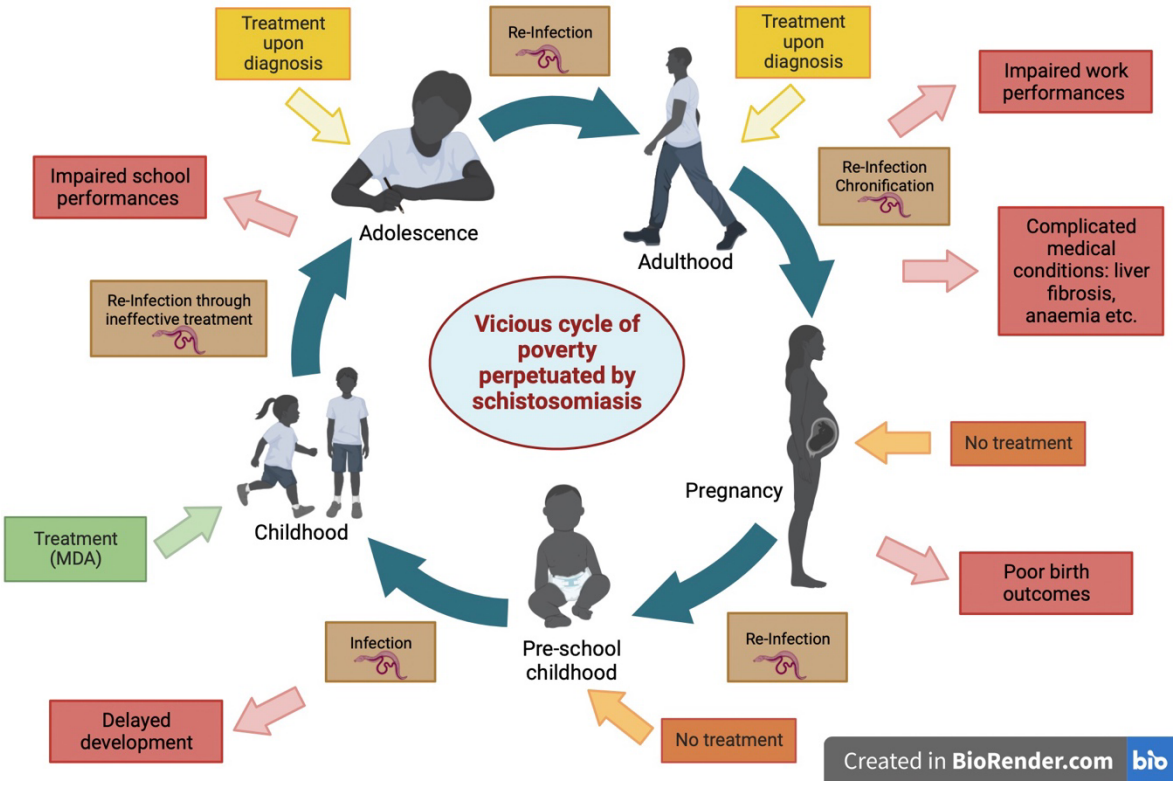


Figure 4 *The vicious cycle of poverty perpetuated by schistosomiasis*
own visualization created with BioRender.com (2023); idea elaborated in cooperation with lab group Fusco (BNITM)

2.9 Madagascar: a country with high schistosomiasis endemicity

The East African country Madagascar is the world's fifth largest island, located off the coast of Mozambique in the Indian Ocean. Its geographically varied landscape ranges from arid regions in the South over fertile temperate central highlands to tropical coastlines (Central Intelligence Agency, 2023).

Madagascar has a long history of settlements from all surrounding continents, resulting in a very diverse population with various ethnicities. After centuries of the native Merina kingdom, it became a French colony that regained independence in 1960 as the third African country. It is now a semi-presidential Republic. Official languages are Malagasy and French. The population of almost 29 billion inhabitants (2023) is very young, nearly 60% are under the age of 25 years (Central Intelligence Agency, 2023).

With a poverty headcount ratio of 80% (2012) and a GDP per capita of 500 current US$ (2021) it is one of the poorest countries in the world. The country's human capital index of

0.4 (2020) indicates the low status of education and health, causing reduced worker productivity (The World Bank, 2022).

I addition, Madagascar is facing social and economic challenges due to climate change. The country has repeatedly been hit by cyclones, storms and droughts in the last years, destroying agricultural land, roads, schools and health centers and increasing the rural to urban migration and the poverty rate (The World Bank, 2023).

2.9.1 Infrastructure and health care system in Madagascar

Madagascar is divided into 6 provinces, 22 regions, 1,597 communes and 17,500 *Fokontany*, the smallest Malagasy administrative unit. As part of the policy of decentralization, this division is part of the political will to bring decision-making, development planning and administration closer together (Ministère de la Santé Publique de Madagascar, 2016).

Infrastructure is a challenge. The urbanization is low, 60% of the population live in rural areas (Central Intelligence Agency, 2023). There are long distances between towns and the road network, of which only 10% is paved, is in poor condition (Ministère de la Santé Publique de Madagascar, 2016). Only one third of the population has electricity (The World Bank, 2022).

Furthermore, access to WASH is a great issue. Improved drinking water sources, such as household connections, public standpipes, boreholes, protected dug wells, protected springs or rainwater collection, are accessible to only 56% of the population and in rural areas the percentage is even lower with 38%. Only 33% of the total population have access to improved sanitation facilities, 22% in rural areas (Central Intelligence Agency, 2023).

Yet another challenge is access to healthcare. The physician density is 0.2 per 1,000 inhabitants (Central Intelligence Agency, 2023). Health services are provided by university hospitals in the biggest cities, regional and district referral hospitals and PHCCs (Ministère de la Santé Publique de Madagascar, 2016). The latter serve the majority of the population. PHCCs, which are rarely staffed by a doctor but are mostly run by midwives or nurses, offer basic health care services and engage in health promotion and education, with priority given to maternal, child and reproductive health. In terms of geographical accessibility, 60% of the population lives more than 5 km from a PHCC, and 20% of Madagascar's areas are very isolated, with the population lacking adequate means of transport (Ministère de la Santé Publique de Madagascar, 2016). This lack of sufficient infrastructure makes the management of general health care for the population, and the control of schistosomiasis in particular, even more challenging.

2.9.2 Schistosomiasis prevalence and elimination attempts in Madagascar

As pointed out previously, schistosomiasis is a disease of poverty and therefore Madagascar, one of the poorest countries in the world, is among the countries with the highest prevalence. It is endemic in 106 out of 113 administrative sanitary districts. In 44 districts the prevalence is 50% and above (**Figure 5**) (ESPEN, 2021a).

The two dominant *Schistosoma* types in Madagascar are *S. mansoni*, using *Biomphalaria* as intermediate snail host and causing the intestinal form, and *S. haematobium*, having *Bulinus* snails as their host and being responsible for the urogenital form. As illustrated in figure 5, their geographic distribution is divided. *S. mansoni* is most prevalent in the south-east and highlands, *S. haematobium* in the north-west along the coast (Deka, 2022).

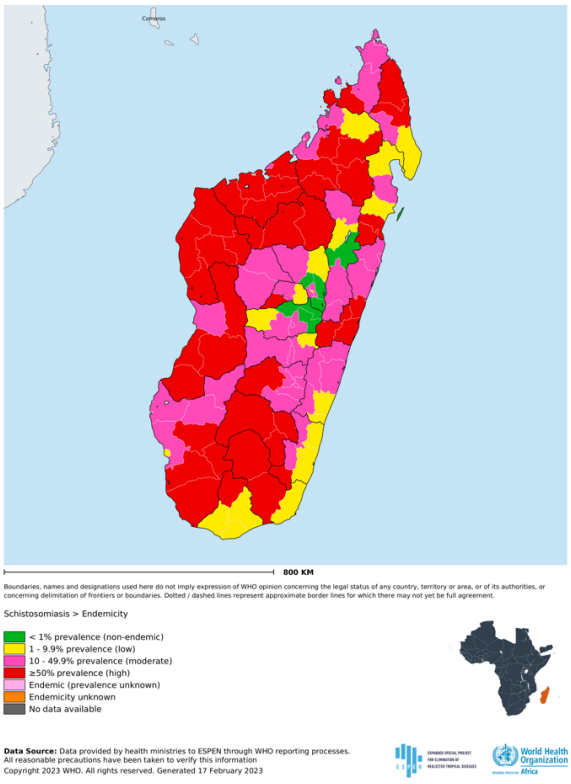


Figure 5 *Endemicity of schistosomiasis in Madagascar in 2021 (ESPEN, 2021a)*
Districts colored in red reported a prevalence of 50% and higher

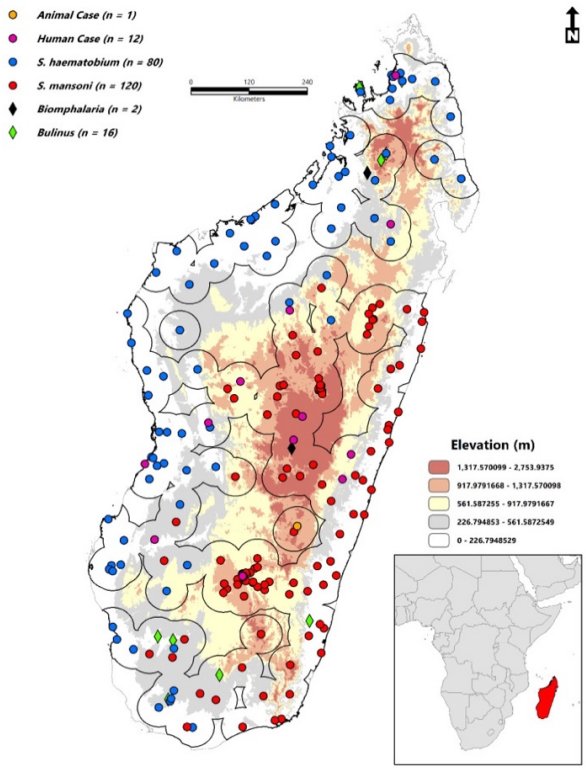


Figure 6 *Geographic distribution of Schistosoma types in Madagascar (Deka, 2022)*
S. mansoni is endemic in areas with red points, S. haematobium is found in areas with blue points

The Malagasy ministry of health has integrated measurements to eliminate schistosomiasis into their health policies. In line with the WHO recommendations it provides a NTD master plan that sets specific, measurable targets for the eradication, elimination and control of all endemic NTDs (Ministère de la Santé Publique de Madagascar, 2016).

The mainstay of the programs are MDA campaigns targeting SAC. Although Madagascar has been among the first countries to start MDA in the 1990s the coverage rates have been low. Even in 2013, only 15% of the people requiring treatment received Praziquantel (Stanford University, 2015). Fortunately, these numbers have improved in the last decade. The latest ESPEN/WHO report from 2021 showed that 90% of the almost 5.5 million SAC and 83% of almost 100.000 adults targeted for PC through the MDA programs were treated (ESPEN, 2021b).

PSAC have not yet been included in MDA campaigns and there is very little data on prevalence among children from this age group available in Madagascar (Sheehy et al., 2021). However, action has been taken in the last months and recently a policy brief has been published to advise for new strategies to fight the disease in the country (Rasoamanamihaja et al., 2023).

2.9.4 The freeBILy study in Madagascar

The dataset analyzed in this thesis was provided by the freeBILy (fast and reliable easy to-use-diagnostics for eliminating bilharzia in young children and mothers) - consortium that conducts trials in Madagascar and Gabon in collaboration with the Universities of Antananarivo and Fianarantsoa, the *Centre d'Infectiologie Charles Mérieux*, the *Centre de Recherches Médicales de Lambaréné*, the Bernhard Nocht Institute for Tropical Medicine (BNITM), the Barcelona Institute for Global Health (ISGlobal), the Eberhard Karls Universität of Tübingen and the Leiden University Medical Center (Hoekstra et al., 2020).

The freeBILy trial carried out in the highlands of central Madagascar is a phase III cluster randomized controlled trial (CRCT) that aims at determining the effectiveness of test-based schistosomiasis treatment (TBST) using the POC-CCA-test for pregnant women and their infants. Its overall purpose is to integrate a TBST into routine maternal and child primary health care programs in order to control schistosomiasis in these vulnerable populations and reduce the long-term burden (Fusco et al., 2021).

