DEPARTMENT OF PUBLIC HEALTH AND INFECTIOUS DISEASES

PhD thesis

Cycle XXXV

Modulation of host immunity to malaria by *Schistosoma haematobium*: observational and experimental investigations

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TO

My parents, Patrice and Alponsine

My husband Dominique and children Ornella and Oswald

In memorium of my colleague and friend Barry/Some Ines

SUMMARY

Malaria represents a major public health problem, with 247 million clinical cases and 619000 deaths reported in 2021, the great majority of which occurring in children and pregnant women in Sub-Saharan African countries and caused by *Plasmodium falciparum* (World Malaria Report 2022). In these countries, diseases caused by helminths such as intestinal nematodes, lymphatic filariae and schistosomes are also endemic but often neglected. Although few studies suggest that co-infection with helminths might modulate the immune response towards intracellular pathogens including *Plasmodium* (Salgame et al. 2013), conclusive evidence is not available. In particular, little is known on the immunomodulatory role of *Schistosoma haematobium*.

The major aim of this thesis was to therefore investigate the impact of *S. haematobium* infection on immunity to malaria combining *in natura*, *in vitro* and *in vivo* approaches.

An observational study *in natura* was conducted in human populations living in rural villages from Burkina Faso, who had been previously shown to have different susceptibility to *P. falciparum* malaria, and where *S. haematobium* is also endemic. Indirect (anti-SEA and anti-SWAP IgM and IgG antibodies) and direct (Circulating Anodic Antigen and circulating DNA) markers of *S. haematobium* infection were measured in plasma samples collected at baseline of a repeated cross-sectional malariological study. As expected, the seroprevalence of specific antibodies (45-72%) was much higher than that of either *S. haematobium* circulating antigen (28%) or DNA (26%). In the multivariate logistic regression analysis of demographic factors that might affect the prevalence of *S. haematobium* infection, all markers showed a clear increase in seroprevalence from children 1-4 years old to 5-9 years old, with a maximum in the age group 10-19 years old and a decrease at older ages. No clear differences were observed between sexes, villages, or ethnicities. In the comparison between cDNA and CAA testing, overall agreement was 85%, with cDNA showing a 73% sensitivity and a 94% specificity compared to CAA; however, the use of a composite reference standard where a sample is considered positive when either one or both direct markers are detected can be envisaged. In the comparison between *S. haematobium* specific antibodies and CAA testing, anti-SEA IgG showed the best diagnostic performance. The association between CAA, as a validated marker of *S. haematobium* infection, and the prospective risk of *P. falciparum* malaria was assessed in the study populations by multivariate regression analysis adjusted for the effect of age, sex, village, ethnicity, and hemoglobin genotype. Results suggest that infection with *S.*

haematobium, or higher infection intensity with this trematode, increases susceptibility to *P. falciparum* malaria, in line with those of a systematic review and meta-analysis (Degarege et al., 2016).

An experimental study has been conducted *in vitro* to evaluate the immunomodulatory properties of *S. haematobium* candidate antigens tetraspanins (TSP) 2, 6 and 23 on cultured Dendritic and T CD4+ Cells from human donors. Results of flow cytometry show that the three tetraspanins were able to induce the expression of DCs maturation markers HLA-DR, CD80 and CD86. Results of Luminex assay show that the three tetraspanins were able to induce the production of inflammatory cytokines IL6, TNFalpha, IL12p70 and IL33, as well as of the Th2 cytokine IL13 and the regulatory cytokine IL10. Finally, results of RT quantitative PCR show that the supernatants of tetraspanin-stimulated DCs were able to induce changes in cytokine gene expression in T CD4+ cells, with different pattern depending on the tetraspanin. Such interesting results deserve confirmation in a larger number of donors.

A second experimental study has been conducted *in vivo* to evaluate the impact of exposure to the known *S. haematobium* immunomodulatory antigen HIPSE on the course of *P. berghei* infection in CD1 mice. Results of two experiments using a parasite clone expressing green fluorescent protein and flow cytometry show a slower growth of parasite density in HIPSE treated versus control mice, although with inconclusive statistical evidence. A second round of experiments including a dose-response component are needed to demonstrate an impact of HIPSE treatment in reducing *P. berghei* parasite density in mice, and therefore a role of this antigen in modulating anti-malarial immune responses.

Novel insights have been provided into the diagnostic value of different plasma biomarkers, into the ability of *S. haematobium* antigens to modulate the host immune response and thereby to affect immunity to malaria, and into the impact of *S. haematobium* infection to affect the prospective risk of *P. falciparum* malaria. Further investigations are needed to confirm the results of this thesis and to characterise immunomodulatory antigens and host responses. Such kind of knowledge has potential for application in products development (drugs and vaccines) and in malaria control. For instance, anti-helminthic mass treatment campaigns in the period preceding the malaria transmission season could be deployed to decrease the incidence of cases of infection/disease, or before malaria vaccination to increase the immune response to the vaccine itself and therefore its effectiveness.

ABBREVIATIONS

ADCC	Antibody-Dependent Cell Cytotoxicity (ADCC)
APC	Allophycocyanin fluorochrom
APCs	Antigens Presenting Cells
ASC	Antibodies Secreting Cells
BSA	Bovine Serum Albumin
CAA	Circulating Anodic Antigen
CD	Cluster of Differentiation
CSP	Circumsporozoite Protein
DNA	Desoxyribonucleic Acid
ELISA	Enzyme Linked Immunosorbent Assay
FcRs	Fc receptors
FITC	Fluorescein isothiocyanate
GC	Germinal Center
GFP	Green Fluorescent Protein
GM CSF	Granulocyte-Macrophage Colony-Stimulating factor
Hb	Hemoglobin
HLA-DR	Human Leukocyte Antigen – DR isotype
LPS	Lipopolysaccharides
MAEBL	Membrane associated erythrocyte binding like protein
MCP	Monocyte chemotactic protein
MFI	Mean Fluorescence Intensity
MHC	Major Histocompatibility Complex
MIF	Migration Inhibitory Factor
NETS	Neutrophils Extracellular Traps (NETS)
NKT	Natural Killer T cell
OPD	o-phenylenediamine dihydrochloride
PE	Phycoerythrin fluorochrom
RBC	Red Blood Cells

ROC	Receiver Operating Characteristic
ROS	Reactive oxygen species (ROS)
RPMI	Roswell Park Memorial Institute medium
SEA	Schistosoma haematobium egg antigen
SNP	Single Nucleotid Protein
SWAP	Schistosoma Worm Antigen Preparation
TCA	Trichloro acetic acid
Tfh	T follicular helper
TGF	Growth Factor
TLRs	Toll like receptors
TNF	Tumor Necrosis Factor
TNFR	Tumor Necrosis Factor Receptor
TRAP	thrombospondin-related anonymous protein
TRAP	Tryptophan Regulated Attenuation Protein
Treg	regulatory T cells
TSP	Tetraspanins
UCP LF	Up converting phosphore lateral flow

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CHAPTER 1. INTRODUCTION

If the global efforts have made it possible to reduce the disease and death rate attributable to malaria through several strategies including prevention diagnosis and treatment, malaria remains a major public health burden. According to World Health Organization, in 2021, there where an estimated 247 million malaria cases in 84 malaria endemic countries with 619 000 deaths and victims under five years old represented 78.9%. 593 000 of these deaths have been enumerated in WHO African regions with 3,3% in Burkina Faso (WHO, 2022). Transmitted by the bite of infective female Anopheles mosquito, malaria is caused by a parasite of the genus Plasmodium. Among the 30 Anopheles species, which transmit the disease, *An. gambiae* s.s, *An. arabiensis* and *An. funestus* are the three main vectors commonly found in Sub-Saharan Africa (Mandala et al., 2021). About *Plasmodium* parasites, six species have been identified to commonly infect humans: the anthroponotic *Plasmodium falciparum*, *P.malariae, P. vivax*, *P. ovale wallikiri* and *P. ovale curtisi*, and the zoonotic *P. knowlesi* (Ansari et al., 2016; Fuehrer & Noedl, 2014; Zaw & Lin, 2017). However other simian parasites, *P. cynomolgi* (Imwong et al., 2019), *P. brasilianum* (Lalremruata et al., 2015) and *P. inui* (Liew et al., 2021) have been observed in humans. Among the cited Plasmodium species, the greatest impact on human health in terms of morbidity and mortality is from *P.falciparum* malaria, which is endemic in most of Sub-Saharan Africa countries and in many tropical's world regions(Doolan et al., 2009). Previous studies in Burkina Faso estimates Plasmodium species distribution. Gnémé and colleagues found that *P. falciparum* was the predominant species with a prevalence of 68.19% followed by *P. malariae* (6.51%) and *P. ovale* (1.08%) (Gnémé et al., 2013). The present research project mainly focuses on *P. falciparum*.

1.1. *Plasmodium* life cycle

Plasmodium are obligate intracellular apicomplexan (Crompton et al., 2014) with a complex life cycle. The asexual cycle takes place in the intermediate vertebrate host and the sexual cycle occur in the definitive host, a female Anopheles mosquito. During a blood meal, the infected mosquito injects an average of 15–123 sporozoites mostly into human dermis or sometimes directly into the blood vessels (Amino et al., 2006). Then, sporozoites migrate into the liver, invade and infect hepatocytes. CSP, TRAP and recently MAEBL are mediators of the

invasion process (Sá et al., 2022; Steinbuechel & Matuschewski, 2009). Inside the hepatocytes, sporozoites extensively multiply over a week's time, increasing in number, and differentiate, giving rise up to 30000 asexual blood stage parasites called, merozoites. This is the pre-erytrocytic phase (Bonam et al., 2021; Crompton et al., 2014; Krettli & Miller, 2001). Merozoites are released into blood and infects erythrocytes (Sato, 2021; Sturm et al., 2006), initiating the erytrocytic phase, origin of malaria disease. In the red blood cell, a single invading merozoite begins schizogony as a ring stage, mature into trophozoite and then undergoes several mitosis and nuclear division to produce the schizont (Gerald et al., 2011). After erythrocyte rupture, 12 to 32 new merozoites are released back to the blood, and the parasites repeat this intraerythrocytic propagation cycle every 48 hours (Sato, 2021). The process of invasion sets in motion malaria clinical symptoms typically 12 days after infection (Koch & Baum, 2016) A small proportion of asexual parasites converts to gametocytes, male (microgamete) and female (macrogamete). During a blood meal on an infected human, the mosquito ingests the sexual parasites and gametocytogenesis continue with some cycle of mitosis in the midgut with the exflagellation of the microgamete to become a gametocyte male. Then occurs the fusion of male and female gametes to become a zygote which progress to an ookinete. Afterwards, the ookinete leaves a basal side to become a proliferative form so called oocyst producing sporozoites (Krettli & Miller, 2001; Liu et al., 2011). The sporozoites escape from the oocyst to invade the mosquito's salivary glands, ready to be injected during the next blood meal (Figure 1).

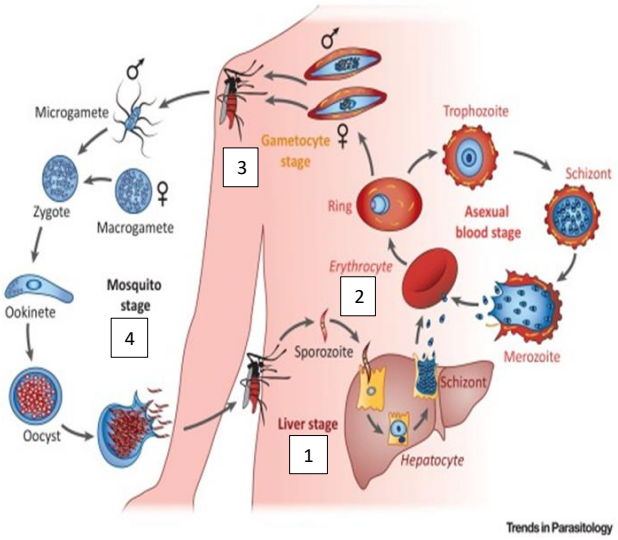

Figure 1 : *Plasmodium falciparum* life cycle. Adapted from *(Maier et al., 2019)*

1.2. Pathogenesis and clinical outcomes

- Invasion of Red Blood Cells

Plasmodium falciparum is known to infect any age Red Blood Cells (RBC), leading to higher parasitemia and thus creating a general correlation between the parasitemia and malaria prognosis (Garcia, 2010). Due to the crucial impact of intraerythrocytic cycle on pathology, the pathway of RBC invasion has been investigated and the process divided into four distinct stages. Process starts with an initial merozoite attachment mediated by the merozoite surface protein (MSP 1), followed by the re-orientation of the merozoite to its apical end, and stronger binding to glycophorin A, B or C or to the complement receptor 1 on the erythrocyte surface. This step is mediated by the erythrocyte-binding-like (EBL) protein or *P. falciparum* reticulocyte-binding protein homologs (PfRh). After that, the formation of a tight or moving junction is observed. Finally, the squeleton of the RBC formed by spectrin tetramers which ends by short actin filaments undergo a weak deformation due to the phosphorylation induced by increased Ca^{2+} leading to a complete invasion and sealing of the parasitophorous vacuole(Koch & Baum, 2016; Warncke & Beck, 2019).

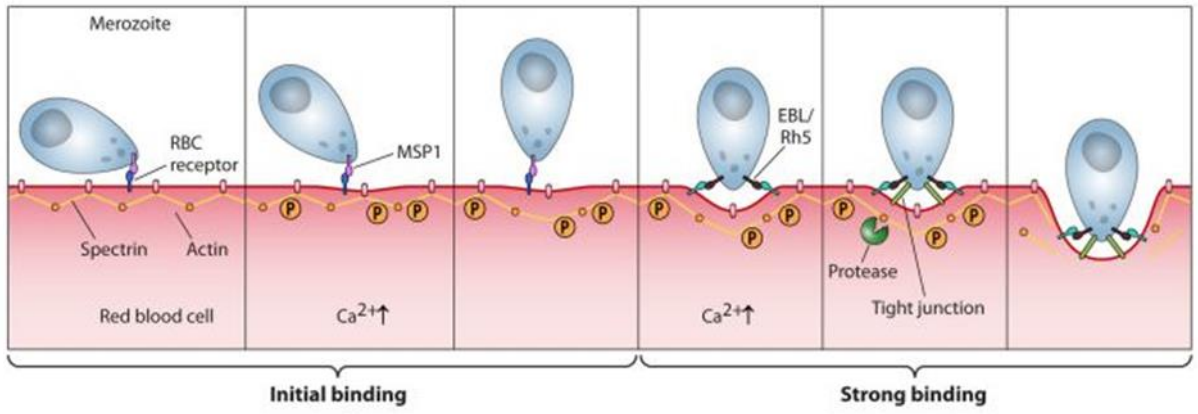

Figure 2: Red blood cell invasion mechanism by *P.falciparum* merozoite adapted from *(Warncke & Beck, 2019)*.

- Impact of invasion on RBCs

Once inside the RBC, parasites use their ability to modify the host cells and some changes, like formation of knobs, cytoadherence and rosetting can be observed during the pathogenesis (Nureye & Assefa, 2020). Indeed, after invasion, parasite encoded proteins such as PfEMP1, KAHRP, PfEMP3 become either associated with the erythrocyte cytoskeleton or inserted into

the host cell membrane leading to a modification of RBCs. While PfEMP1 is responsible of immune invasion and adhesion of infected RBCs to host receptors such as CD 36 (cytoadherence), KAHRP is essential for the formation of an electro dense, cup shaped structure so called knob that deform the erythrocyte surface (reviewed by (Wickham et al., 2001)). PfEMP3 is predicted to facilitate the escape of new generation of merozoites into bloodstream (Pei et al., 2007). PfEMP1 has also been identified as a rosetting ligand allowing infected erythrocytes to bind to uninfected cells, and exacerbating microvascular obstruction (Ashley et al., 2018; Chen et al., 1998). Another mechanism acting in sequestration and thought to be important in pathogenesis is a binding of platelet and infected RBCs so called clumping (Moxon et al., 2011).

- Uncomplicated malaria

From asymptomatic parasites carriage, infection can lead to symptoms, organs dysfunction and death (Patel et al., 2020). According to the severity of the disease, malaria has two presentations. The clinical symptoms of uncomplicated malaria are usually common to other diseases and can be presented as fever, chills, body-aches, headache, cough, and diarrhea, making clinical diagnosis unreliable (Ashley et al., 2018). Parasitaemia < 250000 parasites per µl of blood is also characteristic of uncomplicated malaria (WHO, 2010).

- Severe malaria

For the management of severe malaria, hyperparasitemia is defined as more than 5% erythrocytes parasitized, acknowledging that immune individuals in high transmission areas may tolerate higher parasitemias with few symptoms (WHO, 2010). In severe malaria, possible huge complications starting by some hematological changes like anemia (<8 g/dL), leukocytosis, leucopenia, thrombocytopenia can be observed (Maina et al., 2010). In addition, erythrocytes containing mature parasites can be sequestrated in vessels avoiding clearance in the spleen but leading to microvascular obstruction and endothelial cell injury in the host. The severity of *P falciparum* malaria seems to be positively correlated with the number of sequestered infected RBCs. Sequestration in the brain contributes to coma and cerebral malaria, the outcome in the lungs is respiratory failure (Ashley et al., 2018; Garcia, 2010).

1.3. Immunity

Immunity to malaria is acquired over the time depending on age, exposure and transmission intensity. Pregnant women and children under five years old are more at risk of clinical malaria. However, the immunity acquired in an endemic area is not permanent as it regress and can be lost in case of long period immigration to a non-endemic area. In addition, the complexity of parasite life cycle, the diversity of its surface antigens associated to it abilities to avoid elimination strain the immune system. However, mechanisms involved in host defenses against the parasite have been extensively investigated.

1.3.1. Innate immunity

Parasites are detected as well as in liver stage as in blood stage and the innate response is balance by production of pro-inflammatory and anti-inflammatory cytokines, playing then a key role in protective immunity and in pathogenesis. In response to malaria infection, neutrophils are the first responders to sporozoites injection through the skin(Gowda & Wu, 2018) . Their clearance activities include phagocytosis, production of reactive oxygen species (ROS), and formation of neutrophils extracellular traps (NETS). Activities are mediated by different immunoglobulin binding receptors, FcγRs (Aitken et al., 2018; Feng et al., 2021). The implications of FcγRs have been reviewed and their role in triggering activatory and/or inhibitory signaling pathways, thus in regulating immune response established. Indeed, effectors in DCs maturation and their regulatory activities, FcγRs control either immunogenic or tolerogenic response initiation during the peptide antigenic presentation to T cells. In addition, FcγRs co-operate with Toll-like receptors (TLRs) in controlling levels of IL-12 and IL-10, which are regulatory cytokines (reviewed by (Rj, 2009)).

In response to the liver stage infection, the NKT cells kill the parasites infected hepatocytes with the contribution of the type I IFNs produce (Gowda & Wu, 2018). During the blood stage, the release of hemoglobin digestion product, hemozoin, activates macrophages and DCs for the production of different pro-inflammatory cytokines and chemokines such as interleukin IL6, TNFα, IL12, IL18, monocyte chemotactic protein (MCP) and also anti-inflammatory cytokines and chemokines including IL10 and macrophage migration inhibitory factor (MIF) (Coban et al., 2007).

1.3.2. Adaptive immunity

In an antigen specific defense mechanism, DCs activate CD4+T cells which are polarize under the influence of cytokine milieu into Th1, Th2, Th17, Treg, and Tfh cells (Bonam et al., 2021). The initiation of an early and prompt cell-mediated immune response to *P. falciparum* requires activation of Th1 cells with consequent pro-inflammatory cytokines, mainly IFN-γ (Torre et al., 2002). Th2 cells major cytokine, IL-4 can promote B cell class switching and modulate macrophage responses during infection (Kurup et al., 2019). In patients with uncomplicated *P. falciparum* malaria, IFN-γ represents a key molecule in antimalarial host defense but a more pronounced Th2 driven immune response with a shift towards Th1 responsiveness induced by parasite clearance has been shown (Torre et al., 2002; Winkler et al., 1998). However, malaria parasite specific CD8+ T cells, which have been described in a blood of endemic areas inhabitants and after vaccination recognize pathogen-derived peptides bound to surface MHC class I molecules on APCs or infected cells. NK cells in turn, perceive the signals by cytokines, produced from DCs, monocytes, and/or macrophages.

Activated NK cells secrete inflammatory cytokines such as IL-8, IFN-γ, TNF-α, CCL4, and others, which act as danger signals to alert other immune cells. Also, NK cells perform cytotoxicity as one of their main functions and kill the infected cells either directly or indirectly using perforin and granzyme and antibody-dependent cell cytotoxicity (ADCC), respectively (Bonam et al., 2021).

To clear parasites infection, the immune system simultaneously triggers cellular and humoral responses, which converge to the onset of a long lasting, protective response. The scenario involves an interaction between the Germinal Center (GC) B cells with the activated CD4+T cells in structure of lymphoid organs called follicles. These particular CD4+T cell subset are called the follicular T helper (Tfh) cells. Within the GCs, B-T cell talk leads to B cell activation, which mature through somatic hypermutations in V (D) J Immunoglobulin (Ig) genes as well as the Ig isotype switch, and differentiation into Antibodies Secreting Cells (ASCs). The Tfh cytokines IL-6 and IL-21 have been shown to regulate B cell survival and cell differentiation (Silveira et al., 2018).

1.4. Impact of helminth infection on immunity to malaria

The regions where malaria ride are also at risk of helminths infections. Schistosomiasis, filariasis, and gastrointestinal infection with nematodes are prevalent in Sub–Saharan Africa, south America and certain parts of Asia, where malaria is highly endemic. Some findings show that helminth infection triggers a complex immune response that includes activation of Th2 cytokine producing cell populations of the innate and adaptive immune systems in response to alarmins cytokines. The cytokines produced under the polarization of Th1 and Th2 cells induce functionally distinct subsets of macrophages: classically activated (M1) macrophages and alternatively activated (M2) macrophages, respectively. M1 macrophages secrete proinflammatory cytokines, have high expression of reactive oxygen and nitrogen intermediates and are key effectors against intracellular pathogens. In contrast, M2 macrophages secrete the anti-inflammatory cytokines IL-10 and TGF-β, have high expression of arginase-1, directly promote. wound healing and mediate resistance against helminths (rewied by(Salgame et al., 2013)).

According to a Global Atlas of Helminth Infection, the most widespread helminthic disease is schistosomiasis, which is endemic in 70 developing countries with more than 200 million, infected people worldwide. An estimated 90% of infection occur in Sub-Sahara African populations (GAHI, 2021). Therefore, the impact of co-infection with this helminth species on malaria immunity was addressed by few epidemiological studies. Nevertheless, the various studies conducted have investigated the effect of *S.haematobium* on *Plasmodium* parasitemia, cytokines regulation or malaria occurrence.

Regarding the investigations in Sub-Saharan Africa, in a study in Senegal, Diallo and colleagues did not observe any influence of *S.haematobium* on *Plasmodium* parasitemia, but have found an increased plasma levels of IFN-y and STNF-RII in children and of IFN-y, IL-10, TGF-ß, STNF-RI and STNR-RII in adults showing an age protective effect of *S. haematobium* (Diallo et al., 2004). In another study in Senegal, Briand and colleagues found that children lightly infected with *S.haematobium* had lower *P.falciparum* densities and suggested a negative interaction between to two parasites (Briand et al., 2005). In a study in Mali, Lyke and colleagues have observed an association between *S.haematobium* infection with a delayed clinical malaria onset and lower parasites densities (Kone et al., 2005). Few years later, other investigations also in Mali, suggested an association of *S. haematobium* co-infection with enhanced

protection from febrile malaria in long-term asymptomatic carriers of *P.falciparum* (Doumbo et al., 2014). According to Lemaitre and colleagues in a study in Senegal, a low intensity of *S. haematobium* infection could improve protective immune response to malaria associated with the regulation of specific cytokines production while a high infection could promote the occurrence of malaria related to strong cytokines production (Lemaitre et al., 2014). Based on these findings which suggested on a one hand a synergistic reaction with a protective effect and on another hand antagonistic reactions which exacerbate malaria infection, a systematic review and meta-analysis has been conducted suggesting an association between *S.haematobium* infection and increased prevalence of *P. falciparum* asymptomatic / uncomplicated malaria (Degarege et al., 2016).

However, little is known about the immunological mechanisms involved, and immunomodulatory molecules have been scarcely investigated in this trematode compared to other species of the genus, namely *S. mansoni* (Maizels et al., 2018).

For our contribution to the knowledge on the impact of *S.haematobium* infection in *Plasmodium falciparum* malaria, we have conducted a population-based study in Burkina Faso, our home country. Burkina Faso is endemic for malaria and schistosomiasis. Several epidemiological studies on *Plasmodium falciparum* malaria as well as community-based studies have been previously carried out in certain parts of the country.

1.5. Seroprevalence of helminths infection in populations from Burkina Faso showing different susceptibility to malaria

A possible approach to the investigation of the impact of *S. haematobium* on immunity to *P. falciparum* is the comparison of populations showing different susceptibility to malaria.

The Fulani ethnic group from Burkina Faso has been shown to mount stronger immune responses to *P. falciparum* antigens and to be less susceptible to malaria infection and mild disease than neighbouring Mossi and Rimaibe exposed to comparable levels of malaria transmission (Modiano et al., 1996).

We have previously conducted a retrospective study on the prevalence of IgG against helminth antigens in rural villages of Burkina Faso inhabited by Fulani, Mossi and Rimaibe communities. Findings shows that the seroprevalence of *Strongyloides stercoralis*, *Wuchereria bancrofti* and *Schistosoma haematobium* (Soluble Egg Antigen, SEA) was 5%, 16% and 63% respectively, in line with estimates of infection prevalence in the region for the three parasites (Mangano et

al., 2020) . Anti-SEA IgG prevalence did not show differences between ethnic groups, although lower levels were observed among the malaria resistant Fulani population; it remains to be confirmed whether lower anti-SEA IgG levels are indicative of lower intensity of *S. haematobium* infection in the Fulani (Mangano et al., 2020). Since seroprevalence was highest for *S. haematobium*-specific antibodies, this thesis will focus on the impact of *S. haematobium* infection on immunity to malaria.

1.6. *S.haematobium* parasite, life cycle and pathogenesis

Responsible of urogenital schistosomiasis, *S.haematobium* parasite is a dimorphic blood fluke of the class of trematode whose first host is a fresh water snail of genus *Bulinus.* Mature schistosomes have a length of 7-20 mm, a cylindrical shape with two endings suckers, a blind digestive tract, a complex tegument and reproductive organs (Adenowo et al., 2015). Life cycle of *S.haematobium* is divided into two main stages, one completed in an intermediate snail host and the other in definitive host (humans and other mammals). The life cycle (Figure 2) begins when infected mammal host release parasite eggs through the urine in external environment. When eggs reach fresh water, they form miracidia, hatch and then release free-living ciliated miracidia that infect snail. After infiltration in a snail, the miracidium removes the ciliated plate to become an adult sporocyst. Parasite undergoes asexual replications from mother to daughter sporocysts through successive generations and free swimming cercariae are released into water(Colley & Secor, 2014; Nelwan, 2019; Shebel et al., 2012). During a contact with infected water, cercariae penetrate the skin of human host, shed their forked tail, migrate in a blood via the lungs to the liver and transform into young worms or schistosomula. Schistosomula mature and migrate to the venus plexus of bladder, where they couple and female worms lay eggs, which are secreted in urine to restart the life cycle (Adenowo et al., 2015; Gryseels et al., 2006; Nelwan, 2019).

