THE UNIVERSITY OF YAOUNDE I

FACULTY OF SCIENCES

CENTRE FOR RESEARCH AND TRAINING IN GRADUATE STUDIES IN LIFE, HEALTH AND ENVIRONMENTAL SCIENCES

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Effect Of Repeated Praziquantel Treatment on School-Aged Children In Cameroon Susceptibility To *Schistosoma mansoni* Infection, Immunopathology And Cognitive Impairment

Dissertation submitted in partial fulfilment of the requirements for the award of master's degree in Biochemistry

Option: Biotechnology and Development

By:

SOH BAYECK Etienne Borel

Registration N^{O} 18I2072

Bachelor's degree in Biochemistry

Supervised by:

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DEDICATION

I dedicate this document to my mother, Mrs. Wogang Clementine Landry.

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LIST OF ABBREVIATIONS

AOR:	Adjusted Odds Ratio
BMI:	Body Mass Index
CI:	Confidence Interval
CNPCSSTH:	Cameroon National Program for Control of Schistosomiasis and Soil Transmitted Helminthiasis
ELISA:	Enzyme-Linked Immunosorbent Assay
EPG:	Eggs Per Gram of feces
IFN-γ:	Interferon-gamma
Ig:	Immunoglobulin
IL:	Interleukin
GM-CSF:	Granulocytes Macrophage-Colony Stimulating Factor
KK:	Kato-Katz
LAMP:	Loop-Mediated Isothermal Amplification
MDA:	Mass Drug Administration
M1:	Type 1 Macrophage
M2:	Type 2 Macrophage
OR:	Odds Ratio
PZQ :	Praziquantel
RPA:	Recombinase Polymerase Amplification
S. guinensis:	*Schistosoma guinensis*
S. haematobium:	*Schistosoma haematobium*
S. intercalatum:	*Schistosoma intercalatum*
S. japonicum:	*Schistosoma japonicum*
S. mansoni:	*Schistosoma mansoni*
S. mekongi:	*Schistosoma mekongi*
SEA:	Soluble Egg Antigen
STH:	Soil-Transmitted Helminthiasis
SWA:	Soluble Worm Antigen
TGF-β:	Transforming Growth Factor-beta
Th1:	T helper-1 immune response
Th2:	T helper-2 immune response
TNF-α:	Tumor Necrosis Factor-alpha
Treg:	Regulatory T cells

US:	Ultrasonography
VEGF:	Vascular Endothelium Growth Factor
WHO:	World Health Organization

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SUMMARY

Schistosomiasis, caused by schistosome flatworms, is a significant public health concern in endemic regions, leading to anemia, liver fibrosis, cognitive impairment, and immune modulation. This study evaluates the impact of repeated PZQ treatments on infection dynamics, cognitive outcomes, and immunological responses in SAC from endemic areas in Cameroon. A retrospective study was conducted on SAC from five public schools in *S. mansoni*-endemic villages in Bokito, Cameroon, after ethical clearance renewal (No. 2022/12/1505/CE/NECRHH/SP). Previously collected samples and associated data from children grouped by cumulative received PZQ treatments (0–2 vs. >2 rounds) were analyzed for infection rates, parasite burden, hemoglobin levels, liver fibrosis, cognitive performance, and immunological markers (IgE, IgG4, cytokines) using statistical methods, including Student's t-test, ANOVA, Kruskal-Wallis test, Spearman's Chi-squared test, and multivariate logistic regression. Repeated PZQ treatments significantly reduced heavy infection rates ($p = 0.01$) and parasite burdens ($r = -0.33$, $p = 0.04$) but had limited impact on overall infection prevalence ($p = 0.58$). While repeated treatments did not significantly reduce fibrosis progression ($p = 0.31$), they tended to elevate VEGF levels in participants receiving >2 treatments ($p = 0.04$). Children in the >2 treatments group demonstrated improved hemoglobin levels ($p = 0.004$) and better cognitive performance, reflected in higher academic marks ($p = 0.02$) and class/age progression ratios ($p = 0.01$). Immunologically, repeated treatments enhanced Th2-dominated responses, marked by cytokines such as IL-4 ($p = 0.04$) and IL-33 ($p = 0.03$) and increased levels of IgE ($p = 0.002$), IgG4 ($p = 0.02$), suggesting a balanced immune response with both protective and regulatory roles. Repeated PZQ treatments effectively reduce parasitic burden, alleviate anemia, enhance cognitive performance, and foster a regulated protective immune response in SAC. Despite these benefits, the persistence of liver fibrosis highlights the need for integrative approaches, including antifibrotic therapies and vaccines.

Key words: *Schistosoma mansoni*, Praziquantel, School-age children, Immunopathology, Cognitive impairment.

RÉSUMÉ

La schistosomiase, causée par des vers plats schistosomes, est un problème majeur de santé publique dans les régions endémiques. Elle entraîne anémie, fibrose hépatique, troubles cognitifs et modulation immunitaire. Cette étude examine l'impact des traitements répétés au PZQ sur la dynamique de l'infection, les performances cognitives et les réponses immunitaires chez les enfants d'âge scolaire dans les zones endémiques du Cameroun. Une étude rétrospective a été réalisée auprès d'enfants d'âge scolaire fréquentant cinq écoles publiques dans des villages endémiques à *S. mansoni* à Bokito, Cameroun, après renouvellement de l'autorisation éthique (No. 2022/12/1505/CE/NECRHH/SP). Des échantillons et données précédemment collectés ont été analysés selon le nombre cumulatif de traitements au PZQ reçus (0–2 ou >2 cycles). Les paramètres analysés comprenaient les taux d'infection, la charge parasitaire, les niveaux d'hémoglobine, la fibrose hépatique, les performances cognitives et les marqueurs immunitaires (IgE, IgG4, cytokines), en utilisant des méthodes statistiques telles que le test t de Student, l'ANOVA, le test de Kruskal-Wallis, le test du khi-deux de Spearman et la régression logistique multivariée. Les traitements répétés au PZQ ont réduit significativement les taux d'infections sévères ($p = 0,01$) et la charge parasitaire ($r = -0,33$, $p = 0,04$), mais ont eu un impact limité sur la prévalence globale de l'infection ($p = 0,58$). Bien que ces traitements n'aient pas considérablement ralenti la progression de la fibrose hépatique ($p = 0,31$), ils ont été associés à une augmentation des niveaux de VEGF chez les participants ayant reçu >2 traitements ($p = 0,04$). Les enfants ayant reçu >2 traitements ont montré des niveaux d'hémoglobine améliorés ($p = 0,004$) et de meilleures performances cognitives, reflétées par des notes académiques plus élevées ($p = 0,02$) et de meilleurs ratios de progression classe/âge ($p = 0,01$). Sur le plan immunitaire, les traitements répétés ont renforcé les réponses dominées par Th2, marquées par des niveaux accrus de cytokines comme IL-4 ($p = 0,04$) et IL-33 ($p = 0,03$), ainsi que des augmentations d'IgE ($p = 0,002$) et d'IgG4 ($p = 0,02$), suggérant une réponse immunitaire équilibrée et régulée. Les traitements répétés au PZQ réduisent efficacement la charge parasitaire, atténuent l'anémie, améliorent les performances cognitives et favorisent une réponse immunitaire régulée et protectrice chez les enfants d'âge scolaire. Cependant, la persistance de la fibrose hépatique souligne la nécessité d'approches intégrées, incluant des thérapies antifibrotiques et des vaccins.

Mots-clés : *Schistosoma mansoni*, PZQ, enfants d'âge scolaire, immunopathologie, troubles cognitif

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Parasitic diseases represent a serious public health problem, mainly in developing tropical and subtropical regions. They are categorized as neglected tropical and poverty diseases countries since several of them have not gotten any special attention from the governmental or private sectors **(Hotez *et al.*, 2020)**. Schistosomiasis, also known as bilharziasis, is one of them and is the second most common parasitic disease in the world after malaria **(CDC, 2021)**. It's caused by the platyhelminths of the genus schistosome within the class of trematodes parasites **(Pd *et al.*, 2001).** According to WHO, approximately 251.4 million people are infected and more than 800 million are at risk of infection to schistosomiasis, which results in human morbidity and mortality worldwide **(WHO, 2022a, 2023)**. Out of the 78 countries where transmission has been reported, only 51 endemics with moderate-to-high transmission require preventative chemotherapy for schistosomiasis, in which persons and communities are being targeted for widespread treatment **(WHO, 2023)**. Sub-Saharan Africa bears the greatest proportion of the world's schistosomiasis burden at over 90%.

A consistent feature of human schistosomiasis is that school-aged children (SAC) in endemic areas are more susceptible to reinfection than older individuals **(McManus *et al.*, 2020)**. Despite the possibility that lack of exposure plays a role in older people's low susceptibility to infection, it is now widely acknowledged that age-dependent acquired resistance to reinfection also plays a significant role. This age-related resistance is primarily the result of the development of protective humoral immune responses **(Butterworth *et al.*, 1988; Dunne *et al.*, 1992)**.

Schistosomiasis treatment with PZQ through the MDA is crucial for managing and preventing the diseases **(Cioli *et al.*, 2014),** but it mainly affects the adult worms that can escape the immune system with their skin disguise. Immunity refers here to the immune system’s ability to mainly get rid of the invading larvae and the young worms that are barely affected by PZQ. Many studies have extensively discussed how immunity develops during infection and treatment. Dunne and collaborators proposed that it is specifically a delayed concomitant immunity mechanism, where the immune responses triggered by the adult worms do not harm them but instead target the invading larvae and kill them **(Fitzsimmons *et al.*, 2012b)**. Low level of IgE induced by the specific antigens sequestered from the dying adult worms following the treatment that reacted crosswise with the invasive larvae and conferred immunity **(Fitzsimmons *et al.*, 2012a, 2012b)**.

On the other hand, schistosomiasis infection in SAC is related to educational loss and cognitive deficits that can affect future development. Ezeamama *et al.* showed a positive association between the intensity of infection and cognitive impairment **(Ezeamama *et al.*, 2018)**. Chronic inflammation is suggested to induce these deficits through close communication between the systemic cytokines produced during infection and the central nervous system **(Fung *et al.*, 2012; TSAO *et al.*, 2001; Xie *et al.*, 2019)**.

Our study aimed to highlights the impact of recurrent PZQ treatments on morbidity, cognitive impairment, and immunological responses due to *S. mansoni* infection.

1) Research question

→ How do cumulative praziquantel treatments influence the development of immunological responses in SAC residing in *S. mansoni* endemic regions, and what are the repercussions on their cognitive performances?

2) Research hypothesis

→ Repetitive treatment fosters the development of immunological profiles linked to resistance against reinfection to *S. mansoni* and mitigates the progression of cognitive impairment in infected hosts.

3) Objectives

3.1) General objective

→ To determine the effect of cumulative number of PZQ treatments on infection outcomes, cognitive performances and immunological responses of SAC with *S. mansoni* susceptibility.

3.2) Specific objectives

→ To assess the impact of cumulative PZQ treatments on parasite burden, hemoglobin levels and liver fibrosis in SAC with *S. mansoni* susceptibility.

→ To evaluate the influence of cumulative PZQ treatments on academic performance and academic progress over time in SAC susceptible to *S. mansoni* infection.

→ To investigate the immune modulation in total IgG4 and IgE antibody responses, as well as the levels of GM-CSF, IL-4, IL-2, VEGF, and IL-33 cytokines responses.

LITERATURE REVIEW

CHAPTER I: LITERATURE REVIEW

I. History of schistosomiasis diseases

Human schistosomiasis is an ancient parasitic disease affecting millions, primarily in tropical and subtropical regions. The term "schistosomiasis" was introduced by David Friedrich Weinland in 1858 **(Di Bella *et al.,* 2018)**. The oldest writings on the disease date back to Egyptian medicine from 3000 to 400 BC, with references found in the Ebers papyrus and calcified eggs in mummies from 1200 BC **(Cox, 2002)**. The disease, possibly referred to as 'ā–a-ā', was documented in Egyptian medical papyri from 1500 BC. Prevention methods included avoiding polluted water and wearing linen penile sheaths **(Cox, 2002; Di Bella *et al.,* 2018)**. Professor Marc Armand Ruffer found calcified eggs of Bilharzia haematobium in mummies from the twentieth dynasty **(Di Bella *et al.,* 2018)**. The Bible mentions a curse resembling schistosomiasis in Jericho, linked to well water **(Di Bella *et al.,* 2018)**.

Theodor Bilharz discovered *Schistosoma haematobium* in 1851, leading to the term "bilharzia" **(Aula *et al.,* 2021)**. In 1902, Sir Patrick Manson suspected another Schistosoma species, confirmed in 1915 by Louis Westenra Sambo, identifying *S. mansoni* **(Aula *et al.,* 2021)**. Schistosoma japonicum was described in 1904 by Fujiro Katsurada, and Robert Thomson Leiper elucidated the life cycle of Schistosoma spp. **(Di Bella *et al.,* 2018)**. The disease likely spread from the African great lakes to Egypt during the fifth dynasty of pharaohs. Modern irrigation methods have significantly increased the spread of schistosomiasis, with prevalence rising dramatically in areas with artificial canals **(Di Bella *et al.,* 2018)**.

II. Phylogeny of schistosomiasis diseases

The phylum of Platyhelminthes is a group of animals that have a flat body and a single opening for eating and excreting **(Pd *et al.,* 2001)**. They are parasites that can infect many types of vertebrates, including humans. Their evolutionary history has been studied using molecular and morphological data, allowing us to understand their phylogeny **(Pd *et al.,* 2001)**. The tapeworm *Schistosoma mansoni* belongs to the *Schistosomatidae* family of the class of parasitic Trematodes within the phylum of Platyhelminthes. Indeed, the schistosome genus is subdivided into more than 20 species of which only six are at the origin of the pathology in humans, namely: *S. hematobium, S. mansoni, S. japonicum, S. mekongi, S. intercalatum* and *S. guineensis* **(Webster *et al.,* 2006)**.

III. Epidemiology of schistosomiases

1) Geographic distribution of schistosome species

Due to the uneven distribution of the various intermediate hosts (mollusks) necessary for the transmission, the different schistosome species that cause the two main types of disease, namely intestinal and urogenital schistosomiasis, are unequally distributed throughout the world **(WHO, 2023)**. The geographical distribution of the many schistosome species is displayed in the following **Table 1.**

Table 1: Parasite species and geographical distribution of schistosomiasis **(WHO, 2023)**

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

2) Epidemiology of schistosomiasis diseases in the world

Worldwide, schistosomiasis is considered the second most devastating tropical disease after malaria **(WHO, 2023)**. It is one of the main sources of morbidity and mortality in high-prevalence countries, with major consequences for the health and economy of the population **(CDC, 2021)**. Indeed, more than 240 million cases have been recorded worldwide, and around 800 million people were at risk of contracting the disease in 2019 **(WHO, 2023)**. In 2021, chemoprevention against schistosomiasis was needed in 51 countries **(Figure 1)** for a total of 251.4 million people, including 136 million school-age children and 115.4 million adults **(WHO, 2022b)**. WHO estimates that 11,792 people die from schistosomiasis worldwide each year **(WHO, 2023)**. Additionally, it has been reported that 90% of schistosomiasis cases are in

Africa and are distributed as follows: Nigeria 26.21%, Ethiopia 9.57%, Democratic Republic of the Congo 7.74%, Mozambique 5.82%, Kenya 5.04%, Tanzania 4.32%, Cameroon 4.30%, Uganda 3.66%, Malawi 2.89%, and Ghana 2.88% **(Inceboz, 2022)**.

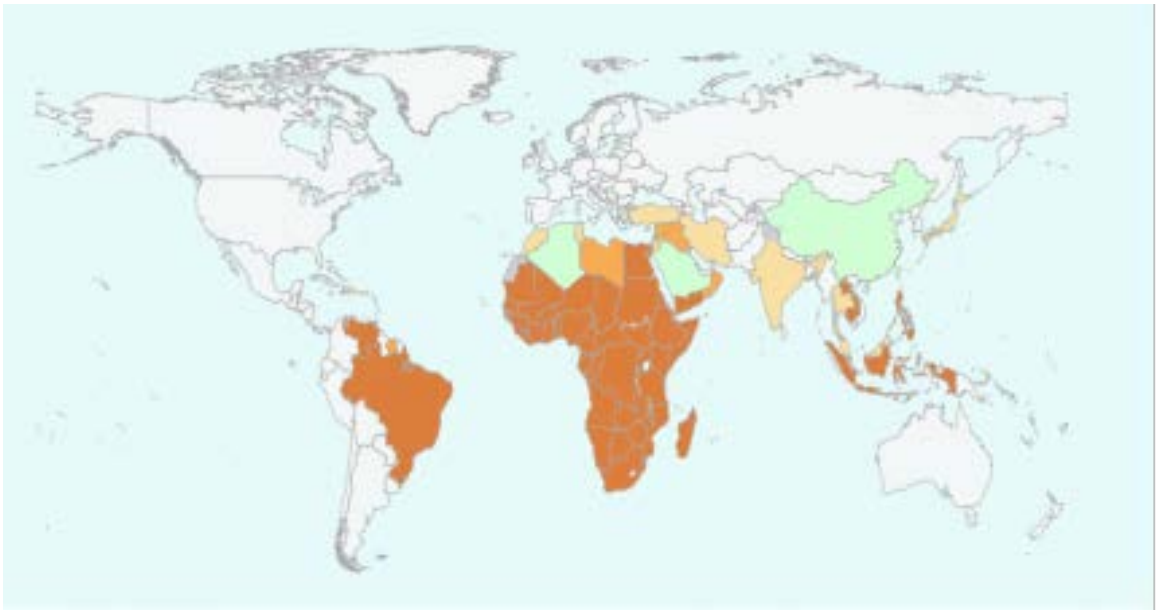


Figure 1: Global praziquantel demand distribution worldwide **(WHO, 2021).**

3) Epidemiology of schistosomiasis diseases in Cameroon

Cameroon is endemic to schistosomiasis, with a prevalence rate ranging from 1.7 to 55.5% **(Njunda *et al.*, 2017)**. According to WHO reports, nearly 5,420,130 people need preventive treatment each year, of whom about 50% are school-aged children **(WHO, 2021)**. More specifically, studies carried out in certain localities have reported prevalences of 34.2% and 4.9% in Loum for urogenital and intestinal schistosomiasis, respectively **(Nkengni *et al.*, 2019)**, and 43% in Magba for urogenital schistosomiasis **(Bong *et al.*, 2022).**

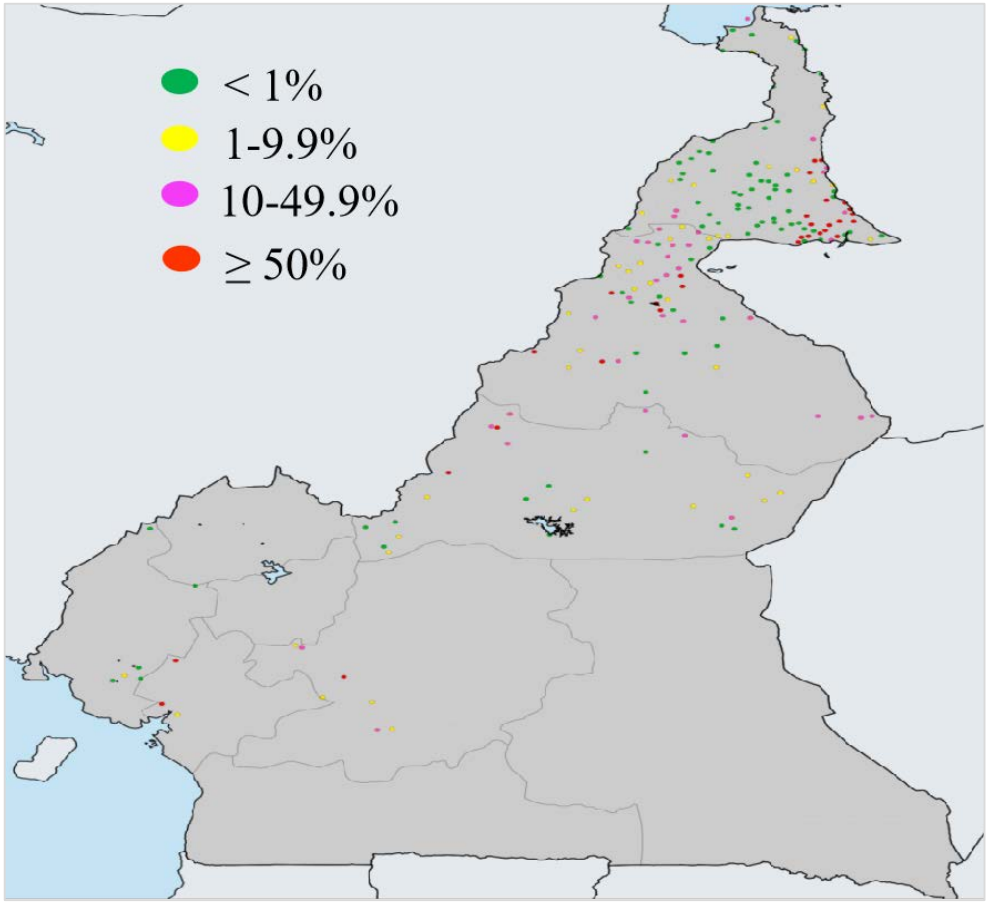


Figure 2: Prevalence of schistosomiasis in Cameroon **(espen.afro.who.int/countries/cameroon)**.

IV. Descriptions of Schistosomiasis life cycle stages

1) Egg stage

Eggs are the result of the copulation between the schistosomes adults worm male and female. They measure between 60 and 400µm and have a subterminal spine whose position can provide information on the species of schistosomes **(Ford and Blankespoor, 1979)**. Of particular interest, **Figure 3** highlights the distinct egg morphology of each Schistosoma species, which can be a valuable tool for species identification.

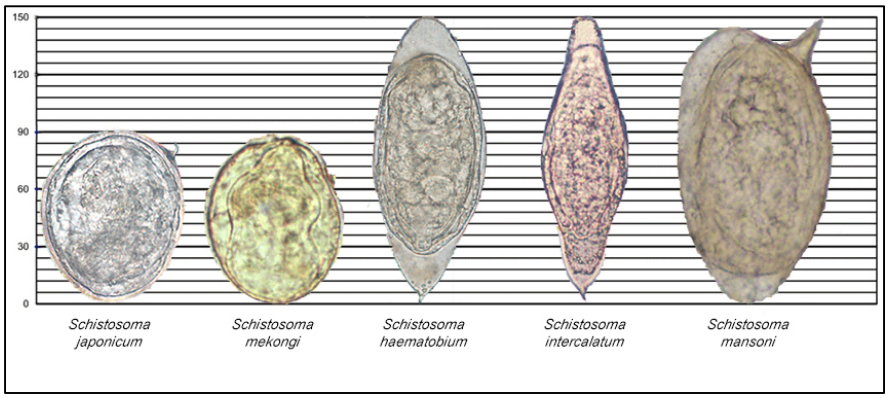


Figure 3: Morphology of different eggs of schistosomes **(CDC, 2021)**.

2) Miracidium stage

Miracidium is a ciliated larva measuring between 150 and 180µm in length. It is pear-shaped and its outer surface is made up of ciliated plates allowing it to swim (**Figure 4**). Miracidium is the residue of eggs hatched in fresh water. It is the form of the parasite that infects the intermediate host, the mollusk, and has a short life due to its limited stock of glycogen (a form of nutrient storage) **(Prah and James, 1978)**. It recognizes and penetrates its suitable snail host by geotropism (orientation with respect to gravity), phototrophism (orientation regarding light) and chemotacticism. The Miracidia of *S. mansoni*, for example, possess a negative geotropism and a positive phototropicism to reach their host; on the other hand, the Miracidias of *S. haematobium* are characterized by an inverse tropism **(Prah and James, 1978)**. In fact, molecules called miraxone (fat acids, amino acids and neuronal products of the molluscum) and serving as signals have been proposed as participating in the attraction of the said miracidium. Once inside the host, the miracidium loses its ciliated plaques, the remaining cells reorganize to set up a lengthy bag that is the first-generation sporocyst **(Mahmoud, 2001)**.

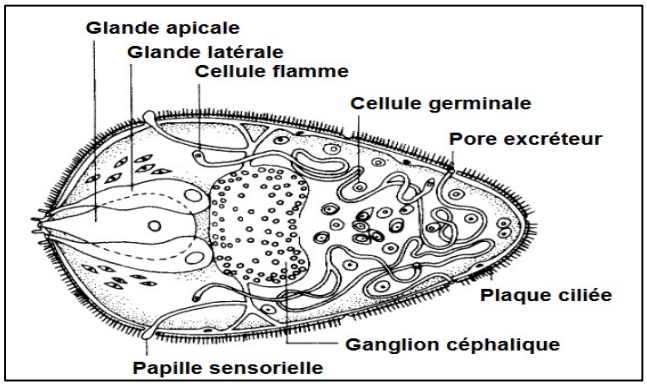


Figure 4: Ultrastructure of a miracidium **(Vanderstraete, 2014).**

3) Sporocyst stage

The sporocyst is a bag containing germinal cells that express the markers of stem cells and are endowed with a significant regenerative capacity **(Figure 5) (Wang *et al.*, 2013)**. These germinal cells will proliferate and result in the bulging of the primary sporocyst into several secondary sporocysts. With the help of its muscle cells and its mobile end, the secondary sporocyst migrates to the internal organs of the molluscum. This latter has a chamber where germinal cells are found that begin to differentiate into cercaria **(Bogitsh *et al.*, 2019)**.