3 Material and Methods

3.1 Materials

3.1.1 Urine collection

Table 2 *Used material for urine collection on site*

Collection kit containing the necessary material	Producer
Sterile 40 mL sterile urine cup for the mother	According to local availabilities
Sterile pediatric urine bag	According to local availabilities
Pre-printed labels for kit (KID) and tube (TID) identification	Charles Mérieux Center of Infectious Disease (CICM)

3.1.2 POC-CCA Rapid test

Table 3 *Diagnostic material*

Diagnostic test	Producer	Batch number	Content	Expiry date
Schisto POC-CCA® cassette-based test kit	ICT INTERNATIONAL, Noordhoek, South Africa	200326039	• 25 x test cassettes each individually packaged • 1 x instructions for use • 25 x pipettes	03/2022
G score panels	Leiden University Medical Center (LUMC), Leiden, Netherlands		Panel of 10 cassettes stored in a light-tight bag	

3.1.3 Drugs and equipment

Table 4 *Used treatment*

Drug/equipment	Producer	Batch number	Expiry date
Praziquantel Tablets USP 600mg	MACLEODS PHARMACEUTICALS LIMITED, Mumbai, India	FPJ805A	11/2020
Praziquantel Tablets USP 600mg	MACLEODS PHARMACEUTICALS LIMITED, Mumbai, India	FPS905B	04/2021
Sterile single-use syringe	According to local availabilities		

3.1.4 Forms and documents

Table 5 *Used forms and study documents*

Document/form	Explanation
Case Report Form (CRF)	Document used in clinical trials to document participant's data in a standardized design that facilitates data collection and analysis. (Latha et al., 2014) For each participant pre-printed personalized CRFs were provided in folders at the study sites.
Laboratory form	Paper form for documentation of the samples. The following two forms were used:
REC-LAB-51-FRA	Identification of mothers' urine samples
REC-LAB-52-FRA	Identification of children's' urine samples
Standard Operating Procedure (SOP)	Document with detailed instructions created for all important steps of a clinical trial. The procedures were explained to the staff in trainings. Refresher trainings were held when new procedures were implemented. The most important SOP used for this thesis are explained below:
SOP-MeC-04-FRA	Procedure for the participant's visit at the study site
SOP-LAB-04-FRA	Procedure for sample (urine) collection of mother and child
SOP-LAB-07-FRA	Procedure for sample analysis explaining how to perform the POC-CCA® test
SOP-MeC-09-FRA	Procedure for standardized treatment of infected participants with Praziquantel

3.1.5 Data banks and software

Table 6 *Used data banks and software*

Data bank / program	Producer	Version
BioRender	https://biorender.com/	2023
Microsoft Excel	Microsoft, Redmond, USA	16.66.1
R®	R Foundation for statistical computing, Vienna, Austria	R 4.1.2 GUI 1.77
R Studio	R Foundation for statistical computing, Vienna, Austria	2022.07.0+548
REDCap® (Research Electronic Data Capture)	Vanderbilt University, Nashville, USA	8.11.6

3.2 Methods

3.2.1 Study design

This study has a cross-sectional design and was embedded in the cluster- randomized controlled clinical phase III trial freeBILy which has the overall objective of determining the effectiveness of a test-based treatment strategy for schistosomiasis in improving health in pregnant women and their children followed-up from birth to age 2 (Fusco et al., 2021).

3.2.1.1 Study area and study site characteristics

The sub-study was conducted at four PHCCs in the region of Amoron'i Mania in the central highlands of Madagascar where *S. mansoni* is endemic (Deka, 2022) (**Figure 6**). Population density differs within the area. Study sites were selected among rural, peri rural and urban communities using information given by local staff on accessibility and condition of roads, infrastructure and access to a hospital nearby.

The study site characteristics are displayed in **Figure 7** and **Table 7**.

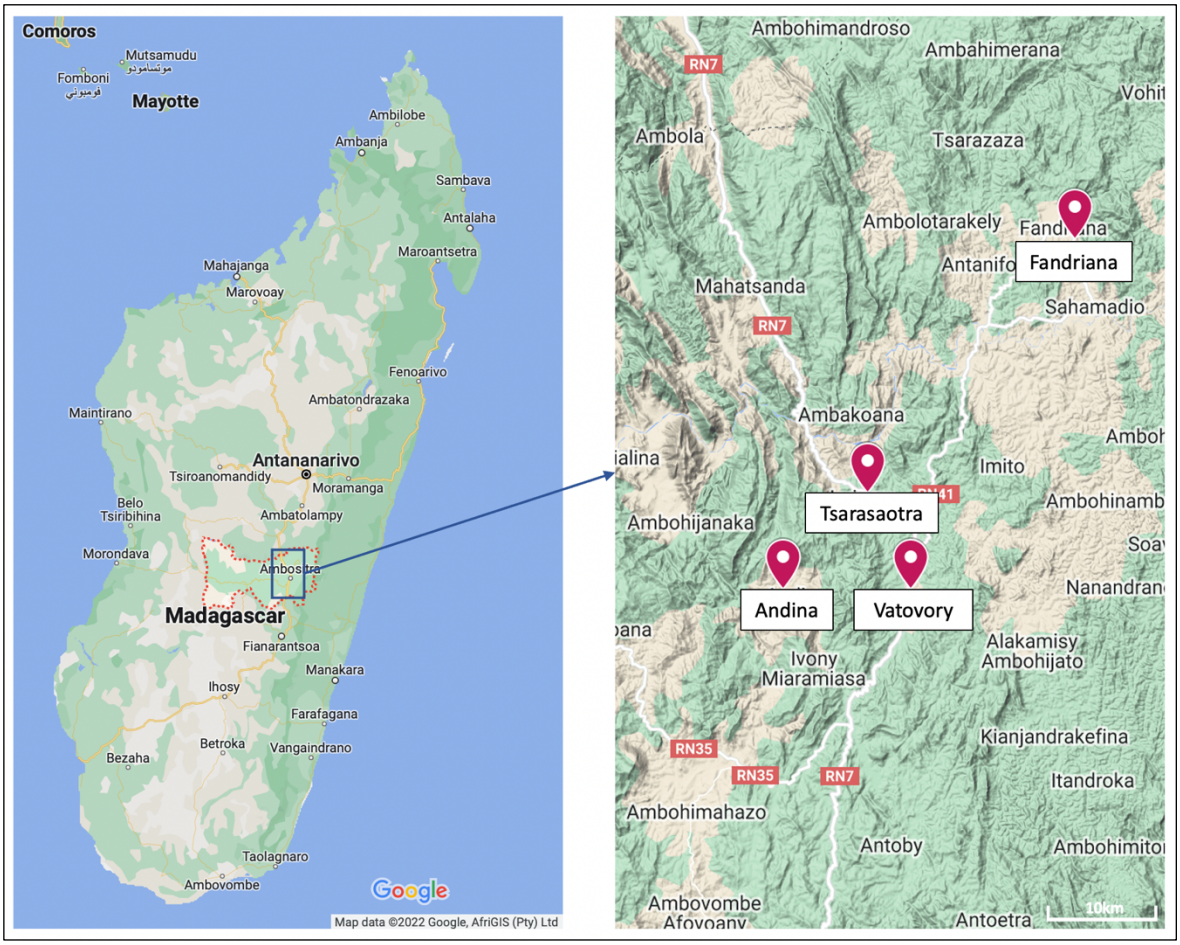


***Figure** 7 Amoron'i Mania region (circled in red) and the location of the four participating study sites within the region (derived from Google Maps, 2022)*

Table 7 Study site characteristics

Study site	Population size (2019)	Type of community
Andina (FA)	14 760 inhabitants	rural
Tsarasaotra (FE)	12 321 inhabitants	peri rural
Fandriana (FK)	29 088 inhabitants	urban
Vatovory (FD)	57 499 inhabitants	urban

3.2.1.2 Study population

At the time of data collection for this sub-study all participants had been already recruited for the overarching freeBILy study. Before the recruitment happened, information sessions were held in the catchment areas of the participating PHCC and eligibility criteria, described in **Table 8**, assessed for participants interested in the study. The women signed an informed consent at the beginning of the study. Their children were automatically enrolled in the study at birth unless the mothers wished to withdraw them.

Table 8 Inclusion and exclusion criteria of the sub-study, adapted from the criteria of the freeBILy study

Inclusion criteria	Exclusion criteria
- Informed consent signed (for mother and child) - Children born from the enrolled mother, including twins - Willingness to comply with the protocol requirements including sampling and treatment for both mothers and children	- Fever (temporary exclusion) - Self-reported epileptic or convulsive episodes - Women who do not live in the area of the CSB - Children born from mothers not enrolled in the study - Children born in a CSB different from the ones in which the mothers were enrolled

3.2.1.3 Data collection and management

Data collection took place from March 2020 to April 2021. After all participants were asked if they wanted to continue their participation or withdraw from the study the next steps of the study visit were explained to them. Sociodemographic background characteristics were collected by local study nurses using a case report form (CRF). Participants were assigned a personalized 12-digit Participant Identification Number (PID) pre-printed on the CRF.

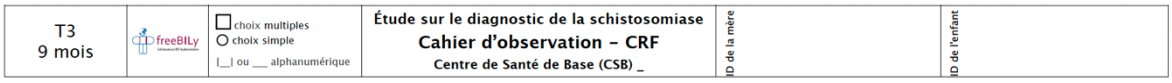


Figure 8 *Top part of the CRF. PIDs were printed on the right for both mothers and children (freeBILy, 2020)*

PIDs contained information of the study site and connected mother and child as explained in **Figure 9**. All CRFs were checked for integrity by the nurse and the supervising doctor following a standard operating procedure (SOP_MeC_04_FRA). The completed CRF data was entered into the database REDCap® via double data entry by two independent operators. During the period of data entry, the data processing and validation was regularly revised by the responsible supervising doctors. In order to guarantee the quality of the data, a query system was implemented within the database. A regular check of the data was carried out at the BNITM. Any discrepancies or inconsistencies were discussed regularly with the local staff and resolved if possible. Cleaned data was extracted in Microsoft Excel.

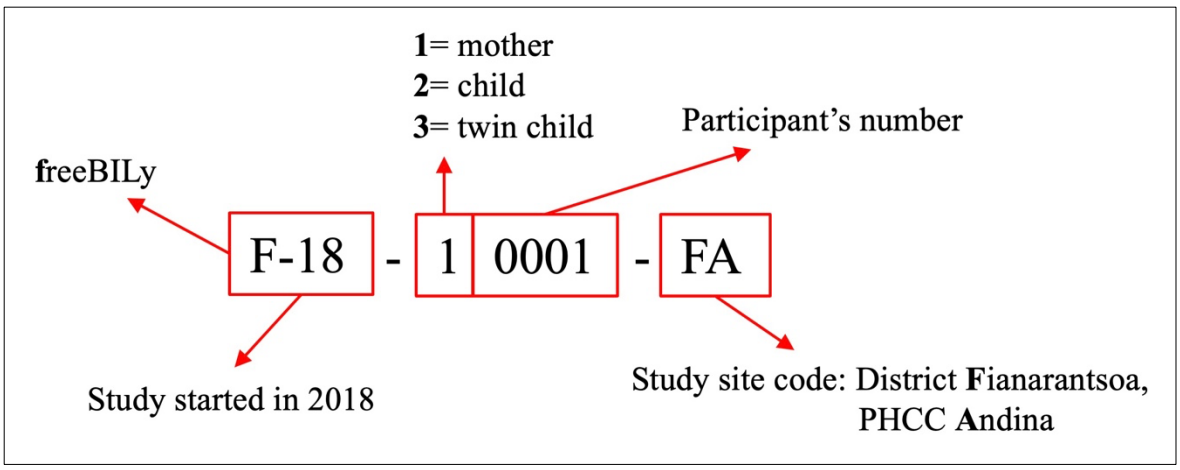


Figure 9 *Composition of a Participant Identification Number (PID), here the example for the first patient recruited in Andina*

3.2.1.4 Sample collection

The SOP-LAB-04-FRA was used to collect the participants' urine sample. Before the collection, the study nurse had to verify the PID and corresponding CRF and laboratory form of both mother and child.

Collection of the child's urine sample was performed by the study nurse or the mother. Briefly, the collection procedure was as following (Mercier and Marseaud, 2015):

- Wash hands thoroughly and place the baby on the back with legs apart.
- Wash the baby's urogenital area and let it dry.
- Take the pediatric urine bag out of the package and place it correctly.

    - For a girl, approach the lower part of the adhesive at the level of the perineum, stick it and finish sticking the adhesive by going up towards the pubis. The pouch should cover the entire urinary area of the child.
    - For a boy, make sure the penis enters the bag completely.
- After application, gently massage the adhesive part and ensure there are no gaps between the bag and the skin.
- Wait for the baby to urinate.
- Remove the bag by gently peeling it off.
- Collect urine out of the bag and perform the rapid test as described below.
- Let the air out and close the bag by sticking the adhesive parts face to face so as to ensure the watertightness.
- Stick the corresponding tube identification number (TID) label on the urine bag.

Mothers' urine samples were collected through self-sampling. More precisely, each woman was given a urine pot and asked to wash their hands before and after filling it with urine, close and return it to the nurse in charge of the labelling. Labelling was performed through TID labels. The nurse should return the urine bag or pot to the collection kit after performing the rapid test. The TID number must be identical to all the KIDs in the kit, the CRF and the laboratory form.

3.2.2.5 Sample analysis – POC-CCA on site

All samples were analyzed directly on site by the study nurses using the Schisto POC-CCA® Rapid test. SOP-LAB-07-FRA based on the test producers instructions was applied (ICT INTERNATIONAL, 2018).

Briefly, the procedure was as following:

- Prepare the material.
    - Check that the bag containing the test is not torn.
    - Ensure that the reagents are at room temperature (20-25°C) before starting.
    - Remove the cassette from its bag just before use.
    - Set the electronic timer for 20 minutes.
- Identify the cassette with the TID of the pot or urine bag.
- Homogenize the urine collection pot by turning it upside down.
- Check that the TID of the urine pot matches the TID on the cassette. If it matches, open the pot or urine bag.