Most of the pathogenesis of *S.haematobium* infection is mainly associated with body reaction to the eggs. Indeed, some days after the first infection, which can be often asymptomatic, a rash or itchy skin is observed. Within 1-2 months of infection, symptoms such as fever, chills, cough, and muscle aches may develop. A systemic hypersensitivity reaction against immature Schistosoma migrating through the body, a katayama syndrome can occur in a delay up to 84 days after primary infection. The chronic infection is classically characterised by the presence of blood in urine (haematuria). Rarely, diarrhoea combined with eosinophilia is observed in

tropical travelers. In addition, gynaecological outcomes can occur. In female subjects, *S.haematobium* infection lead to vaginal bleeding, pain during sexual intercourse, nodule in vulva and ectopic pregnancies while in male subjects, it induces pathology of seminal vesicles, prostate and other organs and even lead to infertility and bladder cancer. In children, infection can cause stunting, and diminished learning ability (Coltart & Whitty, 2015; Ross et al., 2007). Neurological schistosomiasis due to an immune reaction to egg deposition in the brain and spinal cord is common in people from newly endemic area soon after the first infection and symptoms presentation are signs of severe disorder in which prognosis depends on early treatment (Coltart & Whitty, 2015; Ferrari & Moreira, 2011).

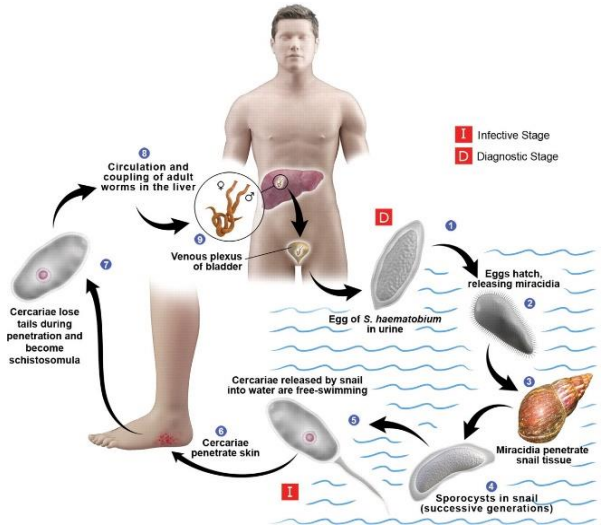

Figure 3: *Schistosoma haematobium* life cycle *(Shebel et al., 2012)*.

1.7. *S.haematobium* diagnosis

Several approaches are used to diagnose *S.haematobium* infection depending to the specific needs of the study. For parasitological diagnosis, urine filtration technique is used for eggs count by microscopy (Knopp et al., 2018). Detection of haematuria in urine is realized using Hemastix strip test (Ibironke et al., 2012). For antigen detection, a rapid diagnostic strip test which sensitivity is not unanimous is used for Circulating Cathodic Antigen (CCA) detection in urine(Obeng et al., 2008) and Up Converting Phosphore Lateral Flow (UCP-LF) assay is used to measure the Circulating Anodic Antigen in urine, serum or plasma (Corstjens et al., 2014). Polymerase Chain Reaction based techniques are used to collect molecular evidence of

S.haematobium infection by the measurement of DNA fragment in urine and of circulating DNA (cDNA) in plasma (Cnops et al., 2013; Ibironke et al., 2011). The serological diagnosis is based on specific antibodies detection in plasma samples (Ibironke et al., 2011). Designed to be applied as a screening test, the sensitivity of serological test is required to be enough. A level of anti-SWAP IgG has been evaluated in serum eluted from dried blood spot to compare the sensitivity and specificity of ELISA test and infection prevalence of *S.haematobium* in Ghana. Anti-SWAP IgG diagnostic specificity was 57% and the sensitivity was 48% compared to four other diagnostic test (Koukounari et al., 2010). Another study in Sudan compared the diagnostic values of urine microscopy and ELISA measurement of anti-SEA IgG level. The diagnostic sensitivity of ELISA was 94.8% and sensitivity was 29.7% (Song et al., 2018).

1.8. *S.haematobium* immunomodulatory molecules

A growing interest is being shown in the immunomodulatory properties of *S.haematobium* molecules. Diverse investigations have allowed the characterization of worm proteins, some derived from mature eggs, others from teguments or other parts. Our attention was drawn to some studies on this subject, and these publications provided the main sources for articulating some of the research questions of the present work.

In effect, Pennington and colleagues have identified multiple variant of proteins derived from *S.haematobium*, which are homologous to IPSE/alpha 1 in *S.mansoni* (M-IPSE), and are called HIPSE. Only detectable on mature eggs, HIPSE have shown an ability to infiltrate the nucleus and to modulate host gene transcription. At molecular level, HIPSE is predicted to play a central role in the control of host-parasite relationship (Pennington et al., 2017). Indeed, HIPSE has been shown to be a potent anti-inflammatory and therapeutic molecule in ifosamide induced hemorrhagic cystitis and bladder pathogenesis associated with bacterial infection. (Mbanefo et al., 2019, 2020), confirming the immunomodulatory properties of the protein.

Sotillo and colleagues have characterised proteomes from adult excretory/secretory products (ES), tegument proteins, egg ES and soluble egg antigen (SEA) of *S.haematobium* (Sotillo et al., 2019). Proteomic analysis of ES product and extracellular vesicle (EVs) showed different proteins including tetraspanins (TSP) (Mekonnen et al., 2020). The family of proteins called tetraspanins are used as vaccine candidates by research teams. Authors have characterized six TSPs from the secretome (including the soluble excretory/secretory products, tegument and extracellular vesicles) of *S. haematobium* (*Sh*-TSP-2, *Sh*-TSP-4, *Sh*-TSP-5, *Sh*-TSP-6, *Sh*-TSP-

18 and *Sh*-TSP-23) for molecular information and other implications (Mekonnen et al., 2022). Vaccine development imply diverse strategies including the selection of key schistosome molecules in the live parasite that are exposed to the host immune system and are essential for parasite survival (Tebeje et al., 2016). Therefore, highest number of unique protein enriched with oxidoreductase activity, ATP binding transferase activity and cytoskeletal protein binding characterizes our proteins of interest. They are located at the interface between the host and the parasite and are believed to play a key role in host immune system modulation and parasite survival (Sotillo et al., 2019) given immunomodulatory properties to these molecules.

CHAPTER 2. AIMS AND OBJECTIVES.

The general aim of this thesis is to investigate the impact of *Schistosoma haematobium* infection on immunity to malaria.

The specific objectives are:

i. To measure biomarkers of *S. haematobium* infection in plasma samples from endemic populations;
ii. To evaluate the diagnostic agreement between biomarkers of *S. haematobium* infection in plasma samples;
iii. To investigate the association between a previously validated plasma biomarker of *S. haematobium* infection and risk of *Plasmodium falciparum* malaria in populations living in areas of co-endemicity;
iv. To characterize immunomodulatory properties of *S. haematobium* candidate antigens (tetraspanins);
v. To assess the impact of exposure to known *S. haematobium* immunomodulatory antigens (HIPSE) on immunity to malaria;

To address these specific objectives, three different studies were undertaken.

The first study is an observational study *in natura* conducted in human populations from Burkina Faso, and addresses objectives i. to iii. Firstly, indirect (anti-SEA and anti-SWAP IgM and IgG antibodies) and direct (Circulating Anodic Antigen and circulating DNA) markers of *S. haematobium* infection were measured in plasma samples. Secondly, the diagnostic agreement between *S. haematobium* indirect and direct markers was evaluated. Thirdly, the association between *S. haematobium* Circulating Anodic Antigen and risk of *P. falciparum* malaria was assessed in the study populations.

The second study is an experimental study conducted *in vitro* and addresses objective iv. Firstly, the ability of three different tetraspanins proteins of *S. haematobium*, TSP2, TSP6 and TSP23, to induce maturation and cytokines production in human Dendritic Cells (DCs) was investigated. Secondly, the ability of above DCs supernatants to induce T helper cells differentiation and cytokine gene expression was assessed.

The third study an experimental study conducted *in* vivo in a murine malaria model, and addresses objective v. Two experiments were performed to evaluate the impact of exposure to the known *S.haematobium* immunomodulatory antigen HIPSE on the course of *P.berghei* infection in mice.

CHAPTER 3. METHODOLOGY

3.1. Observational study *in natura*

3.1.1. Study area and populations

Malaria epidemiological surveys have been conducted in 2007-2008 in two rural villages (Barkoumbilen and Barkoundouba) of shrubby savannah areas of Burkina Faso northeast of the capital town Ouagadougou. The village of Barkoumbilen is inhabited by Mossi and Rimaibè communities, while the village of Barkoundouba is inhabited by Fulani and Rimaibè; given the presence in both villages, the Rimaibè represent therefore an optimal internal control for the study. The two villages are 5 km apart.

Malaria transmission in the area is hyperendemic and seasonal, with a rainy season from June to October. The entomological inoculation rate in the study area is estimated around 100-200 infective bites per person per year. Main malaria vectors are *Anopheles gambiae* ss, *An. arabiensis* and *An. funestus*. *Plasmodium falciparum* is responsible for more than 90% of malaria infections. Previous studies conducted in the area have shown that the entomological inoculation rate is comparable between the different villages (Modiano et al., 1996). The Fulani have been previously reported to be less infected with *P. falciparum*, to have a different genetic background from their neighbours, while the Mossi and Rimaibe show comparable susceptibility to infection and are genetically similar (Lulli et al., 2009; Luoni et al., 2001; Modiano et al., 1996).

3.1.2. Study design

The original epidemiological study consisted of five malariological cross-sectional surveys conducted among all age groups. Participants have been examined by a team of physicians for clinical signs and the axillary body temperature was measured. Subjects exhibiting fever (temperature ≥37.5°C) were treated presumptively with artemether-lumefantrine (coartem). For malaria diagnosis, thin and thick blood slides were prepared from finger pricks, stained with 3% Giemsa according to standard procedure and read independently by two skilled microscopists. The Plasmodium species was identified on the thin blood smear. Readers examined 100 microscopic fields (corresponding to 0.25 µL of blood) from the thick blood smear, parasite counts were converted to numbers of parasites per microliter of blood (assuming a standard count of 8000/µL), and the mean density from 2 readings was used.

A third reader was involved when the 2 readers disagreed about positivity or when estimated densities differed by >30%. In these cases, the mean of the 2 closest density readings was used (Mangano et al., 2015).

During the first survey in August 2007, a 2 mL venous blood sample was collected in ethylenediaminetetraacetic acid (EDTA) tubes at the beginning of the study for buffy coat (used for DNA extraction) and plasma separation (Mangano et al., 2015). Plasma was separated by centrifugation (3min at 2000 rpm), aliquoted and stored at -80°C until immunological assays were performed. Genomic DNA was extracted from whole blood using the Nucleon BACC2 Kit. The rs334 (A → HbA/T → HbS) and rs33930165 (G → HbA /A → HbC) SNPs at the HBB locus were genotyped using the Sequenom MassArray System (Mangano et al., 2015).

A cohort of 452 individuals of all age group that were observed at all 5 cross-sectional surveys were included in the present observational study, that took advantage of archived plasma samples from August 2007.

3.1.3. Ethical approvals

The original epidemiological study received approval from the ethical committees of the Ministry of Health of Burkina Faso and the University of Oxford. Study subjects or their guardians gave written informed consent for participation.

The current observational study with reuse of archived plasma samples received further approval from the ethical committee of the Ministry of Health and Public Hygiene of Burkina Faso.

3.1.4. Enzyme Linked Immunosorbent Assay (ELISA)

ELISA was used to measure indirect markers of *S.haematobium* infection (anti-SWAP IgM, anti-SWAP IgG, anti-SEA IgM and anti-SEA IgG) using a in house procedure adapted from Mutapi and colleagues (Mutapi et al., 1997). Antigens SWAP and SEA were obtained from the Theodor Bilharz Institute (Giza, Egypt), by courtesy of Dr. Faoud Youssif. The concentration of antigens was 5µg/mL for SWAP and 10µg/mL for SEA. Each assay was run in two days. At day 1, antigen was prepared in coating buffer: 100 µL stock solution in 10mL coating buffer (1:100 dilution) for SEA [10 µg/mL]; 50 µL stock solution in 10mL coating buffer (1:200 dilution) for SWAP [5 µg/mL]. 100 µL antigen in coating buffer was added per well in a 96 wells plate and incubated

overnight at 4°C. At day 2, plates were washed three times (X 3) and 200 µL blocking buffer was added per well and incubated 2h at room temperature (blocking step). After a three-time wash, for IgG 90 µL blocking buffer was added per well and 10 µL standards and plasma samples in duplicate, for a final 1:100 dilution; for IgM, 95 µL blocking buffer was added per well and 5 µL standards and plasma samples in duplicate for a final 1:200 dilution (antibody-antigen binding step). Plates were incubated 2h at room temperature. After a three-time washing, revelation step was initiated and 100 µL HRP-anti-human-IgG at 1:1000 dilution in PBS-T (10 µL mAb in 10 mL PBS-T) was added and incubated for 2h at room temperature. Plates were then washed six times, and 100 µL OPD substrate solution was added. After 20 min incubation in dark at room temperature, 25 µL of H2SO4 1 M stop solution was added. Plates were read with VICTOR reader at the absorbance of 490 nm for 1 second. The cut off used for positivity was the mean absorbance plus 2 standard deviation of negative control samples (cutoff = mean OD neg + 2SD neg). 3 positive control samples from infected (eggs in urine) male individuals aged 18-20 years old (courtesy of Prof. Zeno Bisoffi, University of Verona) and 1 negative control, a pool of plasma samples from schistosome naïve Italian male donors across the same age range (courtesy of Prof. David Modiano, University of Rome La Sapienza) have been used. A pool of positive samples has been used to construct a standard curve which was employed to normalise results across plates.

3.1.5. Circulating Anodic Antigens measurement by Up Converting Phospore-Lateral Flow assay

An Up Converting Phospore-Lateral Flow (UCP-LF) assay was used to detect *S.haematobium* the adult worm gut derived antigen Circulating Anodic Antigens (CAA), in plasma samples. A volume of 50 µL of plasma sample was mixed with an equal volume of 4% TCA and centrifuged to well separate the clear supernatant from the pellet. A volume of 50 µL of a run buffer containing 200Mm Tris High Salt Lateral Flow (HSLF) plus 1% BSA was pipetted in a 96 wells plate. 20µL clear TCA supernatant and 50 µL UCP solution were added respectively in each well of the plate. After an incubation of 1 hour at 37°C with shaking (900 rpm), CAA UCP strips were added into the wells. Strips were incubated overnight and scanned with Labrox Upcon Reader for analysis(Corstjens et al., 2014).

3.1.6. Circulating DNA measurement by Real Time PCR

3.1.6.1. DNA extraction from plasma samples

Circulating DNA was extracted from 100 µL plasma samples using the QIA amp Min Elute ccfDNA Mini Kit according to the manufacturer's protocol. Briefly, 100 µL plasma samples were mixed with 900 µL PBS to reach 1mL each in 1.5 mL tubes. Samples were then mixed with 30 µL magnetic bead suspension, 55 µL proteinase K and 150 µL bead binding buffer and incubate 10 min at room temperature while shaking. After a brief spin, samples were placed into a magnetic rack, standed for 1 min, until the solution is clear and the supernatant discarded. Tubes were removed and 200 µL bead elution buffer was added to the bead pellet, vortexed, mixed and transferred into a bead elution tube. Incubate for 5 min on a shaker at room temperature and 300 rpm. Tubes were then placed into a magnetic rack to get clear solution and supernatant transferred into a new bead elution tube. A volume of 300 µL of buffer ACB was added, vortexed and bead pellet discarded. The mixture supernatant-buffer ACB was pipetted into a QIAamp column, centrifuged and the solution collected and discarded. A volume of 500 µL of buffer ACW2 was added to the column, centrifuged and the solution discarded. The column was then placed into a clean elution tube, and incubate, opened lid in a shaker at 56°C for 3min to dry the membrane completely. A volume of 40 µL of ultra-clean water was pipetted onto the center of the membrane, incubate at room temperature for 1 min, centrifuge at 20.000x g for 1 min to elute the DNA.

3.1.6.2. Real Time PCR assay

The semi quantitative standardized polymerase chain reaction (qPCR) analysis was based on the protocol by (Frickmann et al., 2021). The primers used for amplification were: *S.haematobium*- Forward Primer: 5'-GATCTCACCTATCAGACGAAAC-3' (50 pmol/µL), *S.haematobium*- Reverse Primer: 5'-TCACAACGATACGACCAAC-3' (50pmol/µL), *S.haematobium*-Probe: Joe-TGTTGGTGGAAGZGCCTGTTTCGCAA-BMN-Q535 (25pmol/µL) where Z=BMN-Q535. Primers and a probe were added for the detection of Phocid herpesvirus (PhHV) DNA as internal control (PhHV- Forward Primer: 5'-GGGCGAATCACAGATTGAATC-3', PhHV- Reverse Primer: 5'-GCGGTTCCAAACGTACCAA-3', PhHV- Probe: 5' Cy5.5-TTTTTATGTGTCCGCCACCA-BBQ 3') (Gruninger et al., 2023).

The qPCRs analyses were performed in a total volume reaction of 25µL containing 20µL master mix and 5µL of DNA eluate. The Corbett Rotor-Gene 6000 (Qiagen Redwood City, USA) was

used to run the amplification reaction with the following steps: an initial denaturation at 95°C for 15 min, 50 cycles consisting of a denaturation at 95°C for 15s, annealing and elongation at 65°C for 60 s through, a final elongation at 40°C for an additional 20s. cDNA detection was expressed by cycle threshold (Ct) values. The readout resulted from the RotorGene 6000 Software v.7.87 (Qiagen) Results with a clean sigmoid curve within the PCR cycles were considered positive.

3.2. Experimental study in vitro.

3.2.1. Tetraspanins.

The immunomodulatory proteins, tetraspanins TSP2 (1mg/mL), TSP6 (250µg/mL) and TSP23 (125µg/mL), provided by Javier Sotillo at Carlos III Health Institute of Madrid. (Sotillo et al. PLoS Negl Trop Dis 2019) have been used to perform this study. The proteins were purified using Lionex Endotrap HD columns and quantified using Bradford assay with a BSA standard curve.

3.2.2. Dendritic Cells (DCs) generation.

Mononuclear Cells were isolated by Ficoll/Paque density gradient centrifugation from buffy coats obtained from five healthy donors. CD14+ cells were separated performing a positive selection with micromagnetic beads conjugated with mouse anti-human CD14 monoclonal antibody by magnetic cell sorting (MACS) using Miltenyi Biotec kit according to the manufacturer's instructions. The cells were seeded at $1x10^6$ cells/mL. DCs were generated by culture of CD14+ in RPMI 1640 with 10% heat-inactivated foetal bovine serum (FBS), 100U/mL penicillin and 0.1 ng/mL streptomycin with 1000 U/mL rGM-CSF and 1000 U/mL IL-4 for 7 days (Nicolò et al., 2022).

3.2.2.1. DCs Maturation

Maturation of DCs was induced in RPMI 1640 with 10% heat-inactivated foetal bovine serum (FBS), 100U/mL penicillin and 0.1 ng/mL streptomycin and LPS or 10µg/mL TSP2 or 10µg/mL of TSP6 or 10µg/mL TSP23 during 16 hours. Immature DCs or unstimulated have been used as the maturation negative control and LPS as maturation positive control.

3.2.3. Flow cytometry

At the end of the maturation, cells were washed and stained with a mixture of anti-CD83-PE, anti-CD80-FITC, anti-CD86-APC and anti-HLA-DR-APC antibodies (BD Biosciences-Pharmingen), for 30 minutes. Cells were analysed using the ACCURI instrument (BD Biosciences, Franklin Lakes, NJ, USA) using Cflow Plus software (BD Biosciences, Franklin Lakes, NJ, USA). Ten thousand events for each sample were acquired (Nicolò et al., 2022).

3.2.4. Luminex assay

After the 16 hours culture of DCs, supernatants were collected and cytokines levels (IL-1 beta, IL6, IL10, IL12p70, TNF-alpha) estimated using Milliplex Human Th17 Magnetic Bead Panel for Luminex MAGPIX detection system (Affymetrix, eBioscience) following the manufacturer's instructions.

3.2.5. T cells differentiation

Purified CD4+T cells from seven healthy donors were pre-activated by anti-CD3/CD28 antibodies coupled to beads at a 1:1 bead/cell ratio (Gibco, Thermo Fisher Scientific, MA, USA). Preactivated CD4+ T cells (8 or $20x10^4$cells/mL)were then incubated with conditioned medium obtained from cultures of DCs with LPS or tetraspanins (TSP2, TSP6, and TSP23) for 3 days, DCs and T cells were of the same donor (N=3).or they were isolated from different donors (N=2).

3.2.5.1. RNA extraction

T cells RNA extraction was performed using 1mL of TRIzol® (Invitrogen) reagent. 200 µL of chloroform per mL of TRIzol® were added and samples were centrifuged at 17000 rpm for 15 min at 4°C to separate the homogenate into three phases: a clear upper aqueous layer containing RNA, an interphase and a red lower organic layer containing DNA and proteins. The RNA phase was collected, resuspended in 500 µL of isopropanol per mL TRIzol® and precipitated by centrifuging samples at 17000 rpm for 10 min at 4°C. Afterwards, the pellet was washed with 1 mL of 75% and then with 1 mL of 100% ethanol. When the ethanol was evaporated (30' minutes) the pellet was resuspended in H2O RNase-free.

3.2.5.2. RNA quantification

RNA quantity was assessed by the NanoDrop spectrophotometer (ThermoFisher Scientific, Waltman, MA, USA), using 2 µL of sample according to the instrument software's instructions and stored at -80^{o}C.

3.2.5.3. Quantitative RT PCR

A quantity of 1 µg of RNA was used for the reverse transcription (RT) reaction with Prime Script RT reagent Kit Takara (Otsu, Japan). After treatment with DNase for 30 min at room temperature, the cDNA samples obtained were amplified to quantify IFN-gamma, IL-10, IL4,

IL5, IL10, IL 13 and IL-17 mRNA expression. qRT-PCR amplification was carried out using SYBR Premix Ex Taq (Takara) according to manufacturer instructions on a Rotorgene RG-3000 A cycle system (Qiagen) platform. The 18S rRNA housekeeping gene was used as a normalizer and the difference between CT values of the target gene and 18S gene was used to calculate the delta CT. 3The primers used to perform the assay are:

Gene	Forward 5'-3'	Reverse 5'-3'
IFN-gamma	TCGGTAACTGACTTGAATGTCCA	TCGCTTCCCTGTTTTAGCTGC
IL-17	AGATTACTACAACCGATCCACCT	GGGGACAGAGTTCATGTGGTA
IL-10	TCAAGGCGCATGTGAACTCC	GATGTCAAACTCACTCATGGCT
IL-4	ACTTTGAACAGCCTCACAGAG	TTGGAGGCAGCAAAGATGTC
IL-5	CCCACAAGTGCATTGGTGAA	CCTCAGAGTCTCATTGGCTATCAG
IL-13	TGAGGAGCTGGTCAACATCA	CAGGTTGATGCTCCATACCAT
18S	ATTAAGGGTGTGGGCCGAAG	GGTGATCACACGTTCCACCT

3.3. Experimental study *in vivo*

3.3.1. *Schistosoma haematobium* homolog of Interleukin-4-inducing principle of *Schistosoma mansoni* eggs (HIPSE)

Prof. Franco Falcone (University of Geissen, Germany) provided recombinant HIPSE. The integrity and molecular weight of the protein were checked on 15% polyacrylamide gel prior the experiments.

3.3.2. Murine model of malaria infection

A murine malaria model was used to carry out this study. Female CD1 mice weighting 25g were infected with a parasite clone of *Plasmodium berghei* (1 x 10^4 infected Red Blood Cells in 200 µL PBS) expressing green fluorescent protein (GFP clone 107) collected from a donor mouse.

3.3.3. Experimental design

Two experiments were conducted each involving two groups of five female CD1 mice, one treated with HIPSE and one with PBS for control.

3.3.3.1. Experiment 1

Mice were inoculated intra-peritoneally; the experimental group received 25 µg HIPSE/200 µL PBS and the control group, 200 µl PBS. One day later, all mice were infected by intra-peritoneal injection with a parasite clone of *P. berghei* (1 x 10^4 iRBC in 200 µL PBS) expressing GFP. Parasite density was measured at 3, 5 and 7 days post infection by Flow Cytometry.

3.3.3.2. Experiment 2

Mice were also inoculated intra-peritoneally. The experimental group received 25 µg HIPSE/200 µL PBS and the control group, 200 µL PBS. One day later, all mice were infected by intra-peritoneal injection with a parasite clone of *P. berghei* (1 x 10^4 iRBC in 200 µL PBS) expressing GFP. 6 days after infection, mice were inoculated again with the same volume of HIPSE and PBS, to mimic natural conditions where exposure to *S.haematobium* antigens is sustained over time. Parasite density was measured at 3, 5, 6, 7 and 10 days post infection by Flow Cytometry.

3.3.4. Flow cytometry

Parasite density was measured by flow cytometry (CytoFLEX, Beckman Coulter) and expressed as percentage (%) of iRBC.

3.4. Statistical methods

As for the observational study, descriptive analysis of binary variables (e.g. presence/absence of Circulating Cathodic Antigen, CAA) was performed computing frequencies (%) and 95% Confidence Interval (CI) which were shown by barplots; descriptive analysis of quantitative variables (e.g. concentration of CAA) was performed computing median, range, mean and standard deviation and showing boxplots. Correlation between quantitative variables was assessed using Spearman correlation analysis reporting correlation coefficient. Specificity and sensitivity analysis was performed using ROC curve methods. In association analysis, multivariate logistic regression was employed for binary outcome variables reporting Odds Ratio (OR) and its 95% CI, multivariate linear regression was employed for quantitative outcome variables reporting regression coefficient and its 95% CI, and Poisson regression was employed for count outcome variables (e.g. number of infections) reporting Incidence Rate Ratio (IRR) and its 95% CI. Likelihood ratio test between nested regression models was

employed to test the effect of the independent variant of interest. All analyses were performed in STATA v13.00.

As for the experimental study *in vitro*, difference in the distribution of quantitative results (i.e., Mean Fluorescence Intensity, cytokine concentration, cytokine transcript relative amount) between stimulated and unstimulated cells was assessed using Friedman test (Kruskal-Wallis non-parametric rank test for paired data). The fold-change in the value of interest observed in stimulated compared to unstimulated cells was computed for each donor and shown as boxplots. The analysis was performed in GraphPad Prism v.9.0.