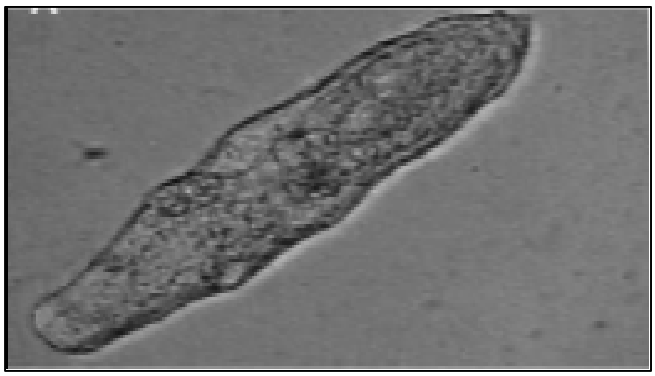


Figure 5: Photography of a secondary sporocyst **(D Negrão-Corrêa *et al.*, 2007).**

4) Cercarial stage

The parasite has a head with an anterior organ called the buccal sucker, a bifurcated locomotor organ that serves as a tail and a median organ called the muscular ventral sucker (**Figure 6**). The cercaria's lifespan is short due to its limited energy store. This is why it must quickly find its definitive host to continue its development **(McKerrow and Salter, 2002)**. It thus migrates and penetrates its host by chemotaxis in the skin **(Haeberlein and Haas, 2008)**. The penetration of cercaria into the skin is also facilitated by the secretion of glands rich in proteolytic enzymes which lyse the extracellular matrix **(Dresden and Asch, 1972)**. After penetration, it undergoes morphological and biochemical modifications which enable it to pass to the schistosomula stage in order to adapt to its new hosts **(El-Ansary, 2003).**

Figure 6: Migrating schistosome cercaria **(Mahmoud, 2001)**.

5) Schistosomula Stage

During skin penetration, cercariae loses its tail and becomes schistosomulae in blood vessels. Nearly 3 to 4 days are required for its passage from the right heart to the lungs. The body of the parasite thus lengthens to be able to move actively in the pulmonary capillaries. Once again, they will be passively transported by the blood in the systemic circulation to reach the liver and the portal system, where these latter will begin the second phase of so-called hepatic maturation. Overall, it lasts between 8 to 10 days and is marked by a set of morphological and biochemical changes (maturation of the esophagus, development of excretory organs, strengthening of the protective integument, acquisition of sexual dimorphism) leading to the adult stage. In addition, the schistosomula have the capacity at this stage to digest erythrocytes in order to feed on hemoglobin **(Mahmoud, 2001)**.

6) Adult worm stage

The female worm is thin and opaque due to hemozoin accumulation in the digestive tract and is approximately 12–16 mm long. Conversely, the male worm is stocky-curved, measures 6 to 12mm long, and forms a gutter covered with irregular spines called the gynecophore canal (**Figure 7**). Worms have a complex integument structure that plays multiple roles, including protection against the host immune system, osmoregulation, and excretion. To complete her sexual maturation and lay her eggs, the female enters the male's gynecophore canal, where she finally lays eggs after copulation with the male worm. The pair of worms migrate to their preferred microhabitats: the mesenteric veins for *S. mansoni* and the bladder plexus for *S. hematobium*. These two environments are directly related to excretory organs and thus facilitate the release of eggs from the host organism **(Kusel *et al.*, 2007)**.

Figure 7: Couple of adult male and female schistosomes in full fertilization **(Ms *et al.*, 2013)**.

V. Vectors agent of schistosomiasis transmission

1) Intermediate host

The intermediate host of the schistosome is a freshwater snail whose genus is specific to the species of schistosome. It ensures the transformation of miracidia into mature sporocytes. However, the miracidium has the ability to penetrate any snail genus, since the development of viable sporocysts only occurs in a specific genus due to specific compatibility (**Table 3**) **(Vanderstraete, 2014)**.

Table 2: Intermediate host of each schistosomes species **(Vanderstraete, 2014)**.

Intermediate host	**Species of schistosomes**
Bulinus	*S. guineensis*
	S. heamatobium
	S. intercalatum
Onconmelania	*S. japonicum*
Biomphalaria	*S. mansoni*
Tricula	*S. mekongi*

2) Definitive host

Human is not the only mammal involved in the complete cycle of the parasite as a definitive host. Indeed, various other mammals, considered as secondary definitive hosts, can serve as reservoirs for the parasite and are often used in preclinical studies for rapid experiments. These include cattle, dogs, domestic pigs, ruminants, monkeys and many rodents **(Di Bella *et al.*, 2018)**. In addition, some cases of zoonotic schistosomiasis have been described in humans **(McManus *et al.*, 2018)**.

VI. Life cycle of *Schistosoma mansoni*

Schistosomes are a digenic parasite which requires an intermediate host which is a mollusk and a definitive host which is mammalian (human, mouse etc.) to accomplish its life cycle. Due to the complex life cycle of all species of schistosomes, transmission requires fresh water to mediate the indirect interaction between hosts (**Figure 8)**.

The eggs shed in the feces of the definitive host hatch upon contact with fresh-water **(1)**, releasing the free-swimming ciliated miracidia that then infect the suitable intermediate snail host **(2) (Mt *et al.*, 2014)**. Inside the snail, the miracidia transform into mother sporocysts (cylindrical structure), which in turn produce daughter sporocysts by asexual reproduction **(3)**. After around 30 days post-infection, cercariae emerge from the daughter sporocysts and are shedding by the snails in response to the light and heat **(4)**. At this stage, the cercariae can penetrate the skin of the definitive host upon skin contact **(5)**. Following penetration of the skin, the cercariae undergo morphological and biochemical changes, transforming into a juvenile form (by cercariae's tails falling), known as the schistosomula, which reaches the blood vessels from the venules **(6)**. Subsequently, they are passively transported to the lungs via the right heart, before reaching the left heart, and then entering the arterial circulation. Schistosomula can then migrate to, and live in, the lower mesenteric veins of the bowel (*S. mansoni*, *S. japonicum*) or the pelvic venous plexus (*S. haematobium*) where they mature into adult male or female worms **(7)**. Once there, the females end up in the male's gynaecophoral canal to form a couple whose main purpose is the production of eggs by the female (about 5 weeks after infection) over a period that in extreme cases may be as long as 20 years. Egg production requires five to seven weeks from the time of the initial infection **(Daniel G. Colley *et al.*, 2014; Gryseels *et al.*, 2006a; McManus *et al.*, 2018)**. Some part of these eggs passes through the intestinal or bladder wall and are eliminated in the feces or urine perpetuating the Life Cycle **(1)**. However, another part is not eliminated and get trapped in several organs (mainly the liver and intestines), inducing a potent granulomatous inflammatory response, responsible for

schistosomiasis pathology **(Daniel G. Colley *et al.*, 2014; Gryseels *et al.*, 2006a; Ross *et al.*, 2002)**.

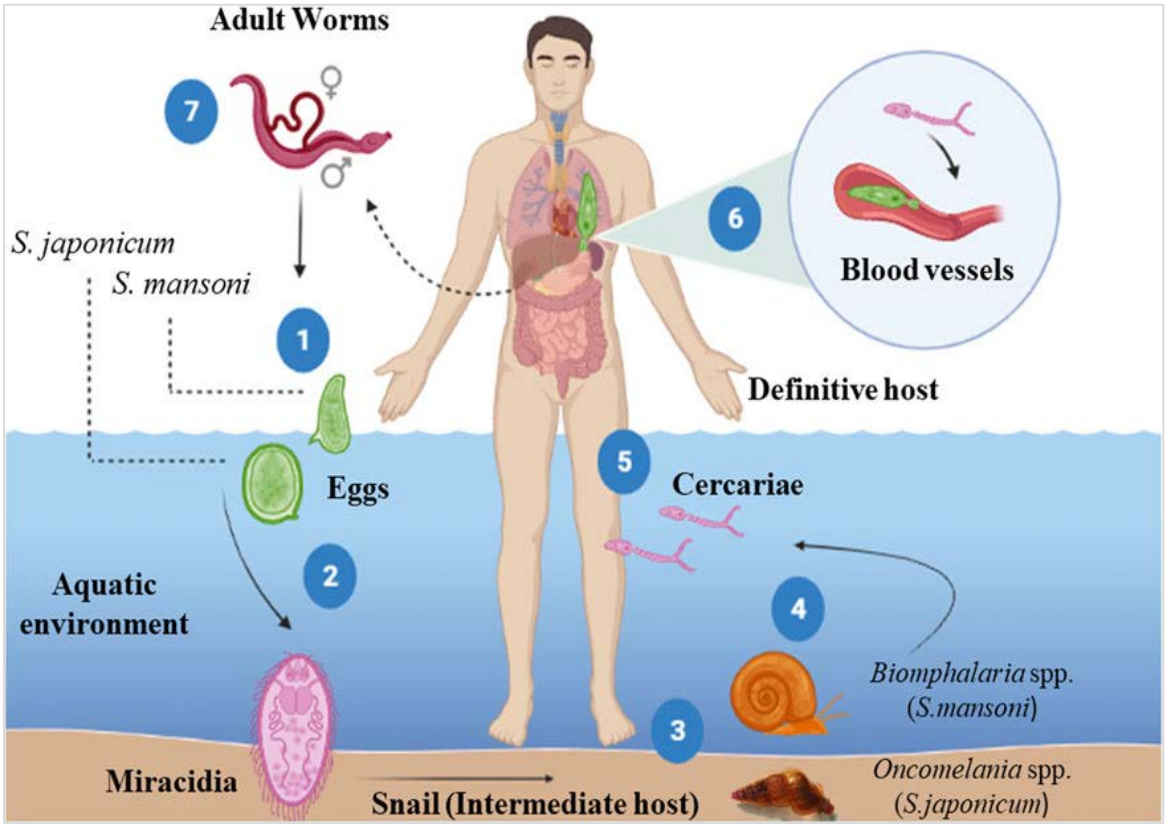


Figure 8: Life cycle of *Schistosoma mansoni* **(Licá *et al.*, 2023)**.

VII. Pathophysiology and clinical manifestation of human schistosomiasis

During its sexual stage in its definitive mammalian host (such as humans, mice, etc.), the worm undergoes different developmental forms in various locations, leading to distinct pathologies across three stages: the acute stage, the active established infection stage, and the chronic infection stage **(McManus *et al.*, 2020)**.

1) Acute schistosomiasis

Before the acute stage itself begins, cercaria dermatitis is observed in response to excreted and secreted products from penetrating cercariae; it is a maculopapular pruritus reaction of the part of the body exposed to cercariae. Clinical manifestations include oedema, itching and scratching of the skin and begin 2 to 3 days after the cercariae enter the host's skin. These signs may occur in individuals exposed to Schistosoma spp. infections for the first time, such as travelers and migrants to schistosomiasis-endemic areas **(McManus *et al.*, 2018)** but occur mainly in individuals frequently exposed to infection in endemic zones **(He *et al.*, 1990;**

Khammo *et al.*, 2002; Lambertucci, 2010; Rj *et al.*, 2003) although it is sometimes unrecognized **(Appleton, 1984)**. The acute stage of schistosomiasis, also known as Katamaya syndrome (for *S. japonicum*) or toxaemic form (for *S. mansoni*), develops during the sexual maturation of the schistosomula into an adult worm as it migrates to the hepatic portal system (3rd and 5th week after exposure and infection) **(Licá *et al.*, 2023)**. Symptoms observed are mainly caused by systemic hypersensitivity reactions in response to released schistosomula migrant's antigen. During primary infection in non-immune individuals, the symptoms include a high fever accompanied by chills, profuse sweating, asthenia, myalgia, headache, and non-reproductive cough **(Bottieau *et al.*, 2006; Schwartz *et al.*, 2000)**. However, acute schistosomiasis is rarely observed in people living in areas endemic to *S. mansoni* or *S. haematobium*. This lack of susceptibility to acute symptoms has been suggested to be due to in-utero desensitization resulting in lowered immune responsiveness to schistosome antigens in infants born to infected mothers **(Secor, 2012)** or to intense regulation of the immune response following repeated exposure to cercariae **(Sanin *et al.*, 2015)**.

2) Establish active infection

Mainly observed in children with a long-standing infection and living in schistosomiasis-endemic areas, the established active infection stage is characterized by a local inflammatory response against parasite eggs and coincides with parasite oviposition (in stool for *S. mansoni* and *japonicum* or in urine for *S. haematobium*) by adult worms **(McManus *et al.*, 2020)**. Clinically, we note intestinal disease and hepatitis with granuloma presence (case of *S. mansoni* and *S. japonicum*) or urogenital disease (*S. haematobium*). The resulting symptoms, which include non-specific intermittent stomach pain, diarrhea, and rectal bleeding, are correlated to the severity of the infection. These gastrointestinal features are often focal with pseudo polyposis and polyposis interspersed with normal bowel. The granuloma formed is a complex, well-organized multicellular structure and constitutes a compromise of the immune system established to contain tissue lesions caused by proteolytic enzymes and immunological substances released by the eggs **(Aula *et al.*, 2021; CDC, 2021; Fairfax *et al.*, 2012; Gryseels *et al.*, 2006b; Hams *et al.*, 2013)**. While the eggs are thought to induce most of the pathology at this stage (Burke *et al.*, 2009)**,** the adult worms seem to hardly trigger any immune response or symptoms. This observation is explained by the ability of adult worms to regenerate their tegmental surface (due to the presence of somatic stem cells) and to bind host antigens to their tegmental surface (thus camouflaging their own antigen surface to the host immune system) **(Daniel G Colley *et al.*, 2014)**. Thus, at this stage of pathology, the death of adult worms and

the reduction of pathology is virtually dependent on deworming by administration of anti-helmetic drugs such as PZQ.

3) Late chronic infection

In most people frequently exposed to infection in endemic areas, the disease evolves to an advanced chronic stage. Mature, patent schistosome infection associated may be intestinal disease, hepatosplenic inflammation, and liver fibrosis in disease due to *S. mansoni and S. japonicum* or urogenital infection (caused by *S. haematobium*) leads to obstructive disease in the urinary and reproductive system **(McManus *et al.*, 2020, 2018)**. At this stage, people who are continuously exposed develop a degree of acquired immunity to new infections; the worm burden is thus greatly reduced, although this is also caused by the natural death of the parasites. Furthermore, an exacerbated granulomatous response against parasite eggs is suggested to be fatal for host tissues (intestinal and hepatic granuloma), as it is tissue-consuming and induces periportal fibrosis accompanied by hepatosplenomegaly for *S. mansoni* and *S. japonicum*. Owing to this immunological down-regulation, while newly formed granulomas are small in size, each previously formed granuloma is progressively replaced by scaring fibrous tissue (collagen deposition), contributing to a reduction in symptom severity **(McManus *et al.*, 2018)**. However, some commonly infected individuals develop only weak immunoregulatory responses to granuloma formation and consequently develop extensive fibrosis and subsequent hepatosplenic disease with periportal fibrosis. Symptoms associated with this later stage of the disease include upper abdominal discomfort with palpable nodules, hard hepatomegaly, splenomegaly, ascites, and hematemesis due to esophageal varices as a complication of portal hypertension, and can rapidly lead to death **(Gryseels *et al.*, 2006b)**. Substantial pulmonary hypertension caused by granulomatous pulmonary arteritis can also occur in patients with advanced hepatic fibrosis disease. The time from initial infection to advanced fibrosis is usually 5-15 years. However, periportal fibrosis can occur in children as young as 6 years, supporting the need for screening and treatment of preschool-aged children **(Gryseels *et al.*, 2006b)**.

VIII. Immune response during schistosomiasis

The immune responses to *Schistosomal* antigens, which includes cytokines and immunoglobulins, likely reflect the variations in the gene expression products of these antigens throughout life cycles and their location within the schistosome organism **(Oettle *et al.*, 2023) (Figure 9)**.

The skin is the first innate immune barrier that cercariae come into contact with **(He *et al.*, 2005)**. It is made up of active cells that secrete cytokines with antibacterial properties **(Piipponen *et al.*, 2020)**. These cells are mainly keratinocytes followed by langerhans and dendritic cells **(Hambrook and Hanington, 2020)**, which are amplified in the presence of antigens, the excreted and secreted products released from penetrating cercariae **(Paveley *et al.*, 2009)**. In this environment, the classically activated M1 macrophages are quickly recruited **(Vannella and Wynn, 2017)**; contributing not only to the production of proinflammatory cytokines **(Shapouri-Moghaddam *et al.*, 2018)** but also involved in the attraction and activation of Th1 cells **(Orecchioni *et al.*, 2019)**, thus constituting an important link between innate and adaptive immunity. In contrast, cercarial excreted and secreted-products also promote the activation of prostaglandin E2 and prostaglandin D2-producing keratinocytes, which are molecules that induce the production of IL10, which favor parasite survival **(Licá *et al.*, 2023)**. In fact, IL10 has been shown to be elevated in patients stimulated with cercarial excreted-secreted product **(Hogg *et al.*, 2003b; Turner *et al.*, 2013)**. However, during migration and sexual maturation of schistosomula, there is a predominance of Th1 response characterized by pro-inflammatory cytokines such as IL1, 2, 6 12, INF g, and TNF **(Egesa *et al.*, 2018b; Zheng *et al.*, 2020)**, which cause systemic and pronounced hypersensitivity from the 3rd to 5th post-infection week **(Gryseels *et al.*, 2006b)**. Th1 and proinflammatory cytokines have been implicated in the antibody-independent killing of schistosomula in animal models **(Wynn *et al.*, 1994)** and the generation of Th1 response has also been associated with protective immunity to *S. mansoni* **(Brito *et al.*, 2000; Cardoso *et al.*, 2008; Mountford *et al.*, 1992)**.

When egg production starts (5th to 6th-week post-infection), the host immune mediators become efficiently polarized to the Th2 profile which is associated with increasing production of anti-inflammatory cytokines including IL4, 5, 9, 10, and 13 **(Burke *et al.*, 2009; MacDonald *et al.*, 2002)**. These changes are in response to soluble egg antigens (SEA) that are composed of a complex mixture of immunostimulatory antigens **(Hams *et al.*, 2013)** that are known for their ability to condition DCs to initiate the induction of the Th2 profile **(Mouser *et al.*, 2019)**, whose

cytokines are anti-inflammatory with the ability to inhibit Th1 responses **(Mewamba *et al.*, 2021)**. The discrepancy between Th1 and Th2 responses is critical and the induction of both Th1 and Th2 is needed to balance effector responses and limit immunopathology. Indeed, immunopathology associated with schistosomiasis in mice **(Hoffmann *et al.*, 2000)** and in humans **(Mbow *et al.*, 2013)** is a result of unregulated and highly polarized Th1 or Th2 immune responses.

When the infection becomes chronic, a large amount of IL-4 and IL-13 is produced **(Kamdem *et al.*, 2018)**. These cytokines have the ability to inhibit type 1 macrophages and activate type 2 macrophages (alternatively activated), mainly via STAT-6 activation **(Enderlin Vaz da Silva *et al.*, 2014; He *et al.*, 2020)**. In fact, M2 macrophages also have anti-inflammatory and immunoregulatory cytokines characterized by low production of IL-1, IL-6, and TNF-a and high production of IL-10 and TGF-β, as well as the chemokines CCL1, 12, 18, 22, and 24 **(Yunna *et al.*, 2020)**. Along with M2 macrophages, Th-reg cells in the chronic stage of infection are responsible for the immunomodulatory responses against Th1 and Th2 responses by also secreting IL10 and TGF-b **(Zheng *et al.*, 2020)**. IL-10 is presumed to be involved in the differentiation of B cells into switched B cells producing IgG4 **(Satoguina *et al.*, 2005)** that can regulate the pathology at the chronic stage of the illness. Indeed, IgG4 compete with IgE for shared antigenic epitopes (mainly from the parasite eggs), but being neither able to recruit and activate mastocyte and basophil cells as IgE do, nor to activate the complement system, they act as powerful inhibitors of the granulomatous response at the chronic stage of the disease **(Aalberse *et al.*, 2009).**

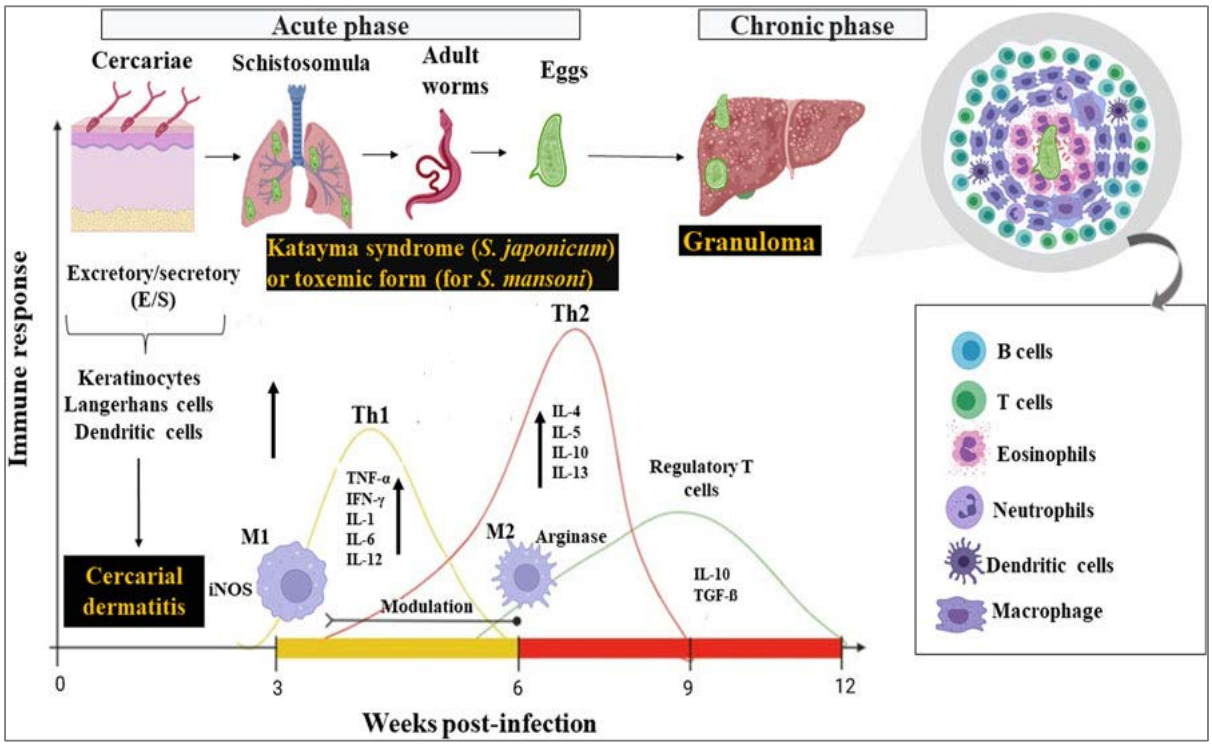


Figure 9: Different immune response profiles during Schistosomiasis infection **(Licá *et al.*, 2023)**.

IX. Impact of schistosomiasis infection on cognitive and academic performance

Cognitive ability encompasses an individual's mental capacity to learn, comprehend, recall, solve problems, grasp abstract concepts, and think creatively **(Shi and Qu, 2022)**. It stands as a pivotal psychological factor influencing the execution of various activities **(Sternberg and Sternberg, 2022)**. This capacity involves the brain's proficiency in storing, processing, and retrieving information, including skills such as attention, memory, and reasoning **(Shi and Qu, 2021)**. In contrast, academic performance typically gauges academic achievement and skill, reflecting the depth of knowledge and learning a student has acquired through training and education **(Shi and Qu, 2022)**.

Within the multifactorial landscape of morbidity in schistosomiasis-endemic areas, cognitive impairment has garnered significant attention, endowed with indirect implications **(Daniel G Colley *et al.*, 2014; Gryseels *et al.*, 2006b; McManus *et al.*, 2020)**. The focus on cognitive impairment arises from the close interaction between inflammatory cytokines from immune cells during infection and the central nervous system **(Daniels *et al.*, 2014; TSAO *et al.*, 2001; Varatharaj and Galea, 2017)**. Many authors have postulated that immunological changes in chronically schistosomiasis-infected hosts are the primary contributors to cognitive impairment

(Ezeamama *et al.*, 2018, 2005; King and Galvani, 2018; Mazibuko and Chimbari, 2020; Nelwan, 2019; Xie *et al.*, 2019). This observed effect results from the passage of systemic cytokines and their receptors through the blood-brain barrier, either via the neural afferent pathway or the circumventricular organ through perivascular space. This passage triggers the production of brain cytokines, modulating neuronal responses and, consequently, cognitive abilities **(Fung *et al.*, 2012; Kasambala *et al.*, 2023a)**.

Academic performance is often considered a tangible outcome that reflects cognitive abilities, concentration, and memory. The alteration in cognitive functioning due to schistosomiasis infection prompts inquiries into its potential repercussions on academic performance. The relationship between cognitive and academic performance has been extensively debated. While the traditional view regards cognitive abilities as foundational for academic development **(Sternberg *et al.*, 2008)**, a meta-analysis of intervention studies challenges this notion. It suggests that direct academic instruction, encompassing subjects like reading and math, substantially enhances cognitive abilities and IQ **(Stockard *et al.*, 2018)**. Additionally, the theory of mutualism challenges the common assumption of a unidirectional influence of cognitive abilities on academic achievement, proposing that various skills become mutually related during human development **(Van Der Maas *et al.*, 2006)**. Finally, the proposition that factors influencing cognitive abilities also impact academic performance, and vice versa, adds complexity to this intricate relationship **(Peng and Kievit, 2020)**.

X. Diagnostic methods

The diagnosis of schistosomiasis is mainly done using three types of tests namely microscopic, immunological and molecular methods.

1) Microscopic methods

Kato Katz (KK) is the most universally applied test for intestinal schistosomiasis. It consists of performing a microscopic examination of a fecal smear to reveal the eggs of the schistosomes decolorized by malachite green. This technique provides a high diagnostic specificity in endemic areas where parasite loads are high. However, the detection limits become low when applied in non-endemic areas or in the diagnosis of early-stage infections where parasitemia is low **(Bärenbold *et al.*, 2017)**.

2) Immunological methods

Immunological methods detect parasite antigens or host molecules in biological fluids. One key technique is ELISA (Enzyme-Linked Immunosorbent Assay), which is used to detect and

measure antigens, antibodies or cytokines in samples. ELISA is widely used in medical diagnostics, research, and quality control because it offers flexible solutions for various needs. Each of the four types of ELISA has unique features suitable for different applications. Choosing the right method depends on the specific needs, sample type, and available resources **(Sulahian *et al.*, 2005)**.

- → **Direct ELISA**: This method involves attaching an antigen directly to a surface and detecting it with an enzyme-labeled antibody. It's simple and quick, needing fewer steps and reagents, but may be less sensitive and specific because the antigen must be well-immobilized on the plate.
- → **Indirect ELISA**: This method detects specific antibodies using a known antigen and an enzyme-labeled secondary antibody. It increases sensitivity by amplifying the signal, as multiple secondary antibodies can bind to a primary antibody. However, it is more complex and requires careful control to avoid cross-reactivity.
- → **Sandwich ELISA**: This method uses two antibodies: a capture antibody fixed to the plate and a detection antibody. It is highly specific, making it ideal for detecting cytokines or antibodies in complex samples. However, it requires well-matched antibody pairs, which can be costly and time-consuming to develop.
- → **Competitive ELISA**: This method involves competition between the sample antigen and a labeled antigen for binding to an antibody. It is useful for detecting small molecules and measuring very low antigen concentrations but can be less precise and needs careful optimization to avoid interference.