- Take a pipette from the kit.
- While pinching the top of the pipette, introduce the pipette into the urine pot.
- Allow the sample to fill up by gently releasing the pipette.
- Holding the pipette at 45° above the circular well, place 2 drops of urine and let it absorb entirely into the specimen pad.
- Start the timer.
- Check that the urine is completely absorbed by the cassette.
- Discard the pipette in an infectious waste bin and close the urine pot.
- After 20 minutes, read the results. Do not wait more than 25 minutes.

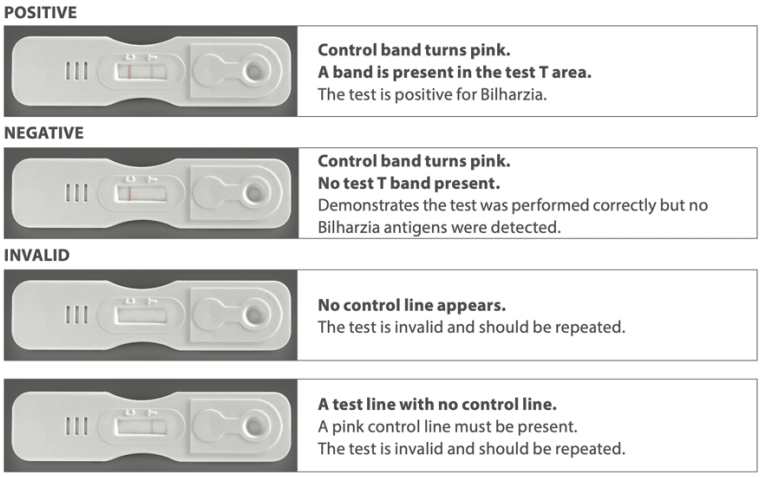


Figure 10 *Interpretation of test result (ICT INTERNATIONAL, 2018)*

If the test was valid and positive, the intensity of the infection was interpreted by using the G score system displayed in **Figure 3**.

To find out which G score to assign to the sample, the test band of the sample was directly compared with the 10 test bands of the G score panel by placing them next to each other. The G score panels were prepared and supplied by the LUMC, who is a partner of the overarching study. In order to preserve integrity, storage in a lightproof box is required. Light could affect the ink on the printed test strips and thus alter the interpretation of results.

3.2.1.6 Treatment

Participants tested positive for Schistosome infection were treated with a single standard dose of praziquantel (PZQ) following the SOP-MeC-09-FRA based on the WHO guidelines: The mothers received a single standard dose of 40 milligrams per kilogram of body weight, administered orally (WHO, 2018). The number of 600 mg tablets required based on body weight was calculated by the nurse.

Children diagnosed as positive received a standard dosage of half a tablet, corresponding to 300 mg PZQ (SOP_MeC_09_FRA, World Health Organization, 2018). The half-tablet was crushed in a clean container by the nurse and then mixed with a little water sweetened with sugar. After that, the nurse or mother administered the drug with a sterile single-use syringe while holding the child.

Each treatment with PZQ was documented in the corresponding CRF in order to be able to monitor adverse events possibly related to the treatment and potential reinfections within the time of the study. All treated participants were monitored for 60 min after administration of PZQ. In the event of emesis within the first 30 min after PZQ administration, the entire dose was repeated.

3.2.2 Data analysis

For data analysis, R® studio version 2022.07.0+548was used (RStudio (Posit)).

3.2.2.1 Descriptive analysis

Univariate analyses were conducted to show the distribution of all test results and explanatory variables for the overall sample. For categorical variables, numbers and percentages were presented, continuous variables were described using median and interquartile range (IQR). Participants with missing test results were not included in the analysis. The exact number and composition of missing values is described in section 4.1.

3.2.2.2 Prevalence analysis

To estimate the overall prevalence of schistosome infection among the study population, unadjusted frequencies of individuals with positive test results as a proportion of all individuals with a valid test result along with exact 95% Confidence Intervals (CIs) were determined.

The distribution of the 10 different POC-CCA testing grades was presented to show the intensity of the infection among the study population.

3.2.2.3 Stratification of the results by sociodemographic factors

Differences in the prevalence by type of community (rural, peri rural, urban), sex (female, male), age groups (6-8 months, 9 months, 10-12 months) and infection status of the mother (infected, non-infected) were examined by comparing 95% CI of prevalence (test for overlapping CI).

3.2.3 Ethical considerations

The overarching freeBILy study was conducted in accordance with the International Conference on Harmonization - Good Clinical Practice guidelines. It was ethically approved both by the National Ethics Committee of Madagascar (ref. no 022-SANP/CERBM of 05/03/2018) and the Ethics Committee of the Hamburg State Medical Chamber in Germany (ref. no PV5966 of 18/03/2019).

All participants received information about the aims of the study and its procedures in the local language Malagasy. The participation was voluntary and on basis of a written informed consent. Informed consent for the participation of mother and child was obtained from the participating mother by signature or, in case of illiteracy, through a thumbprint in the presence of an independent witness. In the event of death of either the mother or the child, the respective other could remain in the study; for the child, a new signature of a custodial parent was required. Participants were free to refuse participation and withdraw the informed consent at any time, without giving reasons.

4 Results

4.1 Missing data values

A total of 517 participants were included in the sub-study; 186 in Andina, 103 in Tsarasaotra, 94 in Fandriana and 134 in Vatovory.

Data pre-processing was performed before analysis to exclude participants with missing test results. Of the 517 child participants initially recruited from the overarching study, 173 were excluded from the statistical analysis for either missing urine samples or missing analysis of the sample. This process resulted in the final study population of 344 children and their 344 mothers eligible for analyses, as illustrated in **Figure 11**.

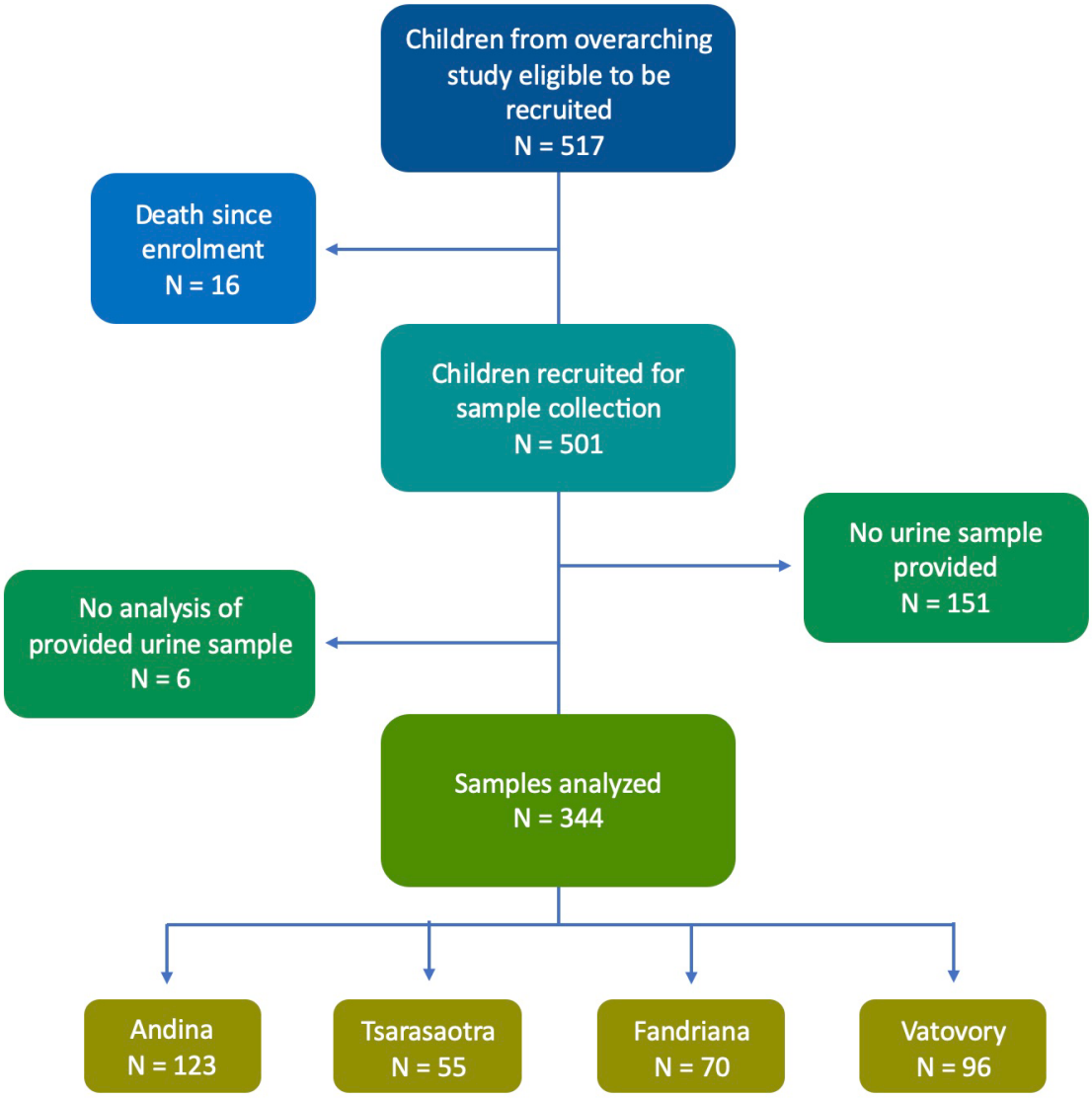


Figure 11 *Data pre-processing and exclusion of participants with missing values*

Specifically, 16 children could not be included because their mothers reported the child's death since the enrolment in the overarching study, hence the children could not participate at the time of sample collection (9 in Andina, 3 in Tsarasaotra and 4 in Vatovory). Another 151 children were excluded because they did not provide urine samples due to not being able or not wanting to urinate during the time of sample collection at the PHCC (49 in Andina, 44 in Tsarasaotra, 24 in Fandriana and 34 in Vatovory). In 6 cases, samples were collected from the children but no rapid test was done (5 in Andina and 1 in Tsarasaotra). No further information on the cause non-performance was given by the nurses.

Regarding other variables, the exact age in months of 85 children is not known due to missing information of the date of birth (23 in Andina, 14 in Tsarasaotra and 48 in Fandriana). These children were still included in the analysis because their mothers were already included during pregnancy as part of the overarching study and it was concluded from the previous dates of the study visits that the children would be within the age range of 6-12 months. Within the study population of mothers, rapid tests were not performed in 10 cases with no further information on the cause given by the nurses.

4.2 Description of the study population

4.2.1 Sociodemographic characteristics of the participants

Out of the 344 participants included in the analysis, 36% (n=123) lived in the rural community Andina, 16% (n=55) came from the peri rural community Tsarasaotra and 48% (n=166) from the two urban communities Fandriana (20%, n=70) and Vatovory (28%, n=96).

Sex was equally distributed with 50% girls (n=171) and 50% boys (n=173).

The participants' age ranged from 6 to 12 months. Median age was 9 months with 50% (n=130) of the study population being 9 months old.

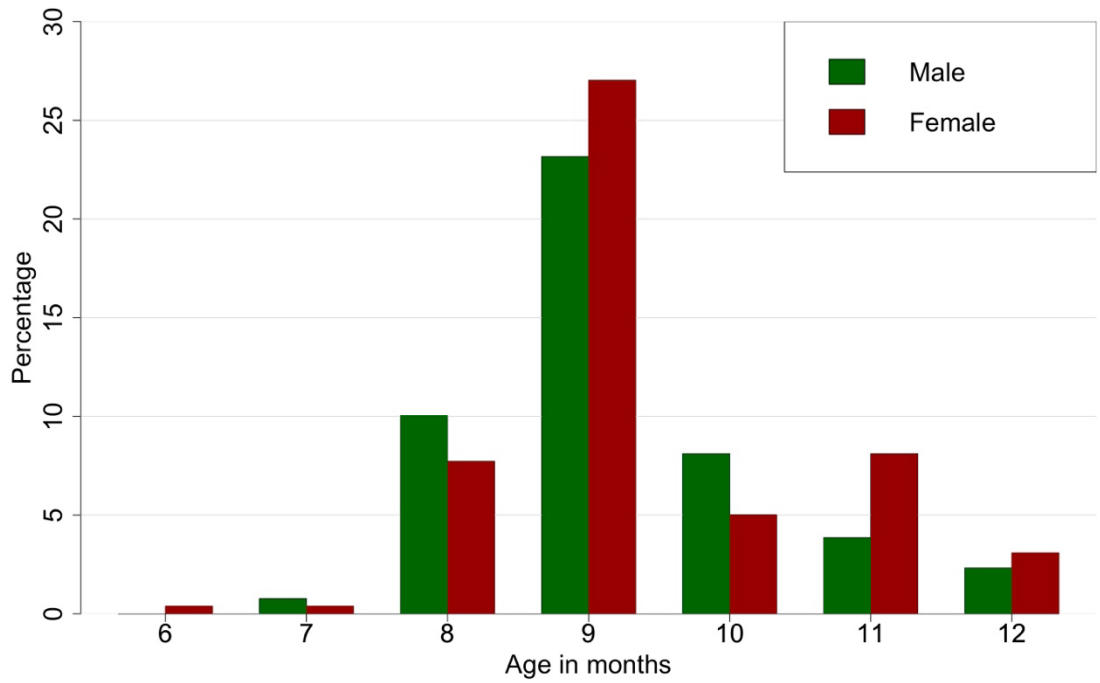
All sociodemographic characteristics of the study population are described in detail in **Table 9**. In **Figure 12**, the exact distribution of age in months for boys and girls is displayed.