As for the experimental study *in vivo*, the percentage (%) of infected Red Blood Cells was compared between treated and non-treated mice at different time points using a mixed-effects restricted maximum likelihood model for repeated measures and missing data, and shown as boxplots. The analysis was performed in GraphPad Prism v.9.0.

CHAPTER 4. RESULTS AND DISCUSSION

4.1. Observational study *in natura*

4.1.1. Markers of *S. haematobium* infection among study populations

4.1.1.1. *S. haematobium* specific antibodies

Proportion of subjects positive to S.haematobium specific antibodies

A descriptive analysis has been performed to assess the variation in the proportion of subjects positive to *S. haematobium* specific antibodies according to age, sex, village and ethnicity.

- **Anti-SWAP IgM**

The overall proportion of subjects positive for anti-SWAP IgM among the study population is 45.14 % (Table 1).

Table 1 shows the proportion of anti-SWAP IgM positive subjects (anti-SWAP IgM positivity) according to age-group (1-4, 5-9, 10-19, 20-39, 40-80 years old), sex (females vs males), village of residence (Barkoundouba vs Barkoumbilen) and ethnicity (Non-Fulani vs Fulani) as well as the results of multivariate association analysis.

As shown in Table 1, anti-SWAP IgM positivity is lowest in children aged 1 to 4 years, it increases in children aged 5 to 9 years, is highest in children aged 10 to 19 years and decreases in subjects older than 20 years (Figure 4); is comparable in females and males although a trend for higher values is observed in females (Figure 5); is comparable in Barkoumbilen and Barkoundouba; is higher in Fulani than Non-Fulani; the comparison of the proportion of anti-SWAP IgM positivity between ethnic groups within each village (Rimaibe vs Mossi in Barkoumbilen, Rimaibe vs Fulani in Barkoundouba) as well as between the same ethnic group living in different villages (Rimaibe in Barkoundouba vs Rimaibe in Barkoumbilen) confirms an independent effect of ethnicity from that of the village (Figure 6, Table 2).

Subjects characteristics		Total population	anti-SWAP IgM positive subjects				Association with anti-SWAP IgM positivity			
		N	N+	Proportion (%)	95% LCL	95% UCL	Odds Ratio	95% CI LCL	95% CI UCL	P-Value
Age-group (years old)	1-4 (ref)	92	16	17.39	9.64	25.14	-	-	-	-
	5-9	105	54	51.43	41.87	60.99	5.35	2.76	10.37	0.000
	10-19	101	63	62.38	52.93	71.83	8.12	4.13	15.98	0.000
	20-39	76	38	50.00	38.76	61.24	4.51	2.19	9.30	0.000
	40-80	58	24	41.38	28.70	54.06	3.35	1.56	7.18	0.001
Sex	Males (ref)	173	68	39.31	32.03	46.59	-	-	-	-
	Females	259	127	49.03	42.94	55.12	1.53	0.99	2.40	0.056
Village	Barkoumbilen (ref)	282	113	40.07	34.35	45.79	-	-	-	-
	Barkoundouba	150	82	54.67	46.70	62.64	0.52	0.23	1.17	0.115
Ethnicity	Fulani (ref)	123	75	60.98	52.36	69.60	-	-	-	-
	Non-Fulani	309	120	38.83	33.40	44.26	0.20	0.09	0.49	0.000
Total		432	195	45.14	40.45	49.83	-	-	-	-

Table 1: Proportion of anti-SWAP IgM positive subjects and association of subject's characteristics with anti-SWAP IgM positivity
This table shows the number of total population (N), the number (N+) and the proportion (%) of anti-SWAP IgM positive subjects and the results of multivariate logistic regression analysis (Odds Ratio and P-value) performed to investigate the association of age-group (1-4 , 5-9, 10-19, 20-39, 40-80 years old), sex (females vs males), village (Barkoundouba vs Barkoumbilen) and ethnicity (Non-Fulani vs Fulani) with anti-SWAP IgM positivity. 95% LCL: 95% Lower Confidence Limit; 95% UCL: 95% Upper Confidence Limit.

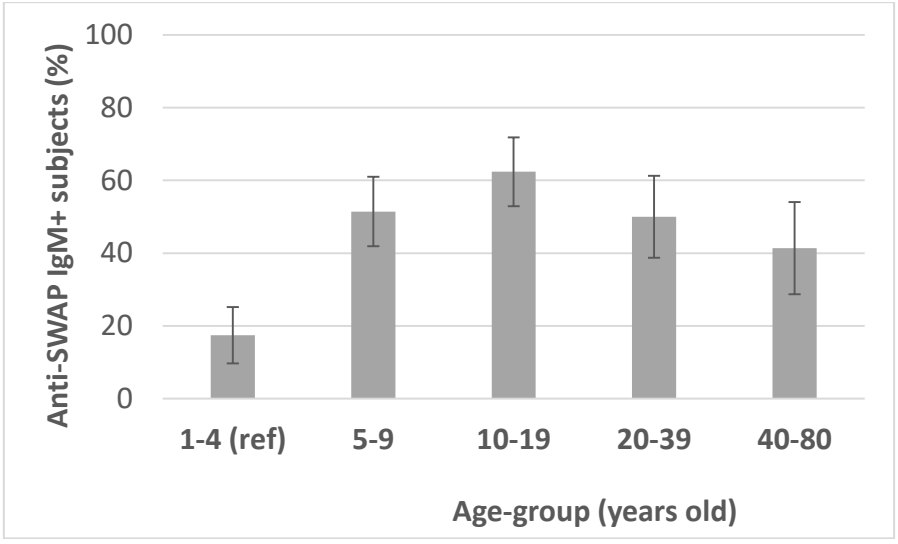

Figure 4: Proportion (%) of anti-SWAP IgM positive subjects according to age-group (years old).
This figure shows the proportion (%) of anti-SWAP IgM positive subjects according to age-group (1-4, 5-9, 10-19, 20-39, 40-80 years old). Error bars indicate the 95% confidence interval of the proportion.

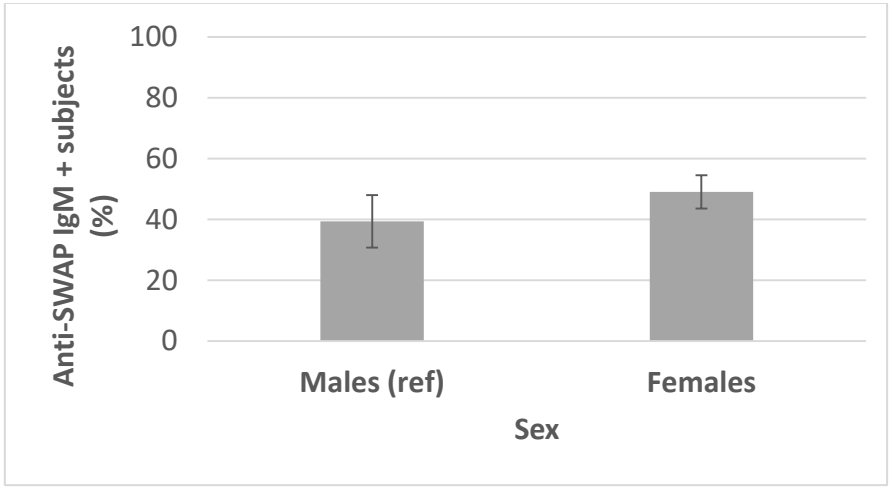

Figure 5: Proportion (%) of anti-SWAP IgM positive subjects according to sex.
This figure shows the proportion (%) of anti-SWAP IgM positive subjects according to sex (males and females). Error bars indicate the 95% confidence interval of the proportion.

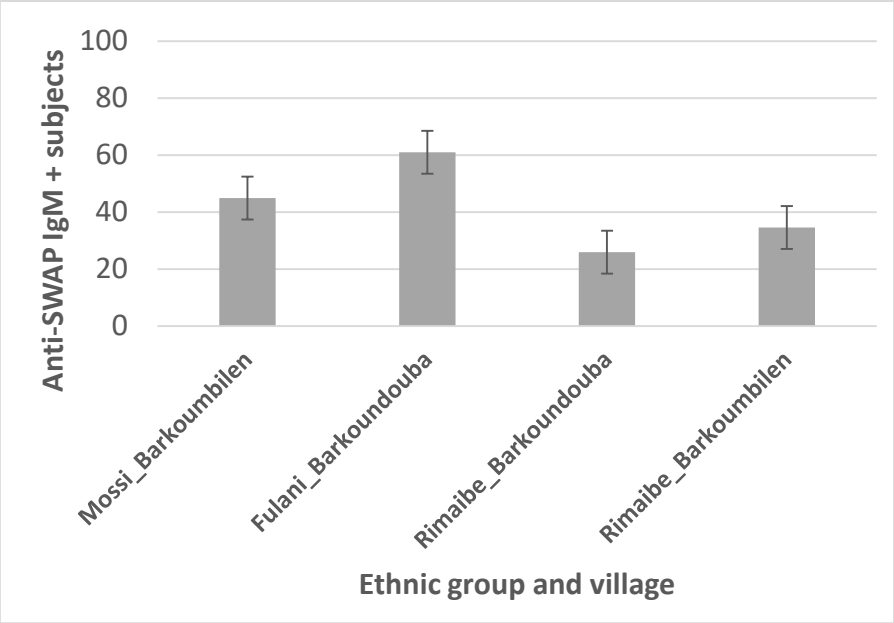

Figure 6: Proportion (%) of anti-SWAP IgM positive subjects according to ethnic group and village.
This figure shows the proportion (%) of anti-SWAP IgM positive subjects according to ethnic group and village (Mossi_Barkoumbilen, Fulani_Barkoumbilen, Rimaibe_Barkoundouba, Rimaibe_Barkoumbilen). Error bars indicate the 95% confidence interval of the proportion.

Village	Ethnic group	Total population	Anti-SWAP IgM positive subjects			
		N	N+	Proportion (%)	95% LCL	95% UCL
Barkoumbilen	Mossi	149	67	44.97	36.98	52.96
	Rimaibe	133	46	34.59	26.51	42.67
Barkoundouba	Fulani	123	75	60.98	52.36	69.60
	Rimaibe	27	7	25.95	9.41	42.49
Total		432	195	45.14	40.45	49.83

Table 2*:* Proportion of anti-SWAP IgM positive subjects according to ethnic group and village.
This table shows the number of total population (N), the number (N+) and the proportion (%) of anti-SWAP IgM positive subjects according to ethnic group and village. 95% LCL: 95% Lower Confidence Limit; 95% UCL: 95% Upper Confidence Limit.

- **Anti-SWAP IgG**

The overall proportion of subjects positive for anti-SWAP IgG among the study population is 55.2 % (Table 3).

Table 3 shows the proportion of anti-SWAP IgG positive subjects (anti-SWAP IgG positivity) according to age-group (1-4, 5-9, 10-19, 20-39, 40-80 years old), sex (females vs males), village of residence (Barkoundouba vs Barkoumbilen) and ethnicity (Non-Fulani vs Fulani) as well as the results of multivariate association analysis.

As shown in Table 3, anti-SWAP IgG positivity is lowest in children aged 1 to 4 years, it increases in children aged 5 to 9 years, is highest in children aged 10 to 19 years and decreases in subjects older than 20 years, although a slight increase is observed in the group aged 40 to 80 years (Figure 7); it is lower in females than males (Figure 8); it is lower in Barkoundouba than Barkoumbilen; it is lower in Non-Fulani compared to Fulani; the comparison of the proportion of anti-SWAP IgG positivity between ethnic groups within each village (Rimaibe vs Mossi in Barkoumbilen, Rimaibe vs Fulani in Barkoundouba) as well as between the same ethnic group living in different villages (Rimaibe in Barkoundouba vs Rimaibe in Barkoumbilen) reveals that Rimaibe show lower values in Barkoundouba than in Barkoumbilen (Figure 9, Table 4).

Subjects characteristics		Total population	anti- SWAP IgG positive subjects				Association with anti-SWAP IgG positivity			
		N	N+	Proportion (%)	95% LCL	95% UCL	Odds Ratio	95% CI LCL	95% CI UCL	P-Value
Age-group (years old)	1-4 (ref)	92	12	13.04	6.16	19.92	-	-	-	-
	5-9	108	67	62.04	52.89	71.19	12.24	5.90	25.38	0.000
	10-19	101	83	82.18	74.72	89.64	37.00	16.55	82.77	0.000
	20-39	81	41	50.62	39.73	61.51	10.26	4.59	22.92	0.000
	40-80	56	38	67.86	55.63	80.09	18.47	7.92	43.07	0.000
Sex	Males (ref)	175	104	59.43	52.15	66.71	-	-	-	-
	Females	263	137	52.09	46.05	58.13	0.58	0.36	0.95	0.029
Village	Barkoumbilen (ref)	283	161	56.89	51.12	62.66	-	-	-	-
	Barkoundouba	155	80	51.61	43.74	59.48	0.29	0.13	0.65	0.002
Ethnicity	Fulani (ref)	123	69	56.10	47.33	64.87	-	-	-	-
	Non-Fulani	315	172	54.60	49.10	60.10	0.35	0.15	0.84	0.018
Total		438	241	55.02	50.36	59.68	-	-	-	-

Table 3: Proportion of anti-SWAP IgG positive subjects and association of subject's characteristics with anti-SWAP IgG positivity.
This table shows the number of total population (N), the number (N+) and the proportion (%) of anti-SWAP IgG positive subjects and the results of multivariate logistic regression analysis (Odds Ratio and P-value) performed to investigate the association of age-group (1-4 , 5-9, 10-19, 20-39, 40-80 years old), sex (females vs males), village (Barkoundouba vs Barkoumbilen) and ethnicity (Non-Fulani vs Fulani) with anti-SWAP IgG positivity. 95% LCL: 95% Lower Confidence Limit; 95% UCL: 95% Upper Confidence Limit.

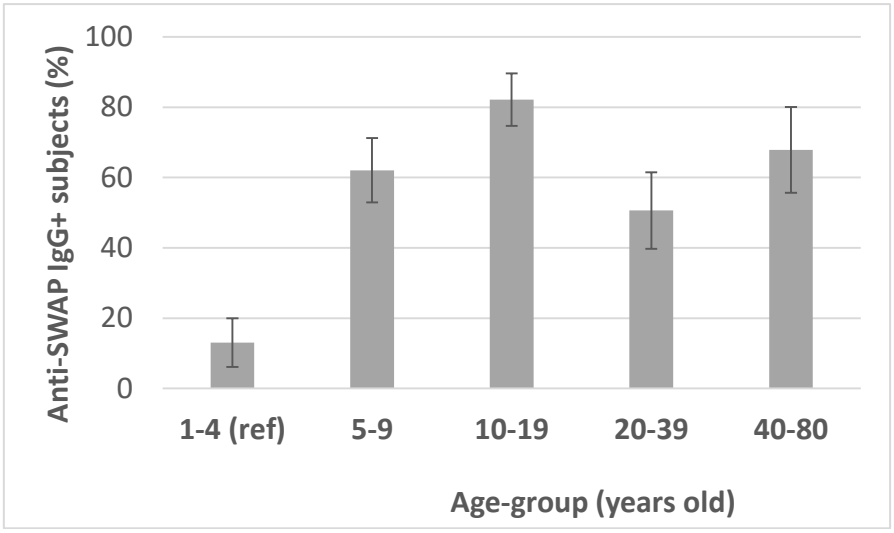

Figure 7: Proportion (%) of anti-SWAP IgG positive subjects according to age-group (years old).
This figure shows the proportion (%) of anti-SWAP IgG positive subjects according to age-group (1-4, 5-9, 10-19, 20-39, 40-80 years old). Error bars indicate the 95% confidence interval of the proportion.

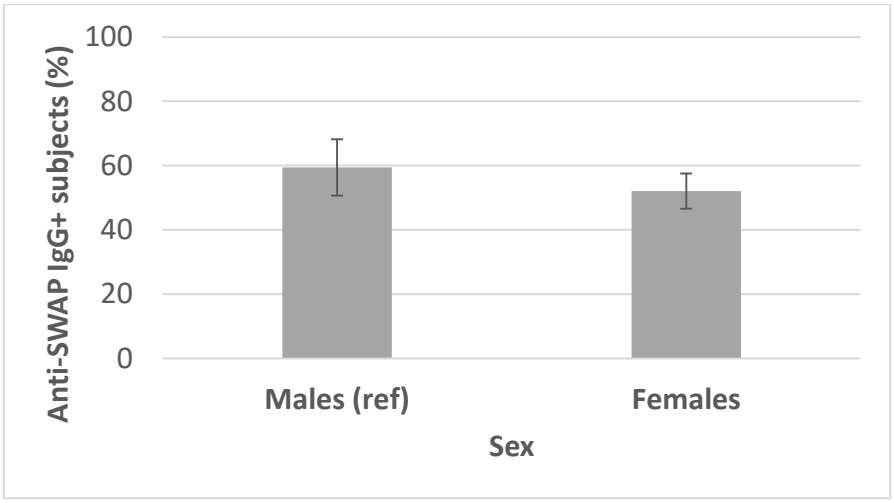

Figure 8: Proportion (%) of anti-SWAP IgG positive subjects according to sex.
This figure shows the proportion (%) of anti-SWAP IgG positive subjects according to sex (males and females), bars indicate the 95% confidence interval of the proportion.

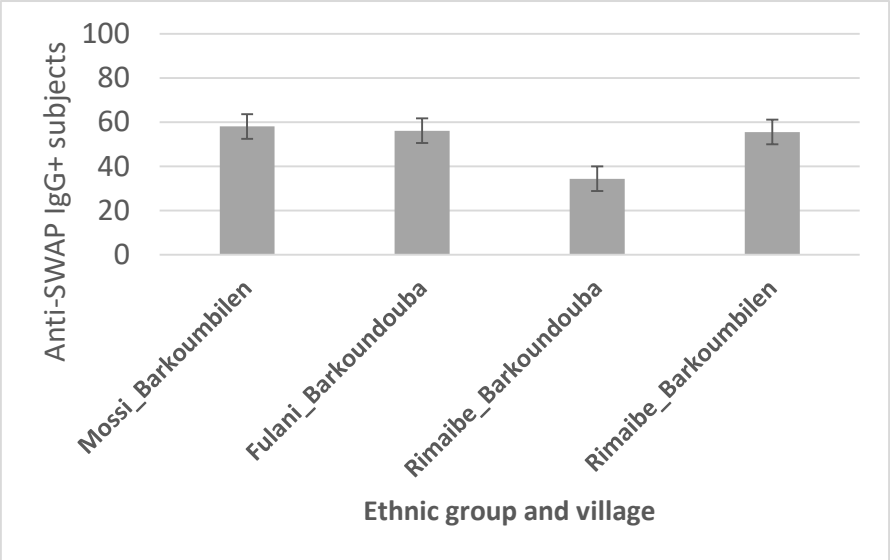

Figure 9: Proportion (%) of anti-SWAP IgG positive subjects according to ethnic group and village.
This figure shows the proportion (%) of anti-SWAP IgG positive subjects according to ethnic group and village (Mossi_Barkoumbilen, Fulani_Barkoumbilen, Rimaibe_Barkoundouba, Rimaibe_Barkoumbilen). Error bars indicate the 95% confidence interval of the proportion.

Village	Ethnic group	Total population	Anti-SWAP IgG positive subjects			
		N	N+	Proportion (%)	95% LCL	95% UCL
Barkoumbilen	Mossi	148	86	58.11	50.16	66.06
	Rimaibe	135	75	55.56	47.18	63.94
Barkoundouba	Fulani	123	69	56.10	47.33	64.87
	Rimaibe	32	11	34.38	17.92	50.84
Total		438	241	55.02	50.36	59.68

Table 4: Proportion of anti-SWAP IgG positive subjects according to ethnic group and village.
This table shows the number of total population (N), the number (N+) and the proportion (%) of anti-SWAP IgG positive subjects according to ethnic group and village. 95% LCL: 95% Lower Confidence Limit; 95% UCL: 95% Upper Confidence Limit.

- **Anti-SEA IgM**

The overall proportion of subjects positive for anti-SEA IgM among the study population is 72.39% (Table 5).

Table 5 shows the proportion of anti-SEA IgM positive subjects (anti-SEA IgM positivity) according to age-group (1-4, 5-9, 10-19, 20-39, 40-80 years old), sex (females vs males), village of residence (Barkoundouba vs Barkoumbilen) and ethnicity (Non-Fulani vs Fulani) as well as the results of multivariate association analysis.

As shown in Table 5, anti-SEA IgM positivity is lowest in children aged 1 to 4 years, it increases in children aged 5 to 9 years, is highest in children aged 10 to 19 years and decreases in subjects older than 20 years (Figure 10); is comparable in females and males (Figure 11); it is comparable in Barkoumbilen and Barkoundouba; it is higher in Fulani compared to Non-Fulani (Figure 12, Table 6).

Subjects characteristics		Total population	anti-SEA IgM positive subjects			Association with anti-SEA IgM positivity			
		N	N+	Proportion (%)	95% LCL	95% UCL	Odds Ratio	95% CI LCL	95% CI UCL	P-Value
Age-group (years old)	1-4 (ref)	91	22	24.18	15.38	32.98	-	-	-	-
	5-9	107	85	79.44	71.78	87.10	11.46	5.93	22.15	0.000
	10-19	100	97	97.00	93.66	100.34	67.48	24.23	187.93	0.000
	20-39	79	66	83.54	75.36	91.72	15.98	7.17	35.62	0.000
	40-80	54	42	77.78	66.69	88.87	11.32	5.12	25.02	0.000
Sex	Males (ref)	171	115	67.25	60.22	74.28	-	-	-	-
	Females	260	197	75.77	70.56	80.98	1.38	0.80	2.36	0.244
Village	Barkoumbilen (ref)	278	191	68.71	63.26	74.16	-	-	-	-
	Barkoundouba	153	121	79.08	72.63	85.53	0.67	0.27	1.67	0.388
Ethnicity	Fulani (ref)	125	99	79.20	72.08	86.32	-	-	-	-
	Non-Fulani	306	213	69.61	64.46	74.76	0.35	0.13	0.96	0.040
	Total	431	312	72.39	68.17	76.61	-	-	-	-

Table 5: Proportion of anti-SEA IgM positive subjects and association of subject's characteristics with anti-SEA IgM positivity.
This table shows the number of total population (N), the number (N+) and the proportion (%) of anti-SEA IgM positive subjects and the results of multivariate logistic regression analysis (Odds Ratio and P-value) performed to investigate the association of age-group (1-4 , 5-9, 10-19, 20-39, 40-80 years old), sex (females vs males), village (Barkoundouba vs Barkoumblien) and ethnicity (Non-Fulani vs Fulani) with anti-SEA IgM positivity. 95% LCL: 95% Lower Confidence Limit; 95% UCL: 95% Upper Confidence Limit.

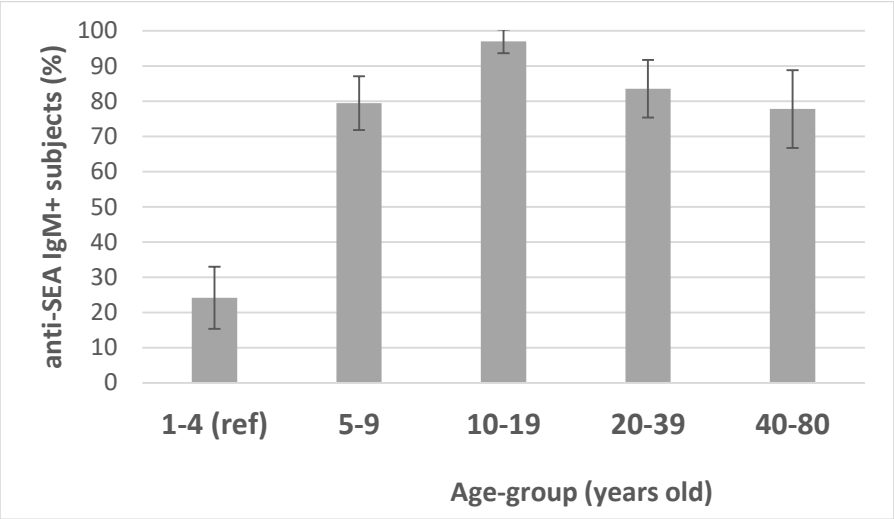

Figure 10: Proportion (%) of anti-SEA IgM positive subjects according to age-group (years old).
This figure shows the proportion (%) of anti-SEA IgM positive subjects according to age-group (1-4, 5-9, 10-19, 20-39, 40-80 years old). Error bars indicate the 95% confidence interval of the proportion.

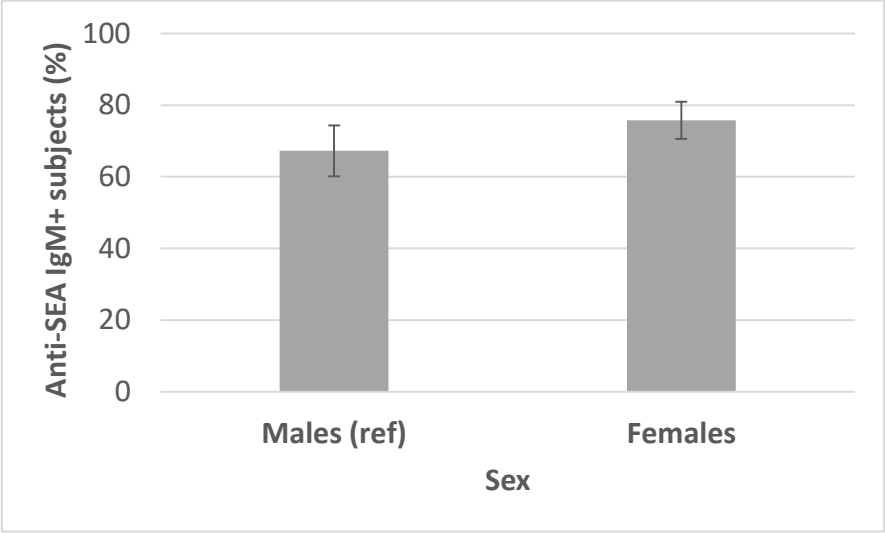

Figure 11: Proportion (%) of anti-SEA IgM positive subjects according to sex.
This figure shows the proportion (%) of anti-SEA IgM positive subjects according to sex (males and females). Error bars indicate the 95% confidence interval of the proportion.

