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Vorstandsvorsitz

Occurrence of Schistosomiasis in Madagascar

Assessing the prevalence of schistosomiasis in pregnant women in Tsiroanomandidy and Ampefy in the Madagascan Highlands using a rapid diagnostic test

Dissertation

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Abbreviations

BNITM	Bernhard Nocht Institute for Tropical Medicine
CAA	Circulating Anodic Antigen
CI	Confidence Interval
CSB	Centre de Santé de Base (Primary Health Care Centres)
DALY	Disability- adjusted life year
EMA	European Medicine Agency
ELISA	Enzyme-linked immunosorbent assay
FGS	Female Genital Schistosomiasis
g	Gram
GCP	Good Clinical Practice
Hb	Haemoglobin
IFAT	Indirect immunofluorescence tests
µl	Microliter
KK	Kato- Katz (Microscopy)
MDA	Mass drug administration
mm	Millimetre
n	Number
NTD	Neglected tropical diseases
NPV	Negative predictive value
OR	Odds Ratio
POC-CCA	Point-of-Care-Circulating Cathodic Antigen
PPC	Paediatric Praziquantel Consortium
PPV	Positive predictive value
PZQ	Praziquantel
RDT	Rapid Diagnostic Test
SAC	School Aged Children
S. guineensis	Schistosoma guineensis
S. haem(atobium)	Schistosoma haematobium
S. intercalatum	Schistosoma intercalatum
SD	Standard deviation
SSA	Sub- Saharan Africa
QALY	Quality Adjusted Life Years
WHO	World Health Organisation

1 Working hypothesis and aim of the study

Prevalence assessment of Schistosomiasis, a water-based disease that affects over 220 million people worldwide, causing chronical damage to the human´s intestinal or urogenital system, is lacking sufficient data for pregnant women, as this population group is usually not included in preventive chemotherapy programmes and accompanying prevalence assessments.

The aim of this study is to provide valuable information about the prevalence of schistosomiasis in pregnant women in two highland locations in Madagascar by using a point-of-care (POC) rapid diagnostic test for one of the schistosome antigens, the circulating cathodic antigen (CCA), in order to identify populations in need for schistosomiasis control measures that include pregnant women, too.

2 Introduction

2.1 Overview

Schistosomiasis, also known as Bilharzia, is the second most frequent parasitic illness (after Malaria) and considered a neglected tropical disease (NTD). It is transmitted to humans via contact to infested water through skin penetration of cercariae of trematodes of the genus *Schistosoma*. Schistosomiasis is estimated to affect over 220 million people world-wide. 779 million people live in exposed areas in 78 countries, mostly in sub-Saharan Africa (WHO 2020).

Schistosomiasis is a serious disease of poverty, associated with a situation of insufficient sanitary systems, that forces people to reach out for unprotected natural open freshwater sources where transmission occurs. In contaminated (by faeces and urine that contain schistosoma eggs) waters, the larvae hatch from the eggs and invade the intermediate host: water snails from the genera *Belinus* and *Biomphalaria*. Inside the snails, the parasite develops to so called cercariae that are released into the water, swim to the human host and penetrate the human skin directly. Inside the human, they migrate into the blood to become adult fluke worms, which form male-female pairs attached to the endothelium in blood vessels close to the liver and intestines or the genitourinary tract. Paired schistosoma constantly release eggs that spread through the whole body and invade different tissues, where they lead to granulomatous long-term damages of tissues and organs.

Cumulative schistosome infections over years result in chronic morbidity manifestations, including hematuria, nutritional deficiencies, anemia and delayed physical and cognitive development in children. Moreover, intestinal schistosomiasis can cause hepatic peri-portal fibrosis and consequently portal hypertension whereas genitourinary schistosomiasis may lead to obstruction and carcinomas of urogenital organs and impairment of female reproductive health (Gurari et al. 2011).

In highly endemic populations, the disease strongly impacts quality of life measured as Quality Adjusted Life Years (QALY) and Disability-Adjusted Life

Years (DALY) (Atun et al. 2015). Madagascar is one of the heavily affected countries with spot checks reporting a prevalence of 77% among school children in the highlands (Schwarz et al. 2014). Some 60- 70% of all infected persons lie within the age range of 5- 14 years (Davies 2009) and pre-school and primary school children are a particularly important population group as infection reservoirs because of their deliberate excretory habits (especially urinating in water) and their many opportunities for water contact in hot climates. However, pre-school children as well as adolescent girls, women of childbearing age and pregnant women are not yet included regularly in massive drug administration programmes. In pregnant women, maternal anaemia caused by the schistosomiasis infection during pregnancy is likely to have negative impacts on the development of the new-born and the child (Haider 2014 et al.).

2.2 Geographical background

Today, the *Schistosoma* trematode worms remain distributed throughout the tropics and flourish wherever freshwater bodies, both natural and man-made, create habitats for the appropriate snail intermediate hosts. Sub-Saharan Africa carries the greatest burden of the disease with 85% of worldwide schistosomiasis infections (Sacolo et al. 2018). Schistosomiasis is acquired in fresh water infested with snail species acting as intermediate host that release larval forms of the parasitic blood flukes. Six *Schistosoma* species can infect humans. In Madagascar, *Schistosoma mansoni* and *Schistosoma haematobium* are endemic*. S. haematobium* causes urinary infections, whereas the other species, predominantly *S. mansoni*, account for intestinal schistosomiasis (Hotez und Kamath 2009). *S. haematobium* is scattered throughout Africa, parts of Arabia, the Middle East, Madagascar and Mauritius. *Schistosoma mansoni* is mainly found in Africa and Madagascar from where it was exported by the slave trade to parts of South America and the Caribbean. Hence, today Brazil still manifests a significant prevalence of *S. mansoni* (Siquiera et al., 2016). *Schistosoma japonicum*, which also causes diseases of the bowel and liver, is found in Asia (especially in China and the Philippines). Minor species are *Schistosoma mekongi* (in the Mekong river on the east border to Thailand), *S.*

intercalatum and *S. guineensis* (both confined to rainforest areas in West and Central Africa). Humans are the main reservoir for *S. mansoni*. Baboons and rodents may be further hosts, whereas many animals are susceptible *to S. japonicum*.

Climate change and global mobility could extend the disease further north or into higher altitudes. High mobility of people and massive migration bear the risk of (re-) introducing schistosomiasis into (previously) non-endemic areas. As an example, Schistosomiasis was discovered on the French island of Corsica in 2014 with DNA analyses suggesting its origin in Senegal (Pennisi 2018). The parasite found in Corsica was a hybrid form between the *Schistosoma haematobium* and *Schistosoma bovis*, this last one being a livestock parasite (Oleaga et al. 2019). Also, increasing numbers of tourists are contracting schistosomiasis (WHO 2020), as eco-tourism and travel "off the beaten track" are becoming more and more popular.

Table 1: Parasite species and geographical distribution of schistosomiasis (WHO 2020)

	Species	**Geographical distribution**
Intestinal schistosomiasis	*S. mansoni*	Africa and Madagascar, the Middle East, the Caribbean, Brazil, Venezuela and Suriname
	S. japonicum	China, Indonesia, Philippines
	S. mekongi	Several districts of Cambodia and the Lao People's Democratic Republic
	S. guineensis and *S. intercalatum*	Rain forest areas of central Africa
Urogenital schistosomiasis	*S. haematobium*	Africa, the Middle East, Corsica (France)

2.3 Lifecycle

2.3.1 Adult Worms and eggs

The parasitosis was first described in 1851 in Egypt by the German doctor and scientist Theodor Bilharz (therefore the diseases´ eponym Bilharziasis), who named the fluke worm "*Distomum haematobium*". Later, the terms ´schistos´ (fissure) and `soma´ (body) were established to describe the male fluke´s shape which holds the female fluke within a layer gap on his ventral side. The adult blood flukes causing Bilharziasis are worm- like parasites (7- 20 mm long) and are called schistosomes. They inhabit parts of the human´s venous system. The worms sometimes live for 30 years, but their normal lifespan is probably 3- 7 years (Squire and Stothard 2014). Being attached mostly to the venous endothelium, the male worm resembles a rolled leaf having a groove on his ventral surface in which the longer and slenderer female is held *in copula*. Males embrace females permanently, but both sexes are actively motile. Adult females lay fertilized eggs in the terminal venules of the preferred host tissues (see image life- cycle). Every fertilized adult female produces hundred to thousand eggs a day and every egg contains a ciliated miracidium, which develops as sort of embryo within a few days (Davies 2009). The eggs of *S. mansoni* have a lateral spur and mainly scatter in the blood vessels of the digestive tract (mesenteric venous plexus), whereas *S. haematobium´*s eggs have a terminal spur and tend to settle in the urinary system (peri vesical venous plexus). Thanks to proteolytic enzymes and through movements of the walls of the hollow viscera involved, about 50 % of the eggs migrate to the lumen of the bladder (*S. haematobium*) or the bowel (*S.mansoni*), from which they escape to the outside world via urination or defaecation (Davies 2009).

2.3.2 Miracidia and aquatic snails

Eggs are released when an infected person urinates (in the case of *S. haematobium*) or defaecates (*S.mansoni*) into the water. In a suitable environment (freshwater and a temperature between 10 – 30 °C), the miracidium larva hatches from the egg and swims actively; guided by light and responding to chemical stimuli produced by the aquatic snails, which are the molluscan intermediate host. These snails can be found in waters from sea

level up to 4200 meters in fresh water with a flow velocity less than 30 cm per second. 10 genera of potential freshwater snail hosts are known, but in general there are two groups (Meulemann 1972): *Schistosoma haematobium* requires an aquatic sinistral turreted snail of the genus *Bulinus*, whereas *Schistosoma mansoni* needs a flat aquatic ´rams-horn` snail, most commonly of the genus *Biomphalaria*. Both genera are aquatic snails, found in many different habitats like ponds, swamps and rivers, as well as in large permanent water bodies such as lakes, irrigation channels and rice fields. The snails can survive out of water for weeks up to months, so that immature infections can be carried through from one wet season to another, maintaining the transmission cycle (Davies 2009). These snails are capable of self- fertilization and egg-laying continues throughout their lifespans; in average 400 days in case of *Bulinus* and 213 days for a *Biomphalaria* snail (Fryer 1986, Meulemann 1972). The miracidium larva is infective to the snails for 8-12 hours (Davies 2009) and penetrates the snail´s body surface mostly via the foot of the snail being assisted by lytic enzymes secreted from the miracidium´s gut (Miller et al. 1980).

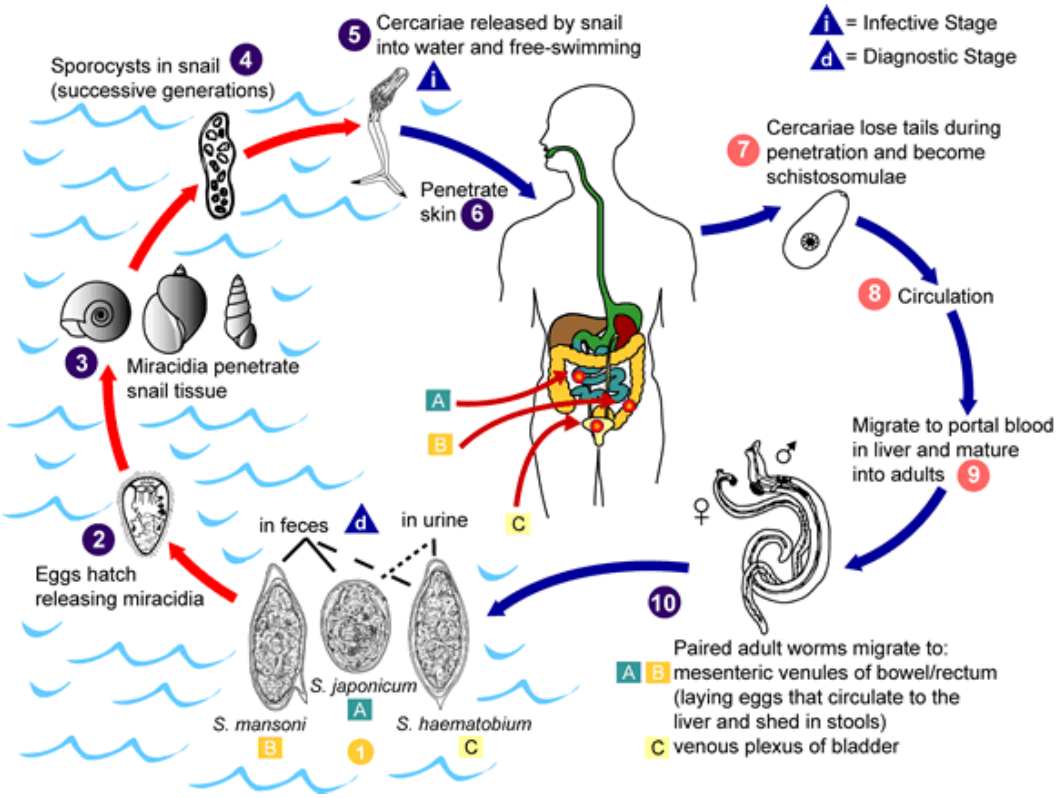
Table 2: Life Expectancy of *S. mansoni* in its different stages (Brazilian Health Ministry, 2014)

PHASE	**DURATION and EVOLUTION**
Mature egg	Within the host, up to 20 days
Miracida	a) within the egg and in solid stool, without exposure to sunlight: up to 5 days, b) after hatching, in humid environment up to 24 hours, c) within the mollusc, 48 hours, before turning into a primary sporocyst.
Primary sporocyst	2 weeks, giving origin to 20- 40 secondary sporocysts
Secondary sporocyst	3-4 weeks to the formation of cercariae; possible return to sporocyst production for a period of several months
Cercariae	Persistence in water up to 2 days; Penetration of the human skin within 15 minutes
Schistosome/adult Worms	27 days to transform into an adult worm, after 40 days first eggs can be found in the stool. Average lifetime is about 5 years, up to 30 years, with excretion of eggs possible for up to 30 years.