Table 9 *Sociodemographic characteristics of the participants in the whole study population and reported by study site: n = 344*

Background characteristics	**n (%)**	**Female sex**	**Age in months**[4]
		n (% total population)	
Total study population	344 (100)	171 (50)	Median: 9 IQR: 9-10 Range: 6-12
Study Sites		**n (% per site)**	
Rural[1]			
Andina (FA)	123 (36)	59 (48)	Median: 9 IQR: 8-9 Range: 7-12
Peri rural[2]			
Tsarasaotra (FE)	55 (16)	22 (40)	Median: 9 IQR: 9-10 Range: 7-12
Urban[3]			
Fandriana (FK)	70 (20)	38 (54)	Median: 9 IQR: 8-9 Range: 6-11
Vatovory (FD)	96 (28)	52 (54)	Median: 9 IQR: 9-11 Range: 8-12

[1] Andina is defined as rural community
[2] Tsarasaotra is defined as peri rural community
[3] Fandriana and Vatovory are defined as urban communities
[4] missing n= 85 (25%) because of missing information of date of birth (T2 visit)
Andina: n=23; Tsarasaotra: n= 14; Fandriana: n= 48; male: 48, female: 37

Figure 12 *Age distribution among study population, N: 259 (75% of overall study population because of missing information of date of birth)*



[1] Details on missing age information: Andina: n=23; Tsarasaotra: n= 14; Fandriana: n= 48; male: 48, female: 37

4.3 Prevalence of Schistosome infection among the study population

4.3.1 Schistosome infection prevalence of the children by POC-CCA

Of the 344 children analyzed, 44,5% (n=153) tested positive for Schistosome infection, 34% (n=118) showed traces on their tests and 21% (n=73) tested negative.

The semi-quantitative grading scale reaching from G1 (negative) to G10 (strongly positive) was applied. With 21% (n=73) of cases, the negative score G1 was the most common result followed by traces (G2 and G3) with 34% (n=118). The mean G score was 3,7 and the median 3 and the IQR 2-4.

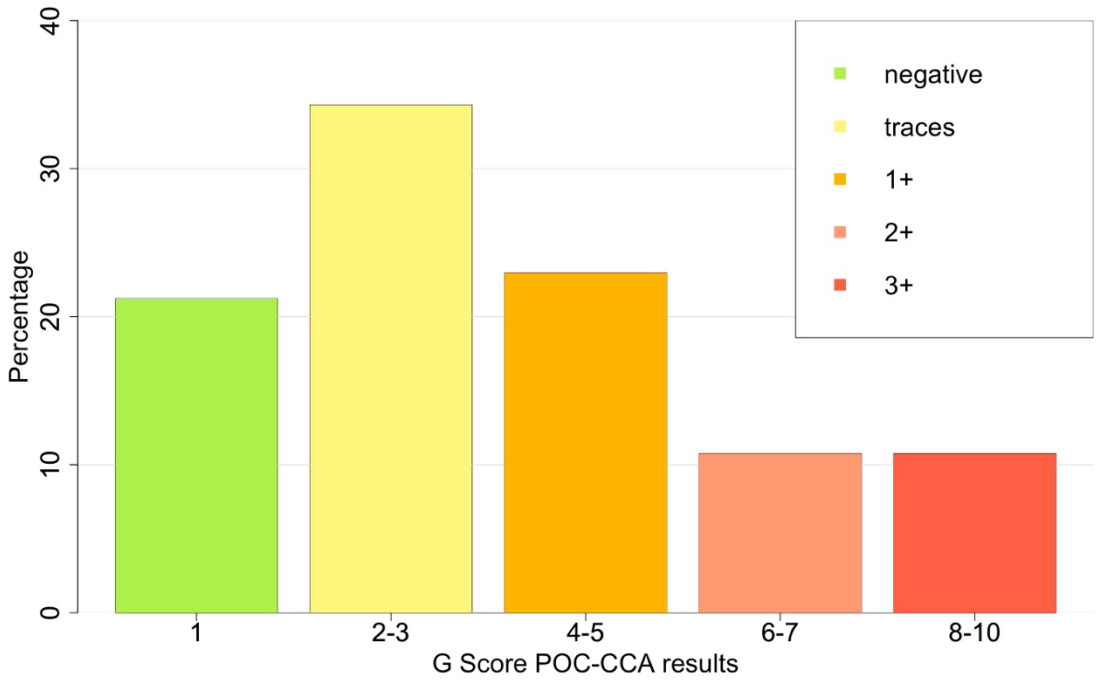
When converted to the visual scale, 23% (n=79) of the children's results were weakly positive (1+), 11% (n=37) moderately (2+) and another 11% (n=37) strongly positive (3+).

The semi-quantitative test results are shown in **Table 10** and visualized in **Figure 13**.

Table 10 *POC-CCA results and prevalence in overall study*

						Traces interpreted as positive		Traces interpreted as negative	
G-Score	**n**	**% (95% CI)**	**Visual Score Classification**	**n**	**% (95% CI)**	**n**	**% (95% CI)**	**n**	**% (95% CI)**
G1	73	21 (17- 25.9)	negative	73	21 (17- 25.9)	73	21 (17- 25.9)	191	55.5 (50.1-60.9)
G2	60	17 (13.6-21.9)	traces	118	34 (29.3- 39.6)	271	79 (74.1-83)		
G3	58	17 (13.1- 21.2)							
G4	50	15 (11-18.7)	1+	79	23 (18.6-27.8)			153	44.5 (39.1- 49.9)
G5	29	8 (5.7- 11.9)							
G6	26	8 (5- 10.9)	2+	37	11 (7.7-14.5)				
G7	11	3 (1.6- 5.6)							
G8	17	5 (2.9- 7.8)	3+	37	11 (7.7-14.5)				
G9	6	2 (0.6-3.8)							
G10	14	4 (2.2- 6.7)							
Total	**344**	**100**		**344**	**100**	**344**	**100**	**344**	**100**

Figure 13 *POC-CCA results visual*



4.3.2 Schistosome infection prevalence of the mothers by POC-CCA

In order to assess potential overlaps between the infectious status of the children and that of their mothers (subsection 4.3.3), the urine samples collected from the mothers were also analyzed by POC-CCA. They resulted in the prevalence displayed in **Table 11** with a positivity rate of 30% (n=99). Traces accounted for 38% (n=128) of the prevalence.

Table 11 *Test results of the participants' mothers*

	Negative n (%) (95% CI)	Traces n (%) (95% CI)	Positive n (%) (95% CI)	Total n (%)
Infection status of mother	107 (32) (27.1 - 37.3)	128 (38) (33.1 - 43.8)	99 (30) (24.8 - 34.9)	334

Missing: n=10

4.3.3 Stratification of Schistosome infection status in different subgroups

At first, a stratification analysis was performed by type of community, in order to compare prevalence among different study sites. The rural site Andina showed the highest positivity rate with 78% (n=96), followed by the peri rural site Tsarasaotra with 27% (n=15). At the two urban sites combined, the participants tested positive in 25% (n=42) of the cases; specifically, the positivity rate was 34% (n=24) in Fandriana and 19% (n=18) in Vatovory (**Table 12**).

When comparing infection status between the two sexes, almost no difference in prevalence was observed with 21% (female: n=36, male: n=37) of each group tested negative. The positivity rate for girls was 43% (n=73), the one for boys was slightly higher with 46% (n=79) (**Table 12**).

When performing a stratification by age, it can be observed that prevalence is highest in the youngest group aged 6 to 8 months with a positivity rate of 62% (n=31). The 9 months old children tested positive in 49% (n=64) and the 10 to 12 months old children in 28% (n=22) (**Table 12**).

Finally, the infection status of the children was cross tabulated with their mothers' infectious status to see if children were more often infected when their mother was positive and less frequently infected when their mother was not. The results can be seen at the bottom of **Table 12**.

The highest percentage (44%, n=47) of children of women who tested negative had a negative test result as well. Vice versa, 60% (n=59) the majority of children of women with positive test results also tested positive.

Table 12 *POC-CCA results and prevalence divided by study sites, sex, age and infection status of the mother*

	Negative n (%) (95% CI)	Traces n (%) (95% CI)	Positive n (%) (95% CI)	Total n (%)
Study site				
Rural				
Andina (FA)	3 (2) (0.5 - 7)	24 (20) (12.9 - 27.6)	96 (78) (69.7 - 85)	123 (100)
Peri rural				
Tsarasaotra (FE)	34 (62) (47.7 - 74.6)	6 (11) (4.1 - 22.2)	15 (27) (16.1 - 41)	55 (100)
Urban				
Fandriana (FK)	12 (17) (9.2 - 28)	34 (49) (36.4 - 60.8)	24 (34) (23.3 - 46.6)	70 (100)
Vatovory (FD)	24 (25) (16.7 - 34.9)	54 (56) (45.7 - 66.4)	18 (19) (11.5 - 28)	96 (100)
	36 (22) (15.7 - 28.7)	88 (53) (45.1 - 60.8)	42 (25) (18.9 - 32.6)	166 (100)
Sex				
female	36 (21) (15.2 - 27.9)	61 (36) (28.5 - 43.3)	74 (43) (35.7 - 51.1)	171 (100)
male	37 (21) (15.5 - 28.3)	57 (33) (26 - 40.5)	79 (46) (38.1 - 53.4)	173 (100)
Age[1]				
6-8 months	9 (18) (8.6 - 31.4)	10 (20) (10 - 33.7)	31 (62) (47.2 - 75.3)	50 (100)
9 months	23 (18) (11.6 - 25.4)	43 (33) (25.1 - 41.9)	64 (49) (40.4 – 58.1)	130 (100)
10-12 months	25 (32) (21.6 - 43.1)	32 (40) (29.6 - 52.1)	22 (28) (18.3 – 39.1)	79 (100)
Infection status of mother[2]				
Negative	47 (44) (34.3 - 53.9)	27 (25) (17.3 - 34.6)	33 (31) (22.3 - 40.5)	107 (100)
Traces	16 (13) (7.3 - 19.5)	55 (43) (34.3 - 52)	57 (44) (35.7 - 53.6)	128 (100)
Positive	7 (7) (2.9 - 14)	33 (33) (24.2 - 43.5)	59 (60) (49.3 - 69.3)	99 (100)

[1] missing n= 85 (25%) because of missing information of date of birth (T2 visit)
Andina: n=23; Tsarasaotra: n= 14; Fandriana: n= 48; male: 48, female: 37
[2] Missing n= 10, no POC-CCA test performed

Lastly, to understand the counterintuitive higher prevalence in younger children aged 6-8 months compared to the ones aged 10-12 months, a cross-tabulation was done comparing the infection status across age groups to the infection status of their mothers, respectively. The mothers of the children aged 6-8 months with high positive percentages of 62% (n=31) also showed high positive rates themselves of 40% (n=20). Children aged 10-12 months with fewer positive results of 28% (n=22) likewise had less infected mothers (15%, n=12 positive) (**Table 13**).

Table 13 *Cross-tabulation: Age groups and infection status of mothers*

Age[1]	Negative n (%) (95% CI)	Traces n (%) (95% CI)	Positive n (%) (95% CI)	Total[1] n (%)	Mother negative	Mother traces	Mother positive
6-8 months	9 (18) (8.6 - 31.4)	10 (20) (10 - 33.7)	31 (62) (47.2 - 75.3)	50 (100)	11 (22) (11.5 - 36)	19 (38) (24.7 - 52.8)	20 (40) (26.4 – 54.8)
9 months	23 (18) (11.6 - 25.4)	43 (33) (25.1 - 41.9)	64 (49) (40.4 – 58.1)	130 (100)	44 (36) (27.6 – 45.3)	45 (37) (28.3 – 46.1)	33 (27) (19.4 – 35.8)
10-12 months	25 (32) (21.6 - 43.1)	32 (40) (29.6 - 52.1)	22 (28) (18.3 – 39.1)	79 (100)	29 (38) (26.9 – 49.4)	36 (47) (35.3 – 58.5)	12 (15) (8.3 – 25.6)

[1] missing n= 85 (25%) because of missing information of date of birth (T2 visit)
Andina: n=23; Tsarasaotra: n= 14; Fandriana: n= 48; male: 48, female: 37

5 Discussion

Schistosomiasis is a neglected tropical disease, predominantly affecting poor and vulnerable communities, resulting in long-term health consequences and disabilities if left untreated (WHO, 2022a). Particularly for children, infection at young age can lead to impaired childhood development (Colley et al., 2014). Moreover, the disease can have a negative socioeconomic impact on the countries in which it is endemic, causing not only an enormous individual burden for the infected person but also a public health problem (King, 2010).

One of the main strategies to control the disease and eliminate it as a public health problem is the use of PC, distributed mainly to SAC through MDA campaigns (WHO, 2022a).

Unfortunately, prevalence data for PSAC worldwide and in Madagascar is scarce (Faust et al., 2020; Sheehy et al., 2021). Traditionally, PSAC have usually not been considered at high risk for the disease and thus, have not been targeted nor integrated into schistosomiasis control programs (Faust et al., 2020).