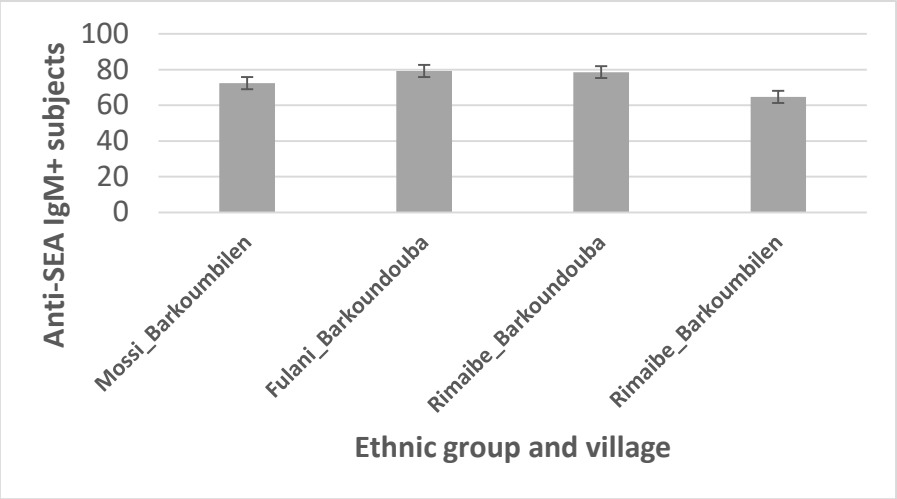

Figure 12: Proportion (%) of anti-SEA IgM positive subjects according to ethnic group and village.
This figure shows the proportion (%) of anti-SEA IgM positive subjects according to ethnic group and village (Mossi_Barkoumbilen, Fulani_Barkoumbilen, Rimaibe_Barkoundouba, Rimaibe_Barkoumbilen). Error bars indicate the 95% confidence interval of the proportion.

Village	Ethnic group	Total population	Anti-SEA IgM positive subjects			
		N	N+	Proportion (%)	95% LCL	95% UCL
Barkounbilen	Mossi	145	105	72.41	65.13	79.69
	Rimaibe	133	86	64.66	56.54	72.78
Barkoundouba	Fulani	125	99	79.20	72.08	86.32
	Rimaibe	28	22	78.57	63.37	93.77
Total		431	312	72.39	68.17	76.61

Table 6: Proportion of anti-SEA IgM positive subjects according to ethnic group and village.
This table shows the number of total population (N), the number (N+) and the proportion (%) of anti-SEA IgM positive subjects according to ethnic group and village. 95% LCL: 95% Lower Confidence Limit; 95% UCL: 95% Upper Confidence Limit.

- **Anti-SEA IgG**

The overall proportion of subjects positive for anti-SEA IgG among the study population is 70.18 % (Table 7).

Table 7 shows the proportion of anti-SEA IgG positive subjects (anti-SEA IgG positivity) according to age-group (1-4, 5-9, 10-19, 20-39, 40-80 years old), sex (females vs males), village of residence (Barkoundouba vs Barkoumbilen) and ethnicity (Non-Fulani vs Fulani) as well as the results of multivariate association analysis.

As shown in Table 7, anti-SEA IgG positivity is lower in children aged 1 to 4 years, it increases in children aged 5 to 9 years, is highest in children aged 10 to 19 years and decreases in subjects older than 20 years, although an increase is then observed in a group aged 40 to 80 years (Figure 13); it is slightly lower in females compared to males (Figure 14); it is slightly lower in Barkoundouba compared to Barkoumbilen; it is comparable in Fulani and Non-Fulani (Figure 15, Table 8).

Subjects characteristics		Total population	anti-SEA IgG positive subjects				Association with anti- SEA IgG positivity			
		N	N+	Proportion (%)	95% LCL	95% UCL	Odds Ratio	95% CI LCL	95% CI UCL	P-Value
Age-group (years old)	1-4 (ref)	90	27	30.00	20.53	39.47	-	-	-	-
	5-9	109	79	72.48	64.10	80.86	6.02	3.27	11.08	0.000
	10-19	103	94	91.26	85.81	96.71	26.80	11.67	61.55	0.000
	20-39	77	59	76.62	67.17	86.07	9.23	4.46	19.10	0.000
	40-80	57	47	82.46	72.59	92.33	12.78	5.54	29.45	0.000
Sex	Males (ref)	178	129	72.47	65.91	79.03	-	-	-	-
	Females	258	177	68.60	62.94	74.26	0.55	0.33	0.93	0.026
Village	Barkoumbilen (ref)	282	199	70.57	65.25	75.89	-	-	-	-
	Barkoundouba	154	107	69.48	62.21	76.75	0.43	0.19	0.99	0.047
Ethnicity	Fulani (ref)	124	88	70.97	62.98	78.96	-	-	-	-
	Non-Fulani	312	218	69.87	64.78	74.96	0.44	0.18	1.06	0.068
Total		436	306	70.18	65.89	74.47	-	-	-	-

Table 7: Proportion of anti-SEA IgG positive subjects and association of subject's characteristics with anti-SEA IgG positivity.
This table shows the number of total population (N), the number (N+) and the proportion (%) of anti-SEA IgG positive subjects and the results of multivariate logistic regression analysis (Odds Ratio and P-value) performed to investigate the association of age-group (1-4 , 5-9, 10-19, 20-39, 40-80 years old), sex (females vs males), village (Barkoundouba vs Barkoumbilen) and ethnicity (Non-Fulani vs Fulani) with anti-SEA IgG positivity. 95% LCL: 95% Lower Confidence Limit; 95% UCL: 95% Upper Confidence Limit

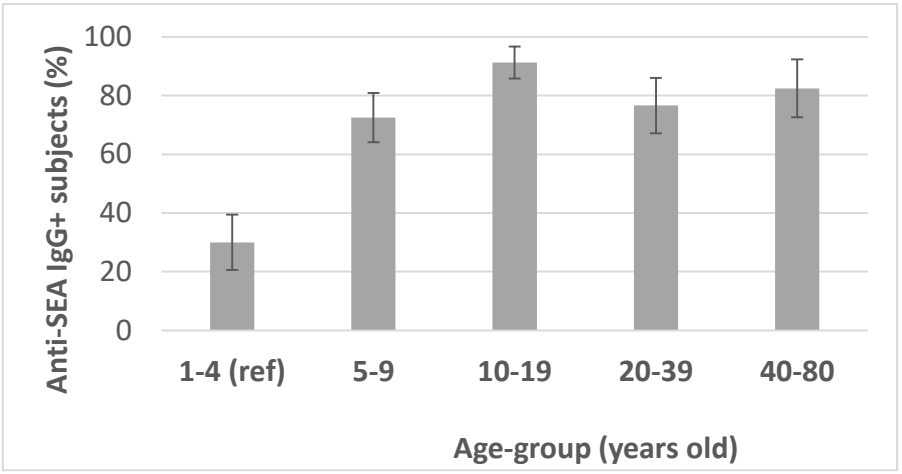

Figure 13: Proportion (%) of anti-SEA IgG positive subjects according to age-group (years old).
This figure shows the proportion (%) of anti-SEA IgG positive subjects according to age-group (1-4, 5-9, 10-19, 20-39, 40-80 years old). Error bars indicate the 95% confidence interval of the proportion.

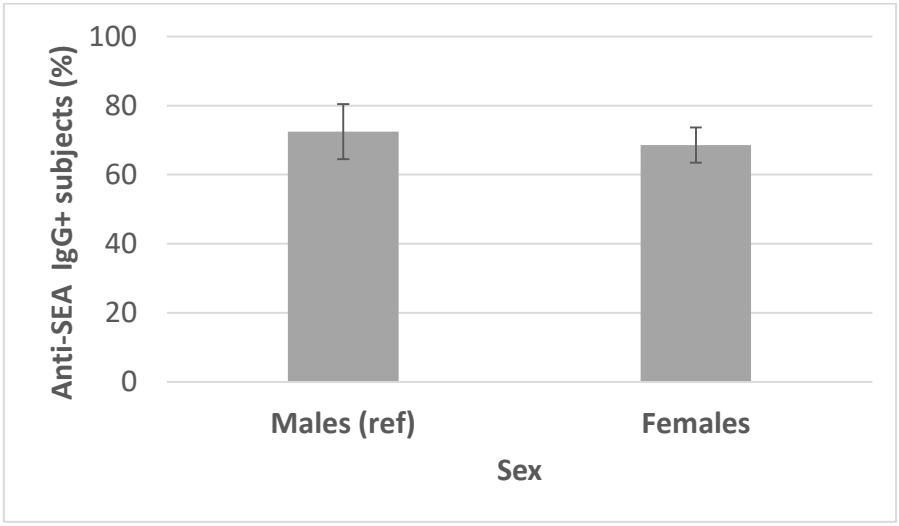

Figure 14: Proportion (%) of anti-SEA IgG positive subjects according to sex.
This figure shows the proportion (%) of anti-SEA IgG positive subjects according to sex (males and females). Error bars indicate the 95% confidence interval of the proportion.

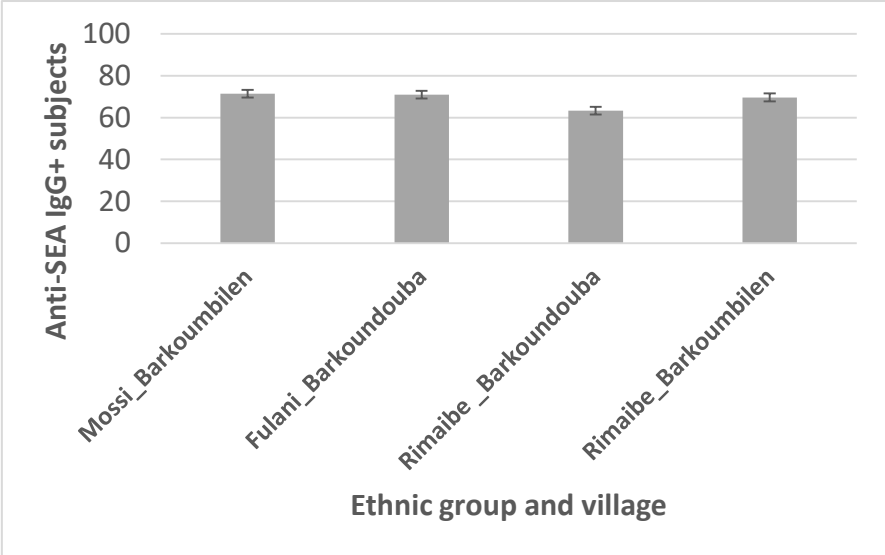

Figure 15: Proportion (%) of anti-SEA IgG positive subjects according to ethnic group and village.
This figure shows the proportion (%) of anti-SEA IgG positive subjects according to ethnic group and village (Mossi_Barkoumbilen, Fulani_Barkoumbilen, Rimaibe_Barkoundouba, Rimaibe_Barkoumbilen). Error bars indicate the 95% confidence interval of the proportion.

Village	Ethnic group	Total population	Anti-SEA IgG positive subjects			
		N	N+	Proportion (%)	95% LCL	95% UCL
Barkoumbilen	Mossi	147	105	71.43	64.13	78.73
	Rimaibe	135	94	69.63	61.87	77.39
Barkoundouba	Fulani	124	88	70.97	62.98	78.96
	Rimaibe	30	19	63.33	46.09	80.57
Total		436	306	70.18	65.89	74.47

Table 8: Proportion of anti-SEA IgG positive subjects according to ethnic group and village.
The table shows the number of total population (N), the number (N+) and the proportion (%) of anti-SEA IgG positive subjects according to ethnic group and village. 95% LCL: 95% Lower Confidence Limit; 95% UCL: 95% Upper Confidence Limit.

- **Patterns of positivity to *S. haematobium* specific antibodies**

In order to describe the pattern of positivity to *S. haematobium* specific antibodies among the study population, the number and proportion of subjects for each possible combination of positivity for anti-SWAP IgM, anti-SWAP IgG, anti-SEA IgM and anti-SEA IgG was computed. Results are shown in Figure 16.

Subjects that do not carry any (0) *S. haematobium* specific antibodies represent 13.6% of the population, those carrying one (1) of the four antibodies represent the 12.5%, those carrying two (2) of the four in various combinations represent the 16.5%, those carrying three (3) of the four, also in various combinations, represent the 27.1%, among which the highest frequency is represented by individuals carrying all antibodies with the exception of anti-SWAP IgM+, and finally those carrying all four (4) antibodies represent 30.4% of the population, the highest proportion.

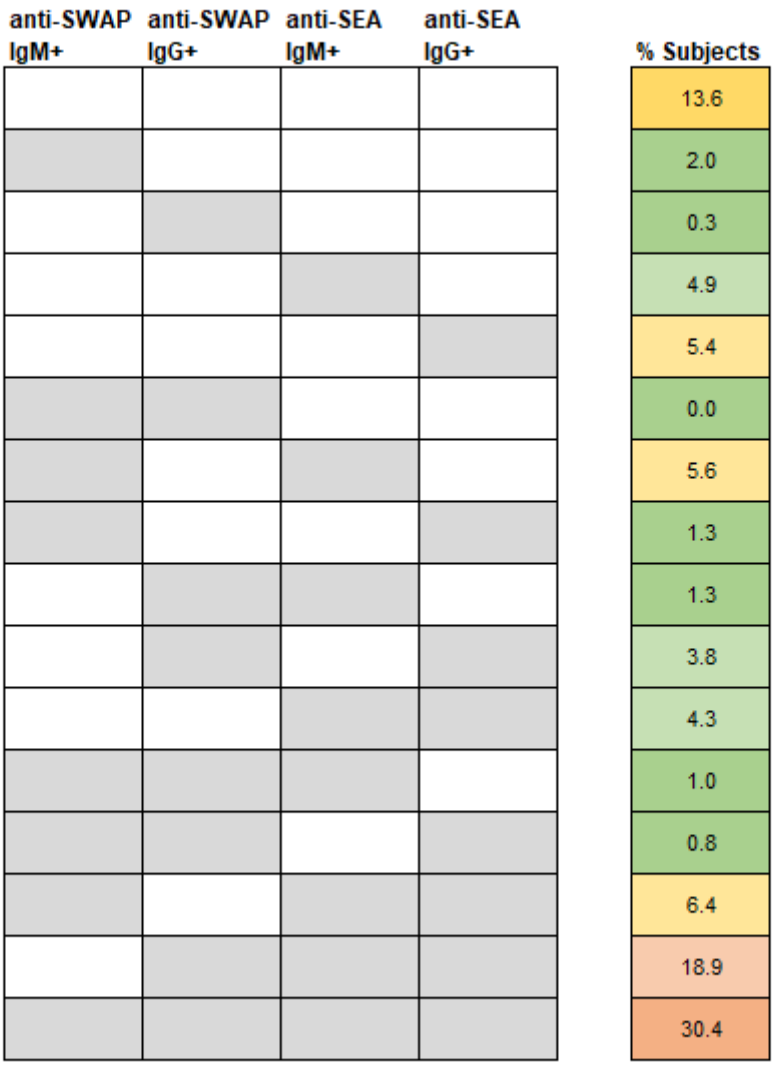

Figure 16: Patterns of positivity to *S. haematobium* antigens.
This figure shows the different possible combinations of positivity for anti-SWAP IgM, anti-SWAP IgG, anti-SEA IgM and anti-SEA IgG and the corresponding proportion (%) of subjects. Positivity to a given antibody is indicated by a grey box. Proportions are heat-map colored from green to orange depending on the range (green: 0-2.5%; light green: 2.6-5%; light yellow: 6-10; yellow: 11-15; light orange: 16-20; orange: >20).

Levels of S. haematobium specific antibodies.

The variation in the level of *S. haematobium* specific antibodies among seropositive subjects has been assessed according to age-group, sex, ethnicity and village using multivariate linear regression association analysis.

- **Anti-SWAP IgM**

The level of anti-SWAP IgM was analysed among seropositive subjects (N+=195).

Table 9 shows the range (minimum-maximum), the median, the mean and standard deviation of anti-SWAP IgM normalised OD (nOD) according to age-group (1-4, 5-9, 10-19, 20-39, 40-80 years old), sex (females vs males), village of residence (Barkoundouba vs Barkoumbilen) and ethnicity (Non-Fulani vs Fulani) as well as the results of multivariate association analysis.

As shown in Table 9, anti-SWAP IgM levels are highest in the age-group 10-19 years old (Figure 17); they are comparable in males and females (Figure 18); they are comparable in Barkoumbilen and Barkoundouba as well as in Fulani and Non-Fulani (Table 10, Figure 19).

Subjects characteristics		anti-SWAP IgM level				Association with anti-SWAP IgM level		
		Range	Median	Mean	Std Dev	Coefficient	Std Error	P-Value
Age-group (years old)	1-4 (ref)	0.36-1.01	0.42	0.48	0.17	-	-	-
	5-9	0.35-1.13	0.52	0.56	0.18	0.03	0.01	0.034
	10-19	0.35-1.09	0.53	0.56	0.18	0.03	0.01	0.022
	20-39	0.35-1.06	0.49	0.53	0.16	0.02	0.01	0.106
	40-80	0.37-0.96	0.59	0.59	0.17	0.04	0.02	0.010
Sex	Males (ref)	0.35-1.13	0.54	0.56	0.19	-	-	-
	Females	0.37-1.06	0.51	0.55	0.17	0.00	0.01	0.984
Village	Barkoumbilen (ref)	0.35-0.93	0.48	0.52	0.14	-	-	-
	Barkoundouba	0.35-1.13	0.56	0.59	0.20	0.00	0.00	0.450
Ethnicity	Fulani (ref)	0.35-1.13	0.55	0.60	0.20	-	-	-
	Non-Fulani	0.35-0.93	0.50	0.53	0.14	-0.01	0.02	0.625

Table 9: Anti-SWAP IgM level according to subjects' characteristics and results of association analysis.
The table shows the anti-SWAP IgM level and the results of linear regression performed to investigate the association of age-group (1-5; 5-9; 10-19; 20-39; 40-80 years old), sex (females vs males), village (Barkoundouba vs Barkoumblien) and ethnicity (Non-Fulani vs Fulani) with anti-SWAP IgM level. Range (minimum-maximum); Median; Mean; Std Dev = Standard Deviation. Coefficient; Std Error = Standard Error; P-Value.

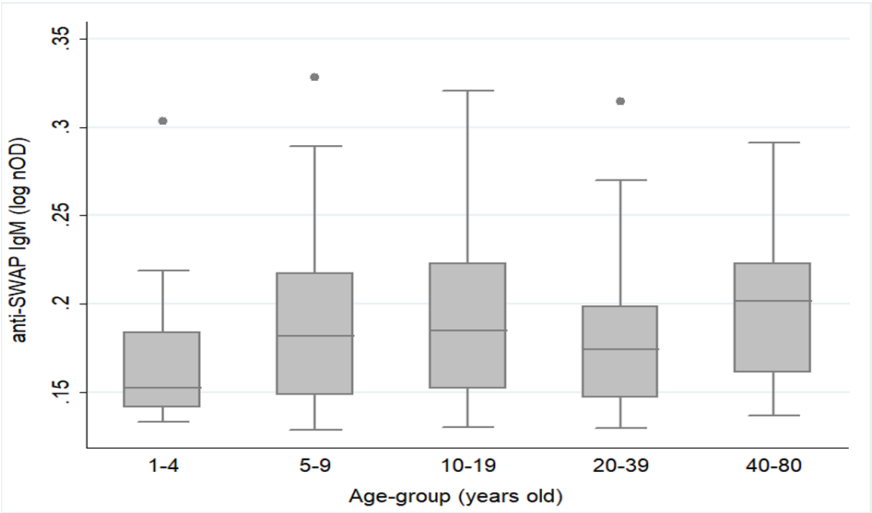

Figure 17: Anti-SWAP IgM level according to age-group (years old).
The figure shows a boxplot of the distribution of anti-SWAP IgM level according to age-group (1-4; 5-9; 10-19; 20-39; 40-80 years old) among positive subjects. The horizontal black line indicates the 50% percentile, the grey box indicates the 25%-75% percentile, the lower whisk indicates the 5% and the upper whisk the 95% percentile respectively, the dots indicate individual's data that lie out of the distribution (outliers).

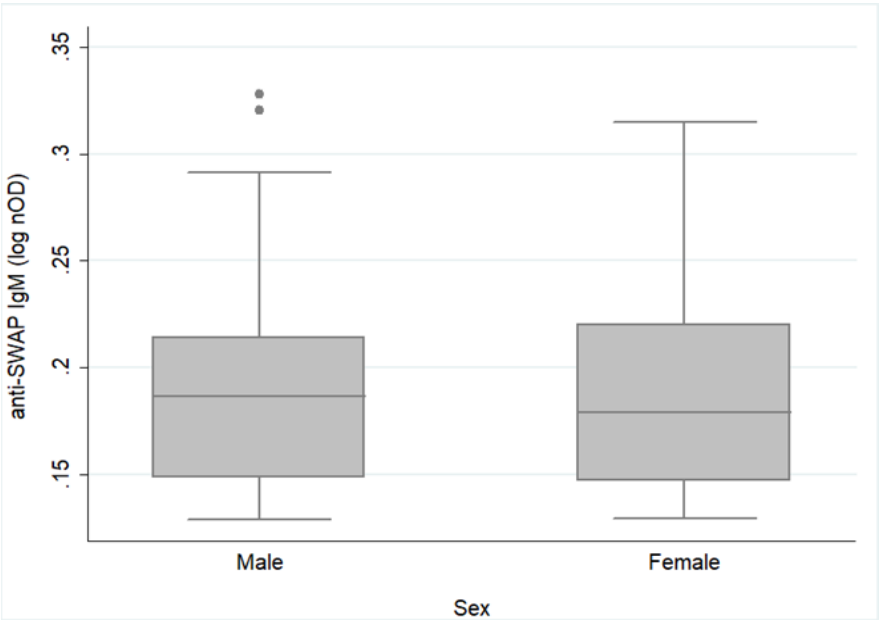

Figure 18: Anti-SWAP IgM level according to sex.
The figure shows a boxplot of the distribution of anti-SEA IgM level according to sex (males and females) among positive subjects. The horizontal black line indicates the 50% percentile, the grey box indicates the 25%-75% percentile, the lower whisk indicates the 5% and the upper whisk the 95% percentile respectively, dots indicate individual's data that lie out of the distribution (outliers).

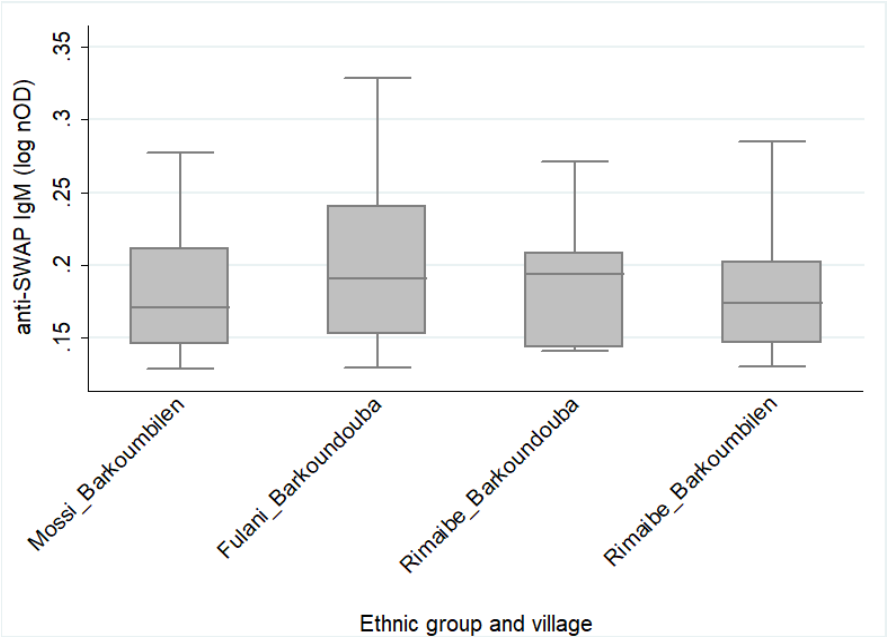

Figure 19: Anti-SWAP IgM level according to ethnic group and village.
The figure shows a boxplot of the distribution of anti-SWAP IgM level according to ethnic group and village (Mossi_Barkoumbilen, Fulani_Barkoundouba, Rimaibe_Barkoundouba, Rimaibe_Barkoumbilen) among positive subjects. The horizontal black line indicates the 50% percentile, the grey box indicates the 25%-75% percentile, the lower whisk indicates the 5% and the upper whisk the 95% percentile respectively.

Village	Ethnic group	Anti-SWAP IgM level			
		Range	Median	Mean	Std Dev
Barkoumbilen	Mossi	0.35-0.89	0.48	0.53	0.15
	Rimaibe	0.35-0.93	0.49	0.52	0.14
Barkoundouba	Fulani	0.35-1.13	0.55	0.60	0.20
	Rimaibe	0.38-0.87	0.56	0.56	0.16

Table 10: Anti-SWAP IgM level according to ethnic group and village.
The table shows the anti-SWAP IgM level according to ethnic group and village. Range (minimum-maximum); Median; Mean; Std Dev = Standard Deviation.

- **Anti-SWAP IgG**

The level of anti-SWAP IgG was analysed among seropositive subjects (N+=241).

Table 11 shows the range (minimum-maximum), the median, the mean and standard deviation of anti-SWAP IgG normalised OD (nOD) according to age group (1-4, 5-9, 10-19, 20-39, 40-80 years old), sex (females vs males), village of residence (Barkoundouba vs Barkoumbilen) and ethnicity (Non-Fulani vs Fulani) as well as the result of multivariate association analysis.

As shown in Table 11, anti-SWAP IgG levels are highest in the age-group 10-19 years old (Figure 20); they are lower in females compared to males (Figure 21); they are lower in Barkoundouba compared to Barkoumbilen as well as in Fulani compared to Non-Fulani (Table12; Figure 22).

Subjects characteristics		anti-SWAP IgG level				Association with anti-SWAP IgG level		
		Range	Median	Mean	Std Dev	Coefficient	Std Error	P-Value
Age-group (years old)	1-4 (ref)	0.40-0.98	0.52	0.60	0.17	-	-	-
	5-9	0.38-1.07	0.67	0.68	0.19	0.02	0.01	0.164
	10-19	0.38-1.12	0.80	0.79	0.18	0.05	0.01	0.000
	20-39	0.38-1.05	0.64	0.67	0.19	0.03	0.02	0.077
	40-80	0.38-0.98	0.67	0.68	0.17	0.02	0.02	0.138
Sex	Males (ref)	0.38-1.12	0.79	0.74	0.18	-	-	-
	Females	0.38-1.07	0.67	0.69	0.20	-0.01	0.01	0.044
Village	Barkoumbilen (ref)	0.38- 1.07	0.75	0.72	0.19	-	-	-
	Barkoundouba	0.38-1.12	0.68	0.69	0.19	0.00	0.00	0.017
Ethnicity	Fulani (ref)	0.38-1.12	0.70	0.70	0.18	-	-	-
	Non-Fulani	0.38-1.07	0.73	0.72	0.19	-0.03	0.02	0.050

Table 11: Anti-SWAP IgG level according to subjects' characteristics and results of association analysis
The table shows the anti-SWAP IgG level and the results of linear regression performed to investigate the association of age-group (1-5; 5-9; 10-19; 20-39; 40-80 years old), sex (females vs males), village (Barkoundouba vs Barkoumbilen) and ethnicity (Non-Fulani vs Fulani) with anti-SWAP IgG level. Range (minimum-maximum); Median; Mean; Std Dev = Standard Deviation. Coefficient; Std Error = Standard Error; P-Value.