Additionally, Circulating Anodic Antigen (CAA) and Circulating Cathodic Antigen (CCA) techniques are employed to detect egg antigens in the serum or urine of infected individuals **(Ross *et al.*, 2002).**

XI. Control and prevention strategies

1) Antivectorial challenges

For the eradication of mollusks, molluscicides were the mainstay of schistosomiasis control up until the 1970s, but newly created drugs for human use eventually took over **(Sturrock, 2001)**. Despite the use of niclosamide, a chemical that is generally reliable, molluscicide has always had significant problems. When these short-lived chemicals are used, aquatic organisms die, but the few surviving snails are able to repopulate the site shortly after disinfection, thus making long-term control of schistosomiasis difficult **(Long, 2018)**. However, local communities are

hesitant to accept yellowish water bodies and fish dying due to molluscicidal effects. Chemical snail control has seen a surge in literature, with plant-derived molluscicides being explored for local development. However, none have achieved high efficacy or mass production. In addition, snail control using predatory or competing organisms like fish or different snail species is still unavailable **(Cioli *et al.*, 2014).**

2) Treatment of schistosomiasis

PZQ, a highly optimized compound, was developed by Merck in Germany to find new tranquillizers. Indeed, it is a derivative of pyrazinosoquinolone, discovered in 1972, and was first used to treat schistosomiasis in animals before being repurposed for humans use **(Chai, 2013; Olliaro *et al.*, 2011)**. This selected product remains unsurpassed and unequaled for its combined high standard of safety and antischistosomal efficacy among countless chemical screened **(Andrews *et al.*, 1983)**. The treatment with a standard dose of 40 mg/kg results in 60–90% cure and 80–95% egg reduction in uncured patients **(Cioli *et al.*, 2014)**. As a potential lack, PZQ does not act on all stages of the parasite. Schistosomes are susceptible for the first few days after infection, and decrease to a minimum around day 28 then resume gradually after weeks 6-7 **(Aa *et al.*, 1986)**. In endemic areas, the presence of immature parasites is likely common, so for a high cure rate, additional chemotherapy was proposed two weeks after the first **(Renganathan and Cioli, 1998; Utzinger *et al.*, 2000).** Short-term adverse reactions are common, usually mild and short-lasting, but the frequency and severity are directly correlated with pretreatment infection intensity, suggesting the implication of dying schistosomes and their products in the mediation of these adverse response. Allergic reactions are rare, but in some instances, they could be attributed to PZQ due to specific desensitization **(Kyung *et al.*, 2011)**. The use of PZQ as the only drug for schistosomiasis treatment may raise the risk of developing resistant schistosomes strain. However, there is no significant resistance observed in the field or in laboratory because the reported cases of decreased drug sensitivity are not of sufficient magnitude to undermine the public health value of PZQ sensitivity, and this testifies to the fantastic quality of PZQ **(Cioli *et al.*, 2014)**. Some factors that may affect the overall cure rates are the immaturity of the worms, the coverage of the MDA, and the immune response of the host. The mechanism of action of PZQ is mysterious. PZQ could induce a massive influx of calcium, which in turn causes muscle contraction and tegument alteration in the parasites **(Greenberg, 2005)**. To do so, it probably acts on a variant subunit of calcium channels, common to other organisms sensitive to PZQ, but the details of the molecular events of its action are inaccurate, and other assumptions are possible **(Pica-Mattoccia *et al.*, 2008)**. PZQ

derivatives have been tested, but none were better than PZQ. It's still unclear which mechanism of action prevents the rational design of improved analogs.

As an alternative to PZQ, oxamniquine is an old drug, safe and effective, but only against *S. mansoni*. Basically, it's a prodrug activated by a *S. mansoni* sulfotransferase, which is also at the heart of the resistance observed with this drug **(Pica-Mattoccia *et al.*, 2006)**. However, biomimetic-based redesigning of oxamniquine derivatives is an ongoing research project with the hope of reaching extended activity for other species of schistosomes **(Cioli *et al.*, 2014)**. Artemisin derivatives are the first-generation of antimalarial drugs but also have antischistosomal properties, especially against immature stages. When they are combined with PZQ, they achieve a better cure rate **(Pérez del Villar *et al.*, 2012)**, but the risk of developing resistant strains of plasmodium has limited their use in co-endemic areas. Many other drugs are not mentioned but still hold promising potential for the development of new antischistosomal drugs.

3) Others control measures

A wiser approach to controlling and eliminating schistosomiasis as a public health problem is the development of other non-medical measures which could prove more rewarding in the long run. The often-recommended integrated approach includes interventions in sanitation, water supply and health education **(WHO, 2021)**. These interventions aim to empower communities with knowledge about the disease's transmission cycle, risk factors, and preventive behaviors. Effective health education programs can raise awareness about the dangers of contact with contaminated water and promote practices like safe water use, proper sanitation, and handwashing with soap **(Phillips *et al.*, 2022)**. This empowers individuals, particularly children, to make informed decisions that minimize their exposure risk. However, successful health education requires a culturally appropriate approach, utilizing engaging methods like interactive workshops, community dialogues, and educational materials tailored to the target audience **(Dawaki *et al.*, 2015)**. Research suggests that integrating health education with WASH interventions can significantly improve long-term behavior change and contribute to sustainable schistosomiasis control efforts **(Phillips *et al.*, 2022)**.

MATERIAL AND METHODS

CHAPTER II: MATERIAL AND METHODS

I. Ethics statement

The renew version of ethical clearance **(No 2022/12/1505/CE/NECRHH/SP)** from the original study was granted by the National Ethic Committee for Research in Human Health (NECRHH) of Cameroon. Subsequently, administrative authorizations were obtained from the ministries of Basic education and public health in Yaoundé (Cameroon), from traditional authorities and schools directors. Information sessions were organized with all selected school directors of the locality and local authorities/representatives such as the Senior Divisional Officer of Bokito, the Chief physician of the Bokito Hospital, the representative of the Ministry of basic education in Bokito, schools Directors and village leaders. With teachers, parents, legal guardians and school children, another information session was organized to obtain parental informed consents and informed assents.

II. Study site and location

The study was carried out in five public schools near contaminated rivers in different villages, namely Bongando, Ediolomo, Kedia, Yoro 1, and Yoro 2, which are all located in the Bokito subdivision in Cameroon's Centre region. The region was found to be highly endemic for *S. mansoni* infection by previous studies **(Kamdem *et al.*, 2018; Moyou-Somo *et al.*, 2003)**. Briefly, Bokito subdivision, is situated in the Mbam and Inoubou Division, approximately 100 km away north from Yaoundé, the country's political capital (**Figure 10**). It is in an intermediate zone between savannah and forest. The local occupations of the inhabitants include fishing and farming. The villages are watered by many streams mainly Lebomo and Okole, and the socioeconomic situation of these neighborhood villages appears to be quite similar.

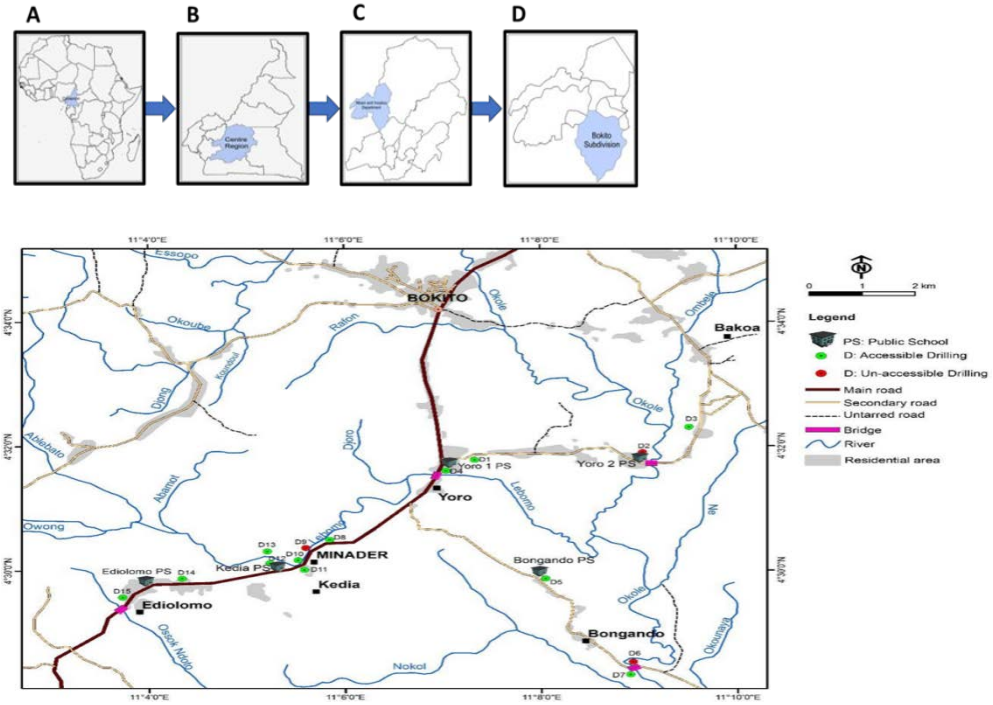


Figure 10: Localization of the study site **(Kamdem, 2020)**

III. Study population and participants

To conduct this study, we have used data and samples from participants enrolled in a larger previous study involving consenting school-aged children from public schools near contaminated rivers in five villages **(Kamdem, 2020)**.

This previous study was cross-sectionally conducted five months after a general mass drug administration (MDA) by the Cameroon National Program for the Control of Schistosomiasis and Soil-Transmitted Helminthiasis (CNPCSSTH) and spanned six months, from September to December 2018 **(Kamdem, 2020)**. The study included school-aged children who had lived in the endemic area for at least six months, were in apparent good health (confirmed by clinical examination), and attended one of the five public schools mentioned. Children showing clinical signs of anemia or testing positive for Hepatitis B/C or Geohelminths were excluded. After administering a questionnaire, participants were screened for schistosomiasis infection using the Kato-Katz (KK) technique and for liver fibrosis using ultrasound (US) **(Kamdem, 2020)**.

In the present research study, which spanned ten months from July 2023 to April 2024, we conducted a retrospective study to investigate the effects of cumulative PZQ treatments (received indefinitely to at least 5 months before the cross-sectional data and sample collections) on *S. mansoni* infection across several domains: infection levels, hemoglobin

levels, liver fibrosis, academic performance, plasma antibody levels, and plasma cytokine levels. Using convenience sampling based on the availability of data, we formed seven groups of participants from the original study who had relevant data on PZQ to address these domains in this nested project (**Figure 11**).

IV. Selection criteria

- → **Inclusion criteria:** All school children attending the aforementioned schools, for whom signed informed consent and assent were obtained, were included in the study.
- → **Non-inclusion criteria:** School children who had resided in the study area for less than six months, or were under medication at the time of the study were not included.
- → **Exclusion criteria**:
    - Children showing clinical signs of anemia or testing positive for Hepatitis B/C or Geohelminths were excluded.
    - School children with missing essential data for analyses or whose collected plasma samples were incomplete were excluded from the study.

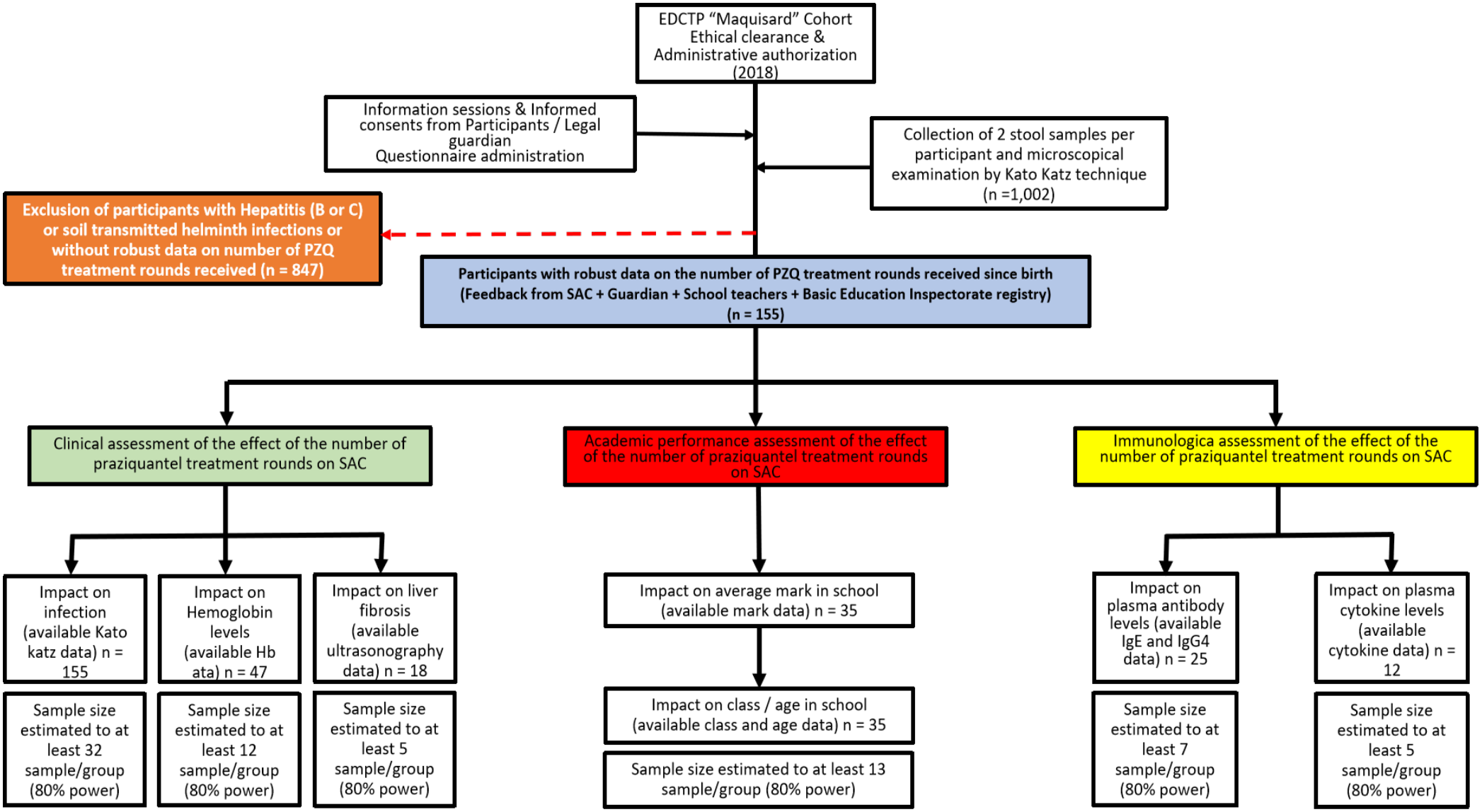

EDCTP "Maquisard" Cohort
Ethical clearance & Administrative authorization (2018)
Information sessions & Informed consents from Participants / Legal guardian
Questionnaire administration
Collection of 2 stool samples per participant and microscopical examination by Kato Katz technique (n =1,002)
Exclusion of participants with Hepatitis (B or C) or soil transmitted helminth infections or without robust data on number of PZQ treatment rounds received (n = 847)
Participants with robust data on the number of PZQ treatment rounds received since birth (Feedback from SAC + Guardian + School teachers + Basic Education Inspectorate registry) (n = 155)
Clinical assessment of the effect of the number of praziquantel treatment rounds on SAC
Academic performance assessment of the effect of the number of praziquantel treatment rounds on SAC
Immunologica assessment of the effect of the number of praziquantel treatment rounds on SAC
Impact on infection (available Kato katz data) n = 155
Impact on Hemoglobin levels (available Hb ata) n = 47
Impact on liver fibrosis (available ultrasonography data) n = 18
Impact on average mark in school (available mark data) n = 35
Impact on plasma antibody levels (available IgE and IgG4 data) n = 25
Impact on plasma cytokine levels (available cytokine data) n = 12
Sample size estimated to at least 32 sample/group (80% power)
Sample size estimated to at least 12 sample/group (80% power)
Sample size estimated to at least 5 sample/group (80% power)
Impact on class / age in school (available class and age data) n = 35
Sample size estimated to at least 13 sample/group (80% power)
Sample size estimated to at least 7 sample/group (80% power)
Sample size estimated to at least 5 sample/group (80% power)

Figure 11: Flow diagram describing the enrolment strategy, examination process and designing of participant groups for each experimental query. For the selection of participants for the assessment of the effect of differential numbers of praziquantel treatment rounds, enrolled participants with provided consent / assent, free from concomitant infections with hepatitis B or C or Geohelminths and presenting with a robust record of their number of PZQ treatment rounds received were selected and their data and samples prepared for downstream analyses. Infection was defined as the presence of *S. mansoni* eggs in the participant's stool, as observed by Kato Katz technique; Liver fibrosis was defined using the liver image pattern (LIP) score. For clinical assessment, participants with PZQ history data were analyzed for the likelihood of having a *S mansoni* infection, as judged by Kato Katz (n=155); probed for the variation in their hemoglobin levels (n = 47) or presence of liver fibrosis (n=41). Cognitive performance depending on the number of rounds of PZQ received was also assessed testing first the average mark of participants depending on their PZQ treatment rounds numbers (n = 35) and seeking for supporting evidence by assessing the class over age ratios distribution in this population (n=35). Finally, immunological assessment of the changes that might be due to the differential numbers of PZQ rounds of treatment received by participants was questioned by either checking the dynamics of the plasma IgE and IgG4 levels (n=25); the plasma cytokine levels for GM-CSF, IL-2, IL-33, IL-4 and VEGF (n=10). Sample size calculation for each experimental query is provided to ensure a minimum power of 80% in these analyses. KK: Kato-Katz, PZQ: Praziquantel, Maquisard: an EDCTP and UK Royal Society funded study on school children from rural Cameroon.

V. Sample size calculation

To ensure robust and statistically powerful analyses, realistic sample sizes for each of the mentioned domains were calculated based on relevant outcomes from previous studies **(Coutinho *et al.*, 2006; Hoekstra *et al.*, 2020; Kasambala *et al.*, 2023b; Ramírez *et al.*, 1996; Sorgho *et al.*, 2017; Yuan *et al.*, 2012).** These sample sizes allowed for the detection of statistically significant effects of repeated PZQ treatment on the targeted outcomes with a high degree of confidence.

The appropriate sample size for each analysis, was determined using the WHO formula **(Lwanga *et al.*, 1991)**. Specifically, a one-sided approach was applied for hypothesis testing for:

- Two population proportions (impact on infection, impact on average mark and class/age in school)

$$n = \backslash frac\left\{\left(Z_{\{1-\alpha\}\sqrt{\{\bar{2}\{P\}(1-\bar{}\{P\})\}}} + Z_{\{1-\beta\}\sqrt{\{P_{1(1-P_1)}+P_{2(1-P_2)}\}}}\right)^2\right\}\{(P_1 - P_2)^2\}$$

Hypothesis testing for two population proportions

- Two population means (impact on Hemoglobin levels, impact on plasma antibody levels, and impact on plasma cytokine levels)

$$n = \backslash frac\left\{2\sigma^{2\left(Z_{\{1-\alpha\}} + Z_{\{1-\beta\}}\right)^2}\right\}\{(\mu_1 - \mu_2)^2\}$$

Hypothesis testing for two population means

- Estimating a sample size with specified relative precision and odd ratio (impact on liver fibrosis). The formula for sample size (n) calculation is as follows:

$$n = \backslash frac\left\{Z^2_{\left\{1-\frac{\alpha}{2}\right\}}\right\}\left\{\left[\backslash log_{e(1-\delta)}\right]^2\right\}\left(\backslash frac\{1\}\left\{P_1^{*(1-P_1^*)}\right\} + \backslash frac\{1\}\left\{P_2^{*(1-P_2^*)}\right\}\right)$$

Estimating a sample size with specified relative precision and odd ratio

We calculated the minimum sample sizes for each group receiving different PZQ treatment schedules using the following assumptions: a significance level of 5% ($\alpha = 0.05$), a 95% confidence level ($z = 1.96$), and a desired power of 80% ($1-\beta = 0.80$). For the fibrosis sample size calculation, a relative precision of 0.96% was assumed. The resulting sample sizes are as follows:

- 32 participants per group for the impact on infection (112/43)
- 12 participants per group for the impact on Hemoglobin levels (29/18)
- 04 participants per group for the impact on liver fibrosis (14/4)
- 05 participants per group for the impact on average mark and class/age in school (27/8)
- 07 participants per group for the impact on plasma antibody levels (7/18)
- 05 participants per group for the impact on plasma cytokine levels (5-6/5-6)

VI. Data collection

1) Sociodemographic data, PZQ treatment, and cognitive performance

These data were investigated and collected at the time of the original project. Each schoolchild was interviewed by a team member, with assistance from a parent or legal guardian and the class teacher. During these interviews, the purpose and methodology of the study were clearly explained. Demographic data such as gender, age, height, weight and frequency of contact with surrounding water were recorded for each schoolchild.

The number of previous PZQ treatments received was documented. To mitigate bias related to potential oversight of the number of PZQ treatments, the information obtained from children was cross-verified with their parents or legal guardians and further confirmed with the local education inspector.

To assess cognitive performance, quarterly average marks were first collected from the class register provided by the teacher counting as the academic performance. Furthermore, each child's age-related grade was modeled as an index of academic progression, considering that age variation within a class due to grade repetition could indicate cognitive abilities **(Sunny *et al.*, 2017)**. This was achieved by recoding the different classes (ranging from SIL to CE2) into numeric scales from 1 to 6, and then calculating the ratio between this recoding and the child's age. These approaches were replicated in all five villages.

2) Blood and stool samples collection and storage

Blood and stool samples were collected during the original project. Whole blood was collected from participants in a private room under aseptic conditions by an experienced phlebotomist, following the procedure described elsewhere **(Hammad *et al.*, 2010).** Specifically, 5ml of blood was drawn using a 21G needle into BD Vacutainer® glass plasma tubes with sodium heparin as an anticoagulant. The samples were stored in coolers with ice packs and transported to Cameroon's National Public Health Laboratory (NPHL) in Yaoundé. In the laboratory, the blood was centrifuged at 3000 rpm for 5 minutes, and plasma samples were aliquoted into 1.5 mL Eppendorf tubes within one hour and stored at -80°C until assays were performed. Sharps containers and well-labeled plastic bags were used for waste disposal.

Two stool samples for the detection of eggs were collected from each participant within a span of 5 days (Kato1 at day 0 and Kato2 at day 5) into a 50 ml pre-labelled and codified screw-cap vial stool container. Subsequently, within three hours, collected stool samples from each of the

five sites were transported to the ImmunoBiology and Helminth Infections (IBHI) unit at the Ministry of Scientific Research and Innovation (MINRESI) in Yaoundé, Cameroon, for parasitological analyses. Upon arrival, sodium azide was mixed with each sample, which was then stored at -20°C for up to 3 days before analysis. This procedure was replicated over the course of two days of stool collection.

VII. Procedure

Except for the ELISA and statistical analysis steps, all the following procedures were originally performed as part of the initial project.

1) Hemoglobin measurement

The Tallquist Hemoglobin Scale was employed to measure hemoglobin levels with meticulous precision following the manufacturer instructions **(Alsayed, 2020)**. Initially, the fingertip was cleansed with an alcohol swab to ensure sterility. A lancet was then used to gently puncture the skin, allowing a drop of blood to form naturally. This drop was carefully absorbed onto an absorbent paper swatch from the Tallquist kit, ensuring an even distribution and avoiding any smearing. After collecting the blood sample, the fingertip was promptly cleaned with a sterile gauze pad to remove any residual blood. An adhesive bandage was then applied to protect the puncture site and prevent contamination.

Once the blood was fully absorbed, the stained paper was compared against the Tallquist Hemoglobin Scale under natural light to maintain accuracy. By aligning the color of the blood stain with the closest match on the scale, the hemoglobin level was determined **(Figure 12).** This process provided a qualitative approximation of hemoglobin concentration. Each measurement was meticulously recorded to ensure that the data was reliable and consistent for further analysis. Throughout the procedure, best practices were adhered to, maintaining the integrity of the results and ensuring the safety of the laboratory environment.

Figure 12: Tallquist haemoglobin scale

2) Parasitological examination of stool sample by Kato Katz technique

The Kato Katz (KK) technique was applied to detect the presence (KK+) or absence (KK-) of parasite eggs. From each stool sample, two KK smears were prepared and independently examined for the detection and quantification of parasites eggs using optical microscopy (Leica Microsystems, DM2000, Germany) at 10X and 40X magnifications, following established protocols **(Borda and Pellegrino, 1971; Katz *et al.*, 1972)**. The prepared KK smears were examined by two experienced laboratory technicians. On a labeled microscopy slide, we used a spatula to transfer 41.7 mg of stool sample on microscopy slide through sieve (square template with 6mm of diameter and 1.5 mm of depth). We pressed a piece of cellophane, which has been soaked overnight in a Kato solution of malachite and glycerol (dilute 0,5g of malachite green powder in 100 ml of distilled water then take 1 ml of malachite stock solution and add to 100 ml 50% of glycerin) on a stool sample; and left for clearing time around 30 to 60 min prior analysis under the light of a microscope. The arithmetic means of eggs counted from the readings (four KK smears per participant) was considered as the participant burden. Additionally, for quality control assessment, 20 randomly selected slides were re-examined by a third laboratory technician for comparison. The eggs counted for each sample were recorded as eggs per gram (EPG), while samples with zero eggs were recorded as negative. The number of *S. mansoni* eggs was counted, recorded, and multiplied by 24 to determine the number of eggs per gram of feces. Infection intensity was classified as light (1–99 EPG), medium (100–399 EPG), or heavy (≥400 EPG) according to the WHO guidelines (WHO, 2022a). **Table 4**

below describes the categorizations of *S. mansoni* infection intensity according to the WHO guidelines.

Table 3: Categorizations of infection intensity for *S. mansoni*.