2.3.3 Intramolluscan stage and cercariae

Within the intermediate host (snail) begins an asexual replicative cycle. Only a small amount of entering miracidia develop to mature mother sporocysts that in turn produce daughter sporocysts (Davies 2009). The replication results a few weeks later in the release of free- swimming minute fork- tailed cercariae that escape out of the snails into the water. The cercariae are about 200 – 500µm long and just visible to the naked eye. They emerge from the sporocyst inside the snail in response to light, generally between 11am and 3pm, at a temperature of 25 to 30 °C and can survive in the water up to 72h (Squire and Stothard 2014), though their normal lifespan is relatively short: 36- 48h, as they are non- feeding organisms and depend on their glycogen reserves. Cercariae are brevifurcate (having a small bifurcation at the tail) and have an oral muscular sucker occupying about one- third of the body. Six pairs of cephalic glands facilitate the emergence from the snail and penetration of the host skin and enzymatic secretions provide an adhesive function as the cercariae shift over the (human) host skin (Davies 2009).

Figure 1: Life Cycle of Schistosomiasis (CDC 2019)



The cercariae then penetrate the human skin or (pharyngeal) mucosa, shed their tails and become schistosomula migrating towards the liver, realizing a notable transition from ´freshwater environment´ towards the `saltwater environment` of the human body (Davies 2009). In intrahepatic portal veins they mature within the time period of 4-6 weeks. After coupling with an appropriate mature worm, they migrate to their final location, the peri vesical (*S. haematobium*) or mesenteric (*S. mansoni*) venous plexus, respectively, where the life cycle restarts, with egg deposition and egg excretion. The time between larval penetration and excretion of eggs (prepatent period) varies from about 4 weeks (*S. mansoni*) to 12 weeks (*S. haematobium*).

We can hence summarize the following life cycle stages:

1) Adult worms produce eggs containing miracidia, eggs are released with stool or urine
2) The eggs release the miracidia (larval form)
3) The miracidia penetrate snail tissue and begin an intramolluscan development, becoming sporocysts within the intermediate host.
4) Cercariae are released from the snails to penetrate human skin.
5) The cercariae become schistosomula in the human tissues, migrate within the vascular system and are transported to the right side of the heart and lungs for further development.
6) In the direction of blood flow, they continue an intravascular route and arrive in the intrahepatic vascular system where maturation into adult worms occurs.

2.4 Clinical impact of Schistosomiasis

2.4.1 Pathology and pathogenesis

Clinical consequences of schistosomiasis are multiple, and they depend on the underlying species. Especially in primary infections, every species´ cercarial

penetration can provoke an itchy skin rash as well as a systemic hypersensitivity reaction to the migrating schistosomula about 4 weeks after infection (Lambertucci 1993, Bottieau 2006, Rocha 1995 et 1996, Cheever 2000). The patient then may present fever, urticaria, eosinophilia, diarrhea, hepato- splenomegaly and cough. This acute complex of symptoms occurs rather in primary infections and is also called Katayama fever or ´acute toxaemic schistosomiasis´, with rising antibody levels and an increase of IgG, IgA and IgM in serum. Additional symptoms like rigors, sweating, general myalgia and lymphadenopathy are possible.

Adult worms themselves cause little or no pathology, though they excrete antigens which are now used as markers for infection and as indicators for therapeutic success (Davies 2009). The circulating cathodic-associated antigen (CCA) and the circulating anodic-associated antigen CAA are in this context important for diagnostics and interpretation for the correlation between circulating antigen levels and number of worms (van Dam et al 1996).

Eggs reach peripheral tissues via the capillary system and the deposition of the eggs lead to a chronic infection, an eosinophilic and granulomatous inflammation and subsequently fibrotic transformation (Cheever et al. 2000). The florid granuloma is a manifestation of delayed hypersensitivity through a T cell-mediated immune response and consists of the schistosome egg surrounded by cellular aggregates of mononuclear phagocytes, eosinophils, neutrophils, lymphocytes, plasma and fibroblasts (Davies 2009).

In conclusion, the pathology of schistosomiasis results from the multiple presence of granulomas, fibroinflammatory egg induced swellings and from fibroblastic transformations obstructing vessels and the capillary system. These late obstructive and fibrous lesions barely respond to antihelminth treatment (Davies 2009). Therefore, hepatosplenomegaly of the early infection due to cell proliferation with florid granuloma and diffuse inflammatory infiltrates is much more likely to be reversible by chemotherapy than the advanced hepatosplenomegaly resulting from fibrovascular pathology with periportal fibrosis and portal hypertension (Lichternberg 1987).

2.4.2 *S. haematobium*: urogenital schistosomiasis

The classic acute sign of urogenital schistosomiasis is recurrent and painless hematuria (blood in urine), as the urinary bladder is the most frequently affected organ. Chronic inflammation of the bladder and ureters lead to ulceration and pseudopapillomas (Cheever et al. 1978) with the symptoms pollakisuria, dysuria, proteinuria and haematuria (Gryseels 1989). Fibrosis and calcification of the bladder and ureters result in hydroureter and hydronephrosis and, in severe cases, kidney failure (Gill et al. 2010) and squamous bladder cancer (Zheng et al. 2012). Rather than directly causing the cancer, infections with *S. haematobium* seem to promote and potentiate carcinogenesis (Davies 2009). The obstructive uropathy predisposes to *Escherichia Coli* or *Salmonella* urinary tract infections, which can result in chronic pyelonephritis and Gram- negative septicaemia (Farid 1970, Farid et al. 1984, Wright et al. 1982). Eggs of *S.haematobium* are commonly present in both male and female genital organs affecting seminal vesicle and vulva, vagina and cervix respectively. Female genitale schistosomiasis (FGS), predominantly caused by *S. haematobium*, remains an often-neglected gynecological disease, as it can affect every female organ without being adequately addressed and recognized by the public and health care systems (Christinet et al. 2016). FGS also seems to have considerable negative impact on female fertility and many reports have suggested since long time an association between ectopic pregnancy and urogenital schistosomiasis (Chen et al. 1989). Alteration of the tubal submucosa is likely to be the pathophysiological mechanism behind ectopic pregnancy whereas tubal obstruction and antispermatozoal antibodies induced by schistosomiasis may lead to female infertility (Christinet et al. 2016).

2.4.3 S. mansoni: intestinal schistosomiasis

With respect to intestinal schistosomiasis, symptoms as abdominal pain, discomfort, and loss of appetite can occur, with a significant association between diarrhoea, blood in the stools and abdominal pain (Gryseels 1989). Focal granulomas and fibrosis can occur in any part of the intestinal tract, predominantly in the rectosigmoid colon, leading seldomly to severe clinical symptoms (Davies 2009). *S. mansoni* infection may present as hepatic

schistosomiasis due to the deposition of numerous schistosoma eggs along the trajectory of the portal vein. It can lead to the formation of the “Symmer´s pipestem fibrosis”, a characteristic liver disease (Cheever 1968) which represents the morphological counterpart of hepatosplenic schistosomiasis, which is clinically characterized by hepato-splenomegaly, portal hypertension (esophageal varices) and variable degrees of pancytopenia (hypersplenism). Heavy worm burden appears to be an important risk factor (Andrade et al., 1997). This periportal hepatic fibrosis is the most important complication of chronic *S. mansoni* infections and hematemesis from gastroesophageal varices is a frequent primary sign of hepato-splenic disease in schistosomiasis and a potentially lethal complication.

2.4.4 Ectopic lesions and neuroschistosomiasis

There are various rare but possible ectopic lesions, for example placental schistosomiasis (Bittencourt et al. 1980) or cutaneous deposition of *S. haematobium* eggs (Girges 1934). Cases of neurological schistosomiasis are also relatively rare but various impacts to the central nervous system (CNS) are possible. Neuroschistosomiasis results from intracerebral granuloma around one or several eggs (Burchard et al. 2011). As for *S. mansoni*, and less frequent in the case of *S. haematobium*, granulomas are occasionally located in the spinal canal, too (Ross et al. 2011). Possible access ways for the schistosomiasis eggs to enter the CNS are arterial embolies and anastomoses between the intervertebral plexus and visceral pelvic and hemorrhoidal veins. Eggs in the CNS can lead to encephalitis with focal or generalised convulsions or cauda- equina or conus medullaris syndrome, as well as a transverse myelitis. Neurological symptoms can occur early on after contact to infested water, due to a cerebral vasculitis after 2 to 6 weeks. (Caumes et al. 2010).

2.4.5 Clinical (differential) diagnosis

Because hematuria in *S. haematobium* infected individuals is the only direct diagnostic symptom, ´classical´ cases of schistosomiasis are rare. Different life stages of the parasite may interpose, making clinical diagnosis often difficult. Acute schistosomiasis must be distinguished from brucellosis, malaria, leptospirosis and many other fever illnesses of uncertain origin. In the chronic

stage, *S. mansoni* must be differentiated from peptic ulcer, pancreatitis, and many forms of dysentery, whereas infections with *S. haematobium* may suggest acute nephritides, renal tuberculosis and cancer of the urogenital tract in general. In areas of medium and high prevalence, schistosomiasis must always be considered as one possible cause of cor pulmonale and of any neurological picture, especially presentations of epilepsy and myelopathy.

2.4.6 Immunity and decrease of prevalence

Visitors to endemic areas often present a clearer clinical description than residents of endemic zones, as there exists a slow (and inefficient) development of acquired immunity in individuals, who are constantly repetitively exposed. Community- based surveys show that prevalence and intensity of infection decrease from school age throughout teenage years and adult decades (Davies 2009). A cell – mediated cytotoxicity process dependent on antibodies and complex of IgE and macrophages can lead to protective immunity directly against cercariae and schistosomula. Also, changing social habits concerning human water contact and tissue fibrosis preventing eggs from reaching the exterior (and from being detected) may explain the decreases of schistosomiasis occurrence in older age groups (Dalton 1976).

2.4.7 Public Health

The socioeconomic and public health effects of chronic schistosomiasis are considerable. The disease disables more than it leads to sudden death; chronic schistosomiasis is likely to affect people´s ability to work and to learn and hence presents a macroeconomic impediment to the progress and development of a country.

Consequences like malnutrition, fatigue, anaemia, impaired cognition and failure to thrive in children present important burdens. The risk for co- infections may rise, for example, schistosomiasis may enhance HIV transmission; particularly *S. haematobium* can increase susceptibility to viral entry through the genital tract (Friedmann et al. 2007, Bustinduy et al. 2014). The death estimates due to Bilharziasis varies considerably (WHO 2018, WHO 2002), surely because of hidden pathologies such as kidney and liver failure, bladder cancer and ectopic pregnancies due to female genital schistosomiasis. In 2000, the

WHO estimated the annual death rate due to schistosomiasis at 200 000 globally (WHO 2020).

2.4.8 Impacts of schistosomiasis in young children

Urogenital disease, maternal and placental inflammation and foetal inflammation may contribute to adverse birth outcomes. Chronic schistosomiasis also causes anaemia in pregnant women and schistosomiasis infection during pregnancy may be as risk factor for low birthweight, preterm birth and stillbirth (Haider et al. 2014). The exposure to schistosome-infested water in preschool-age children was long neglected in epidemiological studies (Bourke et al. 2011) and most schistosomiasis control efforts focused on school age children. However, exposure may happen shortly after delivery through domestic and bathing activities (Garba et al. 2010). Schistosome-associated morbidity in the preschool-age group remains an under-researched area (Knopp et al. 2013) but it is becoming evident that there is substantial morbidity attributable to schistosomiasis in this age group (Sacko et. 2011). Active disease, including anaemia (Magalhaes et al. 2011) haematuria (Coulibaly et al. 2013b) dysuria, bladder wall pathology, hepatosplenomegaly, and diarrhoea (Werf et al. 2003, Sacko et al. 2011) can occur during infancy with the occurrence of advanced fibrotic disease by the time the children enter school- age (WHO 2016).