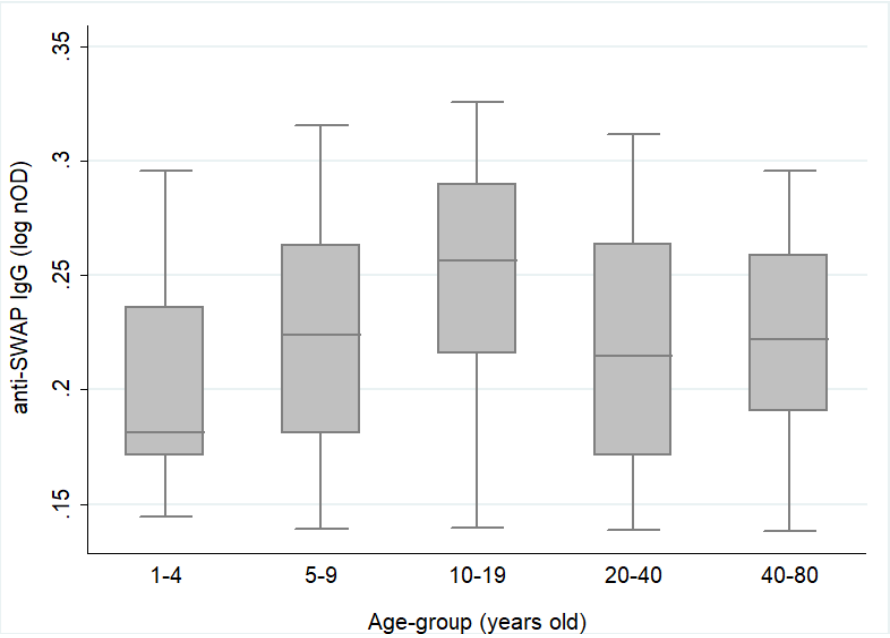

Figure 20: Anti-SWAP IgG level according to age-group (years old).
The figure shows a boxplot of the distribution of anti-SWAP IgG level according to age-group (1-4; 5-9; 10-19; 20-39; 40-80 years old) among positive subjects. The horizontal black line indicates the 50% percentile, the grey box indicates the 25%-75% percentile, the lower whisk indicates the 5% and the upper whisk the 95% percentile respectively.

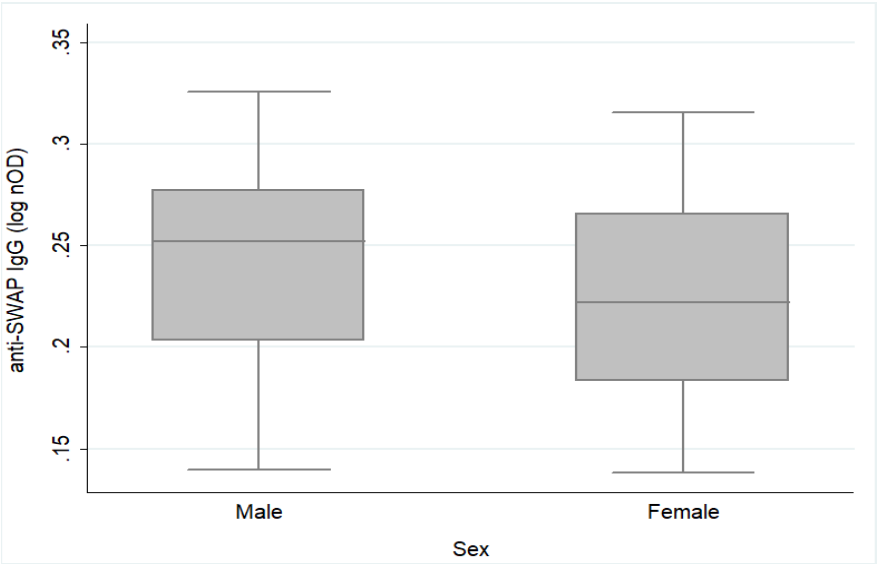

Figure 21: Anti-SWAP IgG level according to sex.
The figure shows a boxplot of the distribution of anti-SWAP IgG levels according to sex (males and females) among positive subjects. The horizontal black line indicates the 50% percentile, the grey box indicates the 25%-75% percentile, the lower whisk indicates the 5% and the upper whisk the 95% percentile respectively.

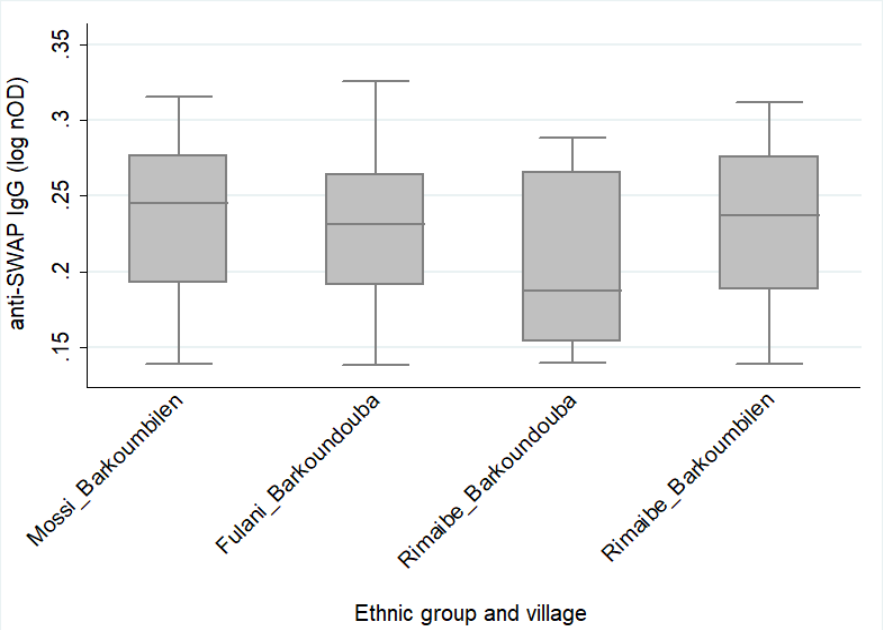

Figure 22: Anti-SWAP IgG level according to ethnic group and village.
The figure shows a boxplot of the distribution of anti-SWAP IgG levels according to ethnic group and village (Mossi_Barkoumbilen, Fulani_Barkoundouba, Rimaibe_Barkoundouba, Rimaibe_Barkoumbilen) among positive subjects. The horizontal black line indicates the 50% percentile, the grey box indicates the 25%-75% percentile, the lower whisk indicates the 5% and the upper whisk the 95% percentile respectively.

Village	Ethnic group	Anti-SWAP IgG level			
		Range	Median	Mean	Std Dev
Barkoumbilen	Mossi	0.38-1.07	0.76	0.73	0.19
	Rimaibe	0.38-1.05	0.73	0.72	0.19
Barkoundouba	Fulani	0.38-1.12	0.70	0.70	0.18
	Rimaibe	0.38-0.94	0.54	0.61	0.21

Table 12: Anti-SWAP IgG level according to ethnic group and village.
The table shows the anti-SWAP IgG level according to ethnic group and village. Range (minimum-maximum); Median; Mean; Std Dev = Standard Deviation.

- **Anti-SEA IgM**

The level of anti-SEA IgM was analysed among seropositive subjects (N+=312).

Table 13 shows the range (minimum-maximum), the median, the mean and standard deviation of anti-SWAP IgG normalised OD (nOD) according to age group (1-4, 5-9, 10-19, 20-39, 40-80 years old), sex (females vs males), village of residence (Barkoundouba vs Barkoumbilen) and ethnicity (Non-Fulani vs Fulani) as well as the result of multivariate association analysis.

As shown in Table 13, anti-SEA IgM levels are highest in the age group 10-19 years old (Figure 23); they are comparable in males and females (Figure 24); they are lower in Barkoundouba compared to Barkoumbilen; they are lower in Non-Fulani compared to Fulani (Table 14, Figure 25).

Subjects characteristics		anti-SEA IgM level				Association with anti-SEA IgM level		
		Range	Median	Mean	Std Dev	Coefficient	Std Error	P-Value
Age-group (years old)	1-4 (ref)	0.4-0.79	0.50	0.54	0.13	-	-	-
	5-9	0.4-1.03	0.67	0.68	0.18	0.04	0.01	0.000
	10-19	0.4-1.04	0.75	0.72	0.17	0.05	0.01	0.000
	20-39	0.4-0.96	0.62	0.62	0.16	0.03	0.01	0.023
	40-80	0.4-0.99	0.64	0.65	0.18	0.03	0.01	0.006
Sex	Males (ref)	0.4-1.01	0.73	0.68	0.17	-	-	-
	Females	0.4-1.04	0.66	0.66	0.18	0.00	0.01	0.908
Village	Barkoumbilen (ref)	0.4-1.04	0.68	0.67	0.19	-	-	-
	Barkoundouba	0.4-1.03	0.66	0.65	0.16	0.00	0.00	0.014
Ethnicity	Fulani (ref)	0.4-1.03	0.66	0.67	0.16	-	-	-
	Non-Fulani	0.4-1.04	0.66	0.66	0.19	-0.02	0.01	0.022

Table 13: Anti-SEA IgM level according to subjects' characteristics and results of association analysis.
The table shows the anti-SEA IgM level and the results of multivariate linear regression performed to investigate the association of age-group (1-5; 5-9; 10-19; 20-39; 40-80 years old), sex (females vs males), village (Barkoundouba vs Barkoumbilen) ethnicity (Non-Fulani vs Fulani) with anti-SEA IgM level. Range (minimum-maximum); Median; Mean; Std Dev = Standard Deviation. Coefficient; Std Error = Standard Error; P-Value.

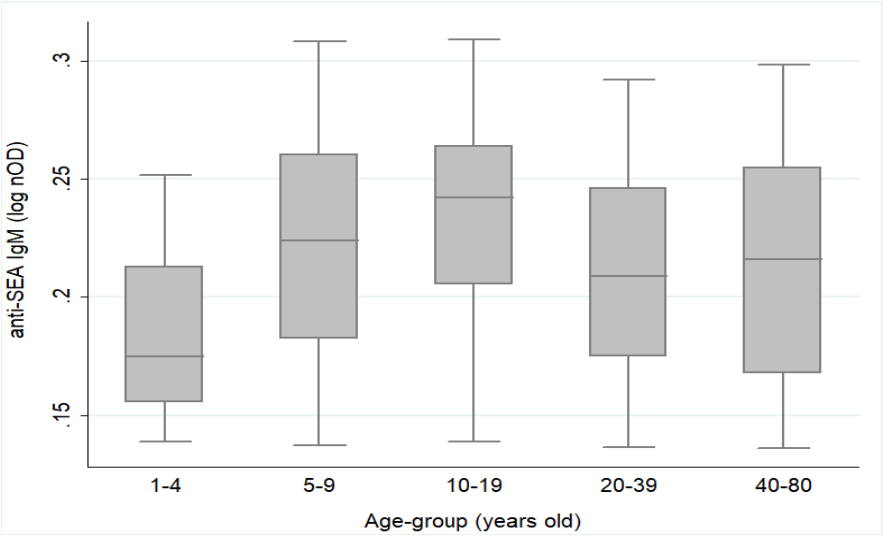

Figure 23: Anti-SEA IgM level according to age-group (years old).
The figure shows a boxplot of the distribution of anti-SEA IgM levels according to age-group (1-4; 5-9; 10-19; 20-39; 40-80 years old) among positive subjects. The horizontal black line indicates the 50% percentile, the grey box indicates the 25%-75% percentile, the lower whisk indicates the 5% and the upper whisk the 95% percentile respectively.

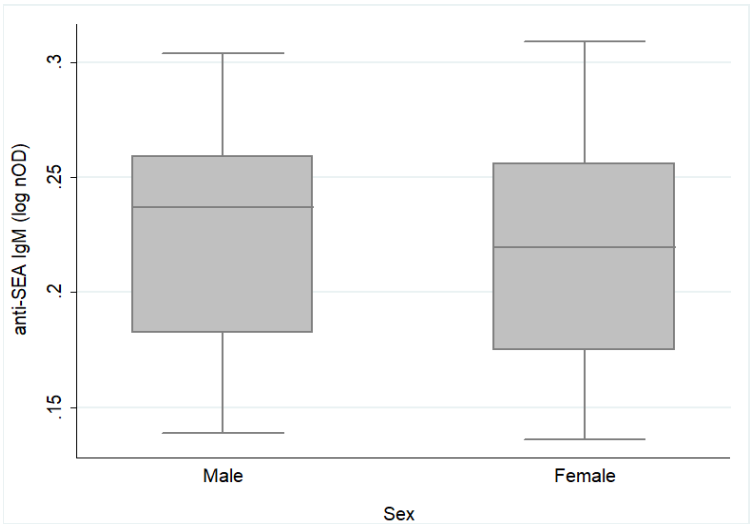

Figure 24: Anti-SEA IgM level according to sex.
The figure shows a boxplot of the distribution of anti-SEA IgM level according to sex (males and females) among positive subjects. The horizontal black line indicates the 50% percentile, the grey box indicates the 25%-75% percentile, the lower whisk indicates the 5% and the upper whisk the 95% percentile respectively.

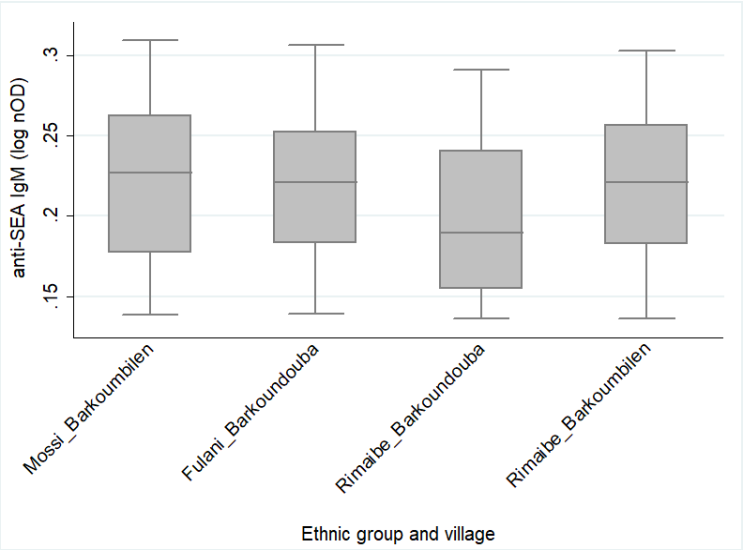

Figure 25: Anti-SEA IgM level according to ethnic group and village.
The figure shows a boxplot of the distribution of anti-SEA IgM level according to ethnic group and village (Mossi_Barkoumbilen, Fulani_Barkoundouba, Rimaibe_Barkoundouba, Rimaibe_Barkoumbilen) among positive subjects. The horizontal black line indicates the 50% percentile, the grey box indicates the 25%-75% percentile, the lower whisk indicates the 5% and the upper whisk the 95% percentile respectively.

Village	Ethnic group	Anti-SEA IgM level			
		Range	Median	Mean	Std Dev
Barkombilen	Mossi	0.38-1.04	0.69	0.68	0.20
	Rimaibe	037-1.01	0.66	0.66	0.17
Barkoundouba	Fulani	0.38-1.03	0.66	0.67	0.16
	Rimaibe	0.37-0.95	0.55	0.58	0.17

Table 14: Anti-SEA IgM level according to ethnic group and village.
The table shows the anti-SEA IgM level according to ethnic group and village. Range (minimum-maximum); Median; Mean; Std Dev = Standard Deviation.

- **Anti-SEA IgG**

The level of anti-SEA IgG was analysed among seropositive subjects (N+=306).

Table 15 shows the range (minimum-maximum), the median, the mean and standard deviation of anti-SWAP IgG normalised OD (nOD) according to age group (1-4, 5-9, 10-19, 20-39, 40-80 years old), sex (females vs males), village of residence (Barkoundouba vs Barkoumbilen) and ethnicity (Non-Fulani vs Fulani) as well as the result of multivariate association analysis.

As shown in Table 15, anti-SEA IgG levels are highest in the age group 10-19 years old (Figure 26); they are lower in females compared to males (Figure 27); they are lower in Barkoundouba compared to Barkoumbilen; they are lower in Fulani compared to Non-Fulani (Table 16, Figure 28).

Subjects characteristics		anti-SEA IgG level				Association with anti-SEA IgG level		
		Range	Median	Mean	Std Dev	Coefficient	Std Error	P-Value
Age-group (years old)	1-4 (ref)	0.24-1.04	0.39	0.51	0.28	-	-	-
	5-9	0.24-1.09	0.91	0.78	0.30	0.07	0.01	0.000
	10-19	0.31-1.10	1.00	0.93	0.19	0.11	0.01	0.000
	20-39	0.24-1.06	0.46	0.54	0.24	0.02	0.02	0.139
	40-80	0.24-1.04	0.63	0.65	0.23	0.05	0.02	0.001
Sex	Males (ref)	0.24-1.10	0.90	0.81	0.28	-	-	-
	Females	0.24-1.10	0.68	0.68	0.29	-0.02	0.01	0.012
Village	Barkoumbilen (ref)	0.24-1.10	0.91	0.78	0.29	-	-	-
	Barkoundouba	0.24-1.09	0.60	0.64	0.27	0.00	0.01	0.040
Ethnicity	Fulani (ref)	0.24-1.09	0.68	0.66	0.27	-	-	-
	Non-Fulani	0.24-1.10	0.87	0.76	0.30	-0.03	0.02	0.040

Table 15: Anti-SEA IgG level according to subjects' characteristics and results of association analysis.
The table shows the anti-SEA IgG level and the results of linear regression performed to investigate the association of age-group (1-5; 5-9; 10-19; 20-39; 40-80 years old), sex (females vs males), village (Barkoundouba vs Barkoumbilen) and ethnicity (Non-Fulani vs Fulani) with anti-SEA IgG level. Range (minimum-maximum); Median; Mean; Std Dev = Standard Deviation. Coefficient; Std Error = Standard Error; P-Value.

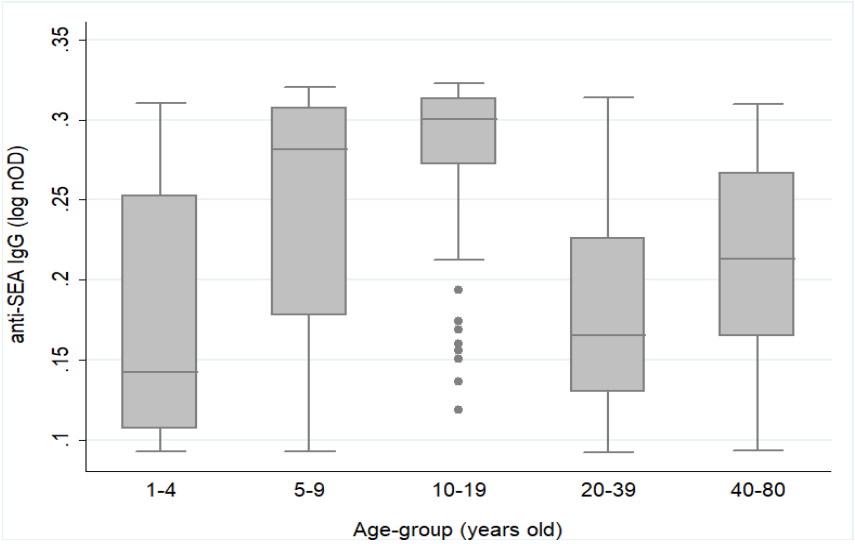

Figure 26: Anti-SEA IgG level according to age-group (years old).
The figure shows a boxplot of the distribution of anti-SEA IgG levels according to age-group (1-4; 5-9; 10-19; 20-39; 40-80 years old) among positive subjects. The horizontal black line indicates the 50% percentile, the grey box indicates the 25%-75% percentile, the lower whisk indicates the 5% and the upper whisk the 95% percentile respectively, dots indicate individual's data that lie out of the distribution (outliers).

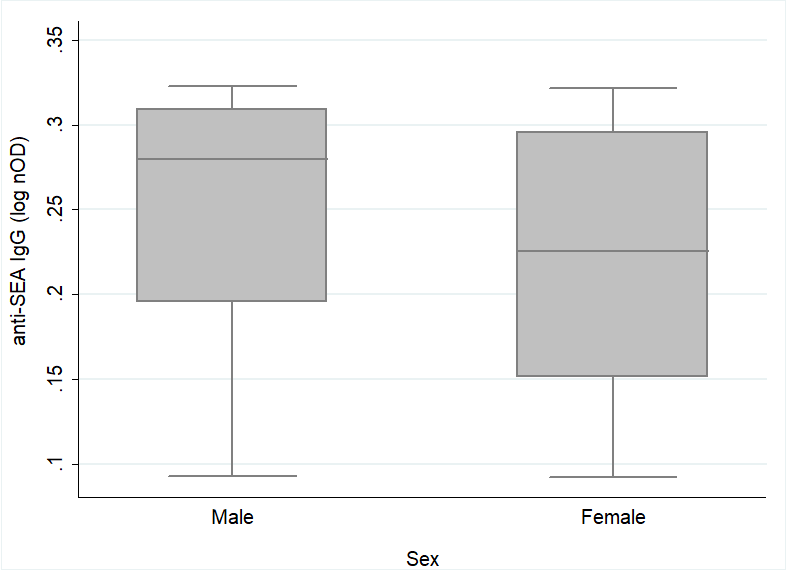

Figure 27: Anti-SEA IgG level according to sex.
The figure shows a boxplot of the distribution of anti-SEA IgG levels according to sex (males and females) among positive subjects. The horizontal black line indicates the 50% percentile, the grey box indicates the 25%-75% percentile, the lower whisk indicates the 5% and the upper whisk the 95% percentile respectively.

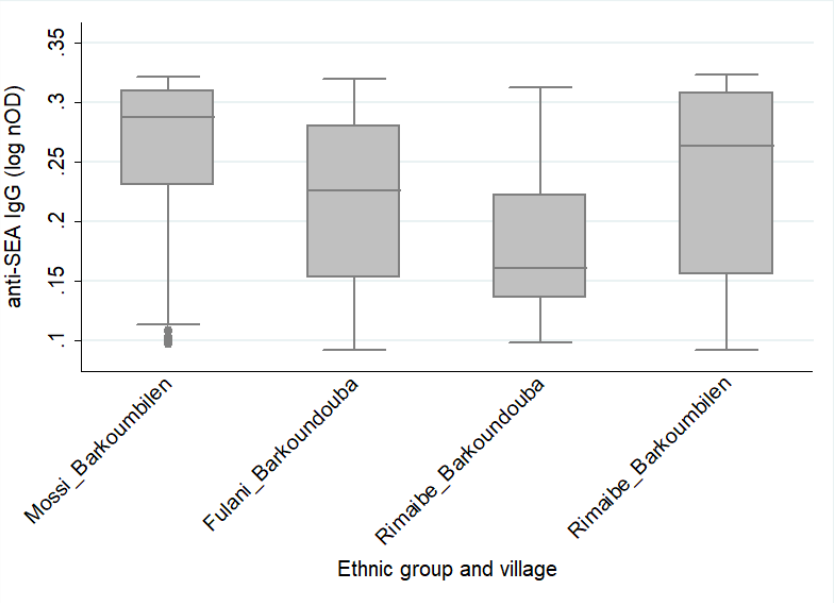

Figure 28: Anti-SEA IgG level according to ethnic group and village.
The figure shows a boxplot of the distribution of anti-SEA IgG levels according to ethnic group and village (Mossi_Barkoumbilen, Fulani_Barkoundouba, Rimaibe_Barkoundouba, Rimaibe_Barkoumbilen) among positive subjects. The horizontal black line indicates the 50% percentile, the grey box indicates the 25%-75% percentile, the lower whisk indicates the 5% and the upper whisk the 95% percentile respectively, dots indicate individual's data that lie out of the distribution (outliers).

Village	Ethnic group	Anti-SEA IgG level			
		Range	Median	Mean	Std Dev
Barkoumbilen	Mossi	0.25-1.10	0.94	0.83	0.26
	Rimaibe	0.24-1.10	0.84	0.74	0.31
Barkoundouba	Fulani	0.24-1.09	0.68	0.66	0.27
	Rimaibe	0.25-1.06	0.45	0.53	0.21

Table 16: Anti-SEA IgG level according to ethnic group and village.
The table shows the anti-SEA IgG level according to ethnic group and village. Range (minimum-maximum); Median; Mean; Std Dev = Standard Deviation.

- **Correlation between antibody levels**

In order to investigate the correlation between levels of different *S.haematobium*-specific antibodies, Spearman correlation analysis was performed for each of the different pairs of anti-SWAP IgM, anti-SWAP IgG, anti-SEA IgM and anti-SEA IgG expressed as logarithmic transformation of normalised OD (log nOD). Results are shown in Figure 29 and Table 17.