In Madagascar, Praziquantel is regularly given to SAC through MDA programs but preventive treatment programs for PSAC have not yet been established in the country, even though the WHO recommends to include all children above 2 years old into MDA (Rasoamanamihaja et al., 2023).

Therefore, to identify whether there is a need to focus more on this young age group, this thesis evaluated the prevalence of schistosome infection among children under one year in the Amoron'i Mania region in the central highlands of Madagascar where *S. mansoni* is known to be endemic.

In the study, 344 children aged 6 and 12 months and their mothers were included in the analysis and tested for schistosome infection using the urine POC-CCA rapid test (ICT INTERNATIONAL, 2018).

As a result, a high prevalence of infection (44.5%) was found among the children. Additionally, in the analysis of the intensity of the schistosome infection, through the semi-quantitative read of the POC-CCA G Score, 11% of the children presented a score of G9-G10, indicating high intensity infections. This is especially alarming with regard to the very young age of the children and hence, the short time of potential infection since their birth. Among the mothers, a prevalence of 30%, measured with the same screening method, was found.

5.1 Discussion of prevalence results

With an overall prevalence of 44.5% among the study population, the findings of this thesis are similar to recent prevalence data for Madagascar published by ESPEN in 2021 with data from the Malagasy National Health Ministry. For the district of Ambositra, where the study sites Andina, Tsarasaotra and Vatovory are located, a prevalence within the range of 10 % to 49 % was estimated (ESPEN, 2021b). Only the district of Fandriana, where the town Fandriana is located, presented a prevalence of less than 10% (ESPEN, 2021b) which is lower than the one estimated in this study (34%). However, a direct comparison between the official national data and the study data cannot be made because of the difference within the study populations (PSAC versus SAC) and the testing methods adopted (POC-CCA vs Kato-Katz technique). Hence, the findings among the young age group of this study are particularly relevant because overall young children are assumed not to be at risk of schistosomiasis infection and thus, are not targeted for treatment. Discovering the similarities between the prevalence results of different age groups and the high prevalence in this study, emphasizes that this assumption should be reconsidered.

5.1.1 Schistosomiasis prevalence in pre-school aged children

Prevalence data for PSAC in Madagascar, and especially for children under one year, is scarce, which makes a direct comparison to current literature difficult. A study conducted with 2-4 year old children in the Marolambo district east of the study region found a prevalence of intestinal schistosomiasis of 67% measured by POC-CCA and 35% measured by Kato-Katz (Sheehy et al., 2021). Studies determining the prevalence of schistosomiasis among PSAC in other sub-Saharan African countries are consistent with these results (Ekpo et al., 2012; Faust et al., 2020; Kalinda et al., 2020; Stothard et al., 2013). Even though prevalence varies because of country specific differences and variation in sensitivity and specificity due to the use of different test methods, all research groups agree on the fact that PSAC should no longer be neglected (Faust et al., 2020). They are at high risk of infection and particularly vulnerable because early childhood infection can negatively affect their development; which is why the WHO has integrated PSAC into the new NTD Roadmap 2021 – 2030 (WHO, 2020).

5.1.2 Differences within subgroups

Additional to the overall prevalence, the infection status among different subgroups of the study population was analyzed. In the following, potential factors influencing the high

prevalence in the highland region as well as the different prevalence results within the subgroups will be discussed.

Rural, peri rural and urban

The analysis allowed to discriminate prevalence across different urbanicity strata. In Andina, a village located in a rural area, a prevalence of 78% was measured. The peri rural site Tsarasaotra showed a prevalence of 27% and the results for two urban sites Fandriana and Vatovory were 34% and 19%, respectively.

Due to limited availability of data for the study sites, the urbanicity classification for this study was adapted to the local context instead of applying the definition proposed by the United Nations (UN) Statistical Commission (UN Statistical Commission, 2020). As seen in studies from other countries, definitions differ across countries because of highly variable national characteristics that distinguish rural from urban (Klohe et al., 2021). Rural areas are commonly defined as 'what is not urban', according to the Food and Agricultural Organization (FAO) of the UN (FAO, 2005; Klohe et al., 2021).

Specifically for this study, the classification was done by local staff on the basis of various characteristics, such as infrastructure, accessibility, population density and other local factors.

The identification of highest prevalence in rural Andina is a finding, which is aligned with the current state of research confirming that schistosomiasis is a disease predominantly affecting poor communities living in rural areas, where agriculture or fishing are the main source of income and access to WASH is limited (WHO, 2022a). In Malagasy rural areas like Andina, only 22% of the population have access to improved sanitation facilities (Central Intelligence Agency, 2023). It is common to use rivers and ponds for clothes and dish washing and bathing which increases the risk to get in contact with infested water. In addition to that, there is an occupational risk factor possibly supporting the high prevalence in rural areas. In the country, 71% of the land is used for agriculture and one of the main sources of income of the population in Madagascar, especially in the highlands, is the cultivation of rice (Central Intelligence Agency, 2023). Rice cultivation is mostly done by hand and involves relevant contact with stagnant water which increases the risk of infection (Nyandwi et al., 2017).

In the two urban study sites Fandriana and Vatovory, prevalence was lower, but, even if lower than in Andina, the frequencies of prevalence are still quite high and far above the 10% threshold for MDA implementation suggested by the WHO guidelines (WHO, 2022c).

The lower prevalence than in rural Andina can probably be attributed to better access to WASH, as well as less occupational contact with water in these urban areas. A systematic literature review investigating schistosomiasis in urban and peri urban settings has shown, that continuous urbanization could, at long sight, contribute to the elimination of schistosomiasis (Klohe et al., 2021). Nevertheless, attention should be kept high because urban schistosomiasis represents a problem as well. Despite the evolving urbanization worldwide, there are other challenges emerging. Fast and unorganized urbanization often results in a lack of sanitary infrastructure and fecal contamination in slum-like new urban areas (Klohe et al., 2021). Moreover, the migration of poor or illiterate people can lead to a low disease awareness and thus, a negative influence on urban prevalence because high educational levels have been shown to be preventive factors for infection (Klohe et al., 2021).

Interestingly, for Tsarasaotra relatively low prevalence results were found. Even though the population density and the distance of approximately 17 km each to the next city Ambositra (Vatovory) are similar compared to Andina, the two sites have very different structural characteristics. While Andina is located in a very remote and hilly area and extremely difficult to access via unpaved roads, Tsarasaotra is located right next to the national road. This better accessibility of the village could explain better access to WASH facilities and a broader choice of different occupations with less water contact due to easier mobility. This is also why Tsarasaotra was classified as peri rural and not rural.

This inconsistent definition of urbanicity might lead to very variable interpretations of the impact of urbanization and its role in disease prevalence.

Sex and gender

Generally, zoonotic diseases such as schistosomiasis affect individuals differently because of sex- and gender-based differences as recently reported by Fusco et al., 2022. Even though research data on the impact of sex and gender in communities affected by schistosomiasis is still scarce, it has been shown that sex and gender play a risk factor for the expression of the disease and the burden it is causing in an individual (Fusco et al., 2022). Hence, in the scope of a One Health approach, the sex and gender perspective should be taken into consideration when implementing disease control strategies (Fusco et al., 2022).

Interestingly, in our study population almost no differences in prevalence results could be observed between female and male children. From this result, it can be assumed that in terms of infection, sex might not play a role but it is more of a gender issue for this age group.

At very young ages, social norms tend to be neutralized and children are apparently treated the same, regardless of their sex. Mothers carry both girls and boys equally to waterbodies, where they are being bathed or placed at the shore to join their mothers during daily activities and chores (Mazigo et al., 2021).

As they grow older, among adult populations, unambiguous differences in prevalence between males and females were found in several studies, revealing a usually higher prevalence of intestinal schistosomiasis in men (Faust et al., 2020; Ayabina et al., 2021; Fusco et al., 2022; Gruninger et al., 2023). A main factor impacting the high prevalence, is the occupational risk exposure. In many countries where schistosomiasis is endemic, it is seen as the father's and man's responsibility to be the main contributor to the family's income. Their occupations, such as fishing or farming, or in Madagascar in particular, rice cultivation involve a high risk of exposure (Gruninger et al., 2023). Nonetheless, in regions where women engage more in water-contact occupations, their risk of infection increases, accordingly (Ayabina et al., 2021).

But in fact, other risk factors, such as the absence of tap water and limited access to WASH in rural or poor communities, often pose a higher risk for women compared to men, as it is, due to cultural habits, commonly seen as their household duty to fetch water and wash clothes and dishes (Ayabina et al., 2021).

Age

Very interestingly, the data show no higher prevalence in older children who supposedly might have easier access to water. Children aged 6 to 8 months showed a prevalence of 31%, whereas the oldest children between 10 and 12 months of age only had a 22% prevalence (**Table 12**). At first, to put these results into context, the high number of missing data has to be considered. Specifically, 25% of the exact dates of birth were missing. Hence, conclusions about prevalence differences among the different age groups only apply for 75% of the study population and should be made with care. Literature about PSAC and SAC has not shown that an age dependency below the age of 12 months is apparent and the association rather becomes pronounced at older ages with increasing prevalence in children until they are targeted by MDA (Bustinduy et al., 2017; Faust et al., 2020; Mazigo et al., 2021). This seems to be more suitable explanation given the increasing risk of infection the more time a child gets in contact with water over the months and years. Among the age groups it can also be seen that older children are more likely to be negative but their mothers as well. Thus,

maybe this association is not caused by age, but rather by the infectious status of the mothers, justified through possibly less risky behaviors, as discussed in the following.
An additional limitation regarding the surprisingly high prevalence in very young children is the questionable lower specificity of the POC-CCA in this age group that will be discussed below in 5.3.3.
Nevertheless, the prevalence among different age groups remains an interesting result, because it shows that even at the very young age of 6 months children can already be affected.

Infectious status of mothers

As displayed in **Table 13**, the results show that children have a higher trend to be infected when their mothers are infected as well (60%). This suggests that there might be factors connecting the infectious status of the mother and the infectious status of her child. Unsurprisingly, it can be assumed that the risk behavior of mothers has an influence on the risk of infection of their children.
All previously discussed factors influencing the risk of infection of a mother, such as socioeconomic background, occupation, education, awareness, access to WASH or living in rural or urban areas, directly impact her risk behavior. Subsequently, a risky behavior rather leads to increased exposure of her child to potentially infested water bodies and hence, results in a higher possibility of infection for the child (Ekpo et al., 2012).

Concluding the discussion of prevalence results among subgroups, it is important to emphasize that the analysis of this study is solely descriptive. The aim of the study and the main research question is to assess the prevalence of PSAC. Hence, the presented prevalence results within different subgroups do not allow to make conclusions about statistical risk factors, but should rather be seen as trends suggesting certain associations. In order to identify real risk factors, a more detailed analysis would be necessary.

5.2 Strengths and limitations - Discussion of methods

This study, embedded in an overarching study, is the first study of this kind ever conducted on this age group in Madagascar. It was achieved to recruit a remarkably high number of participants in the different study sites across the region. This allowed to retrieve important data for prevalence mapping in this region, as well as treatment of a large number of infected PSAC. Furthermore, due to the unique concept of pairing the children with their mothers,

interesting information could be obtained that will allow further analyses regarding risk factors for infection within this age group.

Moreover, the study luckily did not report any severe adverse events (SAE) among the children and mothers after treatment with Praziquantel. The absence of SAEs in this age group, supports the assumption that Praziquantel is a safe drug, even when administered at very young ages (Coulibaly et al., 2017b). Hence, this finding can be another very relevant argument for starting treatment of PSAC and including them into MDA programs.

Nevertheless, there are various limitations, meriting to be discussed in the following.

First of all, the data collection for this study took place between March 2020 and April 2021 and was subsequently affected by the SARS-CoV-2 pandemic. Necessary safety measurements such as lockdowns and distancing rules hindered some women from going to PHCC and community workers were not always able to reach out to them and visit them in their villages in order to invite them to participate in the study, which probably led to a lower number of participants. Also, the accuracy of the test procedure might have been affected because the staff was sometimes scared to stay too long at the PHCC or had to rush to get home before lockdown and hence, they might have performed the test less accurate.

Secondly, some methodological limitations, regarding the sampling of participants and the choice of test methods should be acknowledged when discussing the results.

5.2.1 Sampling of participants

A debatable bias influencing the prevalence results could be the fact that the children participating in this study were born from mothers who were already participants of the overarching study evaluating the implementation of TBST into routine maternal and child primary health care programs. This might also explain the relatively low prevalence of 30% among the mothers compared to the children, because some of these women have received treatment at previous study visits, for example during pregnancy, and did not get reinfected since. In addition to that, they received information on schistosomiasis and the transmission routes in previous study visits which potentially created a higher awareness. Consequently, they might have exposed their children less to potentially infested water than mothers, who have never been in contact with schistosomiasis research, would have done.

5.2.2 The POC-CCA test – strengths and weaknesses

The main strength of the POC-CCA test, specifically compared to the Kato-Katz technique, is the high sensitivity and the simple application not requiring well equipped laboratories with professionally trained staff (Colley et al., 2020; Hoekstra et al., 2020).

For our study, these advantages ensured to reach a high number of participants, even in remote areas, because the collection of urine samples was uncomplicated and less shameful or risky than stool collection or blood drawing.