Schistosoma spp.	Infection categories	Number of eggs	Unit
S. mansoni	Light	1-99	Eggs Per Gram (EPG) of stool
	Moderate	100-399	
	High	≥400	

3) Ultrasonographic examination of participants' abdomen

Participants were examined using a portable ultrasonography device (ultrasound scan Vivid E, General Electric, MEDICAL SYSTEMS, CHINA, CO., LTD, REF 5198983, SN 78856WX2) with convex transducer (SHENZHEN MINDRAY BIO-MEDICAL ELECTRON I CS CO., LTD, SN: AGH86175750) with adjustable frequencies from 2 to 7 Megahertz depending of the corpulence of the participant. All investigations were done by the same radiologist who was unaware of the infection status of the examined participants. The procedure was performed as follows **(Kamdem *et al.*, 2020).** Briefly, participants were placed in the supine position, with the maximum abduction of the right arm to make accessible the right hypochondrium and to increase intercostal space (to improve the acoustic window). After having spread sufficient amount of gel, the probe was place parallel to the intercostal space. To ensure reliable acquisition, a pressure was applied to the probe when scanning the liver. This action decreased tissue thickness between the probe and the ribs. Pathologic lesions associated with *S. mansoni* infection were defined and recorded according to the WHO guidelines on the assessment and quantification of *S. mansoni* morbidity **(Richter *et al.*, 2000)**. Patients with Liver Image Patterns (LIP) grade from C to F was interpreted as positive for liver fibrosis and those with Liver Image Patterns (LIP) grade A or B were considered as negative for liver fibrosis (final score 3 – 14 / LIP C – E) as shown in **Figure 13** below.

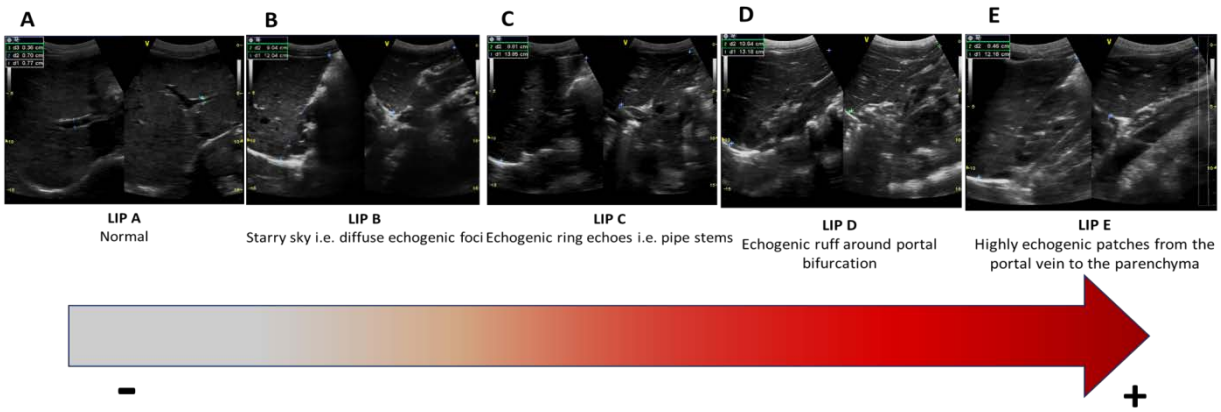


Figure 13: Image patterns with their grades **(Hatz *et al.*, 1992).**

4) Sandwich ELISA test

4.1) Principle

The Sandwich ELISA test adopts a quantitative sandwich enzyme immunoassay technique. A capture antibody specific to the target antibody (Figure 12) or cytokine (Figure 13) is pre-coated onto a microplate. Standards and samples are added to the wells, where the target antibody or cytokine binds to the immobilized capture antibody. After removing unbound substances, a biotin-conjugated antibody specific to the target is added to the wells. Following a wash to remove unbound substances, avidin-conjugated Horseradish Peroxidase (HRP) is added. After another wash to remove unbound avidin-enzyme reagent, a substrate solution is added to each well. Wells containing the target antibody or cytokine, biotinylated detection antibody, and Avidin-HRP conjugate will turn blue. The enzyme-substrate reaction is stopped by adding a stop solution, which turns the color yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of the target antibody or cytokine in the sample. The concentration of antibody or cytokine in the samples can be calculated by comparing the OD of the samples to a standard curve.

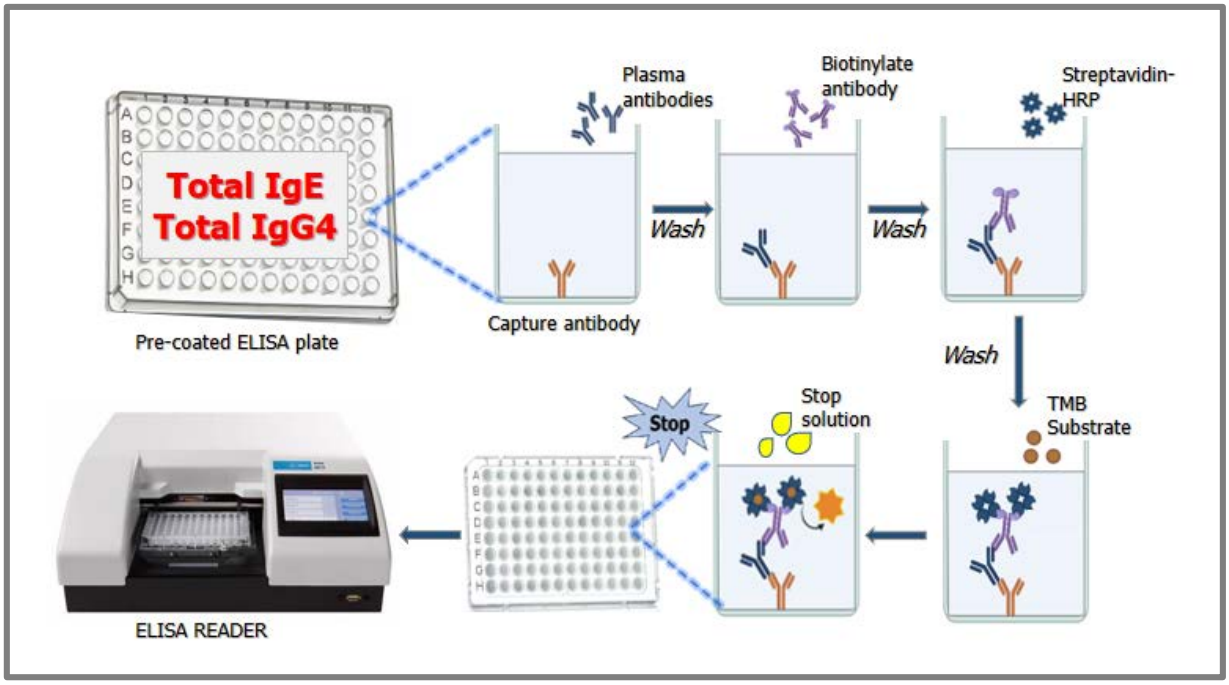


Figure 14: Diagram illustrating the principle of the sandwich ELISA assay for antibodies.

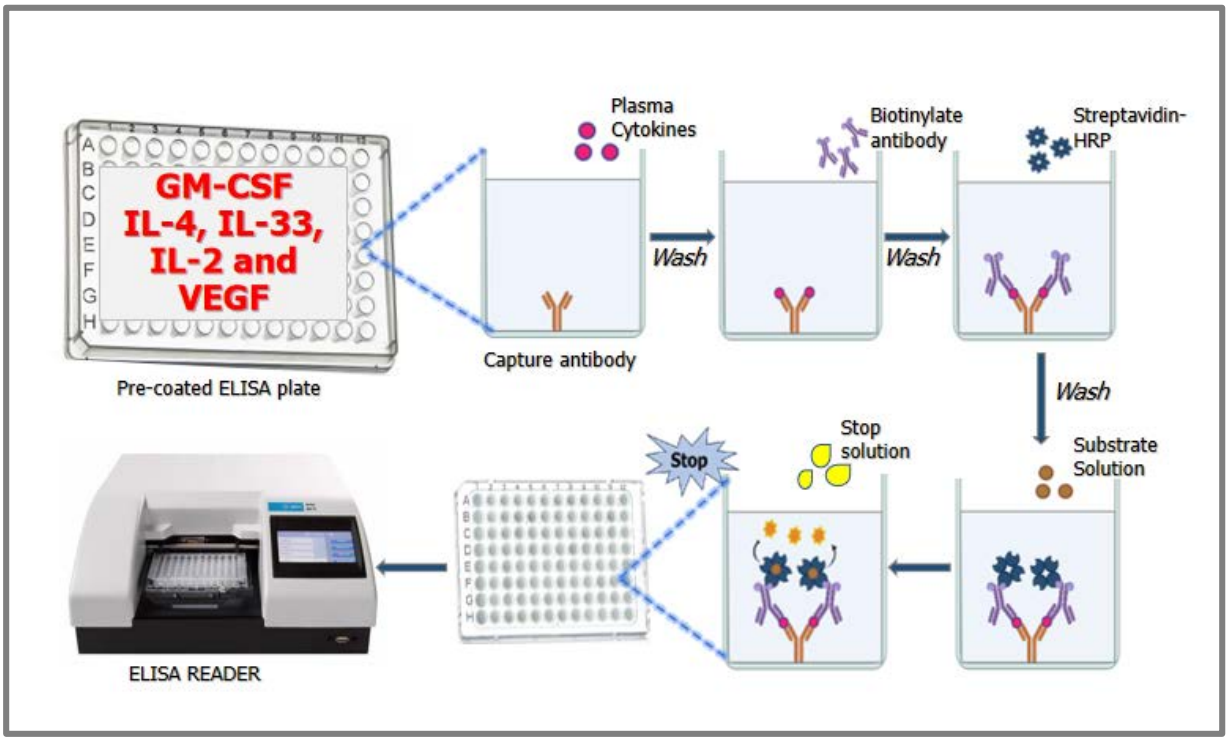


Figure 15: Diagram illustrating the principle of the sandwich ELISA assay for cytokines.

4.2) IgE and IgG4 quantification

Human IgE and IgG4 sandwich ELISA kits (Human IgE ELISA Kit, Catalog Number: EKC31252 and Human IgG4 ELISA Kit, Catalog Number: EKE61042) were used to quantify immunoglobulin levels (total IgE and IgG4) in participants' plasma samples, following the manufacturer's protocol (BIOMATIK). First, all reagents and samples were brought to room temperature and centrifuged after thawing. Reagents, working standards, and samples were prepared according to the manufacturer's instructions. In the pre-coated plate with an anti-isotypic antibody specific to the target antibody, 50 µL of standard and sample were added to the designated well, covered, and incubated for 2 hours at 37°C. After removing the liquid, 50 µL of Biotin-antibody (1x) was added, and the plate was incubated for 1 hour at 37°C. The wells were then washed three times with 200 µL of diluted wash buffer, ensuring complete removal of liquid. Next, 50 µL of HRP-avidin (1x) was added, and the plate was incubated for 1 hour at 37°C. The wash process was repeated five times. Subsequently, 50 µL of TMB Substrate was added, and the plate was incubated for 15-30 minutes at 37°C, protected from light. Finally, 50 µL of Stop Solution was added, and the plate was gently tapped to mix. The optical density (OD) was determined within 5 minutes using a microplate reader set to 450 nm. For result calculation, professional software like "Curve Expert" was used to generate a standard curve. This procedure ensures accurate measurement of immunoglobulin levels using the ELISA kits.

4.3) Cytokines quantification

Human GM-CSF Sandwich ELISA Kit (RayBiotech; LOT: 1009200267), IL-4 Sandwich ELISA Kit (Proteintech, Barcode: 20005524, Cat No: KE00034), IL-2 Sandwich ELISA Kit (Proteintech, Barcode: 40001524, Cat No: KE00017), VEGF Sandwich ELISA Kit (Proteintech, Barcode: 40001452, Cat No: KE00216), and IL-33 Sandwich ELISA Kit (BioLegend, Inc. USA Cat No: 435907) were used to evaluate cytokine concentrations in participants' plasma samples according to the manufacturers' protocols. All reagents and samples were brought to room temperature, and samples were centrifuged after thawing. Standards and samples were prepared and assayed in duplicate. Following the specific kit instructions, 100 µL of standards and samples were added to designated wells pre-coated with the capture antibody, incubated, and washed as required. Detection antibodies and enzyme conjugates were added sequentially, with incubation and washing steps between additions. Substrate solution was added, and the reaction was stopped after the recommended incubation period. The optical density (OD) of each well was measured using a microplate reader set to

450 nm. Standard curves were generated under the same experimental conditions, and these curves were used to determine the cytokine concentrations in the plasma samples. All assayed samples fell within the detection range, ensuring accurate quantification of cytokines.

VIII. Statistical analysis

Excel software was initially used to manually enter all the collected data. A laboratory team member then cross-checked the generated database for potential mistakes (duplications, omissions, errors, etc.). RStudio (version 4.3.1) was subsequently used to perform statistical analyses, and GraphPad Prism (version 10.1.2.324) was used to plot graphs (https://www.graphpad.com/).

Data were summarized using descriptive measures such as means, medians, frequencies, and percentages. The normality of the data was initially checked using the Shapiro-Wilk test. Comparisons between two groups were performed using independent Student's t-tests or their non-parametric equivalent, the Mann-Whitney test. Similarly, ordinary one-way ANOVA or Kruskal-Wallis tests were used for multiple group comparisons depending on the distribution of the data, followed by the Tukey post-hoc test. To evaluate correlations between two continuous variables, Pearson correlation tests were used for normally distributed data and Spearman correlation tests for non-normally distributed data.

The thresholds for severe morbidity were defined based on data from existing literature for prediction analysis **(Alsayed, 2020; Ezeamama *et al.*, 2018; Kamdem, 2020; Sunny *et al.*, 2017; WHO, 2022a)**. Multivariate logistic regressions were therefore conducted to assess the predictive potential of cumulative PZQ treatments on various outcomes, including *S. mansoni* infection (positive KK tick smear), high infection burden (greater than 100 EPG of feces), high hemoglobin levels (greater or equal to 11 g/dL), infection with fibrosis (positive KK tick smear and abdominal ultrasound examination), good academic performance (average mark ≥ 12), and good academic progression (class/age ≥ 0.50). Each model included covariates such as age, sex, BMI, and contact with contaminated water to control for potential confounding factors. For all of the above analysis, a p-value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

CHAPTER III: RESULTS AND DISCUSSION

I. RESULTS

1) Demographic characteristics of the study participants

Six experimental queries were defined to analyze the impact of the cumulative number of PZQ treatments on: infection rate, hemoglobin levels, liver fibrosis, average mark, class/age ratio, plasma antibody levels, and plasma cytokine levels. Table 4 presents the sociodemographic characteristics of the study participants, including age, sex ratio, BMI, and daily frequency of contact with contaminated water, across two groups based on PZQ treatment (0-2 PZQ and >2 PZQ).

Across all six experimental queries, a total of 292 school-aged children, aged 5 to 18 years, were sampled. They were divided into two groups based on the cumulative number of PZQ treatments received since birth (0-2 PZQ and >2 PZQ) as shown in Table 4. The data indicate that age, gender, BMI, and water contact frequency remained relatively consistent across the treatment groups for most analyses (p-value > 0.05, Table 4). However, participants with >2 PZQ treatments were significantly older than those with 0-2 PZQ in the analysis of PZQ's impact on infection (p=0.01, Table 4). These results suggest that the sociodemographic characteristics were well-balanced across the PZQ treatment groups, facilitating a fair comparison in subsequent analyses.

Table 4: Social demographic characteristics of the participants

Variables / **PZQ groups**	**Age in years:** Mean (SD)		**Sex ratio** (Male / Female)		**BMI** Mean (SD)		**Freq contact with water/day** Mean (SD)	
Impact on infection (available Kato Katz data) n = 155								
0-2 PZQ (112)	9.23 (1.79)	**p=0.01**	68/44	p=0.46	15.31 (1.37)	p=0.15	1.85 (0.76)	p=0.57
> 2 PZQ (43)	10.00 (1.66)		23/20		15.67 (1.29)		1.93 (0.73)	
Impact on Hemoglobin levels (available Hemoglobin data) n = 47								
0-2 PZQ (29)	9.86 (1.48)	p=0.67	18/11	p=0.68	15.85 (1.44)	p=0.91	2.04 (0.90)	p=0.13
> 2 PZQ (18)	10.17 (1.65)		8/10		15.96 (1.05)		1.72 (0.66)	
Impact on liver fibrosis (available ultrasonography data) n = 18								
0-2 PZQ (14)	10.36 (2.02)	p=0.12	8/6	p>0.99	16.17 (1.46)	p=0.34	2.14 (0.86)	p=0.44
> 2 PZQ (4)	12.25 (2.36)		3/1		17.00 (1.59)		1.75 (0.95)	

SD: Standard Deviation; BMI: Body Mass Index; Freq: Frequency; n: number of participants; PZQ: Praziquantel; p: p-value.

Table 4: Social demographic characteristics of the participants

Variables / **PZQ groups**	**Age in years:** Mean (SD)		**Sex ratio** (Male / Female)		**BMI** Mean (SD)		**Freq contact with water/day** Mean (SD)	
Impact on average mark and class/age in school (available mark data) n = 35								
0-2 PZQ (27)	9.81 (2.05)	p=0.93	17/10	p=0.68	15.85 (1.63)	p=0.91	2.29 (0.91)	p=0.44
> 2 PZQ (8)	9.75 (1.98)		4/4		15.78 (1.86)		2 (0.92)	
Impact on plasma antibody levels (available IgE and IgG4 data) n = 25								
0-2 PZQ (7)	10.28 (2.36)	p=0.91	3/4	p=0.90	16.07 (1.65)	p=0.82	2.28 (0.95)	p=0.20
> 2 PZQ (18)	10.22 (1.62)		9/9		16.01 (1.05)		1.77 (0.64)	
Impact on plasma cytokine levels (available cytokine data) n = 12								
0-2 PZQ (6)	10.00 (2.44)	p=0.49	3/3	p=0.54	16.16 (1.79)	p=0.84	2.50 (0.83)	p=0.34
> 2 PZQ (6)	10.83 (1.47)		5/1		16.34 (1.30)		2.00 (0.89)	

SD: Standard Deviation; BMI: Body Mass Index; Freq: Frequency; n: number of participants; PZQ: Praziquantel; p: p-value.

2) Effect of repeated PZQ treatments on clinical phenotype

To assess the impact of cumulative PZQ treatment on clinical outcomes, we examined how the number of PZQ treatments received since birth affects *S. mansoni* infection, infection load (measured as eggs per gram of feces, EPG), plasma hemoglobin levels and liver fibrosis (see Figure 16). To meet the conditions for Fisher's Z test, we initially defined three treatment groups, which were later consolidated into two groups for the Mann-Whitney U tests.

Clinically, the percentage of participants infected with *S. mansoni* did not vary significantly across the defined three ranges of received PZQ treatment since birth ($p = 0.58$; Figure 16A). However, participants who received 0–2 PZQ treatments had a significantly higher percentage of moderate infections compared to those who received more than 2 treatments ($p = 0.01$; Figure 16B). Additionally, SAC who had received a higher number of PZQ treatments since birth were less likely to have a high parasite egg burden ($r = -0.33$, $p = 0.04$; Figure 16C). Regarding the effect of PZQ treatment on plasma hemoglobin levels, our study found that an increased number of PZQ treatments since birth was associated with higher hemoglobin levels ($p = 0.004$; $r = 0.41$, $p = 0.003$; Figures 16D and E). Lastly, when evaluating the relationship between PZQ treatment and *S. mansoni* infection associated with liver fibrosis, our results showed no significant difference between participants who received 0-2 PZQ treatments and those who received more than 2 treatments ($p = 0.31$; Figure 16F).

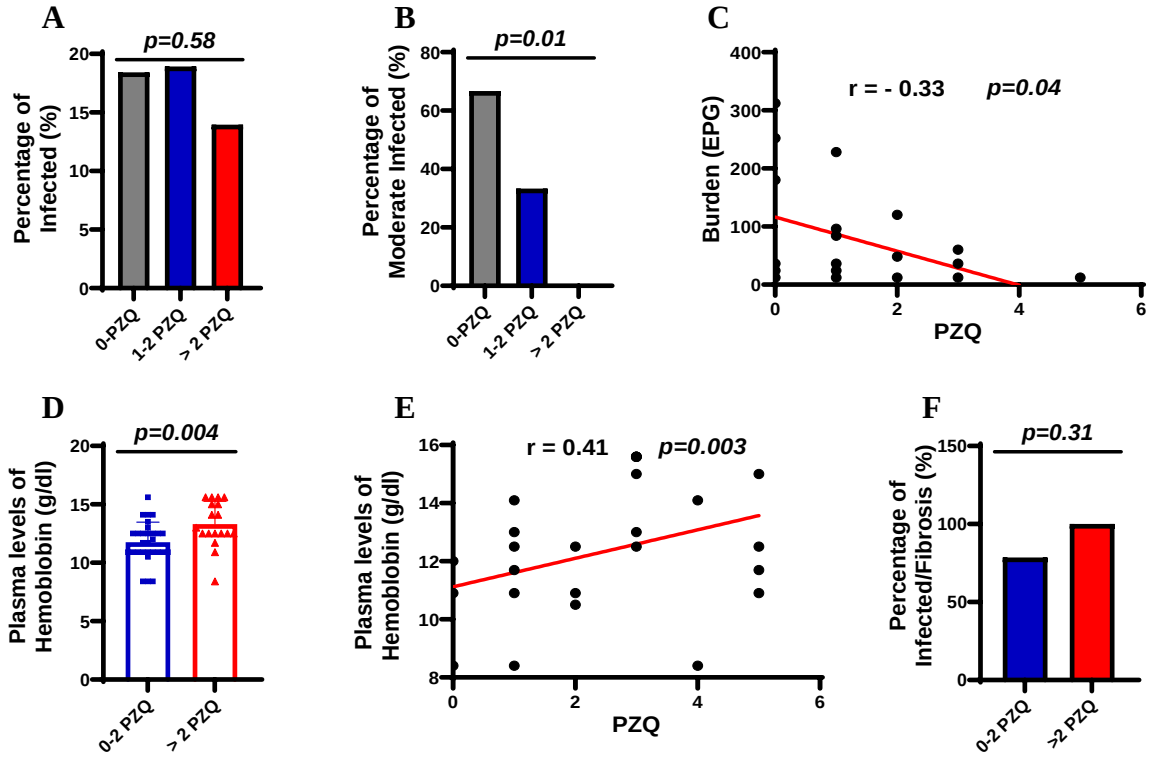


Figure 16: Clinical phenotyping of SAC from schistosomiasis endemic area with history of different numbers of rounds of PZQ treatments. **(A)** Frequencies of all infections with *S. mansoni* across participants with increasing numbers of PZQ treatment rounds. **(B)** Frequencies of higher burden infections with *S. mansoni* (burden ≥ 100 EPG) across participants with increasing numbers of PZQ treatment rounds. **(C)** Spearman correlation between the number of PZQ treatment rounds and the egg burden in all *S. mansoni*-infected participants. **(D)** Average hemoglobin levels in participants with increasing numbers of PZQ treatment rounds, measured using the Kemtec® Tallquist Hemoglobin Scale test paper. **(E)** Spearman correlation between the number of PZQ treatment rounds and hemoglobin levels in SAC. **(F)** Frequencies of *S. mansoni*-infected participants with liver fibrosis distributed by increasing numbers of PZQ treatment rounds. For comparisons of frequencies between groups, the chi-square test for trend (Z test) was applied, and the p-value **(p)** was extracted and displayed for each comparison (A, B, and F). For Spearman correlations, assuming a non-parametric distribution, the correlation coefficient **(r)** and the p-value are shown for each correlation plot (C and E). The simple linear regression line (in red) was plotted to illustrate the overall trend for each correlation plot (C and E). For bar graph comparisons, a non-parametric Mann-Whitney U test was performed (D). **EPG**: Egg Per Gram of feces.

To validate our findings and identify potential risk factors for specific phenotypes, we performed a multivariate logistic regression analysis, examining how the number of PZQ treatments predicts infection rates, infection burden, hemoglobin levels, and fibrosis, while controlling for sociodemographic factors such as age, gender, BMI, and frequency of contact with contaminated water (see Table 5 for a summary).

After accounting for sociodemographic factors, PZQ treatment was not a significant predictor of lower infection rates (AOR = 0.76; p = 0.11, Table 5) or infections associated with fibrosis (AOR = 1.73; p = 0.46, Table 5). However, it did significantly reduce the odds of heavy infection, defined as an egg burden of more than 100 eggs per gram (AOR = 0.16; p = 0.05, Table 5). Additionally, PZQ treatment was associated with higher odds of elevated hemoglobin levels, defined as hemoglobin greater than 11 g/dl (AOR = 2.58; p = 0.03, Table 5). Factors such as age, sex, BMI, and frequency of contact with contaminated water did not significantly influence the odds of severe infection or elevated hemoglobin levels (see Table 5).

Table 5: Assessment of risk factors predisposing to *S. mansoni* infection (positive to KK stick smear), high burden (burden higher that 100 EPG of feces), and infection with fibrosis (positive for both KK stick smear and US examination of abdominal).

	Clinical phenotype assessment of the effect of the number of praziquantel treatment rounds on SAC							
	Predicted probability							
	Being infected		Being heavy infected		Higher haemoglobin		Being infected with liver fibrosis	
Factors	AOR (95% CI)	p-value	AOR (95% CI)	p-value	AOR (95% CI)	p-value	AOR (95% CI)	p-value
Number of PZQ	0.76 (0.53-1.04)	p=0.11	0.16 (0.01-0.61)	**p=0.03**	2.58 (1.22-8.05)	**p=0.03**	1,73 (0.45-14.53)	p=0.46
Age in years	1.31 (1.00-1.74)	**p=0.05**	1.25 (0.50-3.45)	p=0.61	1.88 (0.95-5.16)	p=0.10	0.91 (0.34-2.68)	p=0.86
Gender (Male)	1.15 (0.45-3.04)	p=0.77	1.21 (0.05-23.58)	p=0.89	0.79 (0.11-4.72)	p=0.80	0.28 (0.05-5.61)	p=0.42
Frequency of contacts with water/Day	0.69 (0.37-1.27)	p=0.25	0.25 (0.02-1.26)	p=0.15	0.47 (0.12-1.38)	p=0.20	1.28 (0.21-8.99)	p=0.77
Body Mass index (BMI)	1.38 (0.96-2.05)	p=0.09	2.24 (0.73-11.35)	p=0.22	0.58 (0.24-1.34)	p=0.20	1.36 (0.32-6.89)	p=0.66

Heavily infected: burden higher than 100 EPG; Higher haemoglobin: Haemoglobin level higher than 11 g/dl, EPG: Eggs Per Gram; AOR: Adjusted Odd Ratio; PZQ: Praziquantel; 95% CI: 95% Confidence Interval.