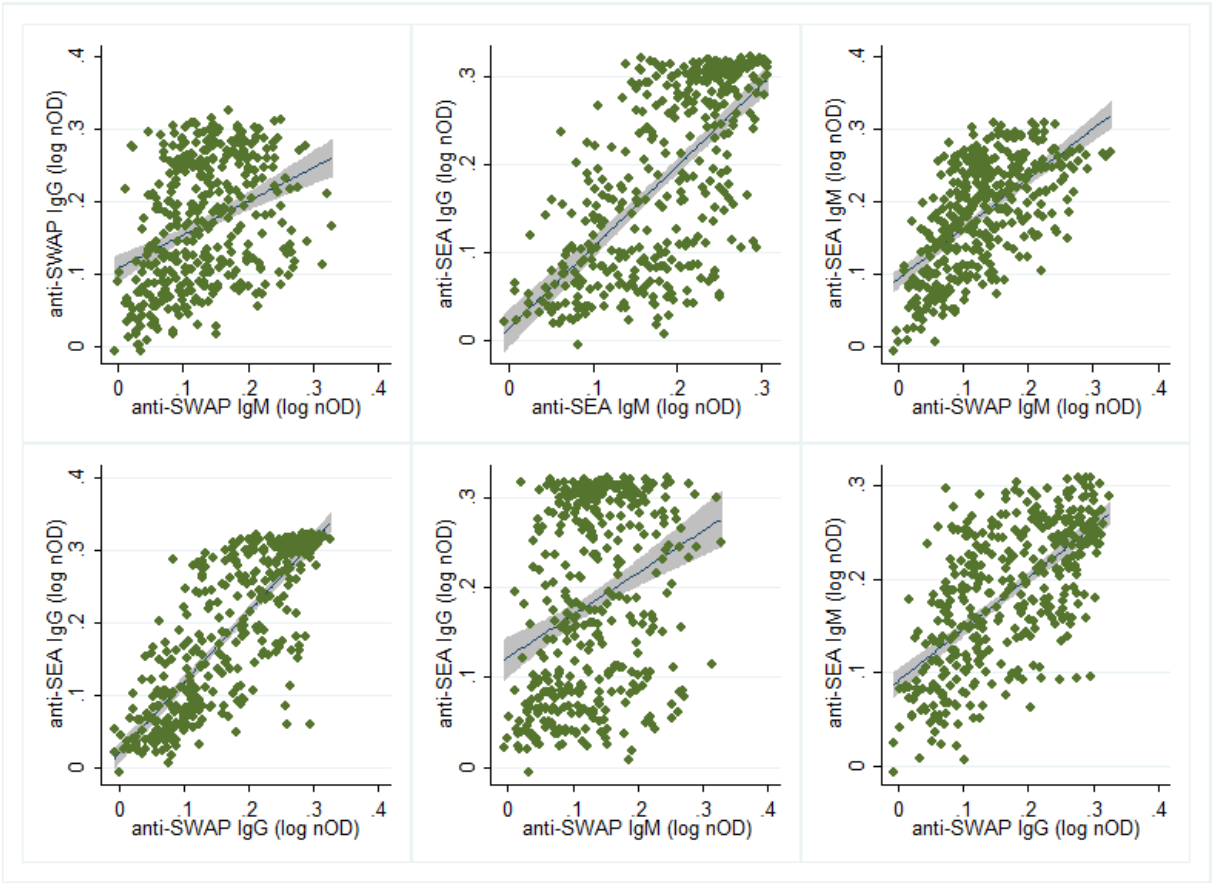

Figure 29: Correlation between *S.haematobium*-specific antibody levels: scatter-plots.
This figure shows scatter plots describing the linear correlation between *S.haematobium*-specific antibody levels (log nOD). The points in green represent the data, the blue line indicates the best linear curve fitted to the data, the grey area indicates the 95% confidence interval of the fitted linear curve.

		anti-SWAP		anti-SEA	
		IgM	**IgG**	**IgM**	**IgG**
anti-SWAP	**IgM**		rho= 0.39 ; p= 0.000	rho= 0.62; p= 0.000	rho= 0.32; p=0.000
	IgG			rho= 0.63; p= 0.000	rho= 0.82; p=0.000
anti-SEA	**IgM**				rho= 0.66; p= 0.000
	IgG				

Table 17: Correlation between *S.haematobium*-specific antibody levels: results of Spearman analysis.
The table shows the results of Spearman correlation analysis (rho: correlation coefficient; p: p-value) between *S.haematobium*-specific antibody levels (log nOD). Grey boxes represent impossible or repetitive combinations.

A significant positive correlation is observed between levels of all pairs of *S.haematobium*-specific antibodies.

The lowest correlation coefficients are observed between levels of anti-SWAP IgM and those of IgG against both SWAP and SEA. Higher correlation coefficients are observed between levels of IgM and IgG against SEA, between levels of IgM against SWAP and SEA, as well as between levels of anti-SWAP IgG and anti-SEA IgM. The highest correlation coefficient is observed between levels of IgG against SWAP and SEA.

4.1.1.2. *S. haematobium* Circulating Anodic Antigen

Proportion of subjects positive for Circulating Anodic Antigen

The overall proportion of subjects positive for CAA (CAA positivity or CAA+) among the study population is 28.3%.

A descriptive analysis has been performed to assess the variation in the proportion of subjects positive for Circulating Cathodic Antigen (CAA) according to demographic factors such as age-group, sex, village and ethnicity.

Table 18 shows the proportion of CAA positive subjects (CAA positivity) according to age-group (1-4, 5-9, 10-19, 20-39, 40-80 years old), sex (females vs males), village of residence (Barkoundouba vs Barkoumbilen) and ethnicity (Non-Fulani vs Fulani) as well as the results of multivariate association analysis.

As shown in Table 18, CAA positivity is lowest in children aged 1 to 4 years, it increases in children aged 5 to 9 years, is highest in children aged 10 to 19 years and decreases in subjects older than 20 years (Figure 30); it is comparable in females and males (Figure 31); it is higher in Barkoumbilen compared to Barkoundouba, while no differences are observed between Non-Fulani and Fulani: Indeed, also when comparing ethnic groups within each village, no differences are observed neither between Mossi and Rimaibe in Barkoumbilen nor between Fulani and Rimaibe in Barkoundouba (Table 19 and Figure 32).

Subjects' characteristics		Total population	Circulating Anodic Antigen positive subjects				Association with CAA positivity			
		N	N+	Proportion %	95% LCL	95% UCL	Odds Ratio	95% CI LCL	95% CI UCL	P-Value
Age-group (years old)	1-4 (ref)	93	4	4.3	0.18	8.42	-	-	-	-
	5-9	107	32	29.9	21.23	38.57	12.83	4.24	38.85	0.000
	10-19	99	58	58.5	48.79	68.21	54.13	17.42	168.23	0.000
	20-39	71	14	19.7	10.45	28.95	6.65	1.98	22.35	0.002
	40-80	54	12	22.2	11.12	33.28	7.52	2.21	25.56	0.001
Sex	Males (ref)	251	54	31.2	25.47	36.93	-	-	-	-
	Females	173	66	26.3	19.73	32.85	0.71	0.41	1.22	0.217
Village	Barkoumbilen (ref)	272	101	37.1	31.36	42.84	-	-	-	-
	Barkoundouba	152	19	12.5	7.24	17.76	0.14	0.04	0.44	0.001
Ethnicity	Fulani (ref)	121	15	12.4	6.53	18.27	-	-	-	-
	Non-Fulani	303	105	24.6	19.75	29.45	0.97	0.27	3.41	0.958
Total		424	120	28.3	24.01	32.59	-	-	-	-

Table 18: Proportion of Circulating Anodic Antigen positive subjects and association of subject's characteristics with CAA positivity.
This table shows the number of total population (N), the number (N+) and the proportion (%) of Circulating Anodic Antigen (CAA) positive subjects and the results of multivariate logistic regression analysis (Odds Ratio and P-value) performed to investigate the association of age-group (1-4, 5-9, 10-19, 20-39, 40-80 years old), sex (females vs males), village (Barkoundouba vs Barkoumbilen) and ethnicity (Non-Fulani vs Fulani) with CAA positivity. 95% LCL: 95% Lower Confidence Limit; 95% UCL: 95% Upper Confidence Limit.

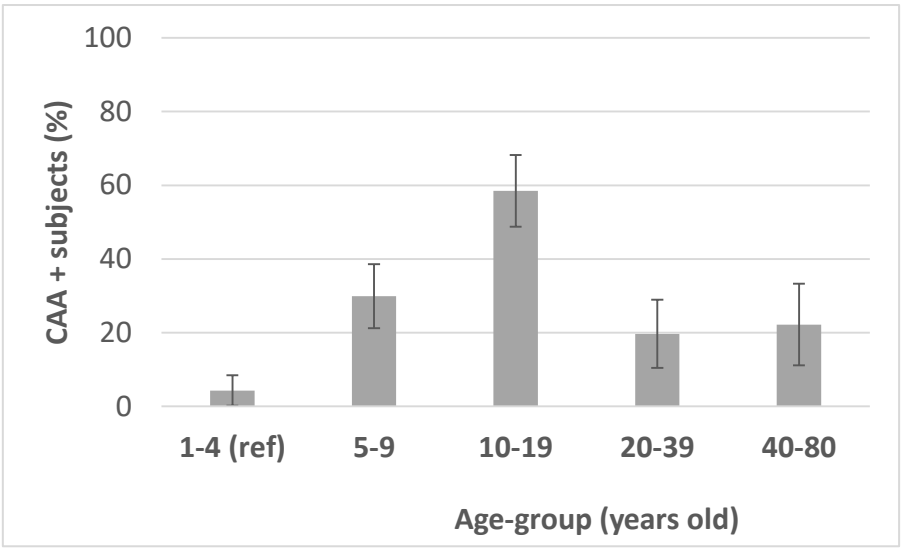

Figure 30: Proportion (%) of CAA positive subjects according to age-group (years old).
This figure shows the proportion (%) of CAA positive subjects according to age-group (1-4, 5-9, 10-19, 20-39, 40-80 years old). Error bars indicate the 95% confidence interval of the proportion.

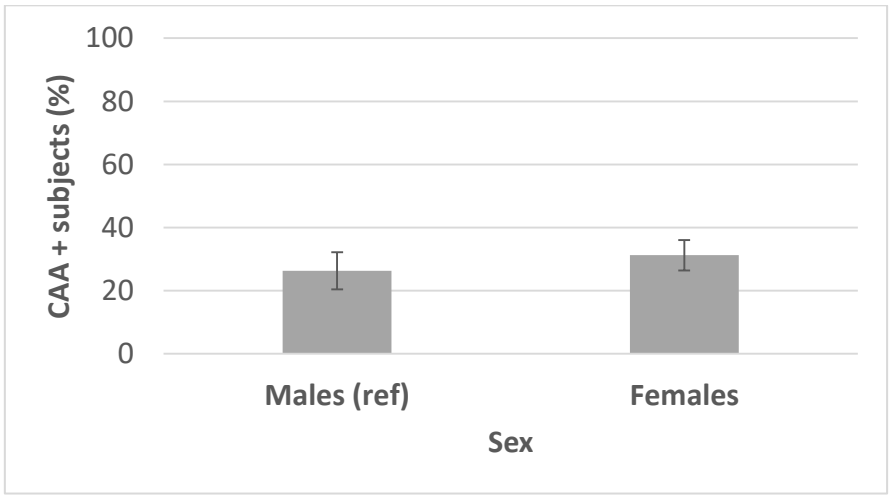

Figure 31: Proportion (%) of CAA positive subjects according to sex.
This figure shows the proportion (%) of CAA positive subjects according to sex (males and females). Error bars indicate the 95% Confidence Interval of the proportion.

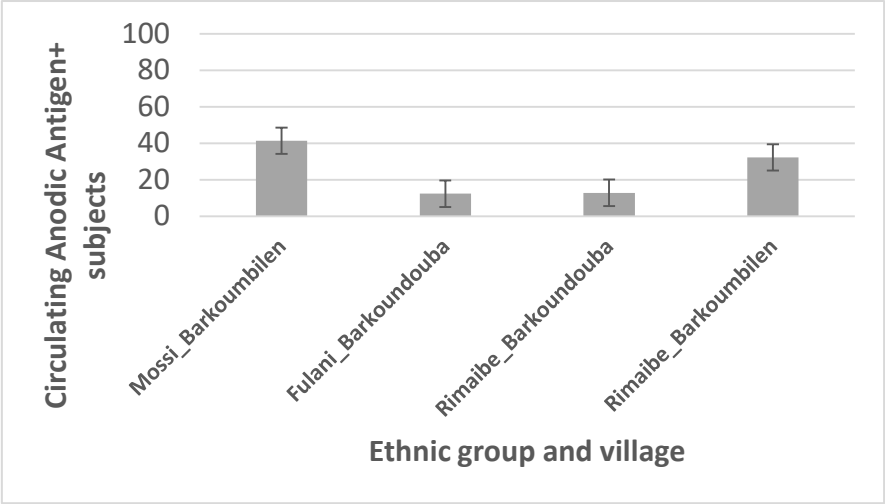

Figure 32: Proportion (%) of CAA positive subjects according to ethnic group and village.
This figure shows the proportion (%) of CAA positive subjects according to ethnic group and village (Mossi_Barkoumbilen, Fulani_Barkoumbilen, Rimaibe_Barkoundouba, Rimaibe_Barkoumbilen). Bars indicate the standard error of the proportion.

Village	Ethnic group	Total population	CAA positive subjects			
		N	N+	Proportion (%)	95% LCL	95% UCL
Barkoumbilen	Mossi	145	60	41.4	33.36	49.40
	Rimaibe	127	41	32.3	24.17	40.43
Barkoundouba	Fulani	121	15	12.4	6.53	18.27
	Rimaibe	31	4	12.9	1.10	24.70
Total		424	120	28.3	24.01	32.59

Table 19: Proportion of CAA positive subjects according to ethnic group and village.
This table shows the number of total population (N), the number (N+) and the proportion (%) of CAA (Circulating Anodic Antigen) positive subjects according to ethnic group and village. 95% LCL: 95% Lower Confidence Limit; 95% UCL: 95% Upper Confidence Limit.

Circulating Anodic Antigen concentration

The variation of the concentration of CAA (pg/ml) among positive subjects (N+=120) has been assessed according to age-group, sex, village and ethnicity.

Table 20 shows the range (minimum-maximum), the median, the mean and standard deviation of the variation of the concentration of CAA (pg/ml) according to age group (1-4, 5-9, 10-19, 20-39, 40-80 years old), sex (females vs males), village of residence (Barkoundouba vs Barkoumbilen) and ethnicity (Non-Fulani vs Fulani) as well as the result of multivariate association analysis.

As shown in Table 20, the concentration of CAA is highest in the age group 10-19 years old (Figure 33); it is comparable in females and males (Figure 34); it is comparable in the villages Barkoundouba and Barkoumbilen as well as among Fulani and Non-Fulani ethnicities (Table 21, Figure 35).

Subjects characteristics		CAA concentration (pg/ml)				Association with CAA concentration		
		Range	Median	Mean	Std Dev	Coefficient	Std Error	P-Value
Age-group (years old)	1-4 (ref)	51.17 - 2608.07	853.55	1091.59	1194.61	-	-	-
	5-9	17.95 - 9900.77	325.38	1702.79	2624.53	566.59	774.84	0.465
	10-19	10.47 - 100047.43	1006.20	4770.57	14318.94	2777.53	790.87	0.000
	20-39	10.74 - 4115.96	152.72	667.88	1293.40	-248.46	899.57	0.783
	40-80	12.55 - 1604.02	39.61	247.52	501.83	-149.49	942.28	0.874
Sex	Males (ref)	11.8 4- 13497.52	692.66	1767.21	2691.40	-	-	-
	Females	10.47 - 100047.43	256.32	3824.85	13493.91	676.741	571.28	0.237
Village	Barkoumbilen (ref)	10.47 - 100047.43	565.68	3289.85	11043.53	-	-	-
	Barkoundouba	11.84 - 5834.60	109.31	820.79	1696.26	-15.111	10.48	0.150
Ethnicity	Fulani (ref)	11.84 - 5834.60	109.31	1014.16	1872.88	-	-	-
	Non-Fulani	10.467- 100047.43	482.13	3168.16	10846.49	-288.82	1101.71	0.793

Table 20: Circulating Anodic Antigen concentration (pg/ml) according to subjects' characteristics and results of association analysis.
The table shows the Circulating Anodic Antigen (CAA) concentration (pg/ml) and the results of multivariate linear regression performed to investigate the association of age-group (1-5; 5-9; 10-19; 20-39; 40-80 years old), sex (females vs males), village (Barkoundouba vs Barkoumbilen) and ethnicity (Non-Fulani vs Fulani) with CAA concentration (pg/ml). Range (minimum-maximum); Median; Mean; Std Dev = Standard Deviation. Coefficient; Std Error = Standard Error; P-Value.

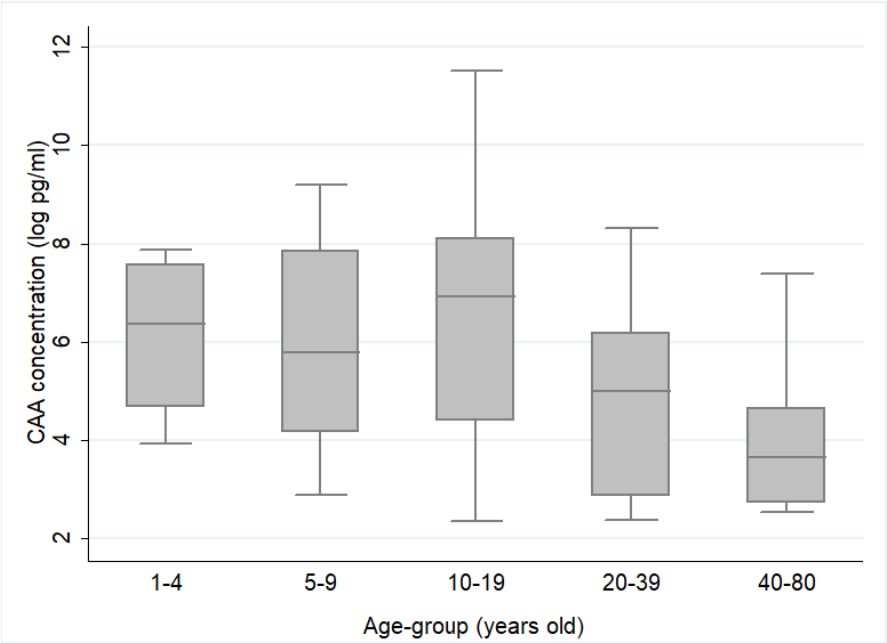

Figure 33: CAA concentration according to age-group (years old).
The figure shows a boxplot of the distribution of CAA concentration according to age-group (1-4; 5-9; 10-19; 20-39; 40-80 years old) among positive subjects. The horizontal black line indicates the 50% percentile, the grey box indicates the 25%-75% percentile, the lower whisk indicates the 5% and the upper whisk the 95% percentile respectively.

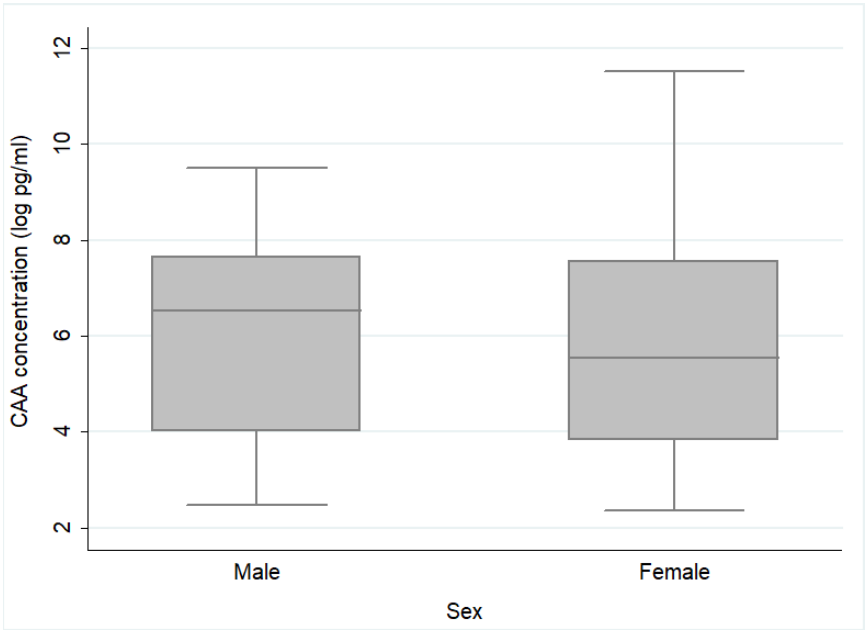

Figure 34: CAA concentration according to sex.
The figure 34 shows a boxplot of the distribution of CAA concentration according to sex (males and females) among positive subjects. The horizontal black line indicates the 50% percentile, the grey box indicates the 25%-75% percentile, the lower whisk indicates the 5% and the upper whisk the 95% percentile respectively.

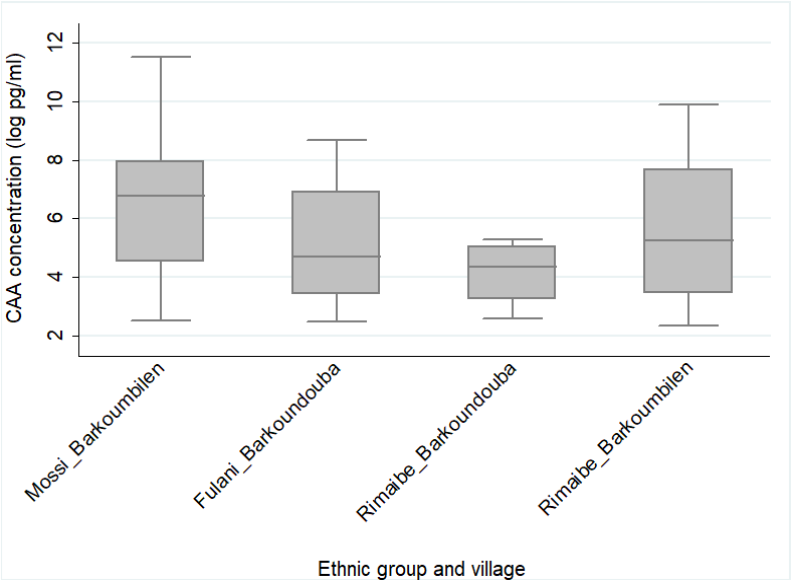

Figure 35: CAA concentration according to ethnic group and village.
The figure shows a boxplot of the distribution of CAA concentration according to ethnic group and village (Mossi_Barkoumbilen, Fulani_Barkoundouba, Rimaibe_Barkoundouba, Rimaibe_Barkoumbilen) among positive subjects. The horizontal black line indicates the 50% percentile, the grey box indicates the 25%-75% percentile, the lower whisk indicates the 5% and the upper whisk the 95% percentile respectively.

Village	Ethnic group	CAA concentration (pg/ml)			
		Range	Median	Mean	Std Dev
Barkoumbilen	Mossi	12.55-100047.43	889.66	4343.96	13995.26
	Rimaibe	10.47-19463.48	188.10	1747.24	3448.01
Barkoundouba	Fulani	11.84-5834.60	109.31	1014.16	1872.88
	Rimaibe	13.53-196.14	86.50	95.67	81.71

Table 21: CAA concentration according to ethnic group and village.
The table shows the CAA concentration (pg/ml) according to ethnic group and village. Range (minimum-maximum); Median; Mean; Std Dev = Standard Deviation.

4.1.1.3. *S. haematobium* circulating DNA

Proportion of subjects positive to S.haematobium circulating DNA

The overall proportion of subjects positive for *S. haematobium* cDNA among the study population is 26% (Table 22). Only two subjects are positive for *S. mansoni* cDNA (0.5%), one of which is also positive to *S.haematobium* cDNA.

A descriptive analysis has been performed to assess the variation in the proportion of subjects positive to *S. haematobium* circulating DNA (cDNA) in plasma according to age, sex, village and ethnicity.

Table 22 shows the proportion of cDNA positive subjects (cDNA positivity) according to age-group (1-4 , 5-9, 10-19, 20-39, 40-80 years old), sex (females vs males), village of residence (Barkoundouba vs Barkoumbilen) and ethnicity (Fulani vs Non-Fulani) as well as the results of multivariate association analysis.

As shown in Table 22, cDNA positivity is lowest in children aged 1 to 4 years, it increases in children aged 5 to 9 years, is highest in children aged 10 to 19 years and decreases in subjects older than 20 years (Figure 36); it is lower in females than males (Figure 37); it is comparable between the two villages Barkoumbilen and Barkoundouba as well as between Fulani than Non-Fulani ethnicities; the comparison of the proportion of cDNA positivity between ethnic groups within each village as well as between the same ethnic group living in different villages) indicate that there are no differences between Mossi and Rimaibe living in Barkoumbilen, while there are no cDNA positive subjects among Rimaibe living in Barkoundouba, possibly due to a limited sample size (N=28), making it challenging to draw conclusions about the comparison with Fulani living in the same village as well as with Rimaibe living in Barkoumbilen (Figure 38, Table 23).

Subjects characteristics		Total population	cDNA positive subjects				Association with cDNA positivity		
		N	N+	Proportion %	95% LCL	95% UCL	Odds Ratio	95% CI LCL	95% CI UCL	P-Value
Age-group (years old)	1-4 (ref)	85	4	4.7	1.7	12.1	-	-	-	-
	5-9	95	37	38.9	29.5	49.3	21.2	6.8	65.8	0.000
	10-19	92	54	58.7	48.2	68.4	55.6	17.5	176.8	0.000
	20-39	72	5	6.9	2.9	15.9	2.4	0.6	9.7	0.233
	40-80	52	3	5.8	1.8	17.0	1.6	0.3	7.6	0.568
Sex	Male (ref)	159	56	35.2	28.1	43.0	-	-	-	-
	Female	237	47	19.8	15.2	25.4	0.44	0.24	0.81	0.008
Village	Barkoumbilen (ref)	253	81	32.0	26.5	38.1	-	-	-	-
	Barkoundouba	143	22	15.4	10.3	22.4	0.00	0.00	.	0.985
Ethnicity	Fulani (ref)	115	22	19.1	12.9	27.5	-	-	-	-
	Non-Fulani	281	81	28.8	23.8	34.4	0.84	0.00	6155222.00	0.983
Total		396	103	26.0	21.9	30.6	-	-	-	-

Table 22: Proportion of *S. haematobium* cDNA positive subjects and association of subject's characteristics with cDNA positivity.
This table shows the number of total population (N), the number (N+) and the proportion (%) of cDNA positive subjects and the results of multivariate logistic regression analysis (Odds Ratio and P-value) performed to investigate the association of age-group (1-4, 5-9, 10-19, 20-39, 40-80 years old), sex (females vs males), village (Barkoundouba vs Barkoumbilen) and ethnicity (Non-Fulani vs Fulani) with anti-SWAP IgM positivity. 95% LCL: 95% Lower Confidence Limit; 95% UCL: 95% Upper Confidence Limit.

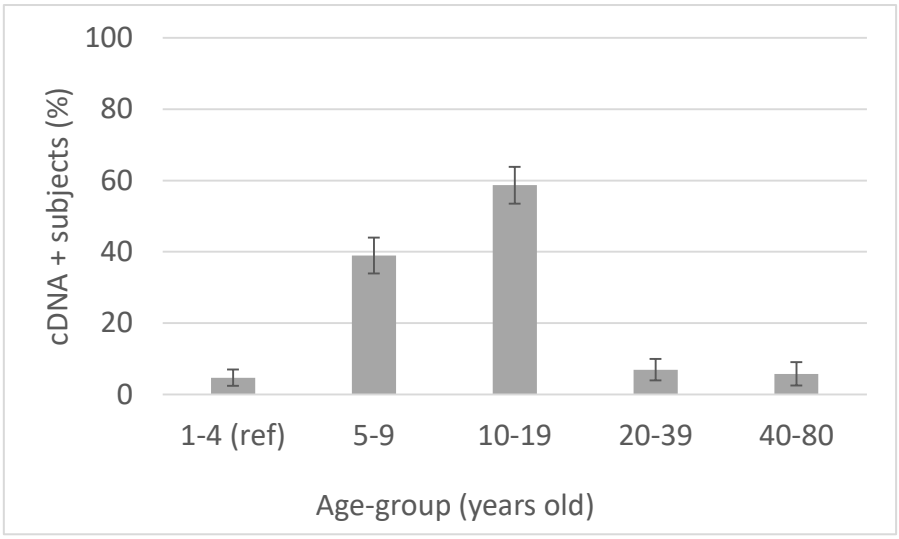

Figure 36: Proportion (%) of cDNA positive subjects according to age-group (years old).
This figure shows the proportion (%) of cDNA positive subjects according to age-group (1-4, 5-9, 10-19, 20-39, 40-80 years old). Error bars indicate the 95% confidence interval of the proportion.