Nevertheless, the tool comes with numerous weaknesses, especially in the age group of the study population, that should be considered when discussing the results. As mentioned previously, the specificity of the POC-CCA is highly variable, which is one of the reasons why the performance of the test has not yet entirely convinced clinicians and researchers to a point where it is widely used in endemic countries and recognized as gold standard for the diagnostic of schistosome infection (Colley et al., 2020).

The easy handling of the rapid test is one of the major advantages but can at the same time cause a bias. The interpretation of the test result is rather subjective and can lead to variation across different readers, especially when interpreting the G-Score with its 10 different grades.

Moreover, the interpretation of the visually faint bands G2 and G3, considered as traces according to the manufacturer, is challenging and often imprecise. Studies have found out that the interpretation of traces depends both on the current prevalence of the study location and the disease control history, specifically MDAs, in the area (Colley et al., 2020). In areas with low-to-very-low prevalence and a long history of successful MDA it is assumed that traces are very likely to be false-positives (Gaspard et al., 2020; Haggag et al., 2019), whereas in areas with a history of only a few years of MDAs in place and a high prevalence by POC-CCA but low by Kato-Katz, traces are estimated to be true-positives in at least 50% (Clements et al., 2018; Ruberanziza et al., 2020) and should be categorized as positive to ensure the treatment of potentially infected patients (Colley et al., 2020).

As there is little prevalence data for PSAC in the study region available and MDAs have not included PSAC in the past, it was difficult to draw conclusions from the traces for the analysis of this study. Hence, it was decided not to interpret traces as positive because the inconsistencies might be due to technical limitation of the investigation tool.

This brings up another important methodological limitation, which is the use of the POC-CCA as a whole for this age group. To date, there is only one study assessing the performance of the POC-CCA test in PSAC, which unfortunately showed poor results (Casacuberta-Partal et al., 2021). This interesting study evaluating the specificity of POC-CCA tested in non-endemic pregnant women and young children in the Netherlands found remarkably high rates of false-positive test results; all confirmed by the highly specific UCP-LF CAA that tested negative in all the non-exposed participants. Specifically in PSAC, who

tested positive by POC-CCA in 47% of the cases when traces were considered negative, the result showed a specificity of only 53% (Casacuberta-Partal et al., 2021). Interestingly, in most of the urine samples that tested positive by POC-CCA, a lower pH average was measured compared to the ones that tested negative. After adding a drop of neutralizing buffer to the test cassette, all samples, except one, turned into negative results (Casacuberta-Partal et al., 2021). Thus, a modification of the buffer could improve the test performance in PSAC. Surprisingly, the positivity rate was highest among PSAC under 9 months, and even increasing towards newborns. This indicates that there might be age-related interfering molecules involved, such as nutritional components via breastfeeding or via the placenta during pregnancy (Casacuberta-Partal et al., 2021).

In regard to these limitations, the results of this study may represent an over estimation of the prevalence and should be viewed with a critical eye. But, since there are only few prevalence data available for this age group, it is still very important to bring attention to this topic. This thesis can be seen as a contribution to new strategies that should be implemented in the future and some of which will be discussed hereafter. However, an additional analysis with a different diagnostic test would clearly be beneficial to gain a more robust picture of the prevalence situation in the country.

5.3 Impact of the results

Despite the limitations, we still see a high prevalence far above the WHO recommended 10% threshold for starting MDA (WHO, 2022c). These results are in line with recent literature focusing on PSAC, calling for more interventions in order to not leave this age group behind (Faust et al., 2020). The data confirm again that PSAC are at high risk of infection and therefore, it is recommended to take action, earlier than later, in order to protect this vulnerable age group.

5.3.1 Possible strategies to integrate PSAC into disease control programs

As the WHO has already recommended in the new NTD Roadmap 2021 - 2030, PSAC should be included in MDA programs from now on, despite the lack of a pediatric formulation of Praziquantel and lacking safety data of Praziquantel among this age group (WHO, 2020). Previous MDA campaigns mainly focused on SAC, who are easily accessible at school. Extension of MDA to PSAC and adults thus requires new strategies and Praziquantel administration door-to-door, in community centers or at churches has been launched in Madagascar since 2020, creating new opportunities but also an increasing

demand of human resources and expenses (Rasoamanamihaja et al., 2023). Since the release of the NTD Roadmap update in 2020, the Malagasy Ministry of Health has not yet updated their country's NTD Master Plan. This year, a yet informal schistosomiasis working group has been established in Madagascar. Their recent publication identifies challenges and opportunities for the implementation of the new WHO guidelines (Rasoamanamihaja et al., 2023). They suggest a three-pillar based strategy including: integrated, multisectoral implementation strategies, political stakeholders' engagement and coordination and strengthening of laboratory, medical and research capacity (Rasoamanamihaja et al., 2023). Within these three pillars they provide concrete ideas and approaches on how to tackle the existing barriers and reach all populations at risk of infection, including PSAC and adults, acknowledging the peculiarities of the country (Rasoamanamihaja et al., 2023). This work is a promising start and will hopefully soon result in official national policy documents building the basis for the implementation of new adapted national schistosomiasis programs. Nevertheless, when focusing on newborns, it is important to mention, that so far, the WHO recommendations expanded to PSAC only applies for children above 2 years, which omits the children affected by schistosomiasis in this study. To mitigate this issue and fill this gap, an implementation of MDA even for children under one year could should be considered. This could prevent early childhood infection and its consequences during development but also comes with a risk, because even though in our study no SAEs were reported, safety data on the treatment of PSAC with Praziquantel is still scarce.

MDA could be integrated into routine child health care services. For example, the combination of already existing public health interventions, such as national immunization campaigns against childhood vaccine-preventable diseases, with the treatment with Praziquantel could set up a sustainable possibility to access PSAC under 2 years (Mutapi, 2015).

Unfortunately, despite the great global effort to achieve universal access to all essential vaccines for all in the last decades, vaccination coverage rates for children in Madagascar remain low and even decreased in the last two decades. While in 2005, 85% of the children aged 12-23 months received adequate immunization with three doses against diphtheria, pertussis and tetanus (DPT), only 55% received full DPT immunization in 2021 (USAID, 2023). A recently published study aiming to understand the reasons for the alarmingly low immunization coverage rate in Madagascar, revealed several obstacles, such as accessibility of health care institutions and availability of healthcare personnel, missing knowledge or misconceptions of the mechanisms and safety of the vaccine and the consequences of the

preventable diseases, confusion due to increasing number of available vaccines, difficult relationships and mistrust towards the healthcare personnel (Ramaroson et al., 2023). These structural, cultural and relational barriers need to be addressed at the soonest to achieve better health for all (Ramaroson et al., 2023).

Alternatively, other approaches, such as door to door distribution or campaigns in the communities, need to be found to successfully reach the children. The currently ongoing program called PEPRAMA (Paving the way for **pe**diatric **pra**ziquantel accessibility in **Ma**dagascar) is focusing on that exact issue. Conducted by the BNITM and local partners in Madagascar, the PEPRAMA study aims to find the most suitable facilities for treatment of PSAC and intents to assess the acceptance among the parents as well as the willingness of health staff to treat very young children (BNITM, 2023). Through the provision of treatment at different facilities including primary health care centers, churches, mobile units in the communities and other public places the research teams have so far achieved remarkably high numbers of treatment uptake (BNITM, 2023). The success of this project underlines the demand for treatment of PSAC in the region and the qualitative part of the study will hopefully result in better insights into new progressive approaches to include PSAC into control programs.

5.3.2 Awareness campaigns and targeting the caretakers

Given the young age of PSAC, the most likely way to success reaching them efficiently is though targeting the adults who are responsible for them. The caretakers of PSAC play a key role in cutting the transmission routes of their children and it is crucial to address their disease awareness (Ekpo et al., 2012; Kibira et al., 2019). This assertion is underlined by the results of this thesis showing that children of infected mothers were more likely to be positive; compared to children of non-infected mothers whose prevalence was lower, suggesting a trend of relation between risk behavior and infectious status of the mother with the prevalence of the children.

Preliminary results from a study conducted with mothers of two-year-old children in Tsarasaotra and three other villages in the Amoron'i Mania and Itasy regions in the highlands of Madagascar in 2022, unfortunately showed a limited knowledge of schistosomiasis. A total of 86 women were interviewed by a local female doctor using structured questionnaires, focus group discussions and in-depth-interviews. Even though all women had heard about schistosomiasis, misconceptions regarding the transmission routes, effects and treatment of the disease were common (Hameister et al., 2022). However, many women indeed reported to be aware of risk behavior, like open defecation or bathing and fetching water in rivers,

but explained that a change of behavior would be difficult due to missing infrastructure. They were rightly complaining about the lack of toilets and safe wells (Hameister et al., 2022, own documentation). In the absence of safer water sources, they have no choice than to expose their children to infested water. In addition to the interviews, structured observations took place at different water bodies in the communities and 20 PSAC were observed during exposure to potentially contaminated water at rivers, lakes and close to rice fields. The children spent sufficient time in the water to get infected and were mostly joining their mothers for daily activities like dishwashing, laundry or bathing and washing as displayed in the following pictures in **Figure 14** (Hameister et al., 2022).

Figure 14 *Typical situations where PSAC are exposed to infested water. All photos taken with oral consent.*
Left: *PSAC playing in shallow water of a small river between rice fields with their mothers, Tsarasaotra 2022, © J. Hameister*
Center: *Mother washing clothes next to a rice field, her children bathing, including at least one PSAC, Ampahimanga, 2022, © J. Hameister*
Right: *Family washing clothes at the shore of Ampefy Lake, PSAC bathing in a tub filled with lake water, Ampefy, 2022, © M.J. Solonirina*

This study, as well as other studies conducted in other countries indicating a lack of awareness among caretakers (Anyolitho et al., 2022; Rassi et al., 2016), once again demonstrate the urgent need to improve the awareness and health education to increase the impact of control strategies for schistosomiasis. It should be considered to integrate more awareness campaigns into national disease control programs. The NTD Roadmap as well emphasized capacity building and awareness as critical actions required to achieve the targets until 2030 (WHO, 2020).

To interrupt transmission routes for PSAC in particular, awareness could, for example, be risen during ANC visits. The WHO recommends at least eight ANC contacts to reduce perinatal mortality and improve maternal health and health care experience (WHO, 2016). Previous recommendations included a minimum of only four visits and most data reporting

still aligns with this recommendation. In Madagascar, neonatal and maternal mortality are still alarmingly high. 24.1% of neonates die before reaching 28 days of age (2021) and 335 women die per 100,000 live births (2017) (USAID, 2023). At ANC, the expectant mothers receive essential services to detect and prevent obstetric complications, as well as information about healthy behaviors during pregnancy, nutrition or breastfeeding. The women also get support for disease prevention, such as HIV testing, mosquito nets in areas where malaria is endemic or immunizations against tetanus (UNICEF, 2022). Thus, health education is already a major part of ANC and such visits represent a suitable occasion for the integration of information on schistosomiasis. Nevertheless, to implement this idea effectively, high ANC attendance is crucial. In 2018, only 50.6% of women aged 15 to 49 attended at least four ANC visits by any health care provider in Madagascar (USAID, 2023). Several factors, such as living in remote areas, education, previous gravidities, occupation and socioeconomic status have an influence on maternal healthcare seeking and ANC attendance, as a survey conducted in the central highlands of Madagascar showed (Andrianantoandro et al., 2021). Moreover, the consultation of traditional birth attendants, *reninjaza* in Malagasy, who also give many advise during pregnancy, is common in Madagascar. *Reninjaza* do not receive academic medical training and their practices and role in maternal health care are seen controversial. Nonetheless, their relevance should be considered, because many pregnant women have a strong interpersonal relationship to these women as they are part of the family or live in the same *Fokontany* where they are trusted and enjoy a high reputation (Andrianantoandro et al., 2021).

Hence, a close and reinforced collaboration between professional and traditional health care workers, as well as national and local authorities, is needed in order to provide low-threshold health care service for all women and open up spaces to raise awareness on schistosomiasis. However, awareness, testing and treatment cannot stand alone in order to reach the targets if the necessary infrastructure is not available. The improvement of WASH is crucial to be able to provide long lasting solutions, not only to achieve elimination of schistosomiasis as a public health problem but also to build resilient communities living in dignity in healthy environments worldwide.

5.4 Future research

The prevalence data generated from this thesis only highlight a very small part of the actual burden of schistosomiasis for PSAC worldwide. To be able to target this age group more specifically, more prevalence data is needed and further research has to be done in the future. Apart from evaluating the prevalence among PSAC in more districts across Madagascar and

in other endemic countries to get a better overview of the endemicity, it is also important to gain a deeper understanding of the efficacy of the test methods utilized to diagnose the infection and the possibilities of their integration into public health programs. Up to date, the Kato-Katz is still seen as the "gold standard" despite its low sensitivity and the POC-CCA shows high variability for specificity. Besides, the POC-CCA test has mostly been evaluated for SAC and adults and its efficacy when used for PSAC remains unclear. As mentioned, research on a new pediatric formulation of Praziquantel is currently ongoing and will hopefully soon be ready to be implemented. Once prevalence mapping will have been expanded and a new pediatric treatment is available it will be critical to comprehend how to successfully reach the communities in order to treat as many affected PSAC as possible and prevent further infections and long-term consequences. Previous strategies were mostly based on SAC, who are easy to be reached and treated in schools. New approaches will need close cooperation with local authorities and community workers to develop interventions together and later, regular evaluation of the effects of the implementation of public health programs for PSAC.