2.4.9 Neurocognition

Schistosomiasis can cause stunting and has also neurocognitive effects on children. Schistosoma infection in school- aged children is associated with educational, learning and memory deficits (Ezeamama et al. 2018). So far, there is no evidence for negative impacts on intelligence and reaction time, but psychometrically assessed cognitive function showed deficits not only in memory and learning domains, but as well in school attendance and achievements, which were lower in non- dewormed children compared to treated and non- infected children (Ezeamama et al. 2018). Early treatment of (young) children in schistosomiasis endemic regions has the potential to alleviate these deficits.

2.5 Schistosomiasis treatment

2.5.1 Chemotherapy

Mass drug administration (MDA) of the anthelminthic drug praziquantel (PZQ) is one of the main control measures against human schistosomiasis. PZQ is the drug of choice against all human affecting schistosome species. It leads to cessation of egg deposition, this by inducing contraction and paralysis of the schistosome worm through a calcium ion- mediated augmentation of schistosome cells´ permeability. Since the new millennium, interventions against schistosomiasis have been escalated and with the impetus of the 2001 World Health Assembly resolution, member states were urged to scale up deworming of school-aged children with praziquantel within the strategy of “preventive chemotherapy” (PCT).

Praziquantel is a well-known and established drug. Initially (in the 1970s) intended for veterinary use, this safe broad-spectrum dewormer became the treatment of choice for schistosomiasis. The drug is formally licensed and labelled for medical use for adults and children (aged 4 and above in years), at 40 or 60 mg/kg dosing for schistosomiasis, although lower dosing is advised for other trematodes. Nevertheless, PZQ is not of much commercial interest as the patent protection has expired (reducing the costs to less than US$ 0.05 per 600 mg tablet) and the affected population groups present no important purchasing power to the pharmaceutical industry. Furthermore, Praziquantel is unable to kill developing schistosomes, does not prevent re- infection and its extensive use may lead to drug resistant parasites in the future (Tebeje et al. 2016).

2.5.2 PZQ for pregnant women and young children

In 2002, the WHO identified all pregnant and lactating women as high-risk group for morbidity and mortality from untreated schistosomiasis, and therefore requiring anthelminthic treatment if infected (WHO 2002). Until then, women of reproductive age and pregnant women were excluded from schistosomiasis mass drug administration (MDA) programs, due to underestimates of prevalence, morbidity and mortality in this population (Chitsulo et al. 2004) and because safety data of praziquantel during breastfeeding and pregnancy has long been scarce (WHO 1998). Several large, well-conducted praziquantel treatment

trials have not found any significant increases in adverse events such as teratogenicity and fetal loss in the treatment groups, suggesting that praziquantel is safe for use in pregnant women (Ndibazza et al. 2010).

Nevertheless, not all countries put the 2002 WHO recommendation (of including women of reproductive age and pre-school children into MDA programs) into practice, and few doctors decide to administrate “off label”, so that preventive treatment coverage in these two population groups is still lower than among school children (WHO 2016). Unfortunately, there has not been a suitable formulation of Praziquantel available for large- scaled MDA- programs targeting babies and toddlers. It is just now that the `Paediatric Praziquantel Consortium´ (PPC) has launched a clinical trial assessing the use of a smaller, orally dispersible, PZQ tablet which contains only the biologically active L- praziquantel (and not the pharmacologically inactive D-enantiomer), has an acceptable taste and is more suitable for use in the target group of preschool-aged children (3 months to 6 years). This program aims to obtain a positive valuation from the European Medicine Agency (EMA) in 2022 for implementing the drug in endemic countries. (PPC 2020).

Despite increasing and annual mass drug administrations (in school-age children), infection rates remain high and re- infections in high prevalence areas are very likely to occur (Njenga et al 2014) which suggests the need for an expanding of the preventive chemotherapy to adults (including pregnant women) and to young children, hence closing this important ‘PZQ treatment gap’ (Stothard et al. 2013).

2.5.3 Vaccines and Vaccinations

Over 100 schistosome antigens have been identified and, with new techniques in biotechnology, much research work (mostly for *S. mansoni*) was done aiming at the production of vaccines against the invasive schistosome stages- though only 3 molecules, *S. mansoni* fatty acid binding protein (Sm14), *S. mansoni* tetraspanin (Sm-TSP-2) and *S. haematobium* glutathione S-transferase (Sh28GST) have entered human clinical trials (Tebeje 2016). However, efficacy, effectiveness and feasibility of vaccination in such a multi staged and complex disease faces much scepticism (Gryseels 2000). Combining genes or antigens

could be a promising approach, yet recent clinical trials like the one in Senegalese children with a recombinant vaccine showed no significant effects so far (Riveau et al. 2018).

2.6 Diagnostic means for schistosomiasis

2.6.1 Direct diagnosis

A direct detection can be realised via hatching tests in which swimming miracidia originating from excreted eggs are visible to the naked eye (Davies 2009). The oogram technique, a quantitative rectal biopsy with division of eggs according to development stages, is another simple diagnostic procedure and commonly used in Brazil (Da Cunha 1982).

Microscopy is the most common used procedure in the field and is currently still considered one of the most cost-effective techniques since it allows the detection of eggs of multiple different parasites with a high specificity. For intestinal schistosomiasis species such as *S. mansoni*, Kato- Katz faecal microscopy (staining a sieved sample of 20-50mg of stool) to determine the number of eggs per gram (epg) remains the standard technique. Infection intensity can be identified as light (1-99epg), moderate (100- 399epg) or heavy (≥400epg), according to the World Health Organization (WHO 2002). However, egg counts vary in time and place and with technicians (Braun-Munzinger et al. 1992).

Another parasitological technique for detecting *S. mansoni* is the saline gradient method by which faeces are subjected to a slow flux of saline solution and filtered through a nylon screen or a porous plaque. Examination of the remaining sediment and quantification of the sample is then realized on a metal plate under a bright field microscope. Concerning the detection by microscopy of *S. haematobium*, the urine filtration microscopy is the technique of choice and represents another efficient and low-cost procedure that can be performed by passing some 10ml of urine through paper filter and then using conventional light microscopy (Ephraim et al. 2014).

Microscopy remains relevant because of its affordability and specificity but is relatively laborious, depends on skilled technicians and is not overly sensitive

and even less sensitive in preschool-aged children and therefore not ideal for mass screening concepts.

2.6.2 Indirect diagnostic techniques

Serological tests have reached good sensitivity and specificity for detecting antibodies to adult worm-, schistosomular-, cercarial- or egg- antigens (Ambroise-Thomas 1976, Coudert et al. 1968, El Aswad Bel D et 2011, Sarhan et al. 2014, Smith et al. 2012, Umaly et al. 1974, Hinz et al. 2017). Numerous detection techniques may be employed, such as enzyme-linked immunosorbent assay (ELISA) or indirect immunofluorescence tests (IFAT), and the presence of antibodies can also be identified with rapid diagnostic tests (Coulibaly et al. 2013). Yet, there is a general disadvantage to all antibody detection techniques that limits their usefulness for the clinician: antibodies can persist for many years, even after parasites have been eliminated for a long time, e.g., through praziquantel treatment, so that serology does not allow differentiating between a current infection (requiring treatment) and a past infection. Moreover, levels of antibodies are generally not correlated to the intensity of infection (worm burden).

2.6.3 PCR

Other methods are DNA-based, such as real-time polymerase chain reaction (PCR)-based techniques; they are increasingly being used for the detection of *Schistosoma* spp. infections (Obeng et al. 2008). These techniques are very sensitive and allow quantitative estimates of the infection load. Using multiplex panels multiple species can be detected. PCR is a highly standardized diagnostic procedure but requires high initial investment and laboratory facilities making it -yet- unsuitable for direct field application.

2.6.4 Radiology and Ultrasonography

Diagnosing schistosomiasis related pathologies (and not the pathogen itself) is possible via radiology and can be employed in various ways; intravenous pyelography to detect bladder and ureteral transformation or obstructive uropathy, portal venography for hepatosplenic bilharziasis, abdominal radiography to identify calcification or computed tomography for

neuroschistosomiasis. Ultrasonography, as a non- invasive, relatively simple and portable diagnostic technique, has become an important alternative to many invasive methods and proves being superior to clinical examination when it comes to e.g., measuring fine hepatic pathologies (Davies 2009). Sonography is the best tool for grading schistosomal periportal fibrosis, portal hypertension, hydronephrosis, urinary bladder wall lesions and renal stones (Abdel-Wahab 1993). Sonographic lesions of periportal fibrosis in *S. mansoni* infections correlated with the numbers of eggs in the stool (Abdel-Wahab et al. 1990). Sonography allows distinguishing schistosomal hepatic fibrosis from cirrhosis and an ultrasonographic scoring system is in clinical use to predict oesophageal varices and the risk of bleeding from them (Abdel Wahab et al. 1993). Sonography, as a cost- effective and relatively easy to operate tool, might become more relevant in low-resources settings such as Madagascar and help to timely detect schistosomiasis induced morbidity and long-term consequences.

2.6.5 Antigen detection tests

In 2012, the World Health Organization proclaimed to overcome the impact of neglected tropical diseases towards 2020. The elimination of schistosomiasis became one of the global health´s priorities, along with the growing interest in developing and establishing new diagnostic tools beyond microscopy or PCR methods (Utzinger et al. 2015). In this context, the detection of schistosoma circulating antigens presents another direct diagnostic approach: Circulating anodic antigen (CAA) and circulating cathodic antigen (CCA) are two glycosaminoglycan-like carbohydrates with unique structures (Bergwerff et al. 1994) released in a regular manner by schistosomes into the host circulation with little day to day fluctuation in serum (de Jong et al. 1989). Circulating Cathodic Antigen (CCA) is one of the main antigens regurgitated by the parasites. Some schistosome eggs release CCA but the major source of this antigen are adult worms. In principle, a quantitative interpretation for tests based on the detection of these molecules is possible since CAA and CCA levels correlate with the number of worms (van Dam et al. 1996, Agnew et al. 1995). Furthermore, the two antigens are cleared within days or few weeks after successful chemother-

apeutical intervention and positive results become negative (Grenfell et al. 2013), making them suitable for treatment success assessment, surveillance and follow-up screenings. Joint research efforts have led to the development of diagnostic tools based on the detection of CCA and CAA. For detecting CCA in urine, a lateral flow assay has been successfully converted into a commercial Rapid Diagnostic Test (RDT), the “Point-of-Care Circulating Cathodic Antigen (POC-CCA) test”.

This RDT has received considerable attention and in 2015 the World Health Organization encouraged its use, especially for detecting *S. mansoni* (WHO 2015). Especially in low-endemicity areas, the POC-CCA Rapid Test has proven higher sensitivity for *S. mansoni* than the Kato-Katz- method (Siqueira et al. 2016) but unfortunately, the sensitivity of the POC-CCA test for *S. haematobium* detection is weak. More recently, the detection of CAA in different body fluids is being investigated. An up-converting phosphor technology based lateral flow (UCP-LF) test detecting CAA (of all *Schistosoma* species) in urine and serum has been developed. This UCP-LF-CAA test represents a quantitative method with exceptional sensitivity and specificity (Corstjens et al. 2014). but still requires laboratory facilities and around 1 ½ days of laboratory work to be performed. Recently, an advanced and robust version of the UCP-LF-CAA test has been developed omitting the need for a cold chain and thus facilitating storage and use in endemic regions and worldwide shipment (Corstjens et al. 2014). A point of care test for detecting CAA antigens is not yet available.

3 Methods

3.1 Study area and study population

The study took place in Madagascar, an African country in the Indian Ocean located 600 kilometres east of Mozambique. With 587.295 square kilometres it is the 4th biggest island of the world and often referred to as the “8th continent” due to its long-isolated development and autonomous evolution of flora and fauna; about 90% of its wildlife is found nowhere else in the world.

The local population of 26,3 Million inhabitants is a mix of 18 ethnic groups, with Austronesian (Southeast-Asian) and Bantu (East African) roots. Madagascar is one of the economically least developed countries (UN 2020) and holds position no. 162 in the Human Development Index ranking (HDI 2019). It is scarcely industrialized, with about 70 to 80% of the working population involved in agriculture activities, such as rice cultivation. This crop plays an important role in the Madagascan society, as rice is served for breakfast, lunch and dinner. There are well elaborated rice terraces throughout the highlands, but the monocultures are harmful to soils and ecosystems. A more environment friendly and less water consuming “System of Rice Intensification” (SRI, introduced in the early 1980s by the French Jesuit Henri de Lauranié) is still less popular than the extensive wet rice cultivation *(horaka),* which requires about 2000 Litres of water for one single kilo rice (Negro 2019). The huge amount of stagnant water provides habitats for bilharziasis transmitting freshwater snails (Davies 2009). Madagascar is a former French colony, politically independent since 1960 and today subdivided in 22 regions. The selected two study regions, Itasy with a population of 897.962 and Bongolava with 674.474 inhabitants (2018 Census Preliminary) are identified as areas of medium and high endemicity of *S. mansoni*, but recent data on the prevalence of *S. mansoni* in the Madagascan highlands are scarce. A cross sectional study in a school in a highland village revealed a PCR detected prevalence of 77% in school children (Schwarz et al. 2014), whereas for infants and pre-school children small-scaled investigations based on microscopy indicate a prevalence of around 40% (Solonirina 2017).