3) Effect of repeated PZQ treatment on cognitive performance.

To evaluate the influence of varying cumulative numbers of PZQ treatments on children's cognitive performance, we first explored the correlation between either average mark or class/age ratio and the number of PZQ treatments. We found a moderate correlation between more rounds of PZQ treatment and academic progress, as measured by the class/age ratio ($r = 0.33$, $p = 0.02$; Figure 17D). However, the correlation with academic performance when measured as average mark was not significant ($r = 0.24$, $p = 0.08$; Figure 17A).

To further explore the influence of cumulative treatments and uncover hidden trends, we considered two treatment groups of PZQ to observe potential variations in cognitive performance. Our analysis showed a significant increase in cognitive performance, measured by both average mark and class/age ratio, among SAC who received more than two PZQ treatments compared to those with up to two PZQ ($p = 0.02$ and $p = 0.01$; Figure 17B and E).

Continuing our investigation, we conducted a multivariate logistic regression analysis, adjusting for potential biases related to sociodemographic factors such as age, sex, BMI, and water contact frequency (Table 6). The results showed that the number of PZQ treatments was a good predictive indicator for both academic performance, defined as an average mark of 12/20 or higher (AOR = 2.39, $p = 0.05$, Table 6), and academic progression, defined as a class/age ratio greater than 0.50 (AOR = 2.33, $p = 0.03$, Table 6).

We then compared cognitive performance, measured by average mark and class/age ratio, across different infection burden categories: uninfected (0 EPG), lightly infected (1-99 EPG), and moderately infected (100-399 EPG) (Figure 17C and F). Uninfected and lightly infected SAC showed significantly better academic performance and progress compared to those moderately infected ($p = 0.009$ and $p = 0.003$ for average marks; Figure 17C, and $p = 0.003$ for class/age ratio; Figure 17F).

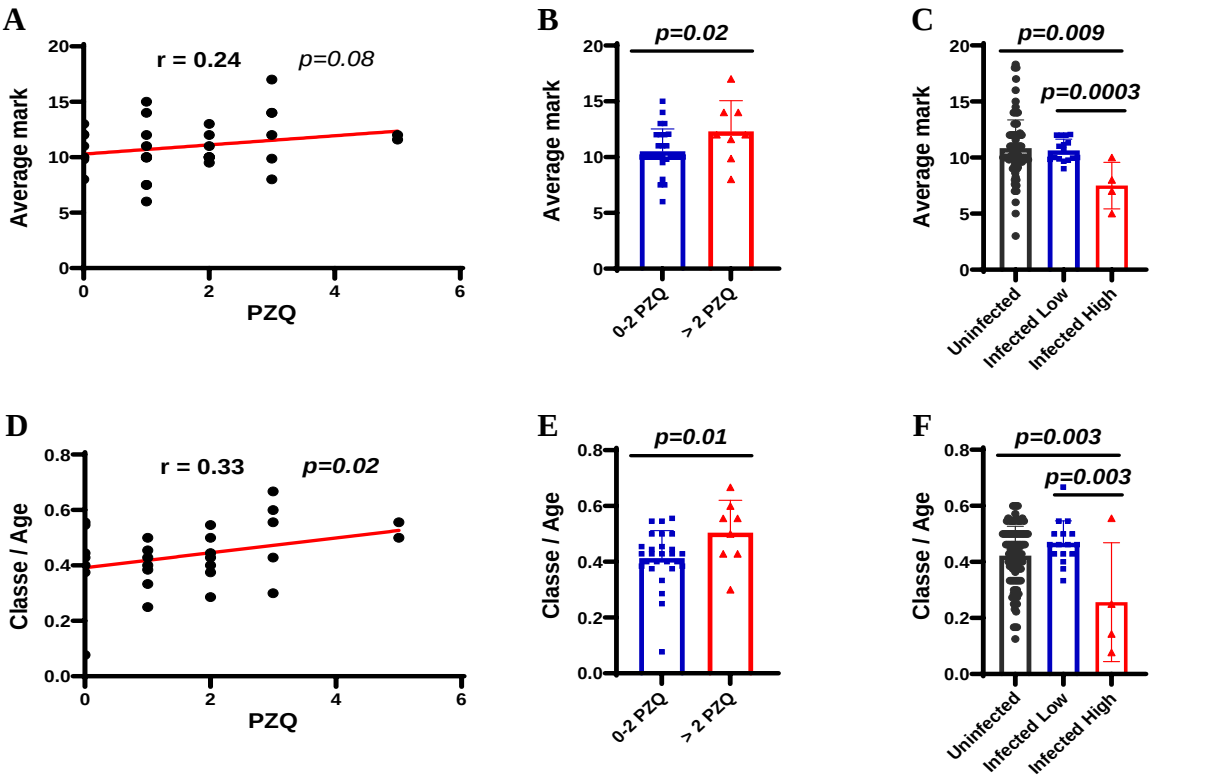


Figure 17: Academic Performance of SAC from schistosomiasis endemic area with history of different numbers of rounds of PZQ treatments. **(A)** Spearman correlation between numbers of rounds of PZQ treatments and the average mark of SAC. **(B)** Average marks of participants with increasing numbers of rounds of PZQ treatments. **(C)** Average mark of participants with increasing grade of *S. mansoni* infection. **(D)** Spearman correlation between numbers of rounds of PZQ treatments and the class/age ratio of SAC. **(E)** Class/age ratio of participants with increasing numbers of rounds of PZQ treatments. **(F)** Classe/age ratio of participants with increasing grade of *S. mansoni* infection. For Spearman correlation, assuming non-parametric distribution, the correlation coefficient **(r)** and the p-value **(p)** are shown for each correlation curve. For bar graph comparisons, a non-parametric Mann-Whitney U test was performed. **Uninfected:** 0 EPG. **Infected Low:** 1-99 EPG. **Infected High:** 100-399 EPG. **EPG**: Eggs per gram of stool.

Table 6: Assessment of risk factors predisposing to *S. mansoni* cognitive impairment

	Clinical phenotype assessment of the effect of the number of praziquantel treatment rounds on SAC			
	Predicted probability			
	Risk factors of better academic performance (Average mark>=12)		Risk factors of unimpaired academic progression (Class/Age > 0.50)	
Factors	AOR (95% CI)	p-value	AOR (95% CI)	p-value
Number of PZQ	2.39 (1.11-7.09)	**p=0.05**	2.33 (1.18-6.16)	**p=0.03**
Age in years	0.24 (0.00-0.59)	**p=0.01**	0.80 (0.47-1.30)	p=0.39
Gender (Male)	0.19 (0.018-1.42)	p=0.12	1.13 (0.20-7.34)	p=0.88
Frequency of contacts with water/Day	3.81 (0.93-24.15)	p=0.09	2.42 (0.77-9.16)	p=0.14
Body Mass index (BMI)	1.13 (0.52-2.74)	p=0.69	0.97 (0.51-1.81)	p=0.93

AOR: Adjusted Odd Ratio; PZQ: Praziquantel; 95% CI: 95% Confidence Interval.

4) Effect of repeated PZQ treatments on the host immune responses.

To investigate the underlying immune modulation in biomarker profiles following cumulative PZQ treatments, we first measured plasma levels of key cytokines and growth factors involved in innate immunity, including Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) **(Becher *et al.*, 2016)**, Interleukin-2 (IL-2) **(Ross and Cantrell, 2018)** , and Vascular Endothelial Growth Factor (VEGF) **(Friedman, 2008)**. No significant differences were observed in GM-CSF and IL-2 levels across the different PZQ treatment groups ($p = 0.46$ and $p = 0.41$, respectively; Figures 18A and 18B). However, children who received multiple rounds of PZQ treatment exhibited significantly higher plasma levels of VEGF ($p = 0.04$; Figure 18C), suggesting a potential role of VEGF in modulating the immune response following cumulative PZQ treatments.

To further characterize the immune profile in SAC who underwent repeated PZQ treatment, we measured plasma levels of Th2-associated cytokines implicated in schistosomiasis activity, specifically Interleukin-33 (IL-33) **(Kamdem *et al.*, 2019)** and Interleukin-4 (IL-4) **(Medhat *et al.*, 1998)**. Children who received increased rounds of PZQ treatment showed significantly elevated plasma levels of IL-33 ($p = 0.03$; Figure 18D) and IL-4 ($p = 0.04$; Figure 18E), suggesting a heightened type-2 immune response.

Moreover, we measured Th2-driven protective immunoglobulin IgE **(Dutra *et al.*, 2002)** and regulatory T-cell-associated IgG4, which is linked to susceptibility **(Satoguina *et al.*, 2008)**. Both IgE and IgG4 levels were significantly elevated in participants with more frequent PZQ treatments ($p = 0.002$ and $p = 0.02$, respectively; Figures 18F and 18G). Importantly, the IgE/IgG4 ratio, a marker of Th2-skewed immunity **(Sousa-Figueiredo *et al.*, 2012)**, was markedly increased in participants receiving more than two PZQ treatments ($p = 0.03$, Figure 18H).

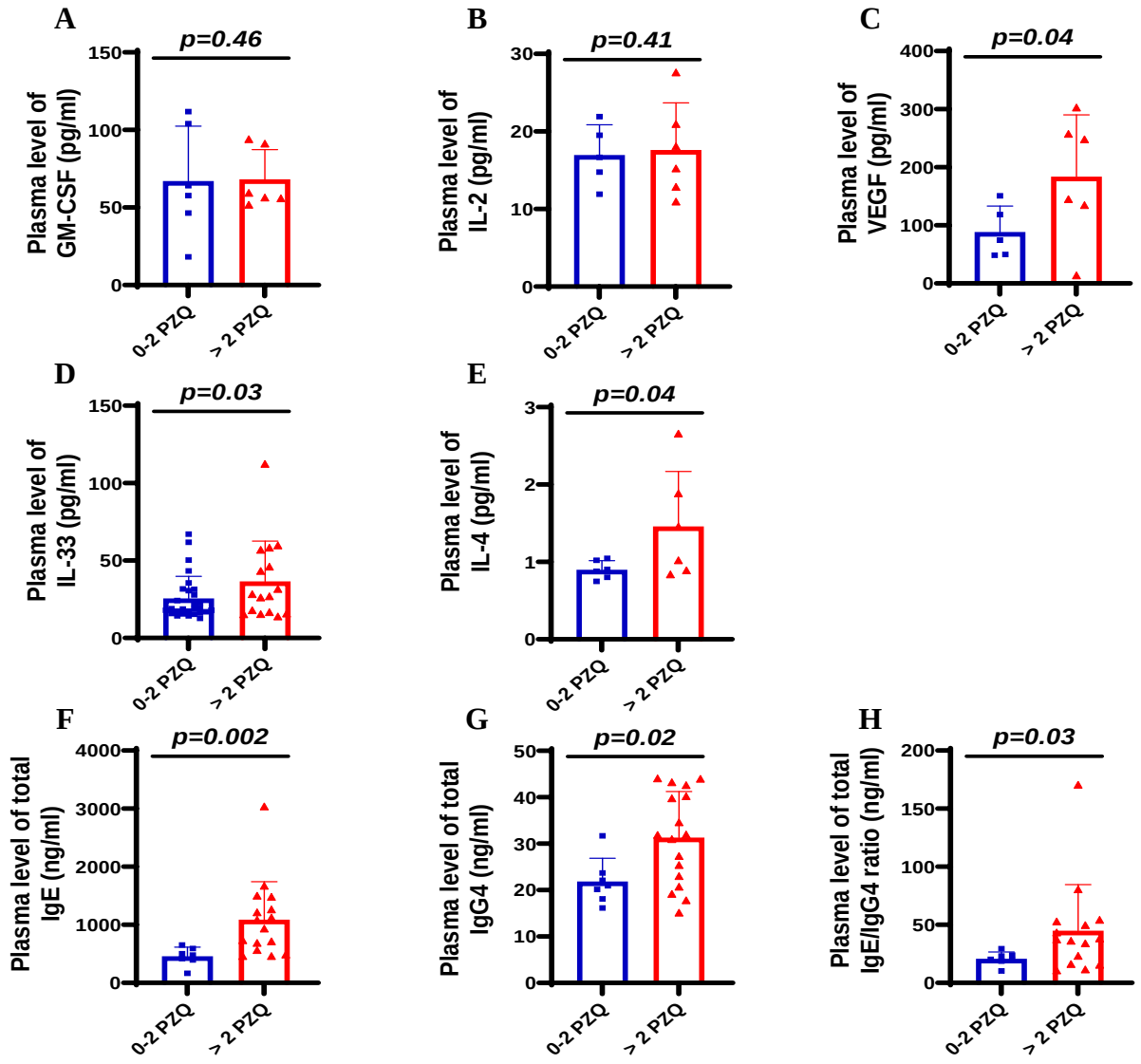


Figure 18: Immunological profiling of SAC from schistosomiasis endemic area with history of different numbers of rounds of PZQ treatments. Comparison of **(A)** plasma levels of the innate myeloid canonical cytokine GM-CSF, **(B)** the adaptive canonical cytokine IL-2 and **(C)** the angiogenic profibrotic factor VEGF between SAC depending on the number of rounds of PZQ treatments received, to explore the differential bases of altered susceptibility to reinfection and liver fibrosis onset. **(D)** Comparison of the plasma levels of the alarmin IL-33, negatively associated with *S. mansoni* infection / reinfection (ref) between SAC depending on the number of rounds of PZQ treatments received. **(E)** Comparison of the plasma levels of the Th2 cytokine IL-4 between SAC depending on the number of rounds of PZQ treatments received. Comparison of **(F)** total IgE, **(G)** total IgG4 and **(H)** the resulting IgE/IgG4 ratios, as indicators of protection against reinfection between SAC depending on the number of rounds of PZQ treatments received. Each graph represents the mean with standard deviation. Statistical differences were determined by non-parametric Mann-Whitney U testing with significance at $p<0.05$. **GM-CSF**: Granulocyte-macrophage colony-stimulating factor; **IL**: Interleukin; **VEGF**: Vascular Endothelial Growth Factor.

II. DISCUSSION

Schistosomiasis, second only to malaria in terms of morbidity among tropical diseases, is predominantly marked by complex pathological alteration of the host. These alterations are reported to impact host immunity and foster deleterious processes such as anemia, tissue fibrosis and cognitive disorders, especially in school-age children **(McManus *et al.*, 2018)**. The diseases remain widely spread globally, with a higher propensity of occurrence in tropical areas, particularly in sub-Saharan Africa. Schistosomiasis is therefore an acknowledged public health issue but remains difficult to control. In order to eliminate the disease as a public health problem by 2030, the WHO advocates for regular annual treatment cycles in endemic regions by administration of PZQ **(WHO, 2022a)**. Sustained annual administration of PZQ to SAC has considerably lowered the disease prevalence over the last 50 years **(WHO, 2020)**, owing to the drug ability to cripple the parasite adults' worms and foster killing by the infected host immune system **(Oettle *et al.*, 2023)**. Since regular treatment of at-risk populations with PZQ remains the core global strategy to control the disease, with no vaccine available to date, there is a pressing need to comprehensively understand what the overall benefits and limitations of regularly receiving PZQ are for these SAC.

Preclinical work in this area reported a reduced host susceptibility to reinfection when exposed to schistosomes after receiving several rounds of PZQ treatments and being constantly exposed to the parasite. However, no amelioration of the host fibrogenic response was observed against trapped eggs in the infected liver of mice even after multiple rounds of PZQ treatments **(Nono *et al.*, 2021)**. This raises an important question: how do multiple rounds of annual PZQ treatment, as implemented in national control programs, influence the human host's response to schistosomes? Specifically, this study aimed to investigate the impact of varying numbers of cumulative PZQ treatments on *S. mansoni* infection in SAC, focusing on egg burden, hemoglobin levels, liver fibrosis, cognitive function, and immunopathology.

To achieve this objective, we categorized individuals into two treatment groups: those who received up to two doses of PZQ and those who received more than two doses. This grouping was inspired partially by a study in mice, which found that the third treatment for *S. mansoni* infection significantly impacted health outcomes **(Nono *et al.*, 2021)**. Additionally, our awareness of field treatment practices influenced this clustering. In endemic areas, school-age children often begin receiving treatment from the age of 4 to 5 years, with a median treatment frequency of approximately three **(Stothard *et al.*, 2011)**.

To ensure that downstream effects could be unequivocally attributed to PZQ treatment, we compared sociodemographic data across different PZQ treatment groups. There were no significant differences in age, sex, BMI or the frequency of contact with contaminated water across the PZQ groups, regardless of the analysis axis. This strengthens the basis for attributing observed health outcomes and immune response solely to the effects of repetitive treatment over time, as sociodemographic factors broadly influence global health outcomes **(Mbanefo *et al.*, 2014; Meurs *et al.*, 2013)**.

The analysis of data from this nested project, addressing the first objective related to the clinical aspects of repetitive treatment, revealed that SAC who had received more cumulative PZQ since birth were less likely to have a higher parasite egg burden, but they remained susceptible to infection. These findings align with previous studies, suggesting that while PZQ treatment alone may not fully eliminate the infection or induce sterile immunity, it can improve parasite clearance and/or reduce the host's susceptibility to infection **(van den Biggelaar *et al.*, 2002; Wilson *et al.*, 2011)**. Although an increased number of cumulative PZQ treatments correlate with higher hemoglobin levels, no significant association was found between the number of PZQ treatments and the onset of liver fibrosis. This suggests that while PZQ treatment effectively reduces the worm burden, thereby mitigating blood consumption by adult worms and improving hemoglobin levels, it has a limited ability to prevent or reverse the fibrosis processes. These findings are consistent with research using mouse models, which demonstrated that the fibrosis process continued to develop even in mice undergoing multiple cycles of infection and treatment **(Nono *et al.*, 2021)**. Additionally, an in-vivo trial aimed at investigating the antifibrotic properties of PZQ replicated both early-stage and established fibrosis, finding that PZQ has the potential to reduce the development of fibrosis by half. However, it was ineffective in reversing the fibrosis once it was firmly established **(Nono *et al.*, 2020)**. The persistent trend of fibrosis observed in our study, despite multiple rounds of PZQ treatment, suggests that while frequent treatments in hosts exposed to parasitic eggs may prevent the onset of fibrosis by limiting egg deposition, once the eggs are deposited, additional PZQ treatments do not prevent further fibrosis development.

Additionally, logistic regression models indicated that the number of PZQ treatments could serve as a reliable predictor for a low infection load of *S. mansoni* and higher hemoglobin levels. This effect was not affected by the participants' ages, gender, body mass index or frequency of contact with infested water. However, PZQ did not predict significantly the absence of infection and infection with fibrosis due to *S. mansoni* infection after adjusting for sociodemographic

characteristics of the study participants. This constitute an additional argument previously report in the literature that the treatment itself is insufficient to preclude further reinfection and fibrosis while effectively reduce the reinfection load **(Nono *et al.*, 2021)**. Therefore, establishing the number of PZQ treatments as a mean of predicting the infection burden status of school-aged children in *S. mansoni* endemic areas depends on considering sociodemographic parameters.

The second objective of our study was to evaluate the influence of cumulative PZQ treatments on cognitive performance (academic performance and academic progress) in SAC susceptible to *S. mansoni* infection. Our findings revealed a positive correlation between the number of PZQ treatments and cognitive performance, particularly when measured as the class/age ratio. We also noted a significant improvement in academic performance and progress, measured by quarterly school grades and class/age ratio, in participants who had received more than two PZQ treatments. This result was further confirmed by logistic regression, showing that PZQ increased the odds of achieving an average mark of 12 or higher by 2.39 times, and the odds of having a class/age ratio of at least 0.5 by 2.33 times, regardless of sociodemographic factors. Additionally, children with higher cognitive performance either had light infections or were completely free of *S. mansoni* infection. These findings are consistent with a previous study on *S. japonicum* infection, which found that school-aged children who were cured after treatment performed better on cognitive tests, including those assessing learning and memory, compared to those who remained infected **(Ezeamama *et al.*, 2012)**. The improvement in cognitive performance is likely related to a reduction in reinfection intensity after cumulative PZQ. This alleviates physical discomfort and reduces distractions caused by the presence of worms, anemia, and systemic inflammation. **(Ezeamama *et al.*, 2018; Hoekstra *et al.*, 2020; Kasambala *et al.*, 2023b)**. Given the well-established relationship between cognitive and academic performance and their mutual influence **(Peng and Kievit, 2020; Stockard *et al.*, 2018; Van Der Maas *et al.*, 2006)**, our measures of academic performance and progress likely reflect cognitive performance, which has been shown to be significantly impaired during schistosomiasis infections **(McManus *et al.*, 2018)**.

The final objective of this work aimed to gain deeper insight into the immunological profile underlying the observed phenotype following repeated treatment. Our findings revealed no significant differences in plasma levels of GM-CSF, and IL-2 between the treatment groups, indicating that cumulative PZQ treatments do not substantially affect cytokine production from innate immune cells **(Becher *et al.*, 2016; Ross and Cantrell, 2018)**. This aligns with previous

studies reporting that innate immunity plays a limited role in mediating protection against *S. mansoni* reinfection **(Joseph *et al.*, 2004; Wilson *et al.*, 2011).** The lack of significant changes in these cytokines suggests that PZQ's immune-modulatory effects are predominantly exerted through adaptive rather than innate immunity. However, the observed elevation of VEGF in participants receiving more PZQ treatments may reflect an angiogenic response to tissue repair processes triggered by schistosome-induced damage. VEGF has been implicated in schistosomiasis-associated fibrosis, where it plays a dual role by promoting tissue repair while also contributing to pathological angiogenesis and fibrotic processes **(Friedman, 2008; Tawfeek *et al.*, 2003)**. The persistent elevation of VEGF observed in our study further supports the sustained fibrotic trend in SAC, even after multiple rounds of PZQ treatment.

In the adaptive immune compartment, our study underscores a significant enhancement of Th2-associated responses, particularly marked by elevated plasma levels of IL-33 and IL-4, in participants with higher cumulative PZQ treatments. IL-33, an alarmin cytokine released during tissue injury, has been shown to promote Th2 polarization and enhance immunity against *S. mansoni* **(Kamdem *et al.*, 2018)**. Conversely, its depletion in chronically infected individuals may result from parasite-driven suppression or early utilization during infection **(Kamdem *et al.*, 2019)**. The significant elevation of IL-33 in children receiving repeated PZQ treatments suggests the restoration of this cytokine's regulatory role in tissue repair and Th2 immune activation post-treatment. Similarly, the increase in IL-4, a multifunctional cytokine crucial for Th2 polarization, likely reflects an adaptive response that facilitates immunity **(Punnonen *et al.*, 1997)**. This occurs through mechanisms such as the enhancement of IgE production by B-cells, which mediate the elimination of both juvenile and adult worms **(Fitzsimmons *et al.*, 2012a; Punnonen *et al.*, 1997)**.

The humoral immune response, a hallmark of host defense against schistosomiasis infection, also exhibited significant modulation following repeated PZQ treatments. The observed elevation of both IgE and IgG4, along with an increased IgE/IgG4 ratio, reflects a balanced but complex immune response. IgE, a protective immunoglobulin central to antibody-dependent cellular cytotoxicity (ADCC), targets schistosome larvae and adult worms, contributing to immunity against reinfection **(Fitzsimmons *et al.*, 2012a)**. Its elevation following repeated PZQ treatments aligns with previous reports showing that PZQ boosts IgE-mediated immunity, reducing reinfection intensity **(Dunne *et al.*, 1992)**. Conversely, the increase in IgG4, which is associated with susceptibility to reinfection, may suggests a regulatory mechanism aimed at

mitigating excessive inflammation driven by IgE responses **(Aalberse *et al.*, 2009; Satoguina *et al.*, 2005)**. In fact, IgG4 has been reported to compete with IgE for shared epitopes, but since it cannot bind to mast cells and basophils nor activate the complement system, it acts as a powerful inhibitor of parasite cytotoxicity and granuloma formation **(Figueiredo *et al.*, 2012; Stone *et al.*, 2010)**. The increased IgE/IgG4 ratio observed in participants receiving more than two PZQ treatments is particularly notable, as it is a well-documented marker of resistance to reinfection **(Egesa *et al.*, 2018a; Figueiredo *et al.*, 2012)**. This finding suggests a shift toward a Th2-dominated immune profile, along with regulatory mechanisms that prevent excessive tissue damage. This duality underscores the delicate immunological balance that is crucial in managing schistosomiasis. Excessive Th2 responses can exacerbate granuloma formation and fibrosis through protective IgE production, while regulatory mechanisms, mediated by Tregs through IgG4, prevent severe pathology **(Egesa *et al.*, 2018b; McManus *et al.*, 2018)**.

Interestingly, despite the enhanced clinical outcome, cognitive performance, and protective immunity observed in SAC with more cumulative PZQ treatments, our study underscores the persistent challenge of managing schistosome-induced fibrosis. As corroborated by murine models **(Nono *et al.*, 2021, 2020)**, PZQ was unable to reverse established liver fibrosis, which highlights the chronic nature of schistosome-induced tissue damage. The elevated VEGF levels observed in our study may reflect ongoing tissue repair processes triggered by PZQ treatment, but these mechanisms appear insufficient to counteract the fibrotic processes initiated by schistosome eggs. This underscores the need for adjunct therapies targeting fibrosis, such as antifibrotic agents, and integrative strategies that combine PZQ with vaccines to enhance protective immunity and prevent reinfection.

CONCLISION AND PERSPECTIVE

CONCLUSION

This study highlights the diverse benefits of repeated PZQ treatments for *S. mansoni* infection in susceptible SAC.

- → Cumulative PZQ treatments significantly reduce heavy parasite burdens ($r = -0.33$, $p = 0.04$) and improve hemoglobin levels ($r = 0.44$, $p = 0.003$). However, they have limited impact on infection rate and the progression of liver fibrosis.
- → Cognitively, repeated treatments are associated with enhanced academic performance ($r = 0.33$, $p=0.02$).
- → Immunologically, repeated PZQ administration stimulates a protective Th2-dominated immune response while promoting a regulated immune state characterized by elevated IgE ($p=0.002$) and IgG4 levels ($p=0.02$).