6 Conclusion

Despite emerging evidence from several sub-Saharan African countries, where schistosomiasis is endemic, showing that even very young children can be affected by the NTD and untreated early-childhood infection can lead to long term health consequences and a serious societal burden, data on prevalence of schistosomiasis among PSAC worldwide and in Madagascar is still scarce.

To the current knowledge, this study, embedded in a larger overarching study, is the first to investigate the prevalence of schistosomiasis among PSAC under 1 year of age in Madagascar. As a result, a prevalence of 44.5% was found among the study population.

This undoubtedly confirms the so far unmet need to integrate this vulnerable group into disease control programs. Nevertheless, PSAC are still excluded from systematic MDA programs and there is no registered dosage of treatment with Praziquantel for this age group. Therefore, this thesis calls for the implementation of innovative public health strategies. New approaches strengthening awareness and improving diagnostics and treatment of schistosomiasis among PSAC should be established in order to eventually improve child health and eliminate schistosomiasis as public health problem.

7 Abstract

Background: The WHO is determined to eliminate schistosomiasis, a parasitic neglected tropical disease, as a public health problem worldwide until 2030. Current disease control programs mainly focus on mass drug administration for school-aged children and neglect pre-school-aged children (PSAC), who have generally considered not to be at risk of infection. However, it is known that infection at young age can lead to long term health consequences and impair the childhood development. This thesis aimed to determine the prevalence of schistosomiasis in children under one year in the highlands of Madagascar, where data is still scarce, in order to contribute to new approaches for more inclusive control strategies in the country and worldwide.

Methods: This cross-sectional study was conducted at four primary health care centers in the highlands of Madagascar between March 2020 and April 2021. Urine samples of 344 children under one year of age and their mothers were analyzed using the Point-of-Care Circulating Cathodic Antigen (POC-CCA) test to determine schistosome infection. R® Studio was used to perform statistical analysis and visualization of the results.

Results: Among the study population 344 PSAC, an overall prevalence of 44.5% by POC-CCA was found. The highest prevalence was found in the rural village Andina with 78%. Prevalence differences among different age groups and sex were not distinct within the study population. Children of infected mothers were more likely to be infected than children of non-infected mothers.

Conclusion: The results of the study undoubtably confirm, that the vulnerable age group of PSAC is at risk and should no longer be neglected. To achieve schistosomiasis elimination as a public health problem and improve general child health, new approaches for innovative public health strategies, that include PSAC, need to be implemented, strengthening awareness, diagnostics and treatment of the disease.

Zusammenfassung

Hintergrund: Die WHO hat sich zum Ziel gesetzt, Schistosomiasis, eine parasitäre, vernachlässigte Tropenkrankheit, bis zum Jahr 2030 weltweit als Problem der öffentlichen Gesundheit zu beseitigen. Die derzeitigen Programme zur Krankheitsbekämpfung konzentrieren sich hauptsächlich auf die medikamentöse Massenbehandlung (mass drug administration) von Kindern im Schulalter und vernachlässigen Kleinkinder im Alter vor Schuleintritt, die im Allgemeinen als nicht infektionsgefährdet gelten. Es ist jedoch bekannt, dass eine Infektion in jungen Jahren zu langfristigen gesundheitlichen Folgen führen und die kindliche Entwicklung beeinträchtigen kann. Ziel dieser Arbeit war es, die Prävalenz der Schistosomiasis bei Kindern unter einem Jahr in einer Region im Hochland von Madagaskar zu ermitteln, wo bisher nur wenige Daten vorliegen, um zu neuen Ansätzen für umfassendere Bekämpfungsstrategien in dem Land und weltweit beizutragen.

Methoden: Diese Querschnittsstudie wurde zwischen März 2020 und April 2021 in vier Gesundheitszentren im Hochland von Madagaskar durchgeführt. Urinproben von 344 Kindern unter einem Jahr und ihren Müttern wurden mit dem Point-of-Care Circulating Cathodic Antigen (POC-CCA) analysiert, um eine Schistosomeninfektion festzustellen. Für die statistische Analyse und Visualisierung der Ergebnisse wurde R® Studio verwendet.

Ergebnisse: In der Studienpopulation von 344 Kindern unter einem Jahr wurde eine Gesamtprävalenz von 44,5 % mittels POC-CCA festgestellt. Die höchste Prävalenz wurde in dem ländlichen Dorf Andina mit 78 % festgestellt. Prävalenzunterschiede zwischen den verschiedenen Altersgruppen und Geschlechtern innerhalb der Studienpopulation waren nicht relevant. Bei Kindern infizierter Mütter war die Wahrscheinlichkeit einer Infektion höher als bei Kindern nicht infizierter Mütter.

Schlussfolgerung: Die Ergebnisse der Studie bestätigen zweifelsohne, dass die untersuchte Altersgruppe gefährdet ist und nicht länger vernachlässigt werden sollte. Um Schistosomiasis weltweit langfristig zu beseitigen und die allgemeine Gesundheit von Kindern zu verbessern, müssen neue Ansätze für innovative Strategien im Bereich der öffentlichen Gesundheit umgesetzt werden, die auch die Kleinkinder einbeziehen und die Sensibilisierung, Diagnose und Behandlung der Erkrankung verbessern.

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V Curriculum Vitae

- Lebenslauf aus datenschutzrechtlichen Gründen nicht enthalten -

[1] **Abstract published:**

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VI Appendix: R Studio Code

Descriptive Analysis

```
#Setup
install.packages("DescTools")
install.packages("xlsx")
install.packages(plyr)
install.packages(AMR)
install.packages(psych)
install.packages(epiR)
install.packages(expss)

library(DescTools)
library("xlsx")
library(plyr)
library(AMR)
library(psych)
library (epiR)
library(expss)

library(readxl)
THESE <- read_excel("~/Documents/Doktorarbeit/data/THESE.xlsx")
View(THESE)
THESE <- read_excel("~/Documents/Doktorarbeit/data/THESE.xlsx")
View(THESE)

### Table 1 ###

#csb proportion
table(THESE$t0_a00_csbcode_c)
round(prop.table(table (THESE$t0_a00_csbcode_c))*100, 2)

#sex
table(is.na(THESE$t3_f01_sex_d))
table(THESE$t3_f01_sex_d)
val_lab(THESE$t3_f01_sex_d) = lab_num("female 2
                                      male 1")
round(prop.table(table (THESE$t3_f01_sex_d))*100, 2)

#age
table(is.na(THESE$`Age of child (months)`))
#some data is missing (n=85), so I calculate the percentage of missing data from the set (24,7 Percent)
BinomCI(85, 344, conf.level = 0.95, method=c("clopper-pearson"))*100


table(THESE$`Age of child (months)`)
summary(THESE$`Age of child (months)`)
round(prop.table(table(THESE$`Age of child (months)`))*100)

## bar plot for sex and age distribution in percent

# Set margins to adjust the plot area
par(mar = c(5, 6, 4, 4))  # c(bottom, left, top, right)

# Calculate the total number of participants
total_participants <- sum(age_table)

# Calculate the percentage table
percentage_table <- prop.table(age_table) * 100
```

```
# Create a bar plot for age distribution by sex with percentages
bp <- barplot(
  percentage_table,
  beside = TRUE,
  xlab = "Age in months",
  ylab = "Percentage",
  col = c("darkgreen", "darkred"),
  axis.lty = 1,
  args.legend = list(x = "topright", bty = "n"),
  ylim = c(0, 30)  # Set the y-axis limits
)

# Add light grey horizontal lines in steps of 1 percent behind the bars because they should not cover the bars
abline(h = seq(0, 30, by = 5), col = "lightgrey", lwd = 0.8)
# Add the bars again to bring them TO THE FRONT
barplot(
  percentage_table,
  beside = TRUE,
  col = c("darkgreen", "darkred"),
  add = TRUE,
  axes = FALSE,
  ylim = c(0, 30),
  border = NA
)

# Same for the legend: Add legend in front of the lines
legend("topright", legend = c("Male", "Female"), fill = c("darkgreen", "darkred"), cex = 2)  # Adjust the
value of cex

# Customize font size for axis labels, titles, and legend to make it bigger
par(
  cex.axis = 2,  # Adjust font size for axis labels
  cex.lab = 2,   # Adjust font size for labels
  cex.main = 2,  # Adjust font size for main title
  cex.legend = 2 # Adjust font size for legend
)

## CSB missing values calculation (age)
table((is.na(THESE$`Age of child (months)`)), THESE$t0_a01_csbnom_s)

#Andina
BinomCI(23, 123, conf.level = 0.95, method=c("clopper-pearson"))*100

#Tsarasaotra
BinomCI(14, 55, conf.level = 0.95, method=c("clopper-pearson"))*100

#Fandriana
BinomCI(48, 70, conf.level = 0.95, method=c("clopper-pearson"))*100

### Table 2 ###

#age FA Andina
table(subset(THESE$`Age of child (months)`,THESE$t0_a00_csbcode_c == "FA")) #age FA Andina
summary(subset(THESE$`Age of child (months)`,THESE$t0_a00_csbcode_c == "FA")) #age FA

#age FE Tsarasaotra
table(subset(THESE$`Age of child (months)`,THESE$t0_a00_csbcode_c == "FE"))
summary(subset(THESE$`Age of child (months)`,THESE$t0_a00_csbcode_c == "FE"))

#age FK Fandriana
table(subset(THESE$`Age of child (months)`,THESE$t0_a00_csbcode_c == "FK"))
summary(subset(THESE$`Age of child (months)`,THESE$t0_a00_csbcode_c == "FK"))
```

```
#age FD Vatovory
table(subset(THESE$`Age of child (months)`,THESE$t0_a00_csbcode_c == "FD"))
summary(subset(THESE$`Age of child (months)`,THESE$t0_a00_csbcode_c == "FD"))

#age all CSB für Table 2 and Percentages
table(THESE$`Age of child (months)`, THESE$t0_a00_csbcode_c)
round(prop.table(table(THESE$`Age of child (months)`, THESE$t0_a00_csbcode_c))*100, 2)
round(prop.table(table(THESE$`Age of child (months)`, THESE$t0_a00_csbcode_c),2)*100, 2)

#sex
table (THESE$t3_f01_sex_d, THESE$t0_a00_csbcode_c)
val_lab(THESE$t3_f01_sex_d) = lab_num("female 2
                                      male 1")
round(prop.table(table (THESE$t3_f01_sex_d, THESE$t0_a00_csbcode_c), 2)*100, 2)

### Table 3 ###

val_lab(THESE$t3_f01_sex_d) = lab_num("female 2
                                      male 1")
sub_male <- subset (THESE, THESE$t3_f01_sex_d == "1")
table(sub_male$t3_f01_sex_d)
sub_female <- subset (THESE, THESE$t3_f01_sex_d == "2")
table(sub_female$t3_f01_sex_d)

#csb
table(THESE$t3_f01_sex_d, THESE$t0_a00_csbcode_c)
prop.table(table(THESE$t0_a00_csbcode_c, THESE$t3_f01_sex_d)*100, 2)
round((table(THESE$t3_f01_sex_d))/(sum(table(THESE$t3_f01_sex_d, THESE$t0_a00_csbcode_c)))*100,
2)
table(sub_male$t0_a00_csbcode_c)
prop.table(table(sub_male$t0_a00_csbcode_c))
round((table(sub_male$t3_f01_sex_d,
sub_male$t0_a00_csbcode_c))/(sum(table(sub_male$t0_a00_csbcode_c)))*100, 2)
prop.table(table(sub_female$t0_a00_csbcode_c))
round((table(sub_female$t3_f01_sex_d,
sub_female$t0_a00_csbcode_c))/(sum(table(sub_female$t0_a00_csbcode_c)))*100, 2)

#age
table(is.na(sub_male$`Age of child (months)`))
table(is.na(sub_female$`Age of child (months)`))

table(sub_male$`Age of child (months)`)
summary(sub_male$`Age of child (months)`)

table(sub_female$`Age of child (months)`)
round(table(sub_female$`Age of child (months)`))
summary(sub_female$`Age of child (months)`)
prop.table((table(THESE$t3_f01_sex_d, THESE$`Age of child (months)`))*100,2)
round(prop.table((table(THESE$t3_f01_sex_d, THESE$`Age of child (months)`)))*100, 2)

round((table(sub_female$t3_f01_sex_d, sub_female$`Age of child (months)`))/(sum(table(sub_female$`Age
of child (months)`)))*100, 2)

round((table(sub_male$t3_f01_sex_d, sub_male$`Age of child (months)`))/(sum(table(sub_male$`Age of
child (months)`)))*100, 2)

round((table(sub_male$t3_f01_sex_d, sub_male$`Age of child (months)`))/(sum(table(sub_male$`Age of
child (months)`)))*100, 2)
```