3.2 Distribution of schistosomiasis in Madagascar

As for the distribution of schistosomiasis in Madagascar, generally spoken, and being the traditional assumption, *S. mansoni* is frequent in the central highlands and rather in the (south-) eastern coastal parts of this big island in the Indian Ocean, whereas *S. haematobium* is more common in the lower coastal regions, mainly in the west. Nevertheless, the data visualized in the maps below show a considerable occurrence of urogenital schistosomiasis in the highlands, too, and is suggesting a significant interposition of these two species in one same region. For this mapping, 29 prevalence studies in the time frame of 1965 till 2016 were analyzed by the London Applied and Spatial Epidemiology Research Group (The Global Atlas of Helminth Infections, London School of Hygiene and Tropical Medicine).

Figure 2: Distribution of *S. haematobium*

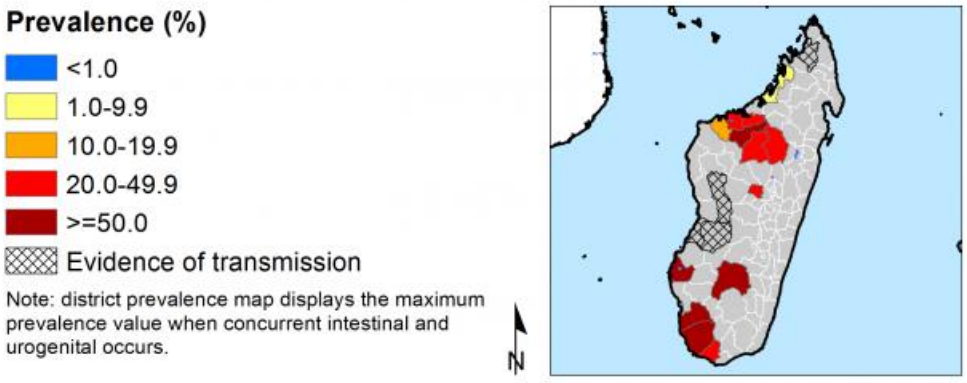
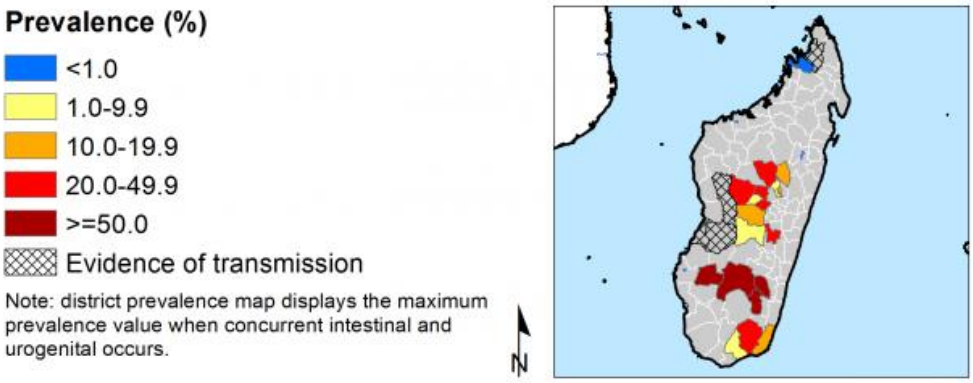


Figure 3: Distribution of *S. mansoni*



3.3 freeBILy – a study into which this sub-study was embedded

This cross-sectional study measuring the prevalence of Schistosomiasis in pregnant women in Madagascar derives from a scientific project called free-BILy: *Fast and reliable easy-to-use-diagnostics for eliminating Bilharzia in young children and mothers*; freeBILy is an international multi-centre consortium focused on improving schistosomiasis diagnostics (https://freebily.eu). In Madagascar, the freeBILy trial aims to determine the effectiveness of a (rapid) Test-Based-Schistosomiasis-Treatment (TBST) strategy for improving health of pregnant women and their infants using the POC-CCA point of care rapid test with praziquantel for treatment in case of a positive test in the intervention arm.

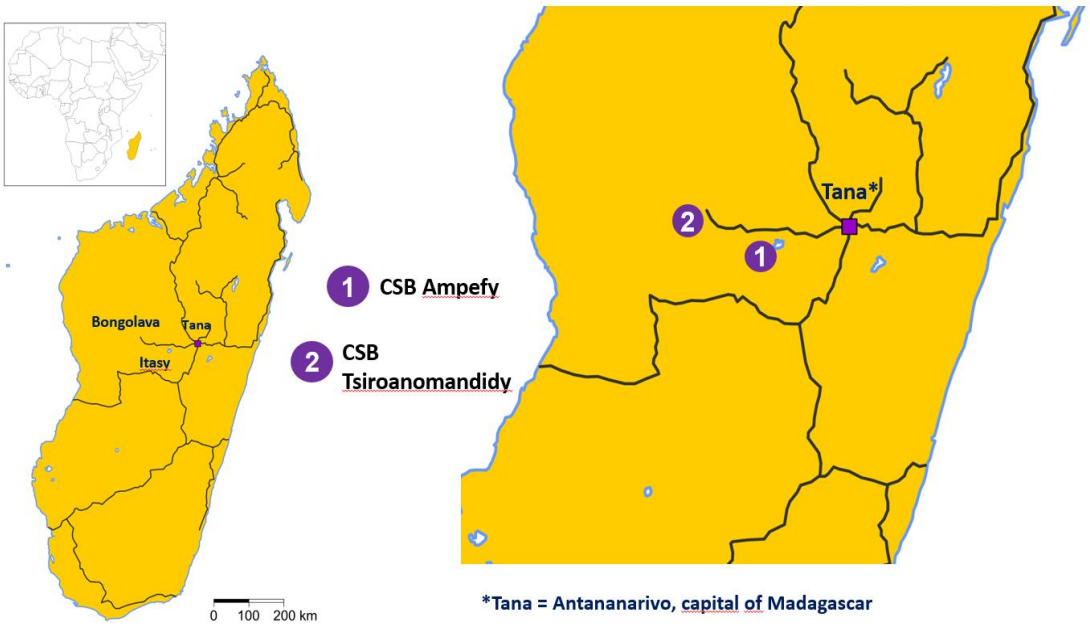
Hence this trial assesses a strategy to include pregnant women and their young children, who are largely excluded from mass drug administration (MDA), in schistosomiasis control programs.

For the freeBILy clinical trial, pregnant women between their 5th and 6th months of pregnancy were recruited in 42 health care posts (centre de santé de base, CSB) within three regions of central Madagascar; Itasy and Bongolava, coordinated and supervised by the University of Antananarivo and the Region Amoroni´Mania being supervised by the University Fianarantsoa. This two-armed cluster randomised controlled phase III trial includes 5200 pregnant women with their offspring and assesses the impact of TBST on child growth and maternal haemoglobin in areas of medium to high endemicity of *Schistosoma mansoni*. The participants are tested with the POC-CCA test. In the intervention arm, a POC-CCA-TBST procedure is offered to women during pregnancy, 9 months after delivery and for their infants at 9 months of age. In the control arm patient care follows national schistosomiasis management guidelines without the POC-CCA-TBST procedure. All participants will be offered the POC-CCA-TBST at 24 months after delivery. The trial is integrated into the routine maternal and child primary health care programmes at the different primary health care centres (Centres de Santé de Base; CSB) in the Madagascan highlands.

The sub-study for this medical doctoral thesis estimates the prevalence of schistosomiasis among pregnant women at two intervention sites, the CSB

Tsiroanomandidy in the Region of Bongolava and the CSB Ampefy in the Region of Itasy, based on Point-of-Care-Circulating Cathodic Antigen test results.

Figure 4: Study region in Madagascar (regions Bongolava and Itasy)



3.4 Sample size

Before starting this sub study, the results obtained by two previous prevalence studies *(*both for *S.mansoni)* realised in highland regions similar to this study´s sites were taken in account to estimate the required sample size. Schwarz et al. in 2014 investigated stool samples of 410 children aged 4-18 years using PCR and obtained a positivity of 77.1%. More recently, in 2017 Solonirina examined the stool of 186 children aged 1-15 years using microscopy and detected a positivity of 40.3%. If we assume the expected POC-CCA-measured-prevalence to be around 60% in both CSBs and want to measure this prevalence with a precision of +/- 10%, we need a sample size of 97 in each site with a confidence level of 95%. The equation to obtain the sample size is the following:

$$\text{Sample size} = \frac{\mathbf{Np(1-p)}}{(d^2/Z^2 1-\frac{\alpha}{2}\times(\mathbf{N-1})+\mathbf{p}\times(\mathbf{1-p})}$$

N = Population Size (1000 000)
p = hypothesized frequency
d = confidence limit (+/- 10%)
Z= Z-value (1,96)

3.5 Preparation

Before starting the recruitment for the large freeBiLy study, each study site required thorough preparation. Every CSB was visited and supervised several times, permission from the authorities were solicited and the necessary material purchased. Preparational visits began early in the morning around 5am starting in the capital of Antananarivo. The CSB Tsiroanomandidy (one of two sites, where this sub-study took place), for example, was then reached at about 9am. Along standardized check- lists of materials in place or still missing (dustbins, chairs, table, etc.) CSBs were equipped. Communication with the staff at site (nurses and doctors on site) was essential for building trust between the study team and the CSB-staff. At noon, the same procedure could be realised in the village of Ampefy (the second site of this sub study) and at its CSB. These visits were both time consuming as indispensable as it was fundamental to keep the project going and the communities informed about the upcoming recruitment.

Study staff was recruited by our partners from the University of Antananarivo, and the *Centre d´infectiologie Charles Mérieux* (CICM), located in the capital, and the University of Fianarantsoa. Nurses and mid- wives were then appointed to work in the CSB side by side with the already existing personal, which naturally required much information, constant communication and good coordination. Previously, our staff participated in various training sessions. In November 2018, a first week of theorical and practical exercises and formation with all the staff was organized. Each study nurse had to pass a minimum of training before starting to work at the CSB. That meant the successful passing of a multiple choice and open questions exam (especially designed for this course) about the study and one other test concerning good clinical practice (GCP). Furthermore, we practiced the interviewing and recruitment process, the right performance of the POC-CCA rapid test and the correct processing of data from case report form to database. During the many practical simulations and role plays, we stressed the relevance of GCP and ethics in clinical research and investigation, putting special focus on the informed consent and the importance of not pushing the women into participation, by giving them enough time to think about the explanation and information provided by the study nurse and by always stating that withdrawal is possible at any time and without sanctioning.

At the end of this training week, after successfully passing the final written exam, every participant got a GCP certificate. Subsequently, one- to- one training took place in the respective health care posts, practicing all the procedures and steps of the recruitment process. Again, it was very important to emphasize the meaning and importance of the informed consent and the ethical principles as well as to train the right performance of the techniques and tasks, such as the procedure of realizing the rapid test and the proper filling of the case report form.

3.5.1 SOP´s and training and recruitment

During the study preparation phase, a Standardised Operating Procedure (SOP) was developed for each clinical trial procedure. An SOP is a manual for a given procedure defining how to carry out the procedure in a standardised way. It is a written instruction on which every person involved in the field- and in this case at the health care posts- can easily rely on. The SOP´s related to the inclusion, the recruitment procedure and the SOP on use of the POC-CCA test in the field were especially important for this thesis.

The whole process of theorical and practical formation, together with the infrastructural (purchasing of materials and setting up working places) and bureaucratic (seeking permissions from the authorities, such as the Committee of Ethics in Hamburg and Madagascar) preparation lasted from September 2018 to March 2019. Information sessions were coordinated between our study nurses and the CSB staff to provide information about the research project to all women during their pregnancy visits. After an additional preparational workshop for our staff, the recruitment for the freeBiLy study began in April 2020. The CSBs for this sub study, Tsiroanomandidy and Ampefy, recruited participants from May 2019 till February 2020. In Ampefy, the first participant was enrolled on 21st May 2019, with the last woman recruited on 4th February 2020. The first interview in Tsiroanomandidy took place the 13th of June 2019 and the last recruitment was on 3rd February 2020.

From the start of the study at a CSB, all eligible pregnant women were asked to participate. For pregnant women under the age of 18, the informed consent was additionally signed by the mother or the father of the pregnant woman. For illit-

erate women, a fingerprint was affixed on the informed consent and an impartial witness signed. Study inclusion of pregnant women under the age of 18 was only possible if both consents were given (by parent of the underaged pregnant woman and by the pregnant woman herself). At recruitment, pregnant women were provided with all the information concerning the study and an informed consent was read and signed. Reimbursement for transportation were provided. Study participation could be withdrawn at any time and single study procedures could be rejected without any reason.