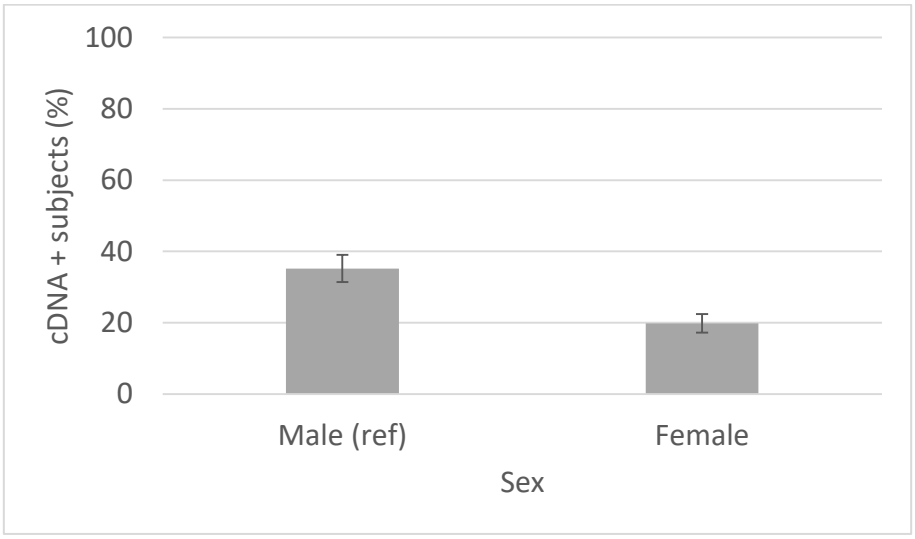

Figure 37: Proportion (%) of cDNA positive subjects according to sex.
This figure shows the proportion (%) of cDNA positive subjects according to sex (male and female). Error bars indicate the 95% Confidence Interval of the proportion.

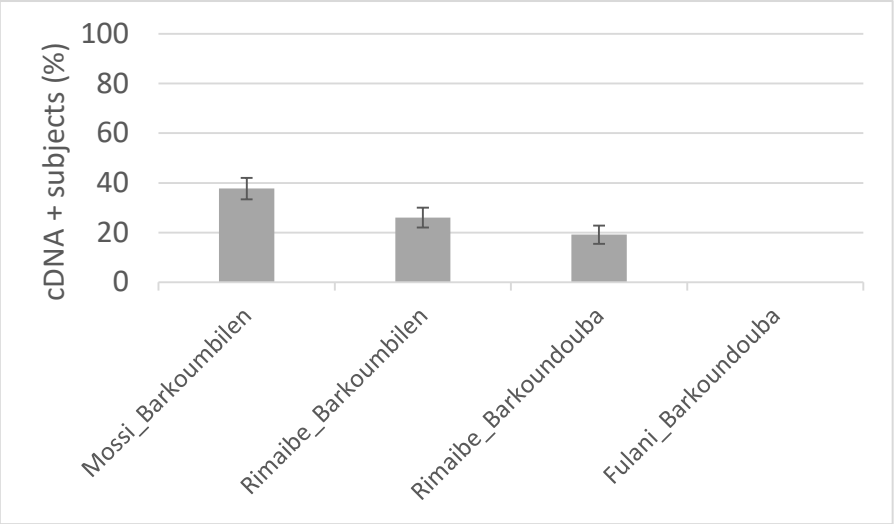

Figure 38: Proportion (%) of cDNA positive subjects according to ethnic group and village.
This figure shows the proportion (%) of cDNA positive subjects according to ethnic group and village (Mossi_Barkoumbilen, Rimaibe_Barkoumbilen, Rimaibe_Barkoundouba, Fulani_Barkoundouba). Bars indicate the standard error of the proportion.

Village	Ethnic group	Total population	cDNA positive subjects			
		N	N+	Proportion %	95% LCL	95% UCL
Barkoumbilen	Mossi	130	49	37.7	29.7	46.4
	Rimaibe	123	32	26.0	18.9	34.6
Barkoundouba	Fulani	115	22	19.1	12.9	27.5
	Rimaibe	28	0	0.0	-	-
Total		396	103	26.0	21.9	30.6

Table 23: Proportion of cDNA positive subjects according to village and ethnic group.
This table shows the number of total population (N), the number (N+) and the proportion (%) of cDNA positive subjects according to ethnic group and village. 95% LCL: 95% Lower Confidence Limit; 95% UCL: 95% Upper Confidence Limit.

4.1.2. Diagnostic agreement between markers of *S. haematobium* infection

The second objective of this thesis is to assess the agreement between measurements of different plasma biomarkers in indicating whether a subject is infected with *S. haematobium* or not. In order to do that, we have chosen the measurement of the Circulating Anodic Antigen (CAA) as the gold standard, i.e. the best available test, as it is a validated diagnostic marker (Corstjens et al., 2008, 2014), and used it as a reference for evaluating the diagnostic value of measuring *S. haematobium* circulating DNA (cDNA) as well specific antibodies against *S. haematobium* antigens (anti-SWAP IgM, anti-SWAP IgG, anti-SEA IgM and anti-SEA IgG).

4.1.2.1. Evaluation of *S. haematobium* circulating DNA.

The overall agreement between results (positive/negative) of cDNA and CAA testing was evaluated, as well as the sensitivity and specificity of cDNA measurement compared to the reference CAA one.

Table 24 shows the comparison of results of the two biomarker measurements.

The overall agreement between the two tests, i.e. the proportion of samples that show the same result (concordant samples), which is calculated as the sum of the true positives and the true negatives divided by the total number of samples, was 85%.

The sensitivity of cDNA compared to CAA, i.e. the proportion of true positives identified by cDNA testing, was 70%. The specificity of cDNA compared to CAA, i.e. the proportion of true negatives identified by cDNA testing, was 90%.

CAA	cDNA		
	N-	N+	Total
N-	257[a]	27[b]	284
N+	32[c]	76[d]	108
Total	289	103	392

Table 24: Comparison of results of cDNA and CAA tests.
The table shows results of cDNA testing stratified by results of CAA reference testing. N+: number of positive samples. N-: number of negative samples. a: true negatives. b: false positives. c: false negatives. d: true positives.

The sensitivity of cDNA testing is unsatisfying, as a 70% sensitivity means a 30% probability of false negative results, and underlying causes are worthy of further investigation. Whether cDNA sensitivity varies according to CAA level was verified as shown in Table 25. It can be observed that the highest proportion of false negative cDNA results is for samples with CAA concentration in the lowest 10-99 pg/ml range (level1), such proportion decreases for samples with CAA concentration in the 100-999 pg/ml range (level 2) and 1000-9999 pg/ml range (level 3) and reaches zero for samples with CAA concentration in the 10000-100000 pg/ml range (level 4). It is possible to hypothesize that such limit in sensitivity could be overcome by employing a larger volume of plasma for DNA isolation.

CAA level	cDNA			
	N-	N+	Total	% False negatives
0	257	27	284	-
1	18	21	39	46%
2	8	20	28	29%
3	6	30	36	17%
4	0	5	5	0%

Table 25: Results of cDNA testing according to CAA level.
The table shows results of cDNA testing stratified by CAA concentration ranges. N+: number of positive samples. N-: number of negative samples. % False negatives: percentage of false negatives in the stratum with respect to the total number of false negatives.

Regarding the 90% specificity of cDNA testing, a closer examination of the 10% 27 false positives results led to the observation that 16 of them had actually a CAA concentration of 0 pg/mL, while the remaining 11 had a CAA concentration of 0.3-9.4 pg/mL. The cutoff concentration used for CAA positivity was indeed 10 pg/mL. Considering the sequence-specificity of Real Time PCR primers and probes used for *S. haematobium* cDNA amplification, this observation suggest lowering the cutoff for CAA positivity to any measurable concentration (>0 pg/mL); with such cutoff for CAA positivity, the sensitivity and specificity of cDNA would increase to 73% and 94% respectively.

A possibility that could be envisaged on the basis of the present data, and taking into consideration that both tests involve measurement of direct parasite biomarkers, is the use of a

composite reference standard where a sample is considered positive when either cDNA and/or CAA can be detected.

4.1.2.2. Evaluation of *S. haematobium*-specific antibodies

To evaluate the potential role of *S. haematobium*-specific antibodies as indirect diagnostic biomarkers, we first investigated whether the distribution of antibody levels varies according to CAA positivity. Figure 39 and 40 show histograms and boxplots, respectively, of antibody levels (logarithm of normalised OD, log nOD) among CAA negative (CAA-) and CAA positive (CAA+) subjects, while Table 26 shows the results of non-parametric Kruskal-Wallis equality-of-populations rank test.

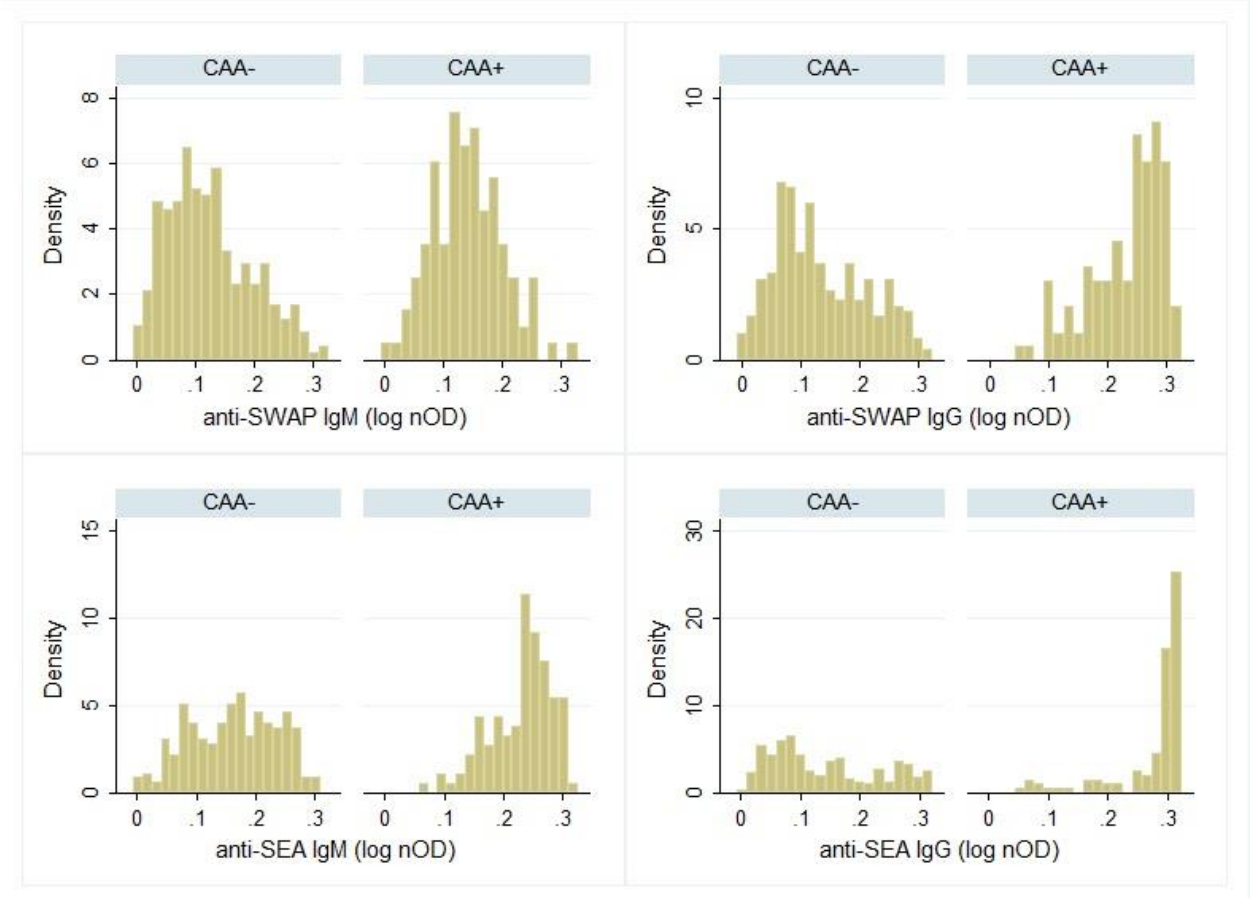

Figure 39: Histograms of *S.haematobium*-specific antibody levels among CAA- and CAA+ subjects.
This figure shows histograms of anti-SWAP IgM, anti-SWAP IgG, anti-SEA IgM and anti-SEA IgG levels (log nOD) among CAA negative (CAA-) and CAA positive (CAA+) subjects.

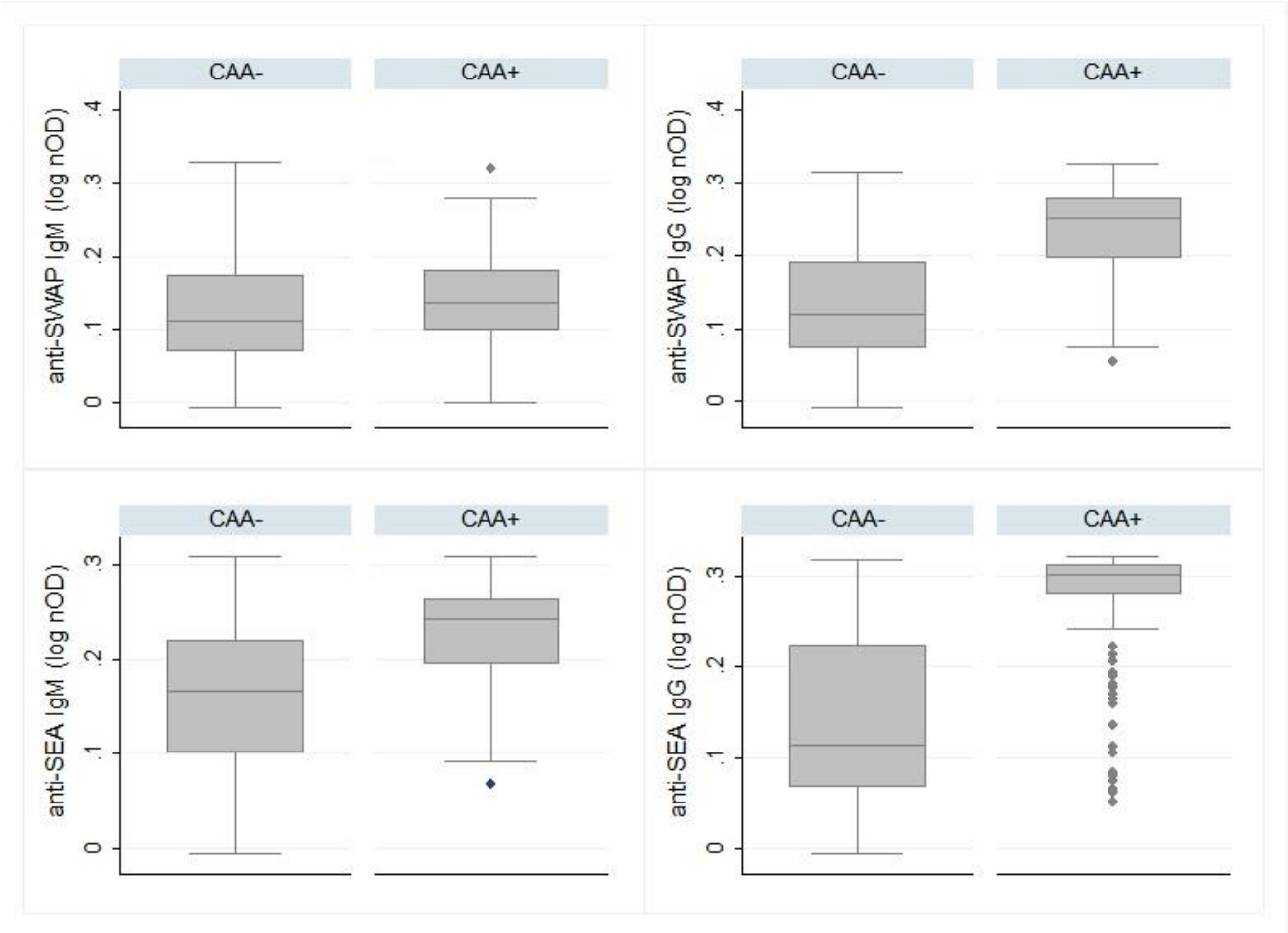

Figure 40: Boxplots of *S. haematobium*-specific antibody levels among CAA- and CAA+ subjects.
This figure shows boxplots indicating the distribution of anti-SWAP IgM, anti-SWAP IgG, anti-SEA IgM and anti-SEA IgG levels (log nOD) among CAA- and CAA+ subjects. The horizontal black line indicates the 50% percentile, the grey box indicates the 25%-75% percentile, the lower whisk indicates the 5% and the upper whisk the 95% percentile respectively, the dots indicate individual's data that lie out of the distribution (outliers).

	Distribution according to CAA Positivity			
	anti- SWAP		anti-SEA	
	IgM	IgG	IgM	IgG
Chi-square	7.42	108.32	70.47	144.87
P-value	0.006	0	0	0

Table 26 : Results of non-parametric Kruskal-Wallis equality-of-populations rank test.
This table shows the results (Chi-square; P-value) of non-parametric Kruskal-Wallis equality-of-populations rank test performed to compare the distribution of anti-SWAP IgM, anti-SWAP IgG, anti-SEA IgM and anti-SEA IgG levels (log nOD) according to CAA positivity.

The observation of histograms and boxplots indicate that *S. haematobium* specific antibody levels show a different distribution among CAA+ and CAA+ subjects, with higher values among positive subjects. This observation is confirmed by the results of of Kruskal-Wallis equality-of-populations rank test. The highest Chi-square value, indicating strongest association with CAA positivity, is observed for anti-SEA IgG level followed by anti-SWAP IgG level and anti-SEA IgM level, while he lowest Chi-square value is observed for anti-SWAP IgM level.

We then investigated whether the distribution of antibody levels varies according to CAA concentration ranges, or CAA levels. Figure 41 shows boxplots of antibody levels among subjects showing different ranges of CAA concentration (pg/ml): negative (0-10 pg/ml; level 0), low positive (10-100 pg/ml; level 1), positive (100-1000 pg/ml; level 2), high positive (1000-10000 pg/ml; level 3), very high positive (>10000 pg/ml; level 4). Table 27 shows the results of non-parametric Kruskal-Wallis equality-of-populations rank test.

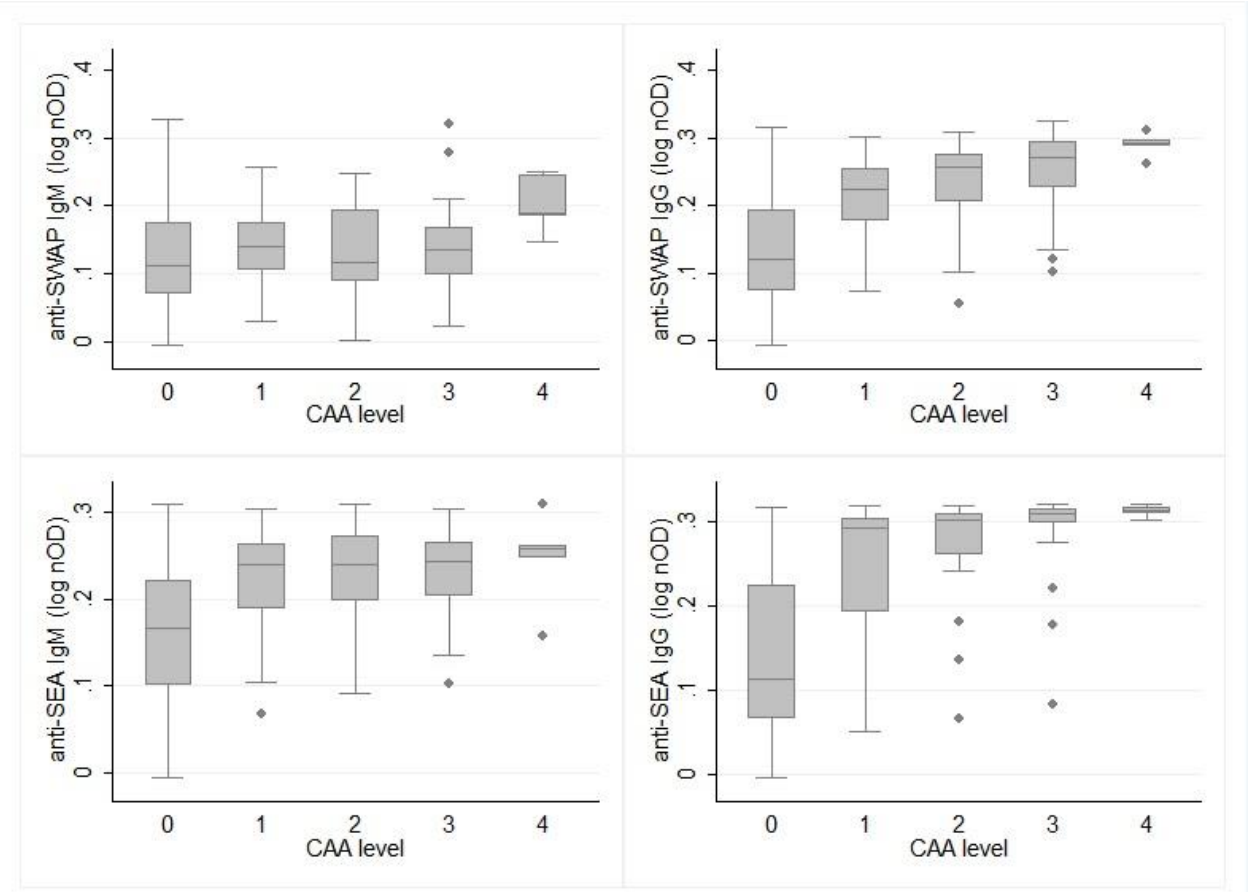

Figure 41: Boxplots of *S.haematobium*-specific antibody levels according to CAA level.
This figure shows boxplots indicating the distribution of anti-SWAP IgM, anti-SWAP IgG, anti-SEA IgM and anti-SEA IgG levels (log nOD) according to CAA level. The horizontal black line indicates the 50% percentile, the grey box indicates the 25%-75% percentile, the lower whisk indicates the 5% and the upper whisk the 95% percentile respectively, the dots indicate individual's data that lie out of the distribution (outliers).

	Distribution according to CAA level			
	anti- SWAP		anti-SEA	
	IgM	IgG	IgM	IgG
Chi-square	12.51	115.18	71.37	153.42
P-value	0.014	0.000	0.000	0.000

Table 27: Results of non-parametric Kruskal-Wallis equality-of-populations rank test.
This table shows the results (Chi-square; P-value) of a non-parametric Kruskal-Wallis equality-of-populations rank test performed to compare the distribution of anti-SWAP IgM, anti-SWAP IgG, anti-SEA IgM and anti-SEA IgG levels (log nOD) according to CAA level.

The observation of boxplots indicates that, for all *S. haematobium*-specific antibodies, the levels increase with the CAA concentration range, although this is particularly evident for IgG antibodies. Indeed, the results of Kruskal-Wallis equality-of-populations rank test show the highest Chi-square values for anti-SEA IgG levels followed by anti-SWAP IgG levels and anti-SEA IgM levels, while the lowest Chi-square value is observed for anti-SWAP IgM levels.

Furthermore, we investigated the correlation between antibody levels (log nOD) and CAA concentration (pg/ml) among CAA positive subjects. Figure 42 shows scatter plots while Table 28 show the results of Spearman correlation analysis.

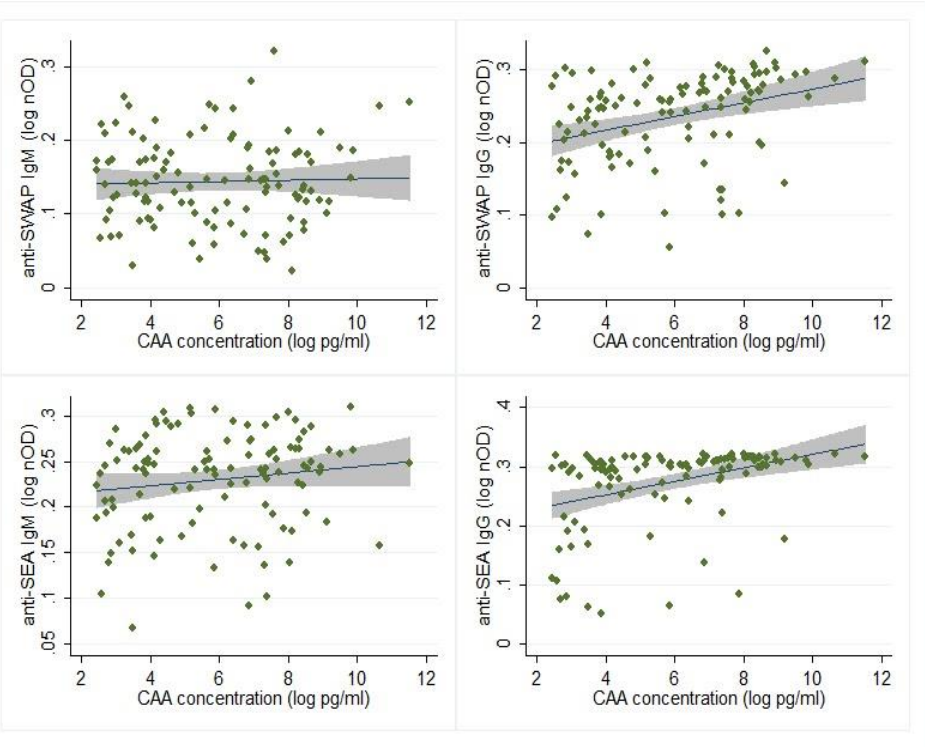

Figure 42: Correlation between *S.haematobium*-specific antibody levels and CAA concentration: scatter plots.
This figure shows scatter plots describing the linear correlation between *S.haematobium*-specific antibody levels (log nOD) and CAA concentration (log pg/ml). The points in green represent the data, the blue line indicates the best linear curve fitted to the data, the grey area indicates the 95% confidence interval of fitted linear curve.

	anti-SWAP		anti-SEA	
	IgM	**IgG**	**IgM**	**IgG**
Concentration CAA	rho= 0.01; p= 0.938	rho= 0.40; p= 0.000	rho= 0.17; p= 0.076	rho= 0.46; p= 0.000

Table 28: Correlation between *S.haematobium*-specific antibody levels and CAA concentration: results of Spearman analysis.
This table shows the results of Spearman analysis (rho: correlation coefficient, p: p-value).

The observation of scatter plots as well as of the results of Spearman correlation analysis indicates that a positive correlation exist between CAA concentration and *S.haematobium*-specific IgG levels, while such correlation does not exist with IgM levels. The highest rho value, indicating the strongest correlation, is observed for anti-SEA IgG. These results are in line with what previously observed about the association between CAA concentration ranges and CAA positivity with antibody levels.