Collectively, these findings suggest that early and consistent adherence to treatment provides substantial health and developmental benefits for SAC, even without achieving complete immunity or resolving fibrosis.

PERSPECTIVE

Building on the insights from this study, future research should prioritize the following areas:

- → **Integrated therapeutic strategies:** Investigate the efficacy of combining PZQ with antifibrotic agents or adjuvant therapies to mitigate liver fibrosis and improve long-term clinical outcomes. The potential integration of vaccines targeting schistosome antigens should also be explored to enhance immunity and reduce reinfection rates.
- → **Expanded cognitive studies:** Conduct comprehensive analyses to explore the specific cognitive domains affected by schistosomiasis and its treatment, such as memory, attention, and executive functions.
- → **Longitudinal cohort studies:** Implement large-scale longitudinal studies to evaluate the long-term impact of PZQ treatment on clinical symptoms, cognitive development, and immune responses, providing a more comprehensive understanding of its benefits.

REFERENCES

REFERENCES

Aa, S., C, F., G, W., Mj, D., 1986. Schistosoma mansoni: chemotherapy of infections of different ages. Experimental parasitology 61. https://doi.org/10.1016/0014-4894(86)90184-0

Aalberse, R.C., Stapel, S.O., Schuurman, J., Rispens, T., 2009. Immunoglobulin G4: an odd antibody. Clin Exp Allergy 39, 469–477. https://doi.org/10.1111/j.1365-2222.2009.03207.x

Ajibola, O., Gulumbe, B.H., Eze, A.A., Obishakin, E., 2018. Tools for Detection of Schistosomiasis in Resource Limited Settings. Med Sci (Basel) 6, 39. https://doi.org/10.3390/medsci6020039

Alsayed, A.A., 2020. Using Tallquist Haemoglobin Scale for Estimating Intraoperative Blood Loss in Liposuction. MPS 10, 17–22. https://doi.org/10.4236/mps.2020.102003

Andrews, P., Thomas, H., Pohlke, R., Seubert, J., 1983. Praziquantel. Med Res Rev 3, 147–200. https://doi.org/10.1002/med.2610030204

Appleton, C.C., 1984. Schistosome dermatitis--an unrecognized problem in South Africa? S Afr Med J 65, 467–469.

Aula, O.P., McManus, D.P., Jones, M.K., Gordon, C.A., 2021. Schistosomiasis with a Focus on Africa. TropicalMed 6, 109. https://doi.org/10.3390/tropicalmed6030109

Bärenbold, O., Raso, G., Coulibaly, J.T., N'Goran, E.K., Utzinger, J., Vounatsou, P., 2017. Estimating sensitivity of the Kato-Katz technique for the diagnosis of Schistosoma mansoni and hookworm in relation to infection intensity. PLoS Negl Trop Dis 11, e0005953. https://doi.org/10.1371/journal.pntd.0005953

Becher, B., Tugues, S., Greter, M., 2016. GM-CSF: From Growth Factor to Central Mediator of Tissue Inflammation. Immunity 45, 963–973. https://doi.org/10.1016/j.immuni.2016.10.026

Bogitsh, B.J., Carter, C.E., Oeltmann, T.N., 2019. Chapter 9-General Characteristics of the Trematoda, in: Bogitsh, B.J., Carter, C.E., Oeltmann, T.N. (Eds.), Human Parasitology (Fifth Edition). Academic Press, pp. 149–174. https://doi.org/10.1016/B978-0-12-813712-3.00009-6

Bong, G.G.B., Metiendjo, V., Mimfoumou, G.H.A., Ngounouh, C.T., Nguwoh, P.S., Bong, G.G.B., Metiendjo, V., Mimfoumou, G.H.A., Ngounouh, C.T., Nguwoh, P.S., 2022. Urinary schistosomiasis prevalence and risk factors among school children at matta-barrage in the Tikar Plain of Magba, West Region, Cameroon: A situational analysis in rural area. World Journal of Advanced Research and Reviews 14, 532–540. https://doi.org/10.30574/wjarr.2022.14.3.0596

Borda, C.E., Pellegrino, J., 1971. An improved stool thick-smear technique for quantitative diagnosis of Schistosoma mansoni infection. Rev Inst Med Trop Sao Paulo 13, 71–75.

Bottieau, E., Clerinx, J., de Vega, M.R., Van den Enden, E., Colebunders, R., Van Esbroeck, M., Vervoort, T., Van Gompel, A., Van den Ende, J., 2006. Imported Katayama fever: clinical and biological features at presentation and during treatment. J Infect 52, 339–345. https://doi.org/10.1016/j.jinf.2005.07.022

Brito, C.F., Caldas, I.R., Coura Filho, P., Correa-Oliveira, R., Oliveira, S.C., 2000. CD4+ T cells of schistosomiasis naturally resistant individuals living in an endemic area produce interferon-gamma and tumour necrosis factor-alpha in response to the recombinant 14KDA Schistosoma mansoni fatty acid-binding protein. Scand J Immunol 51, 595–601. https://doi.org/10.1046/j.1365-3083.2000.00710.x

Burke, M.L., Jones, M.K., Gobert, G.N., Li, Y.S., Ellis, M.K., McManus, D.P., 2009. Immunopathogenesis of human schistosomiasis. Parasite Immunol 31, 163–176. https://doi.org/10.1111/j.1365-3024.2009.01098.x

Butterworth, A.E., Fulford, A.J., Dunne, D.W., Ouma, J.H., Sturrock, R.F., 1988. Longitudinal studies on human schistosomiasis. Philos Trans R Soc Lond B Biol Sci 321, 495–511. https://doi.org/10.1098/rstb.1988.0105

Cardoso, F.C., Macedo, G.C., Gava, E., Kitten, G.T., Mati, V.L., de Melo, A.L., Caliari, M.V., Almeida, G.T., Venancio, T.M., Verjovski-Almeida, S., Oliveira, S.C., 2008. Schistosoma mansoni tegument protein Sm29 is able to induce a Th1-type of immune response and protection against parasite infection. PLoS Negl Trop Dis 2, e308. https://doi.org/10.1371/journal.pntd.0000308

CDC, 2021. Parasites-Schistosomiasis [WWW Document]. URL https://www.cdc.gov/parasites/schistosomiasis/index.html (accessed 7.9.23).

Chai, J.-Y., 2013. Praziquantel treatment in trematode and cestode infections: an update. Infect Chemother 45, 32–43. https://doi.org/10.3947/ic.2013.45.1.32

Cioli, D., Pica-Mattoccia, L., Basso, A., Guidi, A., 2014. Schistosomiasis control: praziquantel forever? Mol Biochem Parasitol 195, 23–29. https://doi.org/10.1016/j.molbiopara.2014.06.002

Colley, Daniel G., Bustinduy, A.L., Secor, W.E., King, C.H., 2014. Human schistosomiasis. Lancet 383, 2253–2264. https://doi.org/10.1016/S0140-6736(13)61949-2

Colley, Daniel G, Bustinduy, A.L., Secor, W.E., King, C.H., 2014. Human schistosomiasis. The Lancet 383, 2253–2264. https://doi.org/10.1016/S0140-6736(13)61949-2

Coutinho, H.M., Acosta, L.P., Wu, H.W., McGarvey, S.T., Su, L., Langdon, G.C., Jiz, M.A., Jarilla, B., Olveda, R.M., Friedman, J.F., Kurtis, J.D., 2006. Th2 Cytokines Are Associated with Persistent Hepatic Fibrosis in Human Schistosoma japonicum Infection.

Cox, F.E.G., 2002. History of human parasitology. Clin Microbiol Rev 15, 595–612. https://doi.org/10.1128/CMR.15.4.595-612.2002

Daniels, B.P., Holman, D.W., Cruz-Orengo, L., Jujjavarapu, H., Durrant, D.M., Klein, R.S., 2014. Viral Pathogen-Associated Molecular Patterns Regulate Blood-Brain Barrier Integrity via Competing Innate Cytokine Signals. mBio 5, e01476-14. https://doi.org/10.1128/mBio.01476-14

Dawaki, S., Al-Mekhlafi, H.M., Ithoi, I., Ibrahim, J., Abdulsalam, A.M., Ahmed, A., Sady, H., Nasr, N.A., Atroosh, W.M., 2015. The Menace of Schistosomiasis in Nigeria: Knowledge, Attitude, and Practices Regarding Schistosomiasis among Rural Communities in Kano State. PLoS ONE 10, e0143667. https://doi.org/10.1371/journal.pone.0143667

Di Bella, S., Riccardi, N., Giacobbe, D.R., Luzzati, R., 2018. History of schistosomiasis (bilharziasis) in humans: from Egyptian medical papyri to molecular biology on mummies. Pathog Glob Health 112, 268–273. https://doi.org/10.1080/20477724.2018.1495357

Dresden, M.H., Asch, H.L., 1972. Proteolytic enzymes in extracts of Schistosoma mansoni cercariae. Biochim Biophys Acta 289, 378–384. https://doi.org/10.1016/0005-2744(72)90089-7

Dunne, D.W., Butterworth, A.E., Fulford, A.J.C., Curtis Kariuki, H., Langley, J.G., Ouma, J.H., Capron, A., Pierce, R.J., Sturrock, R.F., 1992. Immunity after treatment of human schistosomiasis: association between IgE antibodies to adult worm antigens and resistance to reinfection. Eur. J. Immunol. 22, 1483–1494. https://doi.org/10.1002/eji.1830220622

Dutra, W.O., Correa-Oliveira, R., Dunne, D., Cecchini, L.F., Fraga, L., Roberts, M., Soares, A.M., Webster, M., Yssel, H., Gollob, K.J., 2002. Polarized Th2 like cells, in the absence of Th0 cells, are responsible for lymphocyte produced IL-4 in high IgE-producer schistosomiasis patients. BMC Immunology.

Egesa, M., Lubyayi, L., Jones, F.M., van Diepen, A., Chalmers, I.W., Tukahebwa, E.M., Bagaya, B.S., Hokke, C.H., Hoffmann, K.F., Dunne, D.W., Elliott, A.M., Yazdanbakhsh, M., Wilson, S., Cose, S., 2018a. Antibody responses to Schistosoma mansoni schistosomula antigens. Parasite Immunol 40, e12591. https://doi.org/10.1111/pim.12591

Egesa, M., Lubyayi, L., Tukahebwa, E.M., Bagaya, B.S., Chalmers, I.W., Wilson, S., Hokke, C.H., Hoffmann, K.F., Dunne, D.W., Yazdanbakhsh, M., Labuda, L.A., Cose, S., 2018b. Schistosoma mansoni schistosomula antigens induce Th1/Pro-inflammatory cytokine responses. Parasite Immunol 40, e12592. https://doi.org/10.1111/pim.12592

El-Ansary, A., 2003. Biochemical and immunological adaptation in schistosome parasitism. Comp Biochem Physiol B Biochem Mol Biol 136, 227–243. https://doi.org/10.1016/s1096-4959(03)00124-6

El-Faham, M.H., Wheatcroft-Francklow, K.J., Price, H.P., Sayers, J.R., Doenhoff, M.J., 2017. Schistosoma mansoni cercarial elastase (SmCE): differences in immunogenic

properties of native and recombinant forms. Parasitology 144, 1356–1364. https://doi.org/10.1017/S0031182017000658

Enderlin Vaz da Silva, Z., Lehr, H.-A., Velin, D., 2014. In vitro and in vivo repair activities of undifferentiated and classically and alternatively activated macrophages. Pathobiology 81, 86–93. https://doi.org/10.1159/000357306

Ezeamama, A.E., Bustinduy, A.L., Nkwata, A.K., Martinez, L., Pabalan, N., Boivin, M.J., King, C.H., 2018. Cognitive deficits and educational loss in children with schistosome infection—A systematic review and meta-analysis. PLOS Neglected Tropical Diseases 12, e0005524. https://doi.org/10.1371/journal.pntd.0005524

Ezeamama, A.E., Friedman, J.F., Acosta, L.P., Bellinger, D.C., Langdon, G.C., Manalo, D.L., Olveda, R.M., Kurtis, J.D., McGarvey, S.T., 2005. Helminth infection and cognitive impairment among Filipino children. The American Journal of Tropical Medicine and Hygiene 72, 540–548. https://doi.org/10.4269/ajtmh.2005.72.540

Ezeamama, A.E., McGarvey, S.T., Hogan, J., Lapane, K.L., Bellinger, D.C., Acosta, L.P., Leenstra, T., Olveda, R.M., Kurtis, J.D., Friedman, J.F., 2012. Treatment for Schistosoma japonicum, Reduction of Intestinal Parasite Load, and Cognitive Test Score Improvements in School-Aged Children. PLOS Neglected Tropical Diseases 6, e1634. https://doi.org/10.1371/journal.pntd.0001634

Fairfax, K., Nascimento, M., Huang, S.C.-C., Everts, B., Pearce, E.J., 2012. Th2 responses in schistosomiasis. Semin Immunopathol 34, 863–871. https://doi.org/10.1007/s00281-012-0354-4

Figueiredo, J.P., Oliveira, R.R., Cardoso, L.S., Barnes, K.C., Grant, A.V., Carvalho, E.M., Araujo, M.I., 2012. Adult worm-specific IgE/IgG4 balance is associated with low infection levels of *Schistosoma mansoni* in an endemic area: *IgE/IgG4 balance in* Schistosoma mansoni *infection*. Parasite Immunol 34, 604–610. https://doi.org/10.1111/pim.12001

Fitzsimmons, C.M., Jones, F.M., Pinot de Moira, A., Protasio, A.V., Khalife, J., Dickinson, H.A., Tukahebwa, E.M., Dunne, D.W., 2012a. Progressive Cross-Reactivity in IgE Responses: an Explanation for the Slow Development of Human Immunity to Schistosomiasis? Infection and Immunity 80, 4264–4270. https://doi.org/10.1128/iai.00641-12

Fitzsimmons, C.M., Jones, F.M., Stearn, A., Chalmers, I.W., Hoffmann, K.F., Wawrzyniak, J., Wilson, S., Kabatereine, N.B., Dunne, D.W., 2012b. The Schistosoma mansoni tegumental-allergen-like (TAL) protein family: influence of developmental expression on human IgE responses. PLoS Negl Trop Dis 6, e1593. https://doi.org/10.1371/journal.pntd.0001593

Ford, J.W., Blankespoor, H.D., 1979. Scanning electron microscopy of the eggs of three human schistosomes. Int J Parasitol 9, 141–145. https://doi.org/10.1016/0020-7519(79)90105-x

Friedman, S.L., 2008. Mechanisms of hepatic fibrogenesis. Gastroenterology 134, 1655–1669. https://doi.org/10.1053/j.gastro.2008.03.003

Fung, A., Vizcaychipi, M., Lloyd, D., Wan, Y., Ma, D., 2012. Central nervous system inflammation in disease related conditions: Mechanistic prospects. Brain Research 1446, 144–155. https://doi.org/10.1016/j.brainres.2012.01.061

Greenberg, R.M., 2005. Are Ca2+ channels targets of praziquantel action? Int J Parasitol 35, 1–9. https://doi.org/10.1016/j.ijpara.2004.09.004

Gryseels, B., Polman, K., Clerinx, J., Kestens, L., 2006a. Human schistosomiasis. Lancet 368, 1106–1118. https://doi.org/10.1016/S0140-6736(06)69440-3

Gryseels, B., Polman, K., Clerinx, J., Kestens, L., 2006b. Human schistosomiasis. Lancet 368, 1106–1118. https://doi.org/10.1016/S0140-6736(06)69440-3

Haeberlein, S., Haas, W., 2008. Chemical attractants of human skin for swimming Schistosoma mansoni cercariae. Parasitol Res 102, 657–662. https://doi.org/10.1007/s00436-007-0807-1

Hambrook, J.R., Hanington, P.C., 2020. Immune Evasion Strategies of Schistosomes. Front Immunol 11, 624178. https://doi.org/10.3389/fimmu.2020.624178

Hammad, S.M., Pierce, J.S., Soodavar, F., Smith, K.J., Al Gadban, M.M., Rembiesa, B., Klein, R.L., Hannun, Y.A., Bielawski, J., Bielawska, A., 2010. Blood sphingolipidomics in healthy humans: impact of sample collection methodology. Journal of lipid research 51, 3074–3087.

Hams, E., Aviello, G., Fallon, P.G., 2013. The schistosoma granuloma: friend or foe? Front Immunol 4, 89. https://doi.org/10.3389/fimmu.2013.00089

He, Y., Gao, Y., Zhang, Q., Zhou, G., Cao, F., Yao, S., 2020. IL-4 Switches Microglia/macrophage M1/M2 Polarization and Alleviates Neurological Damage by Modulating the JAK1/STAT6 Pathway Following ICH. Neuroscience 437, 161–171. https://doi.org/10.1016/j.neuroscience.2020.03.008

He, Y.-X., Salafsky, B., Ramaswamy, K., 2005. Comparison of skin invasion among three major species of Schistosoma. Trends Parasitol 21, 201–203. https://doi.org/10.1016/j.pt.2005.03.003

He, Y.X., Yu, Q.F., Yu, P., Mao, C.S., Hu, Y.Q., 1990. Penetration of Schistosoma japonicum cercaria into host skin. Chin Med J (Engl) 103, 34–44.

Hoekstra, P.T., Casacuberta-Partal, M., van Lieshout, L., Corstjens, P.L.A.M., Tsonaka, R., Assaré, R.K., Silué, K.D., Meité, A., N'Goran, E.K., N'Gbesso, Y.K., Amoah, A.S., Roestenberg, M., Knopp, S., Utzinger, J., Coulibaly, J.T., van Dam, G.J., 2020. Efficacy of single versus four repeated doses of praziquantel against Schistosoma mansoni infection in school-aged children from Côte d'Ivoire based on Kato-Katz and POC-CCA: An open-label, randomised controlled trial (RePST). PLoS Negl Trop Dis 14, e0008189. https://doi.org/10.1371/journal.pntd.0008189

Hoffmann, K.F., Cheever, A.W., Wynn, T.A., 2000. IL-10 and the dangers of immune polarization: excessive type 1 and type 2 cytokine responses induce distinct forms of lethal immunopathology in murine schistosomiasis. J Immunol 164, 6406–6416. https://doi.org/10.4049/jimmunol.164.12.6406

Hogg, K.G., Kumkate, S., Anderson, S., Mountford, A.P., 2003a. Interleukin-12 p40 secretion by cutaneous CD11c+ and F4/80+ cells is a major feature of the innate immune response in mice that develop Th1-mediated protective immunity to Schistosoma mansoni. Infect Immun 71, 3563–3571. https://doi.org/10.1128/IAI.71.6.3563-3571.2003

Hogg, K.G., Kumkate, S., Mountford, A.P., 2003b. IL-10 regulates early IL-12-mediated immune responses induced by the radiation-attenuated schistosome vaccine. Int Immunol 15, 1451–1459. https://doi.org/10.1093/intimm/dxg142

Hotez, P.J., Aksoy, S., Brindley, P.J., Kamhawi, S., 2020. What constitutes a neglected tropical disease? PLoS Negl Trop Dis 14, e0008001. https://doi.org/10.1371/journal.pntd.0008001

Inceboz, T., 2022. One Health Concept against Schistosomiasis: An Overview, in: Inceboz, T. (Ed.), Infectious Diseases. IntechOpen. https://doi.org/10.5772/intechopen.106912

Joseph, S., Jones, F.M., Walter, K., Fulford, A.J., Kimani, G., Mwatha, J.K., Kamau, T., Kariuki, H.C., Kazibwe, F., Tukahebwa, E., Kabatereine, N.B., Ouma, J.H., Vennervald, B.J., Dunne, D.W., 2004. Increases in human T helper 2 cytokine responses to Schistosoma mansoni worm and worm-tegument antigens are induced by treatment with praziquantel. J Infect Dis 190, 835–842. https://doi.org/10.1086/422604

Kamdem, S., 2020. Identification of Regulatory Factors of Liver Fibrosis in Schistosomiasis6 diseased Children in Cameroon. University of Cape Town, Cap Town.

Kamdem, S.D., Konhawa, F., Kuemkon, E.M., Meyo Kamguia, L., Tchanana, G.K., Nche, F., Oumarou, A., Hamza, M., Ouratou, Y., Tcheutchoua, M.N., Ghislain Essomba, R., Ngogang, M.P., Kengne, M., Netongo, P.M., Ondigui, B.E., Okomo Assoumou, M.C., Brombacher, F., Nono, J.K., 2019. Negative Association of Interleukin-33 Plasma Levels and Schistosomiasis Infection in a Site of Polyparasitism in Rural Cameroon. Front. Immunol. 10, 2827. https://doi.org/10.3389/fimmu.2019.02827

Kamdem, S.D., Kuemkon, E.M., Kamguia, L.M., Tchanana, G.K., Konhawa, F., Nche, F., Oumarou, A., Hamza, M., Essomba, R.G., Kengne, M., Ondigui, B.E., Assoumou, M.C.O., Brombacher, F., Nono, J.K., 2020. An ultrasound-based referential of body height-adjusted normal liver organometry in school children from Bokito in rural Cameroon. Sci Rep 10, 2773. https://doi.org/10.1038/s41598-020-59613-z

Kamdem, S.D., Moyou-Somo, R., Brombacher, F., Nono, J.K., 2018. Host Regulators of Liver Fibrosis During Human Schistosomiasis. Frontiers in Immunology 9, 2781. https://doi.org/10.3389/fimmu.2018.02781

Kasambala, M., Mukaratirwa, S., Vengesai, A., Mduluza-Jokonya, T., Jokonya, L., Midzi, H., Makota, R.B., Mutemeri, A., Maziti, E., Dube-Marimbe, B., Chibanda, D., Mutapi, F., Mduluza, T., 2023a. . Front Immunol 14, 1139912. https://doi.org/10.3389/fimmu.2023.1139912

Kasambala, M., Mukaratirwa, S., Vengesai, A., Mduluza-Jokonya, T., Jokonya, L., Midzi, H., Makota, R.B., Mutemeri, A., Maziti, E., Dube-Marimbe, B., Chibanda, D., Mutapi, F., Mduluza, T., 2023b. The association of systemic inflammation and cognitive functions of pre-school aged children residing in a Schistosoma haematobium endemic area in Zimbabwe. Front. Immunol. 14, 1139912. https://doi.org/10.3389/fimmu.2023.1139912

Katz, N., Chaves, A., Pellegrino, J., 1972. A simple device for quantitative stool thick-smear technique in Schistosomiasis mansoni. Rev Inst Med Trop Sao Paulo 14, 397–400.

Khammo, N., Bartlett, A., Clothier, R.H., Whitfield, P.J., 2002. The attachment of Schistosoma mansoni cercariae to human skin cells. Parasitology 124, 25–30. https://doi.org/10.1017/s003118200100885x

King, C.H., Galvani, A.P., 2018. Underestimation of the global burden of schistosomiasis. The Lancet 391, 307–308. https://doi.org/10.1016/S0140-6736(18)30098-9

Kloos, H., Gazzinelli, A., Bethony, J., Alvares, M.C.B., Guerra, H.L., Gazzinelli, G., Fraga, L.A.D.O., Prata, A., Correa-Oliveira, R., Loverde, P.T., Silveira, A.M.S., 2002. High levels of IgG4 to Schistosoma mansoni egg antigens in individuals with periportal fibrosis. The American Journal of Tropical Medicine and Hygiene 66, 542–549. https://doi.org/10.4269/ajtmh.2002.66.542

Kusel, J.R., Al-Adhami, B.H., Doenhoff, M.J., 2007. The schistosome in the mammalian host: understanding the mechanisms of adaptation. Parasitology 134, 1477–1526. https://doi.org/10.1017/S0031182007002971

Kyung, S.Y., Cho, Y.K., Kim, Y.J., Park, J.-W., Jeong, S.H., Lee, J.-I., Sung, Y.M., Lee, S.P., 2011. A paragonimiasis patient with allergic reaction to praziquantel and resistance to triclabendazole: successful treatment after desensitization to praziquantel. Korean J Parasitol 49, 73–77. https://doi.org/10.3347/kjp.2011.49.1.73

Lambertucci, J.R., 2010. Acute schistosomiasis mansoni: revisited and reconsidered. Mem Inst Oswaldo Cruz 105, 422–435. https://doi.org/10.1590/s0074-02762010000400012

Licá, I.C.L., Frazão, G.C.C.G., Nogueira, R.A., Lira, M.G.S., Dos Santos, V.A.F., Rodrigues, J.G.M., Miranda, G.S., Carvalho, R.C., Silva, L.A., Guerra, R.N.M., Nascimento, F.R.F., 2023. Immunological mechanisms involved in macrophage activation and polarization in schistosomiasis. Parasitology 150, 401–415. https://doi.org/10.1017/S0031182023000021

Long, L., 2018. Invasive Species Highlight: Apple Snails. SOLitude Lake Manag. Full-Serv. Lake Pond Manag. URL https://www.solitudelakemanagement.com/invasive-species-highlight-apple-snails/ (accessed 6.16.25).

MacDonald, A.S., Straw, A.D., Dalton, N.M., Pearce, E.J., 2002. Cutting edge: Th2 response induction by dendritic cells: a role for CD40. J Immunol 168, 537–540. https://doi.org/10.4049/jimmunol.168.2.537

Mahmoud, A.A.F. (Ed.), 2001. Schistosomiasis, Tropical medicine. Imperial College Press ; distributed by World Scientific Pub. Co, London : River Edge, NJ.