3.5.2 Inclusion criteria

Two times per week, early in the morning, the nurses and midwifes hold sessions of information, education and communication (IEC) about the disease of schistosomiasis and the character of the clinical study. Didactic material and printed information sheets about schistosomiasis and the rapid diagnostic test were previously elaborated and then presented and provided to the women gathering and attending for the perinatal care consultations at the CSB. Our personal, sometimes aided by the physician in charge, gave these talks to the group of pregnant women and subsequently, there was always a segment of question and answers, which in general showed substantial curiosity, interest and acceptance towards the topic and the study. Women interested in participating in the study were individually checked if they fulfilled the study´s recruitment criteria; for this substudy in Ampfey and Tsiroanomandidy, the inclusion and exclusion criteria of the overarching large freeBILy study were applied:

Inclusion criteria

- Pregnant woman between her 5th and 6th month of pregnancy
- Informed consent signed (also from the parents for minors)
- Expected residency in the area of the study site for the next 24 months
- Willingness to comply with the protocol requirements including sampling, treatment and follow- up visits

Exclusion Criteria

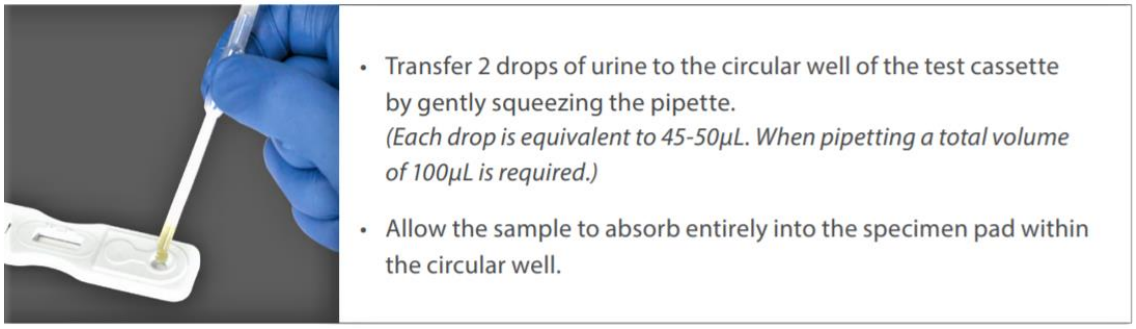
- Woman under 16 years old
- Non- pregnant woman
- Pregnant woman who has not completed her 4th month of pregnancy
- Pregnant woman beyond her 6th month of pregnancy
- Woman who does not live in the area of the respective CSB

Following the SOP for the informed consent, recruitment and inclusion, women willing to participate and fulfilling the criteria, were then asked to sign the informed consent- this only after an accurate session of at least 30 minutes of reading and explaining the content of the information sheet to the participant, always allowing questions or withdrawal at every moment. Once having signed the consent paper, the woman was invited to answer the questions of the Case Report Form (CRF). The CRF is the recruitment sheet for each participant and contains basic sociodemographic information such as age, educational level, residency and ethnicity. Of course, the test result is listed, too (see annex). Before checking the vital signs (temperature, blood pressure) and the level of haemoglobin, the woman was asked to provide some urine for the POC- CCA assay. Again, every participant was informed that withdrawing the informed consent was possible at any time and without giving reasons and that they could also refuse to undergo certain study procedures (such as giving blood or faeces within the freeBILy study).

3.6 POC-CCA Test: Display and Interpretation

The POC-CCA tests were purchased from Rapid Medical Diagnostics, South Africa. They were performed at the study sites by skilled study nurses after various sessions of training and always according to the manufacturer's instruction. The following three figures illustrate the procedure of the POC-CCA test and the interpretation of the result.

Figure 5: Procedure of the Rapid Test (Rapid Medical Diagnostics 2020)



After the application of urine to the circular well of the test cassette, the CCA-antigen possibly present in the sample binds to a monoclonal antibody immobilized on the sample membrane. This solution (urine together with the antibody-CCA-antigen-complex) subsequently runs over the strip. If the CCA- antigen is present and had bound to the monoclonal antibody, the antigen- antibody complex attaches to another monoclonal antibody immobilized at the test line, where a pink- coloured line develops indicating test-positivity. The second line is a procedural control, that should always appear ensuring the test works correctly.

Figure 6: Interpretation of the results (Rapid Medical Diagnostics 2020)

The result is to be read 20 minutes after application of urine. However, visual interpretation of the test is by its nature observer dependant, especially in low-intensity infections when the test lines are weak (Casacuberta et al. 2019). For semiquantitative assessment, in this study we used a standardised visual scor-

ing scheme of the POC- CCA cassettes, a grading system called G-scores. It consists of a series of artificial cassettes containing pink inkjet stripes of different intensities on a scale from 1 to 10 (see figure 7) This system allows to grade the POC-CCA test line by visual comparison. A significant positive correlation between the G- scores and the amount of eggs per gram of faeces has been observed (Casacubierta et al. 2019).

Figure 7: G- Scores. 10 POC-CCA cassettes consisting of artificially produced strips with different test line intensities (Casacubierta et al. 2019)

3.7 Data management and statistical analysis

The data collection sheet for each study participant (Case Report Form, CRF, see annex), with sociodemographic information and the test result, were filled by the nurses at the CSB. The CRF had to be well designed, understandable and simple and at the same time provide all the necessary information. An example of a Case Report Form is attached to this work. A copy of each filled CRF-sheet remained at the health care centre and the original paper was sent to the capital Antananarivo where data were manually entered into a REDcap data base. REDcap is an easy to handle web application, run by a non-profit organization, that supports online and offline data capture for research studies and operations. Two mechanisms were applied to avoid or at least minimize mistakes and errors of illogicality. First, the REDcap system itself detects formal and logical errors such as impossible entries of dates and impossible values for

the vital signs, for example. Also, spaces left blank in the data base form (but eventually fulfilled on the CRF´s paper) were recognized. Second, there was always a double data entry, so that the information of the first data entry was checked by the second data entry and incongruencies could easily be noticed, verified and corrected. Data entry staff was trained in workshops for these procedures. With some difficulties at the beginning, all these procedures (fulfilling the data sheets, transportation of the CRF towards the capital, data management and entry) were realized properly as our staff was gaining routine and confidence. Data extraction for this thesis was realized by the department for data management of the Bernhard Nocht Institute for Tropical Medicine (BNITM) in Hamburg, Germany, using the program Stata version 14.2 after consent from the Madagascan and the German PI had been obtained.

3.7.1 Assessing the true (adjusted) prevalence: review and calculation

Test positivity is not necessarily the same as actual infection with schistosomiasis, as the frequency of positive tests not only depends on prevalence, but also on the characteristics of the test, such as sensitivity and specificity (Rogen et. al. 1978). Therefore, we calculated an adjusted prevalence:

$$\boldsymbol{True\ Prevalence\ (TP)} = \frac{Apparent\ Prevalence\ + \text{Specifity}\ - 1}{Sensitivity\ + Specificity\ - 1}$$

Sensitivity is understood as the chance that the test will detect a positive case among those who really are infected. Specificity describes the chance of getting a negative test result when applied to someone non-infected:

$$\boldsymbol{Sensitivity} = \frac{true\ positives}{true\ positives + false\ negatives} \qquad \boldsymbol{Specificity} = \frac{true\ negatives}{true\ negatives + false\ positives}$$

Only in case of a perfect test (sensitivity = specificity = 1) the apparent prevalence would be equal to true prevalence. To obtain these characteristics, data on sensitivity and specificity from 12 comparisons of the POC- Test with a reference test were searched in literature and then analysed, for example

concerning a study in Brasil that compared the performance of the POC-CCA Test with the conventional Kato- Katz- microscopy and the saline gradient method in a low-endemicity population. Faecal and urine samples of 141 individuals were analysed (Siquiera et al. 2016). Based on these findings we calculated an adjusted prevalence as an estimate for the “true” prevalence of schistosomiasis in our study region. All of these studies examined the performance only in testing for *S.mansoni,* because the parasite antigen test for urogenital schistosomiasis is broadly stated as not accurate (Ochodo 2015).

3.7.2 Simulation of positive and negative predictive values

Depending on the true prevalence, the sensitivity and the specificity of the test and using the Bayes theorem, we designed an Excel tool that allows to simulate positive and negative predictive values and consequently the proportion of false positives among all positives and equally the proportion of false negatives among all negatives, according to the following formula for the positive predictive value;

$$\mathrm{PPV} = \frac{\mathrm{sensitivity} \times \mathrm{prevalence}}{\mathrm{sensitivity} \times \mathrm{prevalence} + (1 - \mathrm{specificity}) \times (1 - \mathrm{prevalence})}$$

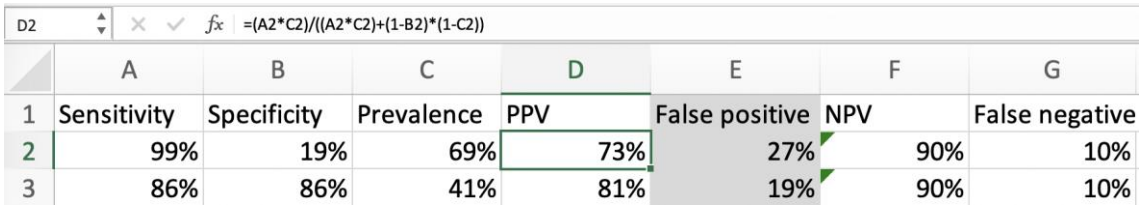
and the negative predictive value:

$$\mathrm{NPV} = \frac{\mathrm{specificity} \times (1 - \mathrm{prevalence})}{(1 - \mathrm{sensitivity}) \times \mathrm{prevalence} + \mathrm{specificity} \times (1 - \mathrm{prevalence})}$$

The Excel sheet contained 3 columns for entering sensitivity, specificity and prevalence in % figures and 4 subsequent columns with embedded formula that displayed the corresponding positive predictive value (PPV), negative predictive value (NPV) with the corresponding proportions of false positives and false negatives (Figure 8).

Figure 8: Excel tool for calculating PPV and NPV

with corresponding proportions of false negatives and false positives in dependence of sensitivity, specificity and prevalence



	A	B	C	D	E	F	G
1	Sensitivity	Specificity	Prevalence	PPV	False positive	NPV	False negative
2	99%	19%	69%	73%	27%	90%	10%
3	86%	86%	41%	81%	19%	90%	10%

With the cursor field on D2, the formula field displays the formula for calculating the PPV in the excel sheet.

Ethical Considerations

This study was realised within the test- based- schistosomiasis treatment project freeBILy and was implemented and conducted according to ICH-GCP guidelines. The study was reviewed by the National Ethics Committee of Madagascar (Comité d'Ethique de la Recherche Biomedicale, Ministère de la Santè, Madagascar: No 022 – MSANP/CER) and the Ethical Committee of the "Ärztekammer Hamburg" (Chamber of Physicians). All study information was written in the local language (Malagasy) and informed consent was sought from any volunteers or legal guardians of under 18 years old volunteers (by signature or in case of illiteracy through a thumbprint in the presence of an independent witness) before any study procedures were performed.

4 Results

In this cross-sectional sub study, a total of 294 pregnant woman were recruited and their urine samples were tested for Schistosomiasis using the POC-CCA test. Positive test results were assessed on a semiquantitative scale from 2-10 with the G- scores, estimating the intensity of the infection. Complete data were obtained for all 294 subjects. 285 were diagnosed positive with schistosomiasis based on the POC-CCA urine cassette result and 9 women were tested negative, which leads to the following equation:

$$\frac{285\ tested\ positive}{294\ participants\ in\ total} = 0.969\ test\ positivity$$

Therefore, as illustrated in figure 9, **96.9%** of the women were test positives:

Figure 9: Results of the POC-CCA-RDT- Procedure:

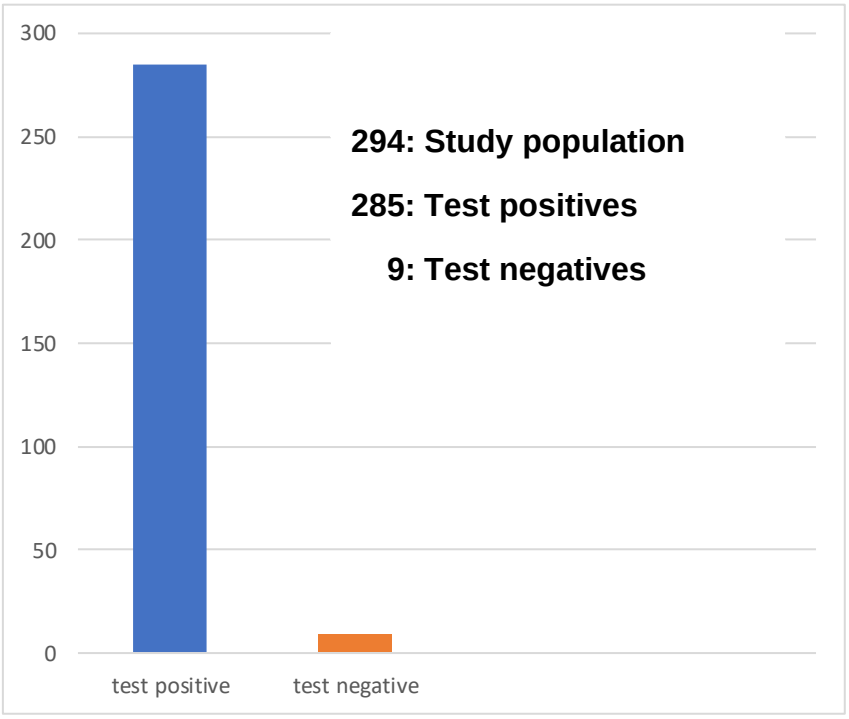


Table 3 shows that the numbers and proportions between the two health care centres are quite similar.