Prevalence calculation analysis

```
# Setup
remove.packages("rstan")
if (file.exists(".RData")) file.remove(".RData")
install.packages("rstan", repos = "https://cloud.r-project.org/", dependencies = TRUE)
Sys.setenv(MAKEFLAGS = paste0("-j",parallel::detectCores()))

install.packages(c("StanHeaders","rstan"),type="source")
library("rstan") # observe startup messages
options(mc.cores = parallel::detectCores())
rstan_options(auto_write = TRUE)

install.packages("DescTools")
install.packages("xlsx") #timed out
install.packages("haven")
install.packages("expss")
install.packages("AMR")
install.packages("ggplot2")

### Load packages
library(DescTools)    # package for estimating binomial CIs
library(xlsx)
library(haven)
library(expss)
library(AMR)
library(ggplot2)

### Load data
library(readxl)
THESE <- read_excel("~/Documents/Doktorarbeit/data/Dataset2 Thesis_data taken out.xlsx")
View(THESE)

### Table 1, calculate CIs for overall population (344)

table (THESE$t3_k02_tdrschistodone_c, THESE$t0_a00_csbcode_c)
table(THESE$`G Score schisto grades adapted`)
table (THESE$`G Score schisto grades adapted`, THESE$t0_a00_csbcode_c)
prop.table1 <- prop.table(table (THESE$`G Score schisto grades adapted`, THESE$t0_a00_csbcode_c))
prop.table2 <- round(prop.table(table (THESE$`G Score schisto grades adapted`,
THESE$t0_a00_csbcode_c),2)*100,2)

# Summary statistics for G Score
g_scores <- Dataset2_Thesis_data_taken_out$`G Score schisto grades adapted`

# Calculate mean, median, and IQR
mean_g_scores <- mean(g_scores)
median_g_scores <- median(g_scores)
iqr_g_scores <- IQR(g_scores)

# Display the results
cat("Mean G Score:", mean_g_scores, "\n")
cat("Median G Score:", median_g_scores, "\n")
cat("IQR G Score:", iqr_g_scores, "\n")


# G1 (73)
BinomCI(73, 344, conf.level = 0.95, method=c("clopper-pearson"))*100

# G2 (60)
BinomCI(60, 344, conf.level = 0.95, method=c("clopper-pearson"))*100
```

```
# G3 (58)
BinomCI(58, 344, conf.level = 0.95, method=c("clopper-pearson"))*100

# G4 (50)
BinomCI(50, 344, conf.level = 0.95, method=c("clopper-pearson"))*100

# G5 (29)
BinomCI(29, 344, conf.level = 0.95, method=c("clopper-pearson"))*100

# G6 (26)
BinomCI(26, 344, conf.level = 0.95, method=c("clopper-pearson"))*100

# G7 (11)
BinomCI(11, 344, conf.level = 0.95, method=c("clopper-pearson"))*100

# G8 (17)
BinomCI(17, 344, conf.level = 0.95, method=c("clopper-pearson"))*100

# G9 (6)
BinomCI(6, 344, conf.level = 0.95, method=c("clopper-pearson"))*100

# G10 (14)
BinomCI(14, 344, conf.level = 0.95, method=c("clopper-pearson"))*100

# Classification traces (G2+G3) = 118
BinomCI(118, 344, conf.level = 0.95, method=c("clopper-pearson"))*100

# Classification 1+ (G4+G5) = 79
BinomCI(79, 344, conf.level = 0.95, method=c("clopper-pearson"))*100

# Classification 2+ (G6+G7) = 37
BinomCI(37, 344, conf.level = 0.95, method=c("clopper-pearson"))*100

# Classification 3+ (G8-G10) = 37
BinomCI(37, 344, conf.level = 0.95, method=c("clopper-pearson"))*100

# Interpretation: traces interpreted as positive (G2-G10=positive) = 271 (G1=negative, s.o.)
BinomCI(271, 344, conf.level = 0.95, method=c("clopper-pearson"))*100

# Interpretation: traces interpreted as negative
# G1-G3 = negative = 191
BinomCI(191, 344, conf.level = 0.95, method=c("clopper-pearson"))*100
# G4-G10=positive = 153
BinomCI(153, 344, conf.level = 0.95, method=c("clopper-pearson"))*100

## Barplot for G Score groups

# Set margins to adjust the plot area
par(mar = c(5, 6, 4, 4))  # c(bottom, left, top, right)

table(THESE$`G Score grouped`)
round(prop.table(table(THESE$`G Score grouped`))*100)

G_Score_grouped <- THESE$`G Score grouped`
G_Score_grouped[G_Score_grouped == "negative"] <- "1"
G_Score_grouped[G_Score_grouped == "trace"] <- "2-3"
G_Score_grouped[G_Score_grouped == "1+"] <- "4-5"
G_Score_grouped[G_Score_grouped == "2+"] <- "6-7"
G_Score_grouped[G_Score_grouped == "3+"] <- "8-10"

# Create a bar plot for G Score groups
```

```
bp <- barplot(
  prop.table(table(G_Score_grouped))*100,
  xlab = "G Score POC-CCA results",
  ylab = "Percentage",
  ylim = c(0, 40),
  col = c("darkolivegreen2", "khaki1", "darkgoldenrod1", "lightsalmon1", "coral2"),
  axis.lty = 1)
# Add grey horizontal lines in steps of 5 percent (background layer)
abline(h = seq(0, 40, by = 10), col = "lightgrey", lwd = 0.8)

# Add bars again to bring them to the front
barplot(
  prop.table(table(G_Score_grouped))*100,
  add = TRUE,
  beside = TRUE,
  col = c("darkolivegreen2", "khaki1", "darkgoldenrod1", "lightsalmon1", "coral2"),
  ylim = c(0, 40),
  border = NA)

# Add legend in front of the lines with adjusted font size and larger color boxes
legend("topright", c("negative", "traces", "1+", "2+", "3+"), pch = 15, col = c("darkolivegreen2", "khaki1",
"darkgoldenrod1", "lightsalmon1", "coral2"), cex = 2, pt.cex = 2)

## For bigger fonts: Customize font size for axis labels, titles, and legend
par(
  cex.axis = 2,   # Adjust font size for axis labels
  cex.lab = 2,    # Adjust font size for labels
  cex.main = 2,   # Adjust font size for main title
  cex.legend = 2  # Adjust font size for legend
)

## Table 2 ## POC-CCA results in Prevalence/study sites (traces pos/traces neg)  subgroups rural/urban

## study sites
table (THESE$`G Score schisto grades adapted`, THESE$t0_a00_csbcode_c)
prop.table(table(THESE$`G Score schisto grades adapted`, THESE$t0_a00_csbcode_c))
round(prop.table(table (THESE$`G Score schisto grades adapted`, THESE$t0_a00_csbcode_c),2)*100,2)

table(THESE$`G Score grouped`, THESE$t0_a00_csbcode_c)
prop.table(table(THESE$`G Score grouped`, THESE$t0_a00_csbcode_c))
round(prop.table(table (THESE$`G Score grouped`, THESE$t0_a00_csbcode_c),2)*100,2)

# CI
# Andina
BinomCI(3, 123, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(24, 123, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(96, 123, conf.level = 0.95, method=c("clopper-pearson"))*100

# Tsarasaotra
BinomCI(34, 55, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(6, 55, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(15, 55, conf.level = 0.95, method=c("clopper-pearson"))*100

# Fandriana
BinomCI(12, 70, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(34, 70, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(24, 70, conf.level = 0.95, method=c("clopper-pearson"))*100

# Vatovory
BinomCI(24, 96, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(54, 96, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(18, 96, conf.level = 0.95, method=c("clopper-pearson"))*100
```

```
# Urban (FK+FD)
BinomCI(36, 166, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(88, 166, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(42, 166, conf.level = 0.95, method=c("clopper-pearson"))*100


## sex
table (THESE$`G Score schisto grades adapted`, THESE$t3_f01_sex_d)
table (THESE$`G Score grouped`, THESE$t3_f01_sex_d)
prop.table(table(THESE$`G Score schisto grades adapted`, THESE$t3_f01_sex_d))
prop.table(table(THESE$`G Score grouped`, THESE$t3_f01_sex_d))
round(prop.table(table (THESE$`G Score schisto grades adapted`, THESE$t3_f01_sex_d),2)*100,2)
round(prop.table(table (THESE$`G Score grouped`, THESE$t3_f01_sex_d),2)*100,2)
val_lab(THESE$t3_f01_sex_d) = lab_num("female 2
                                male 1")
# CIs

#female
BinomCI(36, 171, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(61, 171, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(74, 171, conf.level = 0.95, method=c("clopper-pearson"))*100

#male
BinomCI(37, 173, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(57, 173, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(79, 173, conf.level = 0.95, method=c("clopper-pearson"))*100

## age groups

table(THESE$`Age of child (months)`, THESE$`G Score grouped`)

THESE$age_groups <- age_groups(THESE$`Age of child (months)`, split_at = c(9, 11, 13, na.rm= TRUE))
table(THESE$age_groups)
table(THESE$age_groups, THESE$`G Score schisto grades adapted`)
round(prop.table(table(THESE$age_groups, THESE$`G Score schisto grades adapted`),1)*100, 2)
table(THESE$age_groups, THESE$`G Score grouped`)
round(prop.table(table(THESE$age_groups, THESE$`G Score grouped`),1)*100, 2)

#CIs

# 6-8 months
BinomCI(9, 50, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(10, 50, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(31, 50, conf.level = 0.95, method=c("clopper-pearson"))*100

# 9-10 months
BinomCI(29, 164, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(56, 164, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(79, 164, conf.level = 0.95, method=c("clopper-pearson"))*100

# 11-12 months
BinomCI(19, 45, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(19, 45, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(7, 45, conf.level = 0.95, method=c("clopper-pearson"))*100

## Alternative age groups
THESE$age_groups2 <- age_groups(THESE$`Age of child (months)`, split_at = c(9, 10, 13, na.rm= TRUE))
table(THESE$age_groups2)
table(THESE$age_groups2, THESE$`G Score schisto grades adapted`)
round(prop.table(table(THESE$age_groups2, THESE$`G Score schisto grades adapted`),1)*100, 2)
table(THESE$age_groups2, THESE$`G Score grouped`)
```

```
round(prop.table(table(THESE$age_groups2, THESE$`G Score grouped`),1)*100)

#CIs for alternative age groups

# 6-8 months
BinomCI(9, 50, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(10, 50, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(31, 50, conf.level = 0.95, method=c("clopper-pearson"))*100

# 9 months
BinomCI(23, 130, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(43, 130, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(64, 130, conf.level = 0.95, method=c("clopper-pearson"))*100

# 10-12 months
BinomCI(25, 79, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(32, 79, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(22, 79, conf.level = 0.95, method=c("clopper-pearson"))*100

## infection status of mother

table(is.na(THESE$t3_d05_tdrschistodone_c))
table(is.na(THESE$t3_d05_tdrschistoresult_d))
table(is.na(THESE$t3_d05_tdrschistograde_ADAPTED))
table(THESE$t3_d05_tdrschistograde_ADAPTED)
table(is.na(THESE$`G Score schisto grades adapted`))
table(THESE$t3_d05_tdrschistograde_ADAPTED, THESE$t0_a00_csbcode_c)


## infetction status mother: 0 is negative, 1 is traces, 2 is positive (done as advised by Eva)
THESE$TDR_schisto_mother_groups <- ifelse(!is.na(THESE$t3_d05_tdrschistograde_ADAPTED) &
THESE$t3_d05_tdrschistograde_ADAPTED <= 1,0,
                                ifelse(!is.na(THESE$t3_d05_tdrschistograde_ADAPTED) &
(THESE$t3_d05_tdrschistograde_ADAPTED >=2 & THESE$t3_d05_tdrschistograde_ADAPTED <=3),1,
                                       ifelse(!is.na(THESE$t3_d05_tdrschistograde_ADAPTED) &
THESE$t3_d05_tdrschistograde_ADAPTED >=4,2,NA)))

table(THESE$TDR_schisto_mother_groups, THESE$`G Score grouped`, useNA = "always")

# CIs for crosstabulation infection status mother and child
## Mother negative
BinomCI(47, 107, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(27, 107, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(33, 107, conf.level = 0.95, method=c("clopper-pearson"))*100

## Mother traces
BinomCI(16, 128, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(55, 128, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(57, 128, conf.level = 0.95, method=c("clopper-pearson"))*100

## Mother positive
BinomCI(7, 99, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(33, 99, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(59, 99, conf.level = 0.95, method=c("clopper-pearson"))*100

###Comparison age and infection status mother (cross tabulation done as advised by Eva)

table(THESE$age_groups,THESE$TDR_schisto_mother_groups, useNA = "always")

table(THESE$age_groups2,THESE$TDR_schisto_mother_groups, useNA = "always")

## CIs
```

```
# 6-8 months and infection status mother
BinomCI(11, 50, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(19, 50, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(20, 50, conf.level = 0.95, method=c("clopper-pearson"))*100

# 9 months and infection status mother
BinomCI(44, 122, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(45, 122, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(33, 122, conf.level = 0.95, method=c("clopper-pearson"))*100

# 10-12 months and infection status mother
BinomCI(29, 77, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(36, 77, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(12, 77, conf.level = 0.95, method=c("clopper-pearson"))*100

## Infection status mother table alone without cross tabulation
table(THESE$t3_d05_tdrschistograde_ADAPTED)
BinomCI(107, 334, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(128, 334, conf.level = 0.95, method=c("clopper-pearson"))*100
BinomCI(99, 334, conf.level = 0.95, method=c("clopper-pearson"))*100
```

VII Eidesstattliche Versicherung

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

.....

Jule Hameister