Mazibuko, X.I., Chimbari, M.J., 2020. Parasitic infections and environmental influences on verbal memory and learning potential of isiZulu speaking pre-school children. Journal of Psychology in Africa 30, 471–474. https://doi.org/10.1080/14330237.2020.1821318

Mbanefo, E.C., Huy, N.T., Wadagni, A.A., Eneanya, C.I., Nwaorgu, O., Hirayama, K., 2014. Host determinants of reinfection with schistosomes in humans: a systematic review and meta-analysis. PLoS Negl Trop Dis 8, e3164. https://doi.org/10.1371/journal.pntd.0003164

Mbow, M., Larkin, B.M., Meurs, L., Wammes, L.J., de Jong, S.E., Labuda, L.A., Camara, M., Smits, H.H., Polman, K., Dieye, T.N., Mboup, S., Stadecker, M.J., Yazdanbakhsh, M., 2013. T-helper 17 cells are associated with pathology in human schistosomiasis. J Infect Dis 207, 186–195. https://doi.org/10.1093/infdis/jis654

McKerrow, J.H., Salter, J., 2002. Invasion of skin by Schistosoma cercariae. Trends Parasitol 18, 193–195. https://doi.org/10.1016/s1471-4922(02)02309-7

McManus, D.P., Bergquist, R., Cai, P., Ranasinghe, S., Tebeje, B.M., You, H., 2020. Schistosomiasis-from immunopathology to vaccines. Semin Immunopathol 42, 355–371. https://doi.org/10.1007/s00281-020-00789-x

McManus, D.P., Dunne, D.W., Sacko, M., Utzinger, J., Vennervald, B.J., Zhou, X.-N., 2018. Schistosomiasis. Nat Rev Dis Primers 4, 13. https://doi.org/10.1038/s41572-018-0013-8

Medhat, A., Shehata, M., Bucci, K., Mohamed, S., Dief, A.D.E., Badary, S., Galal, H., Nafeh, M., King, C.L., 1998. Increased Interleukin-4 and Interleukin-5 Production in Response to Schistosoma haematobium Adult Worm Antigens Correlates with Lack of Reinfection after Treatment. Journal of Infectious Diseases 178, 512–519. https://doi.org/10.1086/515630

Meurs, L., Mbow, M., Boon, N., van den Broeck, F., Vereecken, K., Dièye, T.N., Abatih, E., Huyse, T., Mboup, S., Polman, K., 2013. Micro-geographical heterogeneity in Schistosoma mansoni and S. haematobium infection and morbidity in a co-endemic community in northern Senegal. PLoS Negl Trop Dis 7, e2608. https://doi.org/10.1371/journal.pntd.0002608

Mewamba, E.M., Nyangiri, O.A., Noyes, H.A., Egesa, M., Matovu, E., Simo, G., 2021. The Genetics of Human Schistosomiasis Infection Intensity and Liver Disease: A Review. Front Immunol 12, 613468. https://doi.org/10.3389/fimmu.2021.613468

Molu, J.-P., Essome, M.C.N., Monamele, C.G., Njouom, R., 2018. Sero-prevalence of HBsAg in naive HIV-infected patients in a rural locality of Cameroon. BMC Res Notes 11, 39. https://doi.org/10.1186/s13104-018-3159-2

Mountford, A.P., Coulson, P.S., Pemberton, R.M., Smythies, L.E., Wilson, R.A., 1992. The generation of interferon-gamma-producing T lymphocytes in skin-draining lymph nodes, and their recruitment to the lungs, is associated with protective immunity to Schistosoma mansoni. Immunology 75, 250–256.

Mouser, E.E., Pollakis, G., Smits, H.H., Thomas, J., Yazdanbakhsh, M., de Jong, E.C., Paxton, W.A., 2019. Schistosoma mansoni soluble egg antigen (SEA) and recombinant Omega-1 modulate induced CD4+ T-lymphocyte responses and HIV-1 infection in vitro. PLoS Pathog 15, e1007924. https://doi.org/10.1371/journal.ppat.1007924

Moyou-Somo, R., Kefie-Arrey, C., Dreyfuss, G., Dumas, M., 2003. An epidemiological study of pleuropulmonary paragonimiasis among pupils in the peri-urban zone of Kumba town, Meme Division, Cameroon. BMC Public Health 3, 40. https://doi.org/10.1186/1471-2458-3-40

Mt, I., Rm, O., Tn, C., Du, O., Ag, R., 2014. Prevention and control of schistosomiasis: a current perspective. Research and reports in tropical medicine 2014. https://doi.org/10.2147/RRTM.S44274

Nc, L., Ys, L., Da, K.-V., Ii, B., Jt, C., E, B., J, U., P, V., Jr, A., 2016. Assessment of global guidelines for preventive chemotherapy against schistosomiasis and soil-transmitted helminthiasis: a cost-effectiveness modelling study. The Lancet. Infectious diseases 16. https://doi.org/10.1016/S1473-3099(16)30073-1

Negrão-Corrêa, D., Fittipaldi, J.F., Lambertucci, J.R., Teixeira, M.M., Antunes, C.M.D.F., Carneiro, M., 2014. Association of Schistosoma mansoni-Specific IgG and IgE Antibody Production and Clinical Schistosomiasis Status in a Rural Area of Minas Gerais, Brazil. PLoS ONE 9, e88042. https://doi.org/10.1371/journal.pone.0088042

Nelwan, M.L., 2019. Schistosomiasis: Life Cycle, Diagnosis, and Control. Current Therapeutic Research 91, 5–9. https://doi.org/10.1016/j.curtheres.2019.06.001

Njunda, A.L., Ndzi, E.N., Assob, J.C.N., Kamga, H.-L.F., Kwenti, E.T., 2017. Prevalence and factors associated with urogenital schistosomiasis among primary school children in barrage, Magba sub-division of Cameroon. BMC Public Health 17, 618. https://doi.org/10.1186/s12889-017-4539-6

Nkengni, S.M.M., Zoumabo, A.T.C., Soppa, N.P.S., Sizono, A.B.N., Vignoles, P., Tchuenté, L.-A.T., Teukeng, F.F.D., 2019. Current decline in schistosome and soil-transmitted helminth infections among school children at Loum, Littoral region, Cameroon. Pan Afr Med J 33, 94. https://doi.org/10.11604/pamj.2019.33.94.18265

Nono, J.K., Fu, K., Mpotje, T., Varrone, G., Aziz, N.A., Mosala, P., Hlaka, L., Kamdem, S.D., Xu, D., Spangenberg, T., Brombacher, F., 2020. Investigating the antifibrotic effect of

the antiparasitic drug Praziquantel in in vitro and in vivo preclinical models. Sci Rep 10, 10638. https://doi.org/10.1038/s41598-020-67514-4

Nono, J.K., Mpotje, T., Mosala, P., Aziz, N.A., Musaigwa, F., Hlaka, L., Spangenberg, T., Brombacher, F., 2021. Praziquantel Treatment of Schistosoma mansoni Infected Mice Renders Them Less Susceptible to Reinfection. Front Immunol 12, 748387. https://doi.org/10.3389/fimmu.2021.748387

Nyindo, M., Kariuki, T.M., Mola, P.W., Farah, I.O., Elson, L., Blanton, R.E., King, C.L., 1999. Role of adult worm antigen-specific immunoglobulin E in acquired immunity to Schistosoma mansoni infection in baboons. Infect Immun 67, 636–642. https://doi.org/10.1128/IAI.67.2.636-642.1999

Oettle, R.C., Dickinson, H.A., Fitzsimmons, C.M., Sacko, M., Tukahebwa, E.M., Chalmers, I.W., Wilson, S., 2023. Protective human IgE responses are promoted by comparable life-cycle dependent Tegument Allergen-Like expression in Schistosoma haematobium and Schistosoma mansoni infection. PLoS Pathog 19, e1011037. https://doi.org/10.1371/journal.ppat.1011037

Olliaro, P.L., Vaillant, M.T., Belizario, V.J., Lwambo, N.J.S., Ouldabdallahi, M., Pieri, O.S., Amarillo, M.L., Kaatano, G.M., Diaw, M., Domingues, A.C., Favre, T.C., Lapujade, O., Alves, F., Chitsulo, L., 2011. A multicentre randomized controlled trial of the efficacy and safety of single-dose praziquantel at 40 mg/kg vs. 60 mg/kg for treating intestinal schistosomiasis in the Philippines, Mauritania, Tanzania and Brazil. PLoS Negl Trop Dis 5, e1165. https://doi.org/10.1371/journal.pntd.0001165

Olveda, D.U., Inobaya, M.T., McManus, D.P., Olveda, R.M., Vinluan, M.L., Ng, S.-K., Harn, D.A., Li, Y., Guevarra, J.R., Lam, A.K., Ross, A.G.P., 2017. Biennial versus annual treatment for schistosomiasis and its impact on liver morbidity. Int J Infect Dis 54, 145–149. https://doi.org/10.1016/j.ijid.2016.10.001

Orecchioni, M., Ghosheh, Y., Pramod, A.B., Ley, K., 2019. Macrophage Polarization: Different Gene Signatures in M1(LPS+) vs. Classically and M2(LPS-) vs. Alternatively Activated Macrophages. Front Immunol 10, 1084. https://doi.org/10.3389/fimmu.2019.01084

Paveley, R.A., Aynsley, S.A., Cook, P.C., Turner, J.D., Mountford, A.P., 2009. Fluorescent imaging of antigen released by a skin-invading helminth reveals differential uptake and activation profiles by antigen presenting cells. PLoS Negl Trop Dis 3, e528. https://doi.org/10.1371/journal.pntd.0000528

Pd, O., Dt, L., Ra, B., J, M., 2001. Interrelationships and evolution of the tapeworms (Platyhelminthes: Cestoda). Molecular phylogenetics and evolution 19. https://doi.org/10.1006/mpev.2001.0930

Peng, P., Kievit, R.A., 2020. The Development of Academic Achievement and Cognitive Abilities: A Bidirectional Perspective. Child Development Perspectives 14, 15–20. https://doi.org/10.1111/cdep.12352

Pérez del Villar, L., Burguillo, F.J., López-Abán, J., Muro, A., 2012. Systematic review and meta-analysis of artemisinin based therapies for the treatment and prevention of schistosomiasis. PLoS One 7, e45867. https://doi.org/10.1371/journal.pone.0045867

Phillips, A.E., Ower, A.K., Mekete, K., Liyew, E.F., Maddren, R., Belay, H., Chernet, M., Anjulo, U., Mengistu, B., Salasibew, M., Tasew, G., Anderson, R., 2022. Association between water, sanitation, and hygiene access and the prevalence of soil-transmitted helminth and schistosome infections in Wolayita, Ethiopia. Parasites Vectors 15, 410. https://doi.org/10.1186/s13071-022-05465-7

Pica-Mattoccia, L., Carlini, D., Guidi, A., Cimica, V., Vigorosi, F., Cioli, D., 2006. The schistosome enzyme that activates oxamniquine has the characteristics of a sulfotransferase. Mem Inst Oswaldo Cruz 101 Suppl 1, 307–312. https://doi.org/10.1590/s0074-02762006000900048

Pica-Mattoccia, L., Orsini, T., Basso, A., Festucci, A., Liberti, P., Guidi, A., Marcatto-Maggi, A.-L., Nobre-Santana, S., Troiani, A.-R., Cioli, D., Valle, C., 2008. Schistosoma mansoni: lack of correlation between praziquantel-induced intra-worm calcium influx and parasite death. Exp Parasitol 119, 332–335. https://doi.org/10.1016/j.exppara.2008.03.012

Piipponen, M., Li, D., Landén, N.X., 2020. The Immune Functions of Keratinocytes in Skin Wound Healing. Int J Mol Sci 21, 8790. https://doi.org/10.3390/ijms21228790

PNLSHI, 2023. National Programme for the Control of Schistosomiasis and STH. URL https://pnlshi.org/ (accessed 7.9.23).

Prah, S.K., James, C., 1978. The influence of physical factors on the behaviour and infectivity of miracidia of Schistosoma mansoni and S. haematobium. II. Effect of light and depth. J Helminthol 52, 115–120. https://doi.org/10.1017/s0022149x00005228

Punnonen, J., Yssel, H., de Vries, J.E., 1997. The relative contribution of IL-4 and IL-13 to human IgE synthesis induced by activated CD4+ or CD8+ T cells. J Allergy Clin Immunol 100, 792–801. https://doi.org/10.1016/s0091-6749(97)70276-8

Ramírez, R.M., Ceballos, E., Alarcón de Noya, B., Noya, O., Bianco, N., 1996. The immunopathology of human Schistosomiasis-III. Immunoglobulin isotype profiles and response to praziquantel. Mem Inst Oswaldo Cruz 91, 593–599. https://doi.org/10.1590/s0074-02761996000500010

Renganathan, E., Cioli, D., 1998. An international initiative on praziquantel use. Parasitol Today 14, 390–391. https://doi.org/10.1016/s0169-4758(98)01310-6

Richter, J., Hatz, C., Campagne, G., Bergquist, N.R., Jenkins, J.M., Diseases, U.B.S.P. for R. and T. in T., 2000. Ultrasound in schistosomiasis : a practical guide to the standard use of ultrasonography for assessment of schistosomiasis-related morbidity : Second international workshop, October 22-26 1996, Niamey, Niger (No. TDR/STR/SCH/00.1). World Health Organization.

Rj, I., A, B., Mb, B., C, M., Pj, W., 2003. Penetration of human skin by the cercariae of Schistosoma mansoni: an investigation of the effect of multiple cercarial applications. Journal of helminthology 77. https://doi.org/10.1079/JOH2002157

Ross, A.G.P., Bartley, P.B., Sleigh, A.C., Olds, G.R., Li, Y., Williams, G.M., McManus, D.P., 2002. Schistosomiasis. N Engl J Med 346, 1212–1220. https://doi.org/10.1056/NEJMra012396

Ross, S.H., Cantrell, D.A., 2018. Signaling and Function of Interleukin-2 in T Lymphocytes. Annu Rev Immunol 36, 411–433. https://doi.org/10.1146/annurev-immunol-042617-053352

Sanin, D.E., Prendergast, C.T., Bourke, C.D., Mountford, A.P., 2015. Helminth Infection and Commensal Microbiota Drive Early IL-10 Production in the Skin by CD4+ T Cells That Are Functionally Suppressive. PLoS Pathog 11, e1004841. https://doi.org/10.1371/journal.ppat.1004841

Satoguina, J.S., Adjobimey, T., Arndts, K., Hoch, J., Oldenburg, J., Layland, L.E., Hoerauf, A., 2008. Tr1 and naturally occurring regulatory T cells induce IgG4 in B cells through GITR/GITR-L interaction, IL-10 and TGF-beta. Eur J Immunol 38, 3101–3113. https://doi.org/10.1002/eji.200838193

Satoguina, J.S., Weyand, E., Larbi, J., Hoerauf, A., 2005. T regulatory-1 cells induce IgG4 production by B cells: role of IL-10. J Immunol 174, 4718–4726. https://doi.org/10.4049/jimmunol.174.8.4718

SCH-STH, 2023. Atlas SCH-STH – SCH & STH Maps in Cameroon [WWW Document]. URL https://ntdmaps.org/ (accessed 7.9.23).

Schwartz, E., Rozenman, J., Perelman, M., 2000. Pulmonary manifestations of early schistosome infection among nonimmune travelers. Am J Med 109, 718–722. https://doi.org/10.1016/s0002-9343(00)00619-7

Secor, W.E., 2012. The effects of schistosomiasis on HIV/AIDS infection, progression and transmission. Curr Opin HIV AIDS 7, 254–259. https://doi.org/10.1097/COH.0b013e328351b9e3

Shapouri-Moghaddam, A., Mohammadian, S., Vazini, H., Taghadosi, M., Esmaeili, S.-A., Mardani, F., Seifi, B., Mohammadi, A., Afshari, J.T., Sahebkar, A., 2018. Macrophage plasticity, polarization, and function in health and disease. J Cell Physiol 233, 6425–6440. https://doi.org/10.1002/jcp.26429

Shi, Y., Qu, S., 2022. Analysis of the effect of cognitive ability on academic achievement: Moderating role of self-monitoring. Front Psychol 13, 996504. https://doi.org/10.3389/fpsyg.2022.996504

Shi, Y., Qu, S., 2021. Cognitive Ability and Self-Control's Influence on High School Students' Comprehensive Academic Performance. Front Psychol 12, 783673. https://doi.org/10.3389/fpsyg.2021.783673

Sorgho, H., Da, O., Rouamba, T., Savadogo, B., Tinto, H., Ouedraogo, J.-B., 2017. Schistosoma mansoni infection and hematological profile in an endemic foci in Western Burkina Faso.

Sousa-Figueiredo, J.C., Betson, M., Atuhaire, A., Arinaitwe, M., Navaratnam, A.M.D., Kabatereine, N.B., Bickle, Q., Stothard, J.R., 2012. Performance and safety of praziquantel for treatment of intestinal schistosomiasis in infants and preschool children. PLoS Negl Trop Dis 6, e1864. https://doi.org/10.1371/journal.pntd.0001864

Sternberg, R.J., Kaufman, J.C., Grigorenko, E.L., 2008. Applied Intelligence. Cambridge University Press, New York, NY.

Sternberg, R.J., Sternberg, K., 2022. Cognitive Psychology. Cambridge University Press. https://doi.org/10.1017/CBO9780511611445

Stockard, J., Wood, T.W., Coughlin, C., Khoury, C.R., 2018. The effectiveness of direct instruction curricula: A meta-analysis of a half century of research. Review of Educational Research 88, 479–507. https://doi.org/10.3102/0034654317751919

Stone, K.D., Prussin, C., Metcalfe, D.D., 2010. IgE, mast cells, basophils, and eosinophils. Journal of Allergy and Clinical Immunology 125, S73–S80. https://doi.org/10.1016/j.jaci.2009.11.017

Stothard, J.R., Sousa-Figueiredo, J.C., Betson, M., Green, H.K., Seto, E.Y.W., Garba, A., Sacko, M., Mutapi, F., Vaz Nery, S., Amin, M.A., Mutumba-Nakalembe, M., Navaratnam, A., Fenwick, A., Kabatereine, N.B., Gabrielli, A.F., Montresor, A., 2011. Closing the praziquantel treatment gap: new steps in epidemiological monitoring and control of schistosomiasis in African infants and preschool-aged children. Parasitology 138, 1593–1606. https://doi.org/10.1017/S0031182011001235

Sturrock, R.F., 2001. Schistosomiasis epidemiology and control: how did we get here and where should we go? Mem Inst Oswaldo Cruz 96 Suppl, 17–27. https://doi.org/10.1590/s0074-02762001000900003

Sulahian, A., Garin, Y.J.F., Izri, A., Verret, C., Delaunay, P., van Gool, T., Derouin, F., 2005. Development and Evaluation of a Western Blot Kit for Diagnosis of Schistosomiasis. Clin Diagn Lab Immunol 12, 548–551. https://doi.org/10.1128/CDLI.12.4.548-551.2005

Sunny, B.S., Elze, M., Chihana, M., Gondwe, L., Crampin, A.C., Munkhondya, M., Kondowe, S., Glynn, J.R., 2017. Failing to progress or progressing to fail? Age-for-grade heterogeneity and grade repetition in primary schools in Karonga district, northern Malawi. International Journal of Educational Development 52, 68–80. https://doi.org/10.1016/j.ijedudev.2016.10.004

Tawfeek, G.M., Alafifi, A.M., Azmy, M.F., 2003. Immunological indicators of morbidity in human schistosomiasis mansoni: role of vascular endothelial growth factor and anti-soluble egg antigen IgG4 in disease progression. J Egypt Soc Parasitol 33, 597–614.

TSAO, N., HSU, H.P., WU, C.M., LIU, C.C., LEI, H.Y., 2001. Tumour necrosis factor-α causes an increase in blood-brain barrier permeability during sepsis. Journal of Medical Microbiology 50, 812–821. https://doi.org/10.1099/0022-1317-50-9-812

Tufon, K.A., Anong, D.N., Meriki, H.D., Georges, T.D., Maurice, M., Kouanou, Y.S., Bolimo, A.F., Tony, N.J., Kwenti, T.E., Wung, N.H., Nkuo-Akenji, T., 2018. Characterization and assessment of HBV chronically infected patients: Identification of those eligible for treatment in the South West region of Cameroon. PLOS ONE 13, e0203312. https://doi.org/10.1371/journal.pone.0203312

Turner, J.D., Meurs, L., Dool, P., Bourke, C.D., Mbow, M., Dièye, T.N., Mboup, S., Polman, K., Mountford, A.P., 2013. Schistosome infection is associated with enhanced whole-blood IL-10 secretion in response to cercarial excretory/secretory products. Parasite Immunol 35, 147–156. https://doi.org/10.1111/pim.12028

Utzinger, J., N'Goran, E.K., N'Dri, A., Lengeler, C., Tanner, M., 2000. Efficacy of praziquantel against Schistosoma mansoni with particular consideration for intensity of infection. Trop Med Int Health 5, 771–778. https://doi.org/10.1046/j.1365-3156.2000.00646.x

van den Biggelaar, A.H.J., Borrmann, S., Kremsner, P., Yazdanbakhsh, M., 2002. Immune Responses Induced by Repeated Treatment Do Not Result in Protective Immunity to Schistosoma haematobium: Interleukin (IL)–5 and IL-10 Responses. The Journal of Infectious Diseases 186, 1474–1482. https://doi.org/10.1086/344352

Van Der Maas, H.L.J., Dolan, C.V., Grasman, R.P.P.P., Wicherts, J.M., Huizenga, H.M., Raijmakers, M.E.J., 2006. A dynamical model of general intelligence: The positive manifold of intelligence by mutualism. Psychological Review 113, 842–861. https://doi.org/10.1037/0033-295X.113.4.842

Vanderstraete, M., 2014. Structure, Fonction et Evolution des Récepteurs Venus Kinase: rôles dans la reproduction du parasite Schistosoma mansoni.

Vannella, K.M., Wynn, T.A., 2017. Mechanisms of Organ Injury and Repair by Macrophages. Annu Rev Physiol 79, 593–617. https://doi.org/10.1146/annurev-physiol-022516-034356

Varatharaj, A., Galea, I., 2017. The blood-brain barrier in systemic inflammation. Brain, Behavior, and Immunity 60, 1–12. https://doi.org/10.1016/j.bbi.2016.03.010

Wang, B., Collins, J.J., III, Newmark, P.A., 2013. Functional genomic characterization of neoblast-like stem cells in larval Schistosoma mansoni. eLife 2, e00768. https://doi.org/10.7554/eLife.00768

Webster, B.L., Southgate, V.R., Littlewood, D.T.J., 2006. A revision of the interrelationships of Schistosoma including the recently described Schistosoma guineensis. Int J Parasitol 36, 947–955. https://doi.org/10.1016/j.ijpara.2006.03.005

WHO, 2023. Schistosomiasis [WWW Document]. URL https://www.who.int/news-room/fact-sheets/detail/schistosomiasis

WHO, 2022a. WHO guideline on control and elimination of human schistosomiasis. World Health Organization, Geneva.

WHO, 2022b. Schistosomiasis and soil-transmitted helminthiases: progress report, 2021 [WWW Document]. URL https://www.who.int/publications-detail-redirect/who-wer9748-621-632

WHO, 2021. Status of schistosomiasis endemic countries [WWW Document]. URL https://apps.who.int/neglected_diseases/ntddata/sch/sch.html (accessed 7.9.23).

WHO, W.H., 2020. Ending the neglect to attain the Sustainable Development Goals: a road map for neglected tropical diseases 2021–2030.

Wilson, M.S., Cheever, A.W., White, S.D., Thompson, R.W., Wynn, T.A., 2011. IL-10 Blocks the Development of Resistance to Re-Infection with Schistosoma mansoni. PLOS Pathogens 7, e1002171. https://doi.org/10.1371/journal.ppat.1002171

Wynn, T.A., Oswald, I.P., Eltoum, I.A., Caspar, P., Lowenstein, C.J., Lewis, F.A., James, S.L., Sher, A., 1994. Elevated expression of Th1 cytokines and nitric oxide synthase in the lungs of vaccinated mice after challenge infection with Schistosoma mansoni. J Immunol 153, 5200–5209.

Xie, W., Kumar, S., Kakon, S.H., Haque, R., Petri, W.A., Nelson, C.A., 2019. Chronic inflammation is associated with neural responses to faces in bangladeshi children. NeuroImage 202, 116110. https://doi.org/10.1016/j.neuroimage.2019.116110

Yuan, M., Xie, S.-Y., Zhou, X.-J., Hu, F., Liu, Y.-M., Zeng, X.-J., Li, Z.-J., Li, Y.-F., Gao, Z.-L., Li, J.-Y., Chen, H.-G., Lin, D.-D., 2012. [Study on immune status of patients with schistosomiasis japonica in Poyang Lake region II characterization of cellular immune in schistosomiasis patients]. Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi 24, 632–635.

Yunna, C., Mengru, H., Lei, W., Weidong, C., 2020. Macrophage M1/M2 polarization. Eur J Pharmacol 877, 173090. https://doi.org/10.1016/j.ejphar.2020.173090

Zheng, B., Zhang, J., Chen, H., Nie, H., Miller, H., Gong, Q., Liu, C., 2020. T Lymphocyte-Mediated Liver Immunopathology of Schistosomiasis. Front Immunol 11, 61. https://doi.org/10.3389/fimmu.2020.00061

APPENDICES

APPENDICES

1) **Ethical clearance**

2) Research authorization.

REPUBLIQUE DU CAMEROUN
Paix-Travail-Patrie
*****
MINISTERE DE L'EDUCATION DE BASE
*****
DELEGATION REGIONALE DU CENTRE
******
SOUS DIRECTION DES AFFAIRES GENERALES
*****
INSPECTION MEDICO-SCOLAIRE
***
BP : 521 YAOUNDE Tel : 22 22 51 21

N°23/05/MINEDUB/DREB-CS-DAG/IMS

REPUBLIC OF CAMEROON
Peace-Word-Fatherland
*****
MINISTRY OF BASIC EDUCATION
****
REGIONAL DELEGATION OF CENTRE
****
SUB-DIVISION OF GENERAL AFFAIRS
*****
SCHOOL HEALTH INSPECTORATE
****
PO B OX 521 YAOUNDE TEL.: 22 22 51 21

Yaoundé le 03 FEV 2022

V/L: Réf du 12 Janvier 2022

Le Délégué Régional de l'Education de Base pour le Centre

Au
Dr. Justin KOMGUEP NONO
Attaché de recherche à l'Institut de Recherches Médicales et d'Etudes de Plantes médicinales de Yaoundé

Objet: Demande d'une autorisation de recherche Sur la bilharziose dans les écoles de Bokito et de Mbankomo

Docteur,

En accusant réception de votre correspondance susvisée dont l'objet est repris en marge,

J'ai l'honneur de vous informer que je marque mon accord pour votre recherche médicale dans les établissements scolaires suivants : Ecoles publiques de BOKITO (YORO 1 et 2) et de MBANKOMO. Recherche faisant suite à une précédente investigation en 2018 dans les écoles publiques de YORO 1 et 2, de KEDIA, BOGANDO et de TEDJOMOLO.