Table 3: Proportions between the two health care centres

Result TDR	CSB Ampefy	CSB Tsiroanomandidy	Total
Positive	116 (95,1%)	169 (98,3%)	285 (96,9%)
Negative	6 (4,9%)	3 (1,7%)	9 (3,1%)
Total	122 (100%)	172 (100%)	294 (100%)

A summary of the participants´ age and test result is shown in table 4.

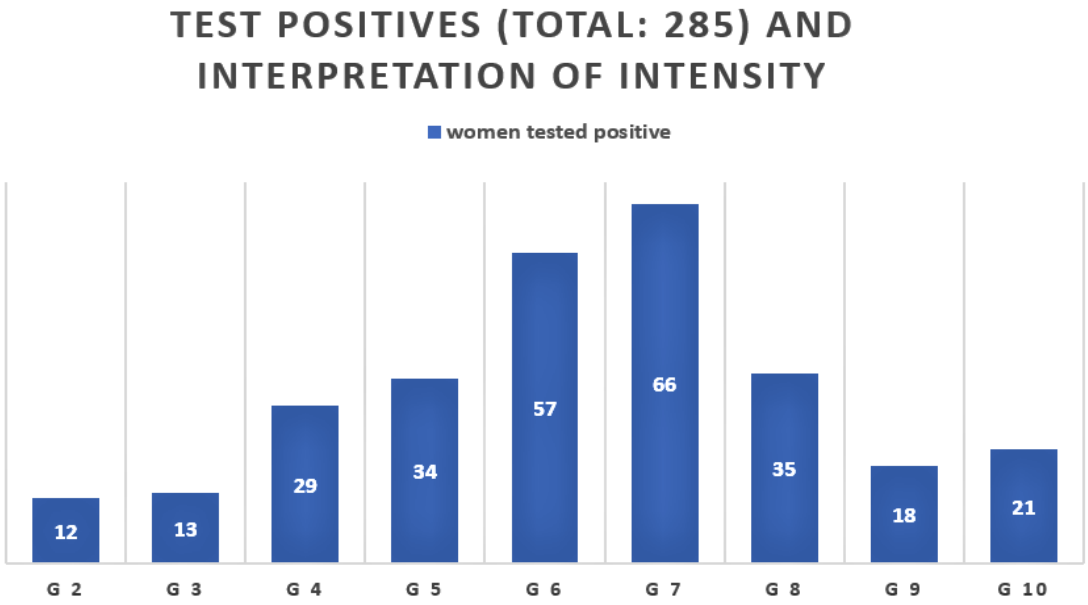
Table 4: Test results and distribution of age

age group	positive	negative	% of test positives
16-21	123	3	97.6%
22-29	110	4	96.5%
30-37	44	1	97,8%
38-43	8	1	88,9%
Total	**285**	**9**	**96,9%**

The youngest participants were of the age of 16 (5 women) and the oldest participants were 43 (2 women). The mean age was 23.9 with a standard deviation of 5.9. Figure 10 shows the distribution of G- Scores as semiquantitative measures of the parasite load (Casacubierta 2019). The intensity´s median of 6 was assessed for 20.0% (n=57) of the positive tested women, 140 women, or 49.1% of the positive tested participants, showed higher

numbers of parasite intensity according to the G- Scores. The 9 test negatives were all identified as G0.

Figure 10: Numbers of positive tested women and G- Scores (2-10)



Test results classified as G2 and G3 can be considered as only traces of Schistosomiasis infection and can be categorized as test negatives (Casacubierta et al. 2019). This applied to the results of our sub study would add 25 test negatives to the 9 G0- test negatives. Consequently, the overall prevalence assessment would be 260/294 with a test positivity of 88.4% (instead of 96.9%).

4.1 Adjusting the prevalence estimate to estimate the true prevalence

The true or adjusted prevalence considering sensitivity and specificity of the test was calculated by the formula presented in the methods part:

$$\textbf{\textit{True Prevalence}}\ (\boldsymbol{TP}) = \frac{Apparent\ Prevalence\ + \text{Specifity}\ - 1}{Sensitivity\ + Specificity\ - 1}$$

However, this formula will easily result in (impossible) values above 100% given the high test-positivity of 0.97 (in the numerator) and lower values of sensitivity (in the denominator). Nevertheless, 12 comparative studies about diagnosing schistosomiasis were found and analysed to get a reliable estimate of the test´s accuracy. These studies (listed also in the review Danso-Appiah et al. 2016) compared the performance of the antigen test with the results of microscopy testing. They were based on the single Kato-Katz procedure, which means the testing of two smears of one stool sample. The results are displayed in table 5. The 2016´ study in Brasil (Siqueira et al.) presents a particularity as the reference test consisted not only in the Kato-Katz microscopy but was combined with the saline gradient-technique.

Table 5:

Accurracy of POC-CCA testing compared with single Kato-Katz testing

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificty (95% CI)
Standley 2010	105	38	1	9	0.99 (0.95-1.00)	0.19 (0.10-0.33)
Coulibaly 2011	230	42	38	249	0.86 (0.81-0.89)	0.86 (0.81-0.89)
T. Tchuenté 2012	247	208	27	231	0.90 (0.86-0.93)	0.53 (0.48-0.57)
Erko 2013	251	158	16	195	0.94 (0.90-0.96)	0.55 (0.50-0.60)
Adriko 2014	114	119	11	176	0.91 (0.85-0.95)	0.60 (0.54-0.65)
Siquiera 2016	13	19	2	107	0.87 (0.62-0.96)	0.85 (0.78-0.90)
		Pooled values:			**0.91 (0.83-0.95)**	**0.60 (0.54-0.66)**

The values obtained by reviewing the literature exhibit in general a high sensitivity but a considerable variable specificity with rather low numbers, this appears to be true also for the triple Kato-Katz testing as displayed in table 6.

Table 6:

Accurracy of POC-CCA testing compared with triple Kato-Katz testing

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificty (95% CI)
Coulibaly 2011	138	2	16	11	0.90 (0.84-0.94)	0.85 (0.58-0.96)
T. Tchuenté 2012	136	37	19	50	0.88 (0.82-0.92)	0.57 (0.47-0.67)
Dawson 2013	37	14	7	22	0.84 (0.71-0.92)	0.61 (0.45-0.75)
Erko 2013	306	103	23	188	0.93 (0.90-0.95)	0.65 (0.59-0.70)
Koukounari 2013	148	2	17	2	0.90 (0.84-0.93)	0.50 (0.15-0.85)
Adriko 2014	155	140	21	153	0.88 (0.82-0.92)	0.52 (0.47-0.58)
		Pooled values:			**0.89 (0.82-0.93)**	**0.62 (0.45-0.75)**

The lowest value of specificity (the highest proportion of false positives) was found in the study of Standley et al. (2010), based on testing schoolchildren from shoreline communities at Lake Victoria, with the following results: 105 true positives, 31 false positives, 1 false negative and 9 true negatives. Therefore, the sensitivity was relatively high with 0.99, CI 95% (0.95- 1.00), whereas the specificity was poor with 0.19, CI 95% (0.10-0.33). If we then integrate the lowest estimated specificity (10% on the low end of the confidence interval) into the formula to obtain the true or adjusted prevalence, the result is the following calculation:

$$\textbf{\textit{True Prevalence}} = \frac{\frac{285}{294} - 0.9}{0.99 - 0.9} = \frac{0.0694}{0.09} = 0.7710 = \mathbf{77.1\%}$$

The simulation of positive and negative predictive values using our Excel tool based on the Bayes theorem and depending on the (raw) prevalence, the sensitivity and the specificity of the test (see methods) gave the following results, summarized in table 7. We took the values of sensitivity, specificity and prevalence from five studies of the 12 mentioned above, as shown in table 7.

Table 7:

Different values of positive and negative predictive values

Sensitivity	Specificity	Prevalence	PPV	False pos.	NPV	False neg.
99% **Standley 2010**	19%	93%	94%	6%	59%	41%
86% **Coulibaly 2011**	86%	49%	86%	14%	86%	14%
94% **Erko 2013**	55%	66%	80%	20%	83%	17%
90% **Koukoun. 2013**	50%	89%	94%	6%	38%	62%
88% **Adriko 2014**	52%	63%	76%	24%	72%	28%

PPV= positive predictive value, NPV= negative predictive value

5 Discussion

This prevalence study provides important information about the occurrence of Schistosomiasis in Madagascar. Positive test results for a schistosomiasis infection were found in 285 women which means 96.9% of all 294 participants. Taking into account the lowest assumed specificity (10%) based on the findings in previous comparative studies, the calculated true or adjusted prevalence would be still 77.1%. That coincides with data previously realised in the highlands of Madagascar, in a region with similar geographical, demographic and socioeconomic characteristics as Ampefy and Tsiroanomandidy (Schwarz et. al 2014): In the village of Andina, the stool of 400 children were examined with the very sensitive PCR- method and test-positivity for infections with *S. mansoni* was equal to the corrected prevalence for schistosomiasis among pregnant women, provided by the POC-CCA test of this study.

The role and importance of diagnostics in schistosomiasis control may seem debatable, particularly in highly infested areas where preventive mass drug administration appears to be the primary control measure. Furthermore, with occurrence of schistosomiasis depending on public hygiene, access to safe water and sanitary infrastructure, one could argue that hygiene-oriented policies and poverty reduction are more important than medical and pharmaceutical measures. Despite these arguments, MDA and targeted-diagnose based treatment appear to be important concepts, especially in low-endemicity regions. Most importantly, to finally eliminate schistosomiasis, pregnant women and pre-schooled children must get access to antihelminth treatment and this requires diagnostic means as they are usually excluded from MDA.

5.1 The rapid test: accuracy, advantages and debilities

As praziquantel is safe, efficacious and not expensive, diagnosis before drug intervention was long considered unnecessary and not cost-effective. There was little interest in research and development of new diagnostic tools beyond the cumbersome and time-consuming microscopy or laboratory requiring PCR

methods. In 2012 the fight against schistosomiasis was reinforced since the World Health Organization proclaimed in it´s 65th Assembly to overcome the impact of neglected tropical diseases towards 2020. The elimination of schistosomiasis has become one of the global health´s priorities. In this context, the need of accurate diagnostic assays became more urgent and new tools were required for individual patient management and for the detection in low- transmission settings. (Utzinger et al. 2015).

Diagnosis becomes more important when endemicity is low as (mass) treatment is more difficult to justify. Targeted treatment of infected persons, based on a rapid antigen test (such as the POC-CCA test), appears to be an alternative to preventive mass drug administration, from which pregnant women and preschool children were and still are excluded. The test-based treatment strategy emphasizes the importance of diagnostics for groups that are not unnecessarily exposed to a drug (pregnant women, small children) but for whom the infection with schistosomiasis presents a treatment requiring condition.

Point-of-care tests have some obvious advantages: they allow obtaining real-time results within minutes, gaining time for providing effective and quality patient care. Such rapid-POC tests are especially relevant in countries of low financial resources and with weak diagnostic laboratory capacities. The urine-POC-CCA test (the rapid test of this study) fulfils these attributes: It is a fast and user- friendly methodology and the procedure requires only a short time. The test is stable at high temperature and works on urine samples which are easier to obtain than faeces (for microscopy). The ability to discriminate people with infection from people without, is – before safety, convenience and cost – the primary criterion that determines the test´s performance and its use in the field. Sensitivity and specificity are the most useful characteristics that define a diagnostic test´s quality (Rogan et al. 1978). Sensitivity is the proportion of positive tests among the samples with condition and specificity is the proportion of negative tests among the samples without the condition. Unfortunately, there is no clear and accurate “gold standard” to reflect the true status of infection with schistosomiasis, but microscopy is the most widely used technique for diagnosing schistosomiasis and the Kato- Katz- method is employed commonly as the reference for detecting intestinal bilharziasis. According to the manufacturer’s

instruction leaflet (Rapid Medical Diagnostics 2020), the POC-CCA-test- sensitivity for *S. mansoni* is 100% in intensities higher than 400 eggs per gram of faeces, however sensitivity can decrease to 70% in individuals with a low burden of infection. It is important to note that no diagnostic test provides perfect results, as there is always a discrepancy between test results and the (unknown) truth.

With regards to a test-based treatment strategy, false positive tests mean that patients are wrongly identified as having the condition and therefore are unnecessarily treated. This study´s findings present a high positive rate (96.9%) suggesting a considerable amount of false positive results. The proportion of false positives would thus quantify to what extent overtreatment took place with women falsely tested positive receiving unnecessary drug treatment. False negative test results on the other hand, lead to no treatment where intervention is indicated. As shown previously in the presentation of the results, simulations of the false positives lead to percentages from 6% up to 24% (whereas the rate of false negatives was between 14% and 72%). With praziquantel being considered as a relatively safe drug that is used for MDA, even high proportions of overtreatment seem justifiable also with regards to the claim, that pregnant women and small children should be integrated into MDA schemes in order to overcome these treatment gaps and finally eliminate schistosomiasis (Stothard et al. 2013).

5.1.1 Detection of *S. haematobium* and disease mapping

Many investigations confirmed the weakness of the test concerning the reliable detection of *S. haematobium*. CCA concentrations are highest in *S. mansoni* infections, whereas the POC-CCA-test shows poor sensitivity and specificity for *S. haematobium* (Ochado et al. 2015). The test is therefore practically limited just to the diagnosis of intestinal schistosomiasis. In the central plateau of Madagascar, *S. mansoni* is the predominant schistosomiasis species, but *S. haematobium* seems to be present, too. At least there are evidence for the occurrence of urinary schistosomiasis in the western and southern hinterland, not only in the western coastal region (Ravaoalimalala et al. 1995). The POC-CCA test is unfortunately not useful to confirm the assumption that urogenital schis-

tosomiasis increasingly occurs in the highlands of the country. Because of the rapid test´s weak ability in detecting *S. haematobium*, POC-CCA- test based mapping would be only reliable for mapping the occurrence of *S.mansoni*.

5.1.2 Cross reactivities and false positives/ negatives

In addition to the test´s weak ability to detect S. haematobium, there are concerns about high rates of false positive results in association with urinary tract infections and haematuria. POC-CCA positive test results were significantly associated with increased leukocytes and haematuria (Homsana et al. 2020). There is also suspicion about a higher proportion of false positives in pregnancy, though data is still scarce. Greter et al. (2016) assessed the performance of the POC-CCA urine cassette in 193 individuals in Chad. Among these adults, 3 women were tested positive by the POC-CCA test and were identified as pregnant. Their microscopy results for schistosomiasis were all negative which raises concerns about a cross-reaction in pregnancy, although further investigation is needed.

5.1.3 Screening, targeted treatment and drug monitoring

Despite these disadvantages and debilities, the POC-CCA test can be a tool for individual diagnostics in *S. mansoni* infested areas, where it can be used in rural CSBs with only basic equipment. The test provides a quicker answer and is easier to use than traditional diagnostic methods (Ochado 2015). Its simplicity of use also makes it a tool for large scale *S. mansoni* control programmes as it allows rapid population screening over large areas. Especially in remote areas it can be used as a primary screening method, as well as a method for a targeted treatment strategy. The POC-CCA test is also suitable for drug monitoring as the test seems to be able to differentiate between past and active infection. Circulating antigens are most likely only present in active infections, so that the POC-CCA test is potentially useful to assess outcome and success of praziquantel treatments.

5.2 Test-based treatment: safety concerns

As test-based treatment strategies aim to amplify treatment with praziquantel to

a wider range of the population, safety concerns that are specific to pregnant women and small children must be considered. The WHO now recommends treating schistosomiasis infected pregnant and lactating women with praziquantel. On the one hand, policy makers recognised that schistosomiasis during pregnancy reaches 60% in some endemic areas and that at-risk women spend much of their life pregnant and lactating, leaving little opportunity to treat women outside these periods (Freer et al. 2017). On the other hand, there is now more safety data for praziquantel during pregnancy and breastfeeding. Several large and well-conducted PZQ treatment trials have not found any significant increases in adverse events such as teratogenicity or perinatal mortality, as shown for example in a randomized double-blind study with over 2500 women pregnant women in Uganda (Ndibazza et al. 2010). Efficacy and safety for praziquantel in small (pre-school) children were equally shown in several trials (Coulibaly et al. 2013a) and the Paediatric Praziquantel Consortium is planning to offer access to a new formulation for small children in endemic African countries in 2022. It is a much smaller tablet with less bitter taste and orally dispersible. This new paediatric formulation (levo-praziquantel 150mg, tested in a full phase I-III clinical development program) showed a good safety profile and will help to fill gaps towards the elimination of schistosomiasis. It is important to underline that the burden of schistosomiasis in adolescents is due to accumulation of repeated infections in early life (King et al. 2008). Furthermore, infection in pregnancy could have effects on the development of the foetus and infant; low birthweight is the adverse birth outcome most associated with maternal schistosomiasis (Freer et al. 2017). Therefore, with safety proven after decades of clinical experience and now supported by trials and studies, these two at-risk groups should not longer be excluded from treatment with praziquantel.

Generally, patient tolerance to praziquantel is good and only minor adverse events such as discomfort, nausea, vomiting, or abdominal pain are reported after administration. Severe allergic reactions are rare. Nevertheless, the treatment of schistosomiasis with PZQ in the presence of another parasitic disease -neurocysticercosis- can rarely cause neurological complications such as convulsions. Cysticercosis is the infection with the larval form of the pork

tapeworm *Taenia solium*, which humans can get infected with when eating insufficiently boiled infected pork meat, frequently in poor hygienic conditions. Within 3 to 4 months the brain can be affected by cysts of 6 to 15mm. Neurocysticercosis is the major cause of later onset epilepsy in adults (Burchard et al. 2011). Neurocysticercosis is generally treated with 1 of 2 drugs, praziquantel or albendazole, but, before cure, administration of praziquantel can initially cause unexpected seizures (Torres et al. 1988). The POC-rapid test is used only for schistosomiasis and does not detect cysticercosis. An improvement of pre-treatment diagnostics of neurocysticercosis in schistosomiasis patients seems necessary, though expensive tools like computer tomography are of course only available in few places in Madagascar and barely affordable as a standard tool. However, it is advisable to increase awareness about this topic given the usual practice of mass drug administration and because new groups of population (pregnant women and young children) are about to receive medication on a regular base against schistosomiasis, too. The POC-CCA tool as part of a test-based treatment program, may be integrated into the routinely health care system in Madagascar, could minimize the risk of severe adverse events by avoiding unnecessary mass drug administration. In any case, not only the rapid test results but every single outcome in medicine should be assessed by using an integral approach. Carefully reviewing the clinical history as well as other important clinical findings before treatment is always important.

5.3 Prevalence assessment

The data obtained by this study provides information about the occurrence of schistosomiasis in pregnant women in two places in the highlands of Madagascar. The frequency of schistosomiasis occurrence may vary from place to place depending on the presence of snail habitats and their contamination with human faeces and urine. Because of water resource development projects or intensified MDA-programs, for example, descriptions of the current status quo and the prevalence situation could soon be invalid (Davies 2009). However, the occurrence of schistosomiasis seems to remain stable in the highlands of Madagascar. Almost 30 prevalence studies within the period of over 50 years

were analysed and they supply the data for a rather consistent and little changing prevalence map.

Most data on schistosomiasis prevalence is based in Kato-Katz or simple sedimentation microscopy. The Kato-Katz concentration is affordable, but still the reagents must be purchased, and laboratory skills are needed. In the prevalence study in the country´s highlands with 410 school children (Schwarz et al. 2014) simple sedimentation microscopy missed about 4/5 of all PCR-confirmed cases. Nevertheless, with all those limitations in mind, estimations of prevalence, even with point-of-care tools, are important to get an overview of the disease´s current impact and they can show where measures of disease control are most needed.

5.4 Schistosoma control strategies

The data of this study is based on the POC-CCA rapid test which is a relatively new tool, and which may improve the control of schistosomiasis in the near future. In the past, transmission control via chemical molluscicides (mainly with niclosamide) has been prioritized, though this was expensive and did not achieve total elimination of snails and their eggs (Davies 2009). Nowadays, the focus is rather on morbidity control, primarily through large-scale drug administration, with praziquantel being relatively cheap and considered safe. Yet, chemotherapy alone has proven to be insufficient, as schistosomiasis has socioeconomic, biological, human behavioural and clinical dimensions, too; the elimination of this disease will not be achieved without greater political and economic efforts and further interventions in health care and sanitary infrastructure. The reduction of intermediate hosts numbers must be continued and intensified (via molluscicides, predator fishes and habitat arrangements), whereas improvements in sanitary systems and access to safe water are essential. A meta-analysis of 44 observational studies (with 90 datasets) found both safe water supplies and adequate sanitation important measures to reduce the odds of infection (Grimes et al. 2014). Safe water resources are identified as closed and clean, such as piped and drinking water. Adequate sanitations forms are latrines, flush toilets and septic tanks. In this context, the WASH (Water, Sanitation and Hygiene) approach is an important concept. Each one of these

three areas is a separated and complex core issue but also depending inalienably on the other two fields of work: Without toilets, water sources become contaminated, whereas the lack of clean water makes basic hygiene measures impossible (UNICEF 2016). Changes in behavioural customs and water contact patterns within the communities are also needed, but they are rather difficult to achieve in the short run. Doing the laundry by the river is often a deep-seated habit (and necessity), and children naturally tend to spend much time playing and swimming in natural waters. In terms of prevention, in Madagascar, nurses, midwifes or doctors regularly realize sessions of information, education and communication (IEC) in the communities and at the health care centres (CSB), this commonly within the scope of perinatal care days with many women and family members attending.

5.5 The POC-CCA test and schistosomiasis control strategies

In sum the need for improvements in diagnostics for schistosomiasis – or even the importance of diagnosing at all- may appear to be at least questionable, especially in high endemicity settings such as the two sites that are the objects of this study. MDA programmes, measures of prevention, improvements in sanitary infrastructure, access to safe water and education might all be of greater help than rapid tests in every remote corner of the country, particularly in regions of very high prevalence, where chances are very high to treat an infected person by applying preventive drug administration.

The remarkably high test-positivity (96.9%) of this study and the calculated values that indicate the “true prevalence” suggest a considerably wide-spread occurrence of the parasite and the data in general evoke the need of increased efforts in disease control. In these settings of high prevalence, preventive chemotherapy seems to be adequate and instrumental – but serious challenges remain. In order to estimate the prevalence of schistosomiasis in a defined geographical area and guide targeted intervention campaigns, accurate diagnostic techniques that serve as mapping tools are needed. The POC-CCA test as sensitive method to detect *S. mansoni*, may be such mapping tool and become an important additional element within the polyvalent fight against schistosomiasis. The POC-CCA test based treatment becomes even more relevant for pregnant

women and small children, as we know that these population groups are still often excluded from MDA interventions. All in all, the POC-CCA test can help in the battle against all the heavy impacts this disease has on the population, as well as on the public health sector and the country´s development in general. Particularly in low-endemicity areas the rapid antigen detection and the test-based treatment can offer an alternative to the large-scale drug administration. The goal is to move from schistosomiasis control towards elimination through the interruption of transmission. The challenge is to expand administration of praziquantel to currently overlooked groups (such as pregnant women) and to apply selective treatment (instead of MDA) wherever it seems appropriate. This strategy requires affordable and accurate diagnostic and drug monitoring tools. The POC-CCA test, with all its limitations, may be able to meet these requirements and be convenient for an individual diagnosis. The POC-CCA test is easier to handle and provides a quicker answer than the Kato-Katz microscopy, which is the current reference standard for intestinal schistosomiasis. The test is recommended as a mapping tool for *S. mansoni* prevalence and data from various comparative studies suggests that a single POC-CCA cassette is more sensitive than stool microscopy using Kato-Katz thick smear for *S. mansoni* diagnosis (Utzinger et al. 2015). The drawback is certainly the POC-CCA test´s weakness in the detection of *S. haematobium*. The detection via urine filtration is more sensitive, by trapping *S. haematobium* eggs on polycarbonate filters – although several filtrations over consecutive days are needed to detect light infections accurately (Utzinger et al. 2015). Nevertheless, the POC-CCA test could help improving the (targeted) treatment of affected pregnant women and young children. Both groups are until now practically excluded from antihelminth medication while equally suffering from physical and neurocognitive morbidity due to this severe, far-reaching but still broadly neglected disease which is schistosomiasis. The WHO guidelines established in 2002 explicitly recommend the administration also to pregnant and lactating women. In 2017, after even more evidence derived from various randomized trials concerning the safety of praziquantel, the WHO recommended the inclusion of pregnant and breastfeeding women in all PZQ-based mass drug administrations, too (Friedman et al. 2018). To finally reach the goal of schistosomiasis elimination, pregnant women

and young children must be included in the treatment programmes and easy to handle diagnostic tools, which are accurate and reliable, are vital.

This study shows a test positivity of 96.9% for schistosomiasis in 294 pregnant women. Although this result remains imprecise because of the imperfect sensitivity and specificity, we can confidently claim that the prevalence of schistosomiasis infections in Ampefy and Tsiroanomandidy is very high and we must state that increasing efforts to battle this occurrence are urgently required.