Ainsi, pour les modalités pratiques, vous voudrez bien prendre l'attache du Médecin Inspecteur Médico-Scolaire et des Inspecteurs d'Arrondissement de BOKITO et de MBANKOMO.

Toutefois, au terme de ladite recherche, une copie de vos travaux est attendue à l'Inspection Médico-Scolaire à toutes fins utiles.

Veuillez agréer, Docteur, l'assurance de ma parfaite considération.

Ampliations :
-MINEDUB/SG/DSSAPPS
-DDEB/MbametInoubou
-DDEB/Mefou et Akono
-Gouverneur /CE
-ARCHIVES / CHRONO

LE DELEGUE REGIONAL,
Le Délégué
TSANGA Jean Blaise

3) Authorization from the regional delegate

REPUBLIQUE DU CAMEROUN
Paix – Travail - Patrie

MINSTERE DE LA SANTE PUBLIQUE

SECRETARIAT GENERAL

DELEGATION REGIONALE DU CENTRE

N° 0291 /AP/MINSANTE/SG/DRSPC

REPUBLIC OF CAMEROON
Peace – Work – Fatherland

MINISTRY OF PUBLIC HEALTH

SECRETARIAT GENERAL

CENTRE REGIONAL DELEGATION

Yaoundé, le 21 FEV 2022

LE DELEGUE REGIONAL
A
Dr NONO KOMGUEP Justin
-Investigateur principal-
Tél: +237 679 55 66 83 / 699 28 83 65

Objet: Accord de principe.

Docteur,

En date du 16 février 2022, vous m'avez adressé une correspondance relative à l'autorisation de mise en œuvre du projet de recherche intitulé : **« Identification of host regulators of tissue fibroproliferative pathology in schistosomiasis-diseased individueals in Cameroon ».**

Au vu de l'intérêt scientifique que revêt l'étude, je ne formule aucune objection à ce que vous procédiez à la collecte de données y relatives dans les ménages et structures éducatives ciblés dans les districts de santé de Mbankomo et de Bafia, région du centre, en collaboration avec les responsables des chefs desdits districts.

Toutefois, cet accord de principe ne vaut pas autorisation de mener l'activité. Vous voudrez par conséquent solliciter une clairance éthique auprès du Comité (National ou du Comité Régional) d'Ethique de la Recherche pour la Santé Humaine et une autorisation administrative de recherche conformément à la Directive ministérielle relative à la mise des projets de recherche au Cameroun.

Veuillez accepter, Docteur, l'expression de ma parfaite considération./-

LE DELEGUE REGIONAL,

Le Délégué
Dr MOUSSI Charlotte
MD - MPH

Ampliations :
- CRERSH-Ce

4) Informed consent

INFORMED CONSENT & ASSENT FORM

I the undersigned, Mr./Mrs./Miss (names)

…….

Having been invited to take part in the study titled: Identification of host regulators of tissue fibroproliferative pathology in schistosomiasis-diseased individuals in Cameroon. Whose coordinator is Dr Justin Komguep Nono of the Institute of Medical Research and Medicinal Plants Studies/Medical Research Centre, Yaoundé, Cameroon

- I have understood the information provided on the study/I was explained on what the study is all about
- I understand the goal and objectives of the study
- I have discussed on all my worries
- I am aware of the risks and benefits of the study
- I am free to stay in the study or not to
- My consent does not exempt the researchers from their responsibilities for I am protected by the law

I accept to take part in the study with respect to the information I received;

- Respond to all questions
- Provide my medical records
- Provide 5 ml of my blood
- Provide samples of my stool and urine
- Undergo ultrasonographic examination (echography)

I accept that my samples collected in this study be used for future studies, as indicated in the information note.

Done in .....on.....

Names, addresses and signatures

Investigator Participant

FORMULAIRE DE CONSENTEMENT ECLAIRE

Je soussigné, Mr/Mme/Mlle (Nom(s) et Prénom(s)

.....……

Avoir été invité à participer au travail de recherche intitulé « Identification des régulateurs contre les pathologies fibroprolifératives tissulaires chez les enfants atteints de schistosomiase

au Cameroun » dont le coordinateur s'appelle Dr Justin Komguep Nono de l'Institut de Recherches Médicales et d'Etudes des Plantes Médicinales

- J'ai bien compris la notice d'information qui m'a été remise concernant cette étude / la notice d'information relative à cette étude m'a été lu et expliqué
- J'ai bien compris le but et les objectifs de cette étude
- J'ai reçu toutes les réponses aux questions que j'ai posées
- Les risques et bénéfices m'ont été présenté et expliques
- J'ai bien compris que je suis libre d'accepté ou de refuser d'y participer
- Mon consentement ne décharge pas les investigateurs de la recherche de leurs responsabilités, je conserve tous mes droits garantis par la loi

J'accepte librement de participer à cette étude dans les conditions précisées dans la notice de l'information ;

- De répondre aux questions d'enquête
- De communiquer les informations médicales
- De donner 5 ml de mon sang
- De donner des échantillons de selles et d'urine
- De faire une échographie

Je donne mon accord pour que le reste des échantillons prélève pour cette étude soient utilisés dans les études ultérieures mentionnées dans la notice d'information.

Fait à ……. Le……

Noms et signatures

_____ _____

Investigateur Participant

PARENTAL CONSENT FOR CHILDREN (FROM 5 TO 20 ANS)

I the undersigned, Mr./Mrs./Miss (names)

……

Having been invited to grant permission for my child, Mr./Mrs./Miss (names)

……

to take part in the study titled: Identification of host regulators of tissue fibroproliferative pathology in schistosomiasis-diseased individuals in Cameroon. Coordinated by Dr Justin Komguep Nono of the Institute of Medical Research and Medicinal Plants Studies/Medical Research Centre, Yaoundé, Cameroon

- I have understood the information provided on the study/I was explained on what the study is all about
- I understand the goal and objectives of the study
- I have discussed on all my worries
- I am aware of the risks and benefits of the study
- My child is free to stay in the study or not to
- His/her consent does not exempt the researchers from their responsibilities for my child protected by the law

I accept for my above-referred-to child to take part in the study with respect to the information I received;

- Respond to all questions
- Provide my child medical records
- Provide 5 ml of my child blood
- Provide samples of my child stool and urine
- Undergo ultrasonographic examination (echography)

I accept that the samples collected in this study be used for future studies indicated in the information notice.

Done in .....on.....

Names, addresses and signatures

Investigator parent/Legal Guardian

CONSENTEMENT PARENTAL DE L'ENFANT de 5 à 20 ANS)

Je soussigné, Mr/Mme/Mlle (Nom(s) et Prénom(s)…….

Avoir été contacté pour donner l'autorisation en vue de la participation de mon enfant Mr/Mme/Mlle (Nom(s) et Prénom(s).....…….

Au travail de recherche intitulé « Identification des régulateurs contre les pathologies fibroprolifératives tissulaires chez les enfants atteints de schistosomiase au Cameroun » dont le coordinateur s'appelle Dr Justin Komguep Nono de l'Institut de Recherches Médicales et d'Etudes des Plantes Médicinales

- J'ai bien compris la notice d'information qui m'a été remise concernant cette étude / la notice d'information relative à cette étude m'a été lu et expliqué
- J'ai bien compris le but et les objectifs de cette étude
- J'ai reçu toutes les réponses aux questions que j'ai posées
- Les risques et bénéfices m'ont été présenté et expliques
- J'ai bien compris que mon enfant est libre d'accepté ou de refuser d'y participer
- Son consentement ne décharge pas les investigateurs de la recherche de leurs responsabilités, mon enfant conserve tous ses droits garantis par la loi

J'accepte librement que mon enfant participe à cette étude dans les conditions précisées dans la notice de l'information ;

- De répondre aux questions d'enquête
- De communiquer les informations médicales
- De donner 5ml de son sang
- De donner des échantillons de selles et d'urine
- De faire une échographie

Je donne mon accord pour que le reste des échantillons prélèves de mon enfant pour cette étude soient utilisés dans les études ultérieures indiquées dans la notice d'information.

Fait à ……. Le……

Noms et signatures

Investigateur parent/Tuteur Légal

5) Questionnaire

FICHE D'ENQUETE

Site:……..

Ecole:……; Classe:……

A- PARTICIPANT

I- DONNEES SOCIODEMOGRAPHIQUES PARTICIPANT

1.Noms et prénoms……

2.Code…… 3. Age……(ans) 4. Sexe…….

5.Temps de résidence…….. ; Moyenne en classe(/20)…..

II- EXPOSITION

6. contact avec l'eau 7. Nombre de fois/jour

-Bains -Matin
-Lessive -Midi
-Jeux -Soir
-WC

III- TRAITEMENT

8. Traitement reçus les 3 derniers mois

PZQ OUI NON
Autres OUI NON Lesquels ?.....
Date dernier traitement avec PZQ……..
Nombre de fois PZQ reçu……..

IV- ANTECEDENTS ET SYMPTOMES

9. Avez-vous déjà eu la bilharziose ? OUI NON
10. Combien de fois ?.....
11. Avez-vous les symptômes de la bilharziose ?
Diarrhée OUI NON
Fréquence selles/jour 1 2 3 4 4+
Brulures/douleurs anales
Douleurs abdominales
Nausées
Vomissement
Sang dans les selles
Vertiges
Fièvre
Dyspnée
Céphalées
Courbatures

V- VACCINS

12. Avez-vous vos vaccins à jour ?

Polio Rougeole
Tuberculose Hepatite B
Autres……..

VI- EFFETS SECONDAIRES DU PRAZIQUANTEL

13. Parmi les effets suivants, lesquels avez-vous ressenti après traitement au PZQ ?

Maux de tête Douleurs musculaires
Etourdissement Pertes d'appétit
Maux d'estomac/crampes Sudation
Nausées/vomissements Aucun
Fatigue Autres

14. Si effet ressenti, avez-vous des lors peur de prendre le PZQ lors des campagnes de traitement ?
OUI NON

VII- LOCALISATION VIS-À-VIS DE LA RIVIERE

15. Quelle distance sépare la maison de la rivière ?

……

16. combien de temps mettez vous pour aller de la maison a la rivière ?

……..

Données collectées par…….

Date……

Signature du superviseur……..

Date……

FICHE D'ENQUETE

Code:……

B- PARENT

I. NIVEAU D'EDUCATION

Aucun Primaire Secondaire

Supérieur Inconnu

II. CONNAISSANCES SUR LA BILHARZIOSES

1.Niveau de connaissance sur la bilharziose
Aucun Faible Modérée Approfondie
2.Avez-vous déjà entendu parler de la Bilharziose ? OUI NON
3. Ou avez-vous entendu parler de la Bilharziose ?
Famille/Amis/Voisins Professionnel de sante
Réunion de quartier Radio/TV
4. Etes-vous concerne par la Bilharziose ? OUI NON
5. Savez-vous que la bilharziose peut à long terme entrainer des dommages graves dans vos vies ? OUI NON

III. CONNAISSANCES SUR LES COMPORTEMENTS A RISQUE

6. Savez-vous comment on contracte la Bilharziose ? OUI NON
7. Parmi les propositions suivantes lesquelles pensez-vous êtres des comportements a risque pour attraper la Bilharziose ?
Rapports sexuels Agriculture
Mauvaise hygiène sanitaire Alimentation
Contact avec l'eau (pêche, bain…) Alcool
Consommation eau contaminée Héréditaire
Echange de plats Autres
Comment est votre système d'évacuation des selles ?
Cours d'eau Fosse septique Toilettes modernes

IV. CONNAISSANCES SUR LA PREVENTION

8. Comment pensez-vous que l'on puisse éviter la Bilharziose ?
En traitant les personnes malades
En traitant tout le monde
En construisant des toilettes moderne
En observant une meilleure hygiène de vie
En traitant les sources d'eau
Abstinence sexuelle/Rapports sexuels protégés
Aucune idee

V. CONNAISSANCES SUR LES SYMPTOMES/CAT

9. Connaissez-vous les symptômes de la Bilharziose ? OUI NON

10.Citez……

VI. CONNAISSANCES SUR LES SYMPTOMES/CAT (Suite)

11. Quand vos enfants ont les symptômes de la Bilharziose. Que faites-vous ?

Attendre que ça finisse seul (pas d'argent)
Aller à l'hôpital consulter
Aller chez un guérisseur traditionnel
Aller acheter les médicaments en pharmacie
Prier
Rien

VII. CONNAISSANCE SUR LE TRAITEMENT

12. Existe-t-il un traitement contre la Bilharziose ?

13. Si oui donnez le nom…….

14. Vos enfants ont déjà reçu le traitement ?

15. Voulez-vous qu'ils continuent de recevoir ?

VIII. CONNAISSANCES SUR LA PROTECTION

16. Vous protégez-vous contre la Bilharziose ?
Comment ?
En évitant les rapports non protégés
En évitant les contacts avec l'eau
En respectant une bonne hygiène de vie
En traitant l'eau à boire
En priant
Rien

IX. STATUT FAMILIAL

17.Combien de personnes vivent en permanence chez vous ?
……

Données collectées par……

Date……

Signature du superviseur……

Date……

Code:……

FICHE D'ECHOGRAPHIE

FICHE D'ENQUETE

Site:……..

C- EXAMINATION ET LABORATOIRE

1. **Code……**

2. Echantillons Recus

-Selles Couleur des selles

-Sang

5. TDRs

- TDR VIH……
- TDR Hepatite B……
- TDR Hepatite C……

3. Examination

Taille(cm)

Poids (kg)

	OUI	NON
Signe d'anémie		
Hépatomégalie		
splénomégalie		

6. Biochimie

ASAT :……..

ALAT :……..

GGT :……

PAL :……..

Bilirubine :……..

Albumine :……

4. Kato Katz

-*S. mansoni* Négatif …… Positif

Charge parasitaire……

-Autres parasites Négatif …… Positif……

Charge parasitaire……

7. ELISA

Total IgE……

Total IgG4……

FICHE D'ECHOGRAPHIE **Code: …**				
Date d'examen (jj/mm/aa)				
Age du participant (date de naissance)				
Sexe				
Taille du participant (cm)				
MODULE 1- EXAMINATION STANDARD				
Foie 1: Parenchyme				**>Score (IP)**
Pas d'anomalie détectée (profil A) -> fin de l'examen				= 0
Considérer séparément : - Surface irrégulière, veines hépatiques déformées, bord hépatique caudal arrondi (x) - Echogénicité hépatique diffuse et accrue, perte de la grande réflectivité des bords des branches portales périphériques, bord caudales hépatique arrondi, possible extinction du son distal (Y) - Toute autre anomalie hépatique, spécifier (Z)				Aucun score
Foyers échogènes diffus <starry sky> (profil B)				=1
Tuyaux de pipes très échogènes <pipe stems> (profil C, Cb)				=2
Anneau échogène autour de la bifurcation porte <ruff> (profil D, Dc, Db, Dcb)				=4
Patchs/taches très échogènes (de veine a parenchyme) (profil E, Ec, Eb, Ecb)				=6
Bandes et stries très échogènes (de veine a surface) (profil F, Fc)				=8
Score IP Total				
Foie 2 : Epaisseur de la paroi des branches de second ordre				**>Score (PT)**
Epaississement péri-portal suspecte non=0 ; oui=1				Score PT préliminaire
Mesures de la paroi péri-portale (diamètre externe - lumen) des branches portales de second ordre (segments de branches portales de 1er ordre)				
Diamètre (mm)	Vaisseau 1	Vaisseau 2	Vaisseau 3 (optionnel)	Score PT intermédiaire
Score Final d'épaississement péri-portal (PT) (0-8)				

Foie 3 : Taille et anomalies				
Surface irrégulière 2=importante	0=aucune	1=légère		
Bord hépatique caudal	0=tranchant	1=arrondi		
Forme du foie	0=convexe/concave	1=déformée		
Taille du lobe gauche….. mm	Score : moyenne + ≤2SD = 0 ; +2 a 4SD = 1 ; +>4SD = 2			
Taille du lobe droit …….mm	Score : moyenne - ≤2SD = 0 ; -2 a 4SD = 1 ; ->4SD = 2			
Foie 4 : Mesure de l'hypertension portale				**>Score (PH)**
Diamètre de la veine porte…….mm	+ ≤2DS = Score 0; +2 a 4DS = Score 4; +>4DS = Score 6			
Veines collatérales …… Varices spléniques …..veines coronaires ≥4mm…… Varices gastro-œsophagiennes….. Varices pancréatico-duodénales…….Veines para-ombilicale entièrement recanalisée(=3mm) …….. Shunt spléno-rénal …….Autres …….Score : 0=non détectée …… 4= présente……..				
Ascites	score : 0=non détectée	3= présente		
Score final d'hypertension péri-portale (PH) (0-13)				
Rate ≤2SD = 0; 2 a 4SD = 1; >4SD = 2		Epaisseur de la vésicule biliaire <4mm = 0 ; >4mm = 1		

6) ELISA procedure

Kit component and storage:

Item	**Specifications**	**Storage**
Micro ELISA Plate (Dismountable)	8 wells ×12 strips	-20°C, 6 months
Reference Standard	2 vials	
Concentrated Biotinylated Detection Ab (100×)	1 vial, 120 uL	
Concentrated HRP Conjugate (100×)	1 vial, 120 μL	-20°C (protect from light), 6 months
Reference Standard & Sample Diluent	1 vial, 20 mL	4°C, 6 months
Biotinylated Detection Ab Diluent	1 vial, 14 mL	
HRP Conjugate Diluent	1 vial, 14 mL	
Concentrated Wash Buffer (25×)	1 vial, 30 mL	
Substrate Reagent	1 vial, 10 mL	4°C (protect from light)
Stop Solution	1 vial, 10 mL	4°C
Plate Sealer	5 pieces	
Product Description	1 copy	
Certificate of Analysis	1 copy	

Other supplied required:

- Microplate reader with 450 nm wavelength filter
- High-precision transfer pipette, EP tubes and disposable pipette tips
- Incubator capable of maintaining 37°C
- Deionized or distilled water
- Absorbent paper
- Beakers and graduated cylinders
- Loading slot for Wash Buffer

Reagent preparation:

1. **Wash Buffer**: Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40℃ water bath and mix it gently until the crystals have completely dissolved.

2. **Biotinylated Detection Ab working solution:** Calculate the required amount before the experiment (100 µL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the stock tube before use, dilute the 100× Concentrated Biotinylated Detection Ab to a 1×working solution with Biotinylated Detection Ab Diluent.

3. **Concentrated HRP Conjugate working solution:** Calculate the required amount before the experiment (100 µL/well). In preparation, slightly more than calculated should be prepared. Dilute the 100× Concentrated HRP Conjugate to a 1× working solution with Concentrated HRP Conjugate Diluent.

4. **Standard serial dilution:**

Take 7 EP tubes, add 500µL of Reference Standard & Sample Diluent to each tube. Pipette 500 µL of the 500 ng/mL working solution to the first tube and mix up to produce a 250 ng/mL working solution. Pipette 500 µL of the solution from the former tube into the latter one according to these steps. The illustration below is for reference. Note: the last tube is regarded as a blank. Don’t pipette solution into it from the former tube.

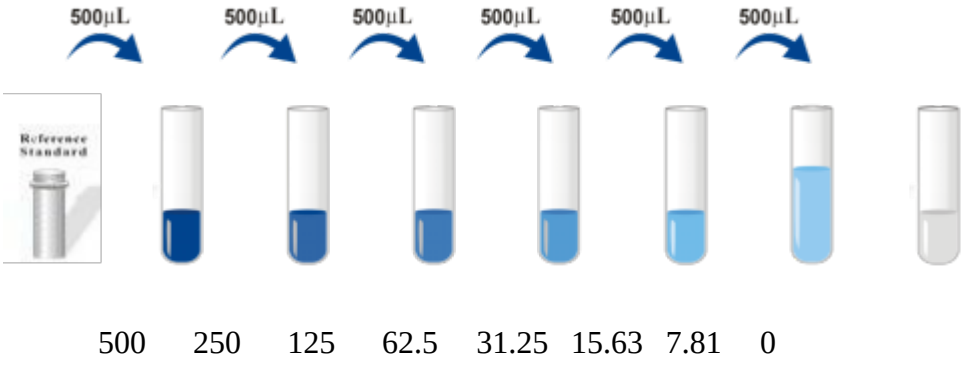


Figure 1: Standard serial dilution

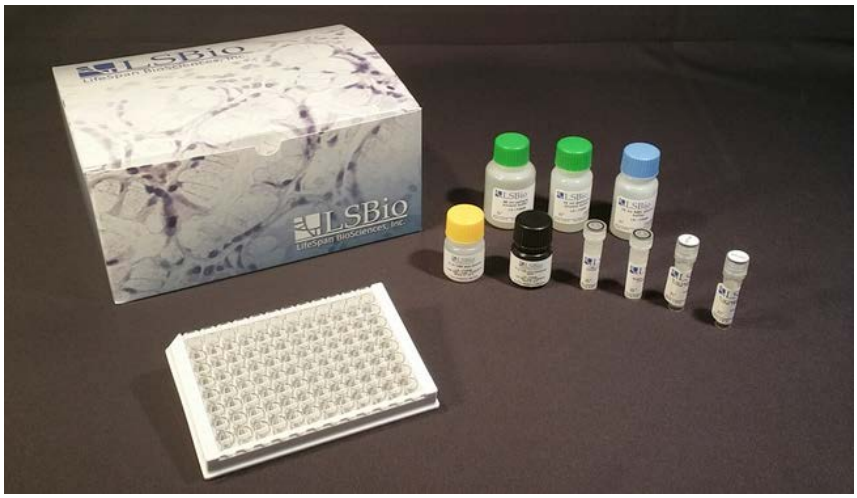
Assay procedure

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate

1. Add the **Standard working solution** to the first two columns: Each concentration of the solution is added in duplicate, to one well each, side by side (100 µL for each well). Add the samples to the other wells (100 µL for each well). Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37℃. Note: solutions should be added to the bottom of the microELISA plate well, avoid touching the inside wall and causing foaming as much as possible.
2. Remove the liquid out of each well, do not wash. Immediately add 100 µL of **Biotinylated Detection Ab working solution** to each well. Cover with the Plate sealer. Gently mix. Incubate for 1 hour at 37°C.
3. Aspirate or decant the solution from each well, add 350 µL of **wash buffer** to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this and other wash steps.
4. Add 100 µL of **HRP Conjugate working solution** to each well. Cover with the Plate sealer. Incubate for 30 min at 37°C.
5. Aspirate or decant the solution from each well, repeat the wash process five times as conducted in step 3.
6. Add 90 µL of **Substrate Reagent** to each well. Cover with a new plate sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but no more than 30 min.
7. Add 50 µL of **Stop Solution** to each well. Note: Adding the stop solution should be done in the same order as the substrate solution.
8. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

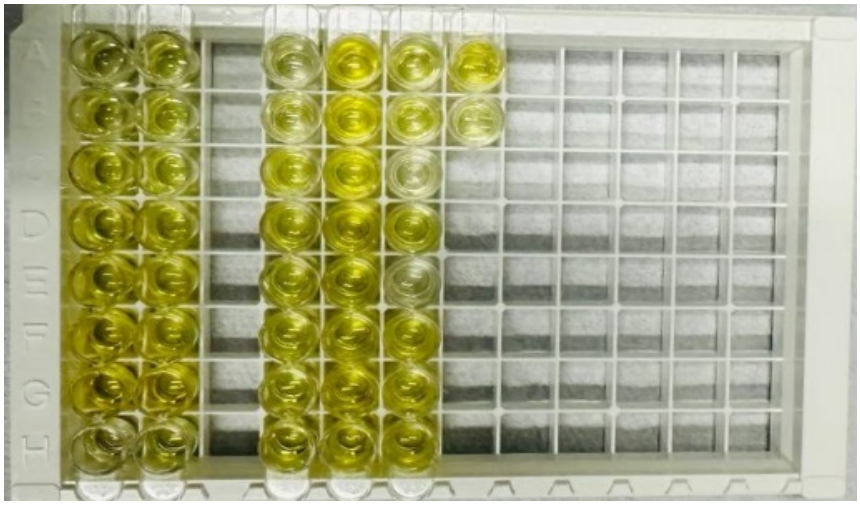
9. Calculation of results: Average the duplicate readings for each standard and sample, then subtract the average zero standard optical density reading. Plot a four-parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis. If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it with an appropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factors including the amounts used in the sample preparation procedure.

7) ELISA equipment

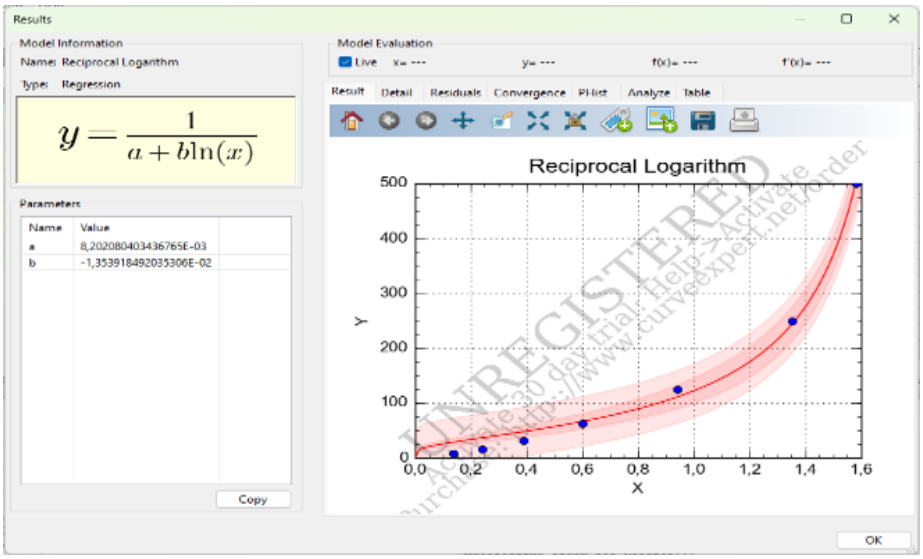


Human IgE ELISA Kit, Catalog Number: EKC31252

ELISA reader used to measure absorbance in samples at the end of experiments



Final 96-well plate after stop solution: An image of the plate following the addition of the stop solution, showing the completed colorimetric reactions.



Reciprocal Logarithmic Standard Curve, generated using Cuve-Expert software to calculate concentrations from absorbance values