6 Summary

Schistosomiasis, as a neglected tropical disease, is present in large parts of Sub-Saharan Africa and Madagascar and causally linked to insufficient sanitary infrastructure. With over 220 million affected people worldwide, this waterborne disease presents a serious public health problem. *Schistosoma mansoni* is responsible for intestinal schistosomiasis and *Schistosoma haematobium* causes urogenital schistosomiasis. Aquatic snails serve as intermediate hosts releasing cercariae that enter the human body through the skin. They penetrate the intestinal or bladder wall and become egg laying worms. Eggs are either passed out via the faeces or the urine to continue the life cycle or remain stuck in the human tissue. This pathogenetic egg deposition in the definitive host provokes granuloma formation with subsequent complications such as periportal fibrosis or obstructive uropathy. Mass drug administration (MDA) with praziquantel is one of the cardinal measures to control schistosomiasis but preventive chemotherapy is until now almost exclusively focused on school-aged children, despite declarations of the WHO to include pregnant women (after 4th month of pregnancy) and young children in MDA- programs, too. As an addition to microscopy and indirect serology, direct diagnostic tools detecting antigens have been established, such as the urine-based point- of- care rapid diagnostic test (POC-RDT) which reacts to the circular cathode- associated antigen (CCA) released by adult schistosome worms. The POC- test can detect both *S. haematobium* and *S. mansoni* for which it seems to be more specific. This study derives from the large scaled and long-term test-based-schistosomiasis treatment project named freeBILy and assesses the occurrence of Schistosomiasis by using the POC-CCA rapid test in 294 pregnant women recruited in 2 health care centres in the central highlands of Madagascar. A test-positivity of 96.9% was found. When calculating the corrected prevalence by taking in account various sensitivity and specificity assumptions, the estimates of prevalence were at least 77.1%, suggesting the need for enhancing schistosomiasis control in pregnant women in the highlands of Madagascar.

6.1 Zusammenfassung

Schistosomiasis, als vernachlässigte Tropenerkrankung, betrifft große Teile von Sub-Sahara-Afrika sowie Madagaskar, steht in direktem Zusammenhang mit unzureichender sanitärer Infrastruktur und ist mit über 220 Millionen betroffenen Menschen weltweit ein ernstes Problem für das Gesundheitswesen. *Schistosoma mansoni* ist verantwortlich für die intestinale Bilharziose, *Schistosoma haematobium* verursacht die urogenitale Bilharziose. Frischwasserschnecken dienen als Zwischenwirte und entlassen Cercarien, die durch die menschliche Haut und in die Venengeflechte des Intestinums oder der Harnblase dringen und zu Eier produzierenden Würmern ausreifen. Die Eier werden über Urin und Stuhl ausgeschieden (und setzen den Kreislauf fort), oder verbleiben im menschlichen Gewebe, wo sie eine Granulombildung provozieren, mit Komplikationen wie der periportalen Fibrose und der obstruktiven Uropathie. Die Massenbehandlung mit Praziquantel ist eine der Hauptmaßnahmen gegen Schistosomiasis, doch diese präventive Chemotherapie ist bisher auf die Gruppe der Schulkinder beschränkt - trotz WHO-Empfehlung, auch Vorschulkinder und Schwangere im letzten Trimenon zu berücksichtigen. Die Diagnostik durch Mikroskopie und Antikörperbestimmung wurde um den Nachweis von Antigenen erweitert, so zum Beispiel mittels eines urinbasierten Schnelltestes für das von den Schistosomen sekretierte *circulated cathode-associated antigene* (CCA). Dieser point-of-care-test erkennt beide Arten, scheint aber spezifischer für *S. mansoni* zu sein. Diese Studie leitet sich von einer großen randomisierten testbasierten Interventions-Kontrollstudie namens freeBILy ab und beurteilt mit Hilfe des POC-CCA-Testes die Verbreitung von Schistosomiasis bei 294 schwangeren Frauen in zwei Gesundheitszentren im Hochland von Madagaskar. 285 Frauen wurden positiv auf die Erkrankung getestet, was eine Testpositivität von 96.9% ergibt. Verschiedene Schätzungen von Sensitivität und Spezifität des Testes zur Berechnung der „wahren Prävalenz“ resultieren in ähnlich hohen Zahlen (nicht unter 77.1%), sodass von einer beträchtlichen Verbreitung der Bilharziose in der Gruppe der schwangeren Frauen in der Region auszugehen ist. Die Untersuchungsergebnisse legen somit die Notwendigkeit einer Intensivierung von Kontroll- und Präventionsmaßnahmen, auch für schwangere Frauen, nahe.

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2002	Abitur
2002- 2004	Social Service in Nicaragua (International Cooperation)
2004- 2006	Studies of Political Science
2006- 2014	Medical School Hamburg Eppendorf
2014- 2015	Practical year in Hamburg (oncology, paediatrics) and Lisbon (surgery)
2016- 2018	Residency in Psychiatry (Lüneburg)
2018- 2019	Field study in Madagascar
2020	Residency in Neurology (Buchholz)

Eidesstattliche Versicherung

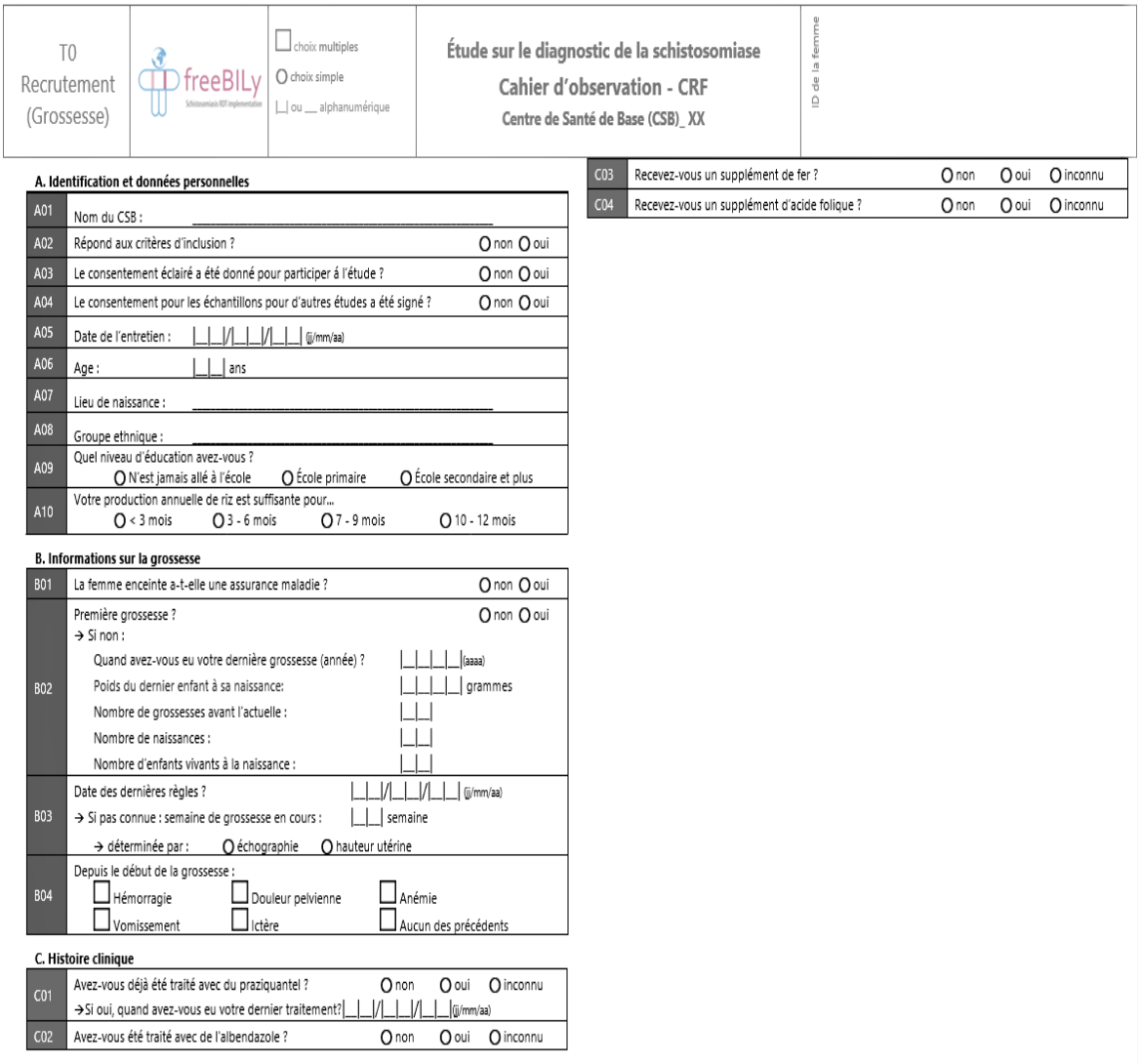
Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), 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.

Hamburg, 25.01.2021.....

Annex

Case Report Form (CRF, French version, as used in the field) for each participant of the study

1st Page



T0 Recrutement (Grossesse)	freeBILy	☐ choix multiples ○ choix simple __ ou ___ alphanumérique	**Étude sur le diagnostic de la schistosomiase** **Cahier d'observation - CRF** **Centre de Santé de Base (CSB)_ XX**	ID de la femme

A. Identification et données personnelles

A01	Nom du CSB : _____
A02	Répond aux critères d'inclusion ? ○ non ○ oui
A03	Le consentement éclairé a été donné pour participer á l'étude ? ○ non ○ oui
A04	Le consentement pour les échantillons pour d'autres études a été signé ? ○ non ○ oui
A05	Date de l'entretien : __/__/____ (jj/mm/aa)
A06	Age : __ ans
A07	Lieu de naissance : _____
A08	Groupe ethnique : _____
A09	Quel niveau d'éducation avez-vous ? ○ N'est jamais allé à l'école ○ École primaire ○ École secondaire et plus
A10	Votre production annuelle de riz est suffisante pour... ○ < 3 mois ○ 3 - 6 mois ○ 7 - 9 mois ○ 10 - 12 mois

B. Informations sur la grossesse

B01	La femme enceinte a-t-elle une assurance maladie ? ○ non ○ oui
B02	Première grossesse ? ○ non ○ oui → Si non : Quand avez-vous eu votre dernière grossesse (année) ? ____ (aaaa) Poids du dernier enfant à sa naissance: ____ grammes Nombre de grossesses avant l'actuelle : __ Nombre de naissances : __ Nombre d'enfants vivants à la naissance : __
B03	Date des dernières règles ? __/__/____ (jj/mm/aa) → Si pas connue : semaine de grossesse en cours : __ semaine → déterminée par : ○ échographie ○ hauteur utérine
B04	Depuis le début de la grossesse : ☐ Hémorragie ☐ Douleur pelvienne ☐ Anémie ☐ Vomissement ☐ Ictère ☐ Aucun des précédents

C. Histoire clinique

C01	Avez-vous déjà été traité avec du praziquantel ? ○ non ○ oui ○ inconnu →Si oui, quand avez-vous eu votre dernier traitement? __/__/____ (jj/mm/aa)
C02	Avez-vous été traité avec de l'albendazole ? ○ non ○ oui ○ inconnu
C03	Recevez-vous un supplément de fer ? ○ non ○ oui ○ inconnu
C04	Recevez-vous un supplément d'acide folique ? ○ non ○ oui ○ inconnu

Case Report Form

2nd page

T0 Recrutement (Grossesse)	freeBILy	☐ choix multiples O choix simple _| ou __ alphanumérique	**Étude sur le diagnostic de la schistosomiase** **Cahier d'observation - CRF** **Centre de Santé de Base (CSB)_ XX**	ID de la femme

D. Diagnostics

	Condition	Test
D01	Tous les centres	Tension artérielle mesurée ? O non O oui → Si oui : Tension artérielle : __|__|__|/|__|__|__| mmHg
D02	Tous les centres	« HemoCue » Hémoglobine mesuré ? O non O oui → Si oui, taux d'hémoglobine : __|__|.|__| g/dl
D03	Tous les centres	Échantillon d'urine reçu ? O non O oui → Si oui : Date de l'échantillonnage : __|__|/|__|__|/|__|__|(j/mm/aa) Bandelette urinaire faite avec du sang dans l'urine ? O non O oui → Si oui : O - O ± O + O ++ O +++ O 5-10 Ery/µL O 50 Ery/µL
D04	Tous intervention centres	TDR schistosoma fait ? O n/a O non O oui → Si oui, résultat : O négatif O positif → Si positif, grade : __|__| (1 to 10)
D05	Tous les centres	Du praziquantel a été donné ? O non O oui
D06	Uniquement centres de formation	Échantillon de selles reçu ? O n/a O non O oui → Si oui : Date de l'échantillonnage : __|__|/|__|__|/|__|__|(j/mm/aa)
D07	Uniquement centres de formation	Prise de sang ? O n/a O non O oui → Si oui : Date de l'échantillonnage : __|__|/|__|__|/|__|__|(j/mm/aa)

E. Informations sur la prochaine visite

E01	Date de la visite T1 :	__|__|/|__|__|/|__|__|(j/mm/aa)

F. Remarques

_____

_____

G. Responsabilités

	Rôle dans l'étude	Initiales	Signature	Date de signature (j/mm/aa)
G01	Infirmière d'étude	__|__|__|	_____	__|__|/|__|__|/|__|__|
G02	ARC	__|__|__|	_____	__|__|/|__|__|/|__|__|
G03	Saisie des données : 1ère entrée	__|__|__|	_____	__|__|/|__|__|/|__|__|
G04	Saisie des données : 2ème entrée	__|__|__|	_____	__|__|/|__|__|/|__|__|
G05	Responsable qualité	__|__|__|	_____	__|__|/|__|__|/|__|__|