Finally, Receiver Operating Characteristic (ROC) analysis was employed to formally quantify the accuracy of *S.haematobium*-specific antibody tests to discriminate between CAA- and CAA+ subjects, i.e. to diagnose CAA positivity or *S. haematobium* infection. This analysis uses the ROC curve, a graph of the specificity versus sensitivity of the antibody test (Figure 43). The ROC curve was generated with no assumptions about the distribution of antibody levels (non-parametric ROC curve) among CAA- and CAA+ subjects. The global performance of a given antibody test is summarised by the area under the ROC curve (AUC), that can be interpreted as the probability that the result value (log nOD) of the antibody test of a randomly selected CAA positive subject will be greater than that of a randomly selected CAA negative subject. The greater the AUC, the better the global diagnostic performance of the antibody test.

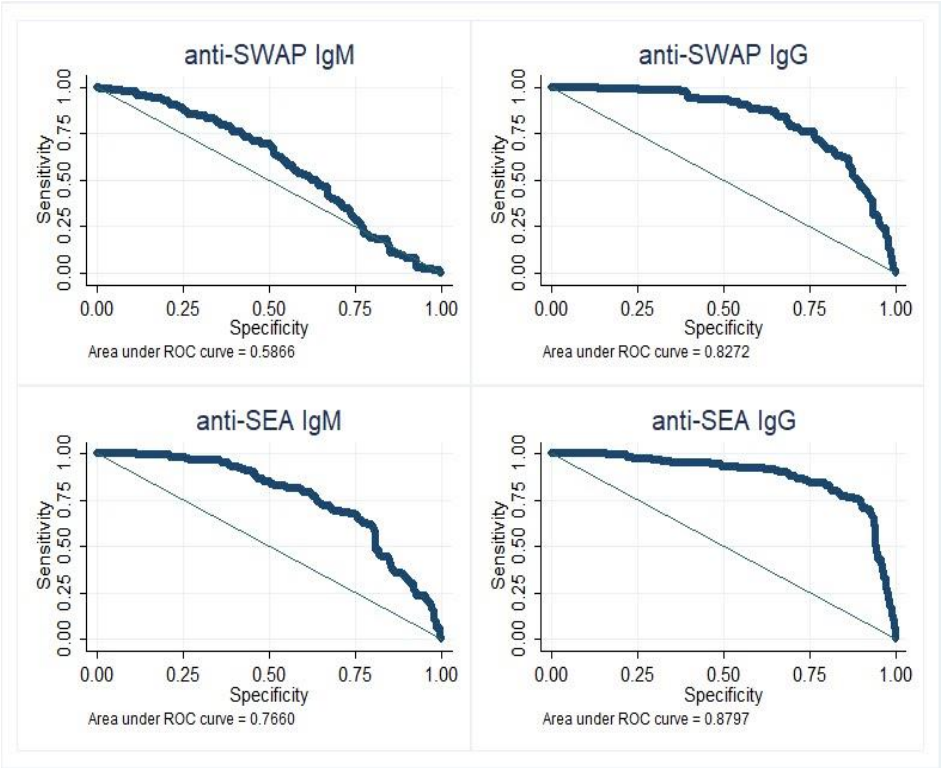

Figure 43: ROC curves of *S.haematobium*-specific antibody tests for the diagnosis of CAA positivity.
The figure shows the ROC curves (specificity vs sensitivity) as well as AUC values for each of the *S. haematobium*-specific antibody tests.

It can be observed that for both SWAP and SEA antigens, specific IgG have a better diagnostic performance than IgM. As anticipated on the basis of previous analyses, the best diagnostic performance (highest AUC value) is shown by anti-SEA IgG.

In ROC analysis, specificity and sensitivity and the overall proportion of correctly diagnosed subjects are estimated for each value (log nOD) of the antibody test used as a cut-off to discriminate CAA- and CAA+ subjects. In general, it is therefore possible to choose the cut-off value depending on what diagnostic feature is preferred.

Since anti-SEA IgG have shown the best diagnostic performance, specificity and sensitivity of an anti-SEA IgG test according to different cut-off values (log nOD) have been estimated and are shown in Figure 44.

The best cut-off was chosen as the value representing the best compromise between different diagnostic features (specificity, sensitivity and overall accuracy being all higher than >80%). The cutoff value of 0.243log nOD leads to 82% specificity, 82% sensitivity and 82% overall accuracy.

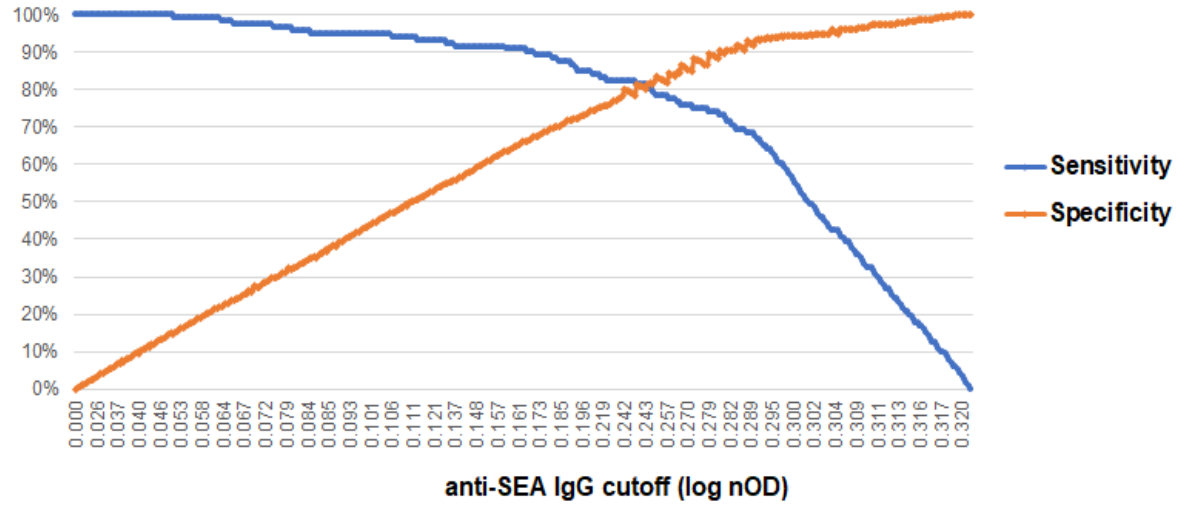

Figure 44: Specificity and sensitivity of anti-SEA IgG antibody test according to different cut-off values (log nOD). This figure shows specificity and sensitivity for all possible cut-off values of anti-SEA IgG (log nOD).

4.1.3. Impact of *Schistosoma haematobium* infection on the prospective risk of *Plasmodium falciparum* parasitaemia.

One objective of the present thesis is to assess whether being infected with *Schistosoma haematobium* affects the prospective risk of *Plasmodium falciparum* parasitaemia among study populations.

In order to do so a repeated-cross sectional design has been employed, which had been previously shown successful in demonstrating the protective effect of haemoglobin S and C (Mangano et al., 2015). Among subjects who participated to five cross-sectional malariological surveys, we measured *S. haematobium* Circulating Anodic Antigen (CAA) in plasma samples collected during the first survey. The association between either the presence (+/-) or the level (i.e. concentration range) of CAA at baseline and the probability of developing *P. falciparum* parasitaemia (yes/no), the total number of infections (0-5), and the mean parasite density (logarithmic transformation) over time has been assessed by logistic, Poisson and linear regression respectively. All regression models were adjusted for age, sex, village, ethnicity and haemoglobin genotype.

The results of association analysis (Tables 29) show, as expected, that age, ethnicity and haemoglobin genotype affect the risk of *P. falciparum* parasitaemia.

Being positive to CAA and higher CAA level increases the odds of getting infected with *P. falciparum* at least once during the study period, but the association does not reach statistical significance.

On the other hand, both CAA positivity and higher CAA level significantly increase the incidence rate of *P. falciparum* over time by about 20% ($p<0.005$).

Finally, CAA positivity and higher CAA level increase *P. falciparum* mean parasite density over five measurements (statistically significant association for CAA level only).

Taken together these observations suggest that infection with *S. haematobium*, or higher infection intensity with this trematode, increases susceptibility to *P. falciparum* malaria independently of age, ethnicity and haemoglobin genotype.

Regression model	Independent variable	*P. falciparum* infection (yes/no)				Number of *P. falciparum* infections (0-5)				Mean *P. falciparum* parasite density (log)			
		OR	LCL	UCL	P-value	IRR	LCL	UCL	P-value	OR	LCL	UCL	P-value
Model 1 (CAA positivity)	Age-group	0.32	0.25	0.4	0.00	0.75	0.71	0.79	0.00	0.70	0.67	0.74	0.00
	Sex	0.71	0.37	1.4	0.31	0.90	0.78	1.03	0.11	0.84	0.73	0.96	0.01
	Ethnicity	17.48	3.74	81.8	0.00	2.00	1.54	2.61	0.00	2.37	1.81	3.11	0.00
	Village	1.01	1.00	1.0	0.17	1.00	1.00	1.01	0.02	1.00	1.00	1.01	0.01
	HBB genotype	0.53	0.36	0.8	0.00	0.92	0.83	1.02	0.11	0.90	0.82	0.99	0.04
	CAA positivity (+/-)	1.79	0.89	3.6	0.10	1.26	1.08	1.46	0.00	1.12	0.96	1.31	0.13
Model 2 (CAA level)	Age-group	0.33	0.25	0.4	0.00	0.75	0.71	0.79	0.00	0.70	0.67	0.74	0.00
	Sex	0.70	0.36	1.4	0.29	0.89	0.78	1.02	0.10	0.84	0.73	0.96	0.01
	Ethnicity	18.14	3.88	84.9	0.00	2.01	1.54	2.61	0.00	2.37	1.81	3.11	0.00
	Village	1.01	1.00	1.0	0.16	1.00	1.00	1.01	0.02	1.00	1.00	1.01	0.01
	HBB genotype	0.53	0.35	0.8	0.00	0.91	0.82	1.02	0.09	0.90	0.82	0.99	0.03
	CAA level	1.28	0.91	1.8	0.16	1.12	1.05	1.19	0.00	1.08	1.01	1.15	0.03

Table 29: Results of association with susceptibility to *P. falciparum* infection.
The table shows the results of multivariate association analysis between *S. haematobium* CAA positivity (model 1) or CAA level (model 2), and *P. falciparum* infection, number of *P. falciparum* infections, and mean *P. falciparum* parasite density. OR: Odds Ratio. IRR: Incidence Rate Ratio. LCL: 95% Lower Confidence Limit. UCL: 95% Upper Confidence Limit

4.2. Experimental study *in vitro*

The immunomodulatory properties of *S. haematobium* antigens (TSP2, TSP6 and TSP23) have been characterized through their ability to: i) induce Dendritic Cells (DCs) maturation and cytokines production; ii) induce T helper cells differentiation and cytokines production.

4.2.1. DCs maturation upon stimulation with tetraspanins TSP2, TSP6, TSP23

4.2.1.1. Expression of maturation markers

DCs obtained from different donors were aliquoted and they were either stimulated with tetraspanins (TSP2, TSP6, TSP23) or LPS (positive control), or non-stimulated (negative control). The expression of DCs maturation markers HLA-DR, CD80, CD83 and CD86 was measured by Flow Cytometry (Mean Fluorescence Intensity, MFI) in three donors (biological replicas). Difference in the distribution of MFI between stimulated and unstimulated DCs was assessed using Kruskal-Wallis non-parametric rank test. The fold-change in MFI of stimulated DCs compared to unstimulated DCs was computed for each donor and is shown in Figure 45.

The three tetraspanins were able to induce the expression of maturation markers HLA-DR, CD80 and CD86. Except for TSP23, they were also able to induce the expression of maturation marker CD83. In particular, TSP2 was able to induce a greater increase in expression, in a comparable way to LPS. Increase in expression by TSP2 was statistically significant for maturation markers CD80 and CD83.

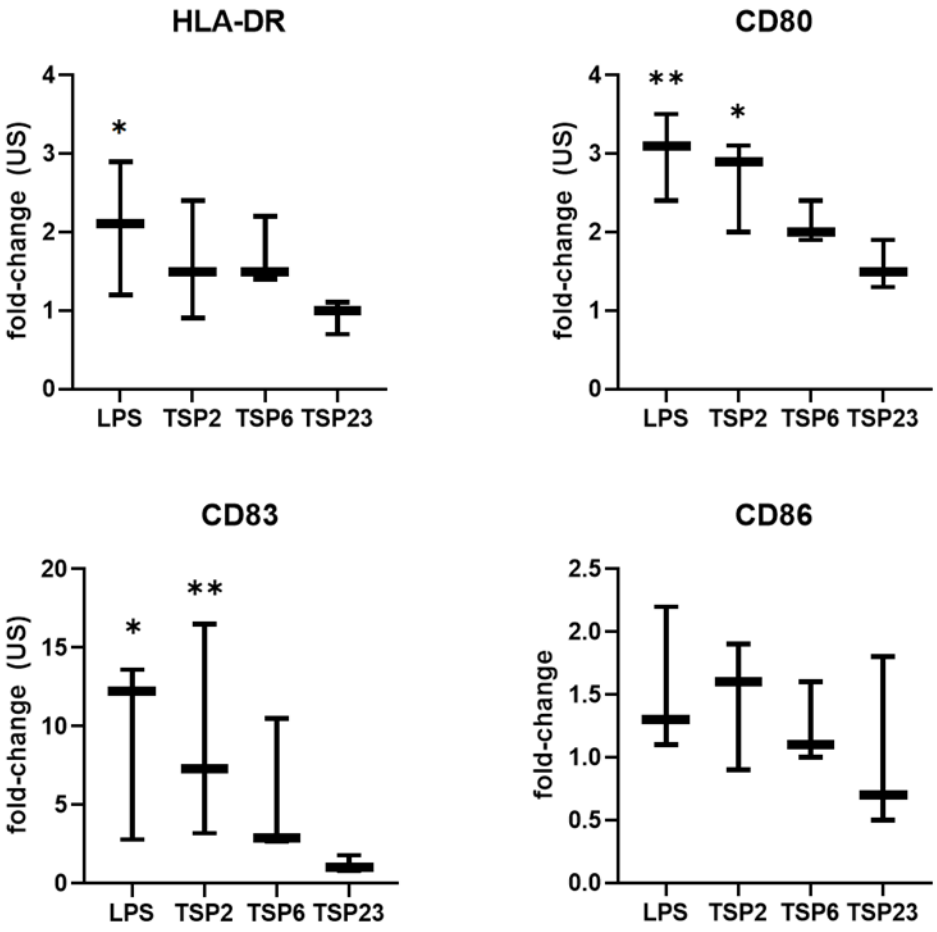

Figure 45: DC maturation markers**.**
The figure shows boxplots (median and 5-95% range) of the fold-change in MFI of stimulated DCs (LPS, TSP2, TSP6, TSP23) compared to unstimulated DCs (US). Asterisks indicated statistical significance of Kruskal Wallis rank test (* p-value≤0.05, ** p-value≤0.01, *** p-value≤0.001).

4.2.1.2. DCs cytokines production.

The concentration of cytokines produced by stimulated and unstimulated DCs was measured in supernatants by Luminex Assay Thelper17 kit (pg/ml) in five donors (biological replicas). Difference in the distribution of cytokine concentrations between stimulated and unstimulated DCs was assessed using Kruskal-Wallis non-parametric rank test. The fold-change in concentration of stimulated DCs supernatant compared to unstimulated DCs supernatant was computed for each donor and is shown in Figure 46.

The three tetraspanins were able to induce the production of inflammatory cytokines IL6, TNFalpha, IL12p70 and IL33, as well as of the Th2 cytokine IL13 and the regulatory cytokine IL10. In particular, TSP2 was able to induce a greater increase in cytokine production, in a comparable way to LPS. Increase in cytokine production by TSP2 was statistically significant for IL6, TNFalpha, IL12p70, IL13 and IL10. Such results deserve confirmation in a larger number of donors using single-cytokine ELISA.

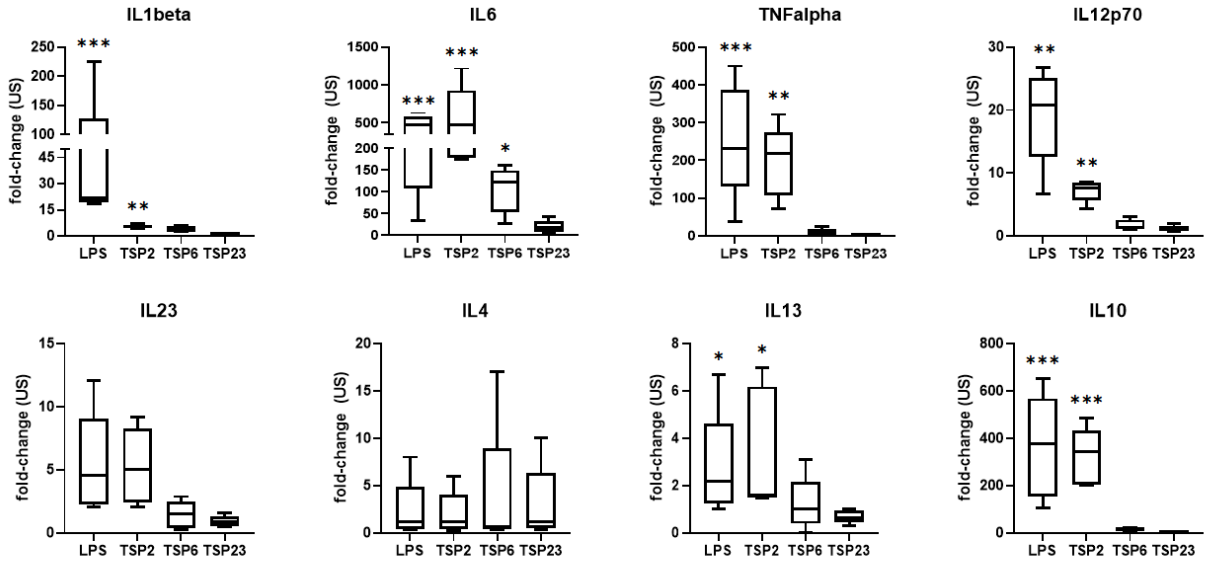

Figure 46 : **DC cytokine production.**
The figure shows boxplots (median and 5-95% range) of the fold-change in concentration of cytokines present in stimulated DCs supernatant (LPS, TSP2, TSP6, TSP23) compared to those present in unstimulated DCs supernatant (US). Asterisks indicated statistical significance of Kruskal Wallis rank test (* p-value≤0.05, ** p-value≤0.01, *** p-value≤0.001).

4.2.2. T helper differentiation upon stimulation with tetraspanins TSP2, TSP6, TSP23.

4.2.2.1. T CD4+ cells cytokine expression.

The cytokine gene expression of pre-activated T CD4+ cells was measured by Retro Transcriptase quantitative Real Time PCR (RTqPCR) using the 2^-ΔCt method and the 18S rRNA as reference gene in three different donors (biological replicas). Difference in the distribution of transcript levels (relative amounts) between T cells co-cultured with tetraspanin-stimulated DC supernatants (TSP2, TSP6, TSP23) and unstimulated T cells (US) was assessed using Kruskal-Wallis non-parametric rank test. The fold-change in transcript relative amount of stimulated T cells compared to unstimulated T cells (US) was computed for each donor and is shown in Figure 47.

The supernatants of tetraspanin-stimulated DCs were able to induce changes in cytokine gene expression in T CD4+ cells, with different pattern depending on the tetraspanin. TSP2 was able to induce an increase in Th1 cytokine IFNgamma and regulatory cytokine IL10 gene expression (significant differences). TSP6 was able to induce an increase in gene expression of Th2 cytokines IL4, IL5 and IL13 (significant differences for IL4 and IL13). To a smaller extent also TSP23 was able to induce an increase in gene expression of Th2 cytokines IL4, IL5 and IL13, as well as of the regulatory cytokine IL10 (non-significant differences, p-value <0.15). Such interesting results deserve confirmation in larger number of donors.

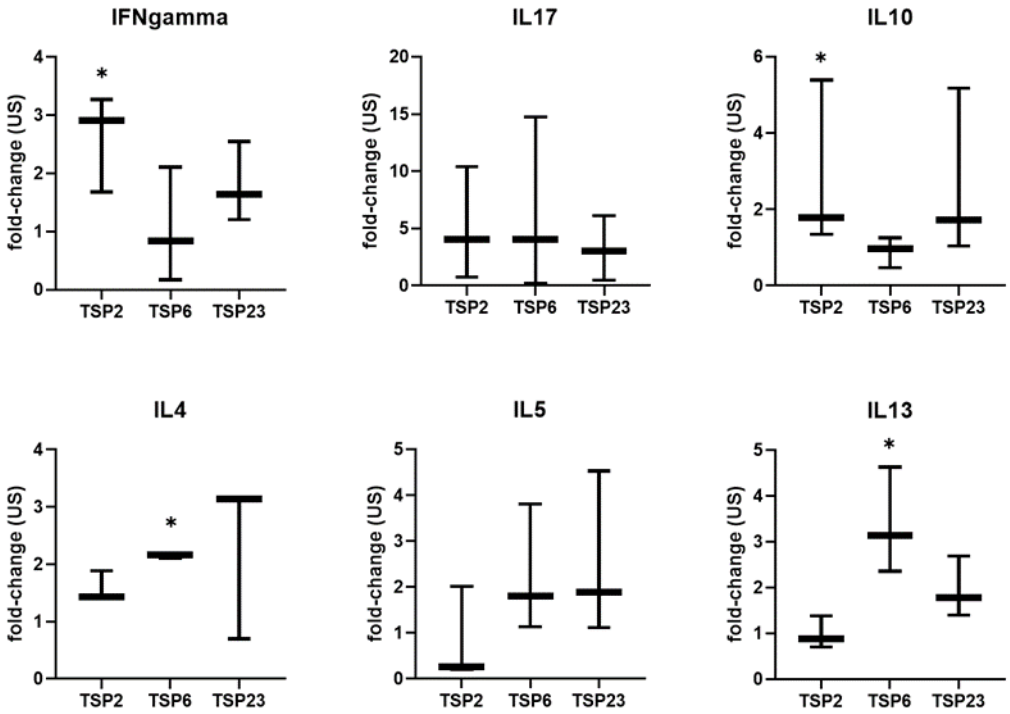

Figure 47 : T CD4+ cells cytokine gene expression.
The figure shows boxplots (median and 5-95% range) of the fold-change in transcript relative amounts of T CD4+ cells co-cultured with tetraspanin-stimulated DCs supernatant (TSP2, TSP6, TSP23) compared to unstimulated T CD4+ cells (US). Asterisks indicated statistical significance of Kruskal Wallis rank test (* p-value≤0.05, ** p-value≤0.01, *** p-value≤0.001).

4.3. Experimental study *in vivo*

This study was carried out to assess the impact of exposure to known *S.haematobium* immunomodulatory antigens (HIPSE) on immunity to malaria in murine model. Two experiments were conducted in CD1 mice where a treatment group was injected with HIPSE and a control group injected with PBS; both groups were then infected with *P.berghei* parasites expressing green fluorescent protein and the growth of parasites density was monitored by flow cytometry.

Table 30 and Table 31 show the results of the first and second experiment respectively.

Treatment group	**Mice**	**% iRBC**			
		Day 3 p.i.	**Day 5 p.i.**	**Day 7 p.i.**	**Day 10 p.i.**
HIPSE	T1	0.3	7.6	X	X
	T2	0	0.1	2.6	X
	T3	0	1.2	5.7	X
	T4	0	2.8	4.1	X
	T5	0	0.3	4.9	4.3
PBS (control)	C1	0.3	10.3	35.2	X
	C2	0.2	7.2	26	X
	C3	0.1	4.7	9.7	X
	C4	0	0.1	3.2	X
	C5	0.4	10.5	X	X

Table 30: Results of experiment 1: distribution of *P.berghei* density over time.
This table shows the variation of *P.berghei* density (%iRBC) monitored after the infection at different time point during the experiment 1 in the experimental group (HIPSE) and the control group (PBS).

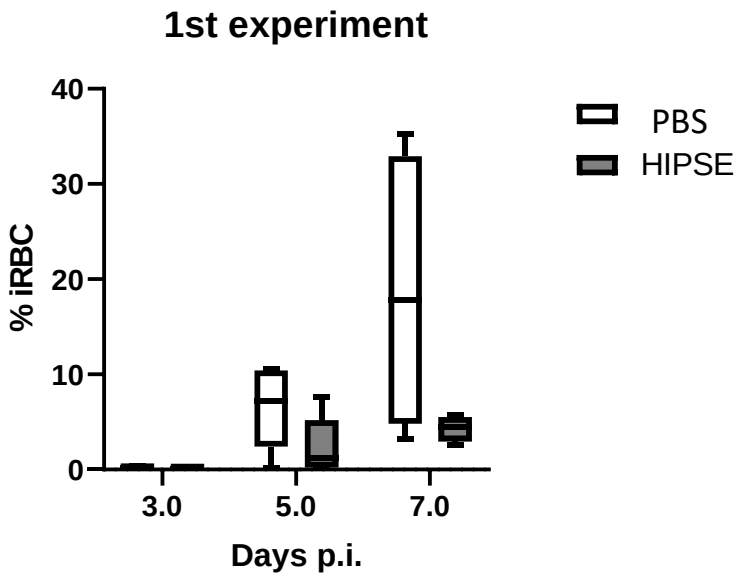

Figure 48: Boxplots of *P.berghei* parasitaemia (%iRBC) in HIPSE and PBS groups over time.
This figure shows boxplots indicating the distribution of *P.berghei* parasitaemia (%iRBC) at days 3, 5 and 7 post infection (days p.i). The horizontal black line indicates the 50% percentile, the blank box (PBS) and the grey box (HIPSE) indicates the 25%-75% percentile, the lower whisk indicates the 5% and the upper whisk the 95% percentile respectively.

Results of experiment 1 showed lower parasite density at all time points and slower growth of parasite density in mice injected with HIPSE compared to PBS, but differences did not reach statistical significance (mixed-effects restricted maximum likelihood model for repeated measures and missing data, p-value=0.0517). Mice started to die on day 7 p.i. in both groups.

Experiment 2 was designed in order to attempt confirming the trend observed in experiment 1 but also to mimic more closely the natural condition in which, being *S. haematobium* a chronic infection, exposure to the protein is sustained over time.

Treatment group	Mice	%iRBC				
		Day 3 p.i.	Day 5 p.i.	Day 6 p.i.	Day 7 p.i.	Day 10 p.i.
HIPSE	T1	0.00	0.02	0.23	6.25	X
	T2	0.00	0.07	0.73	13.46	X
	T3	0.01	0.94	4.56	4.68	X
	T4	0.00	0.17	1.43	1.93	X
	T5	0.02	2.08	7.80	7.1	6.9
PBS	C1	0.06	2.73	5.66	2.4	X
	C2	0.07	3.98	10.17	4.76	22.63
	C3	0.00	0.10	0.96	11.5	18.08
	C4	0.00	0.02	0.26	7.54	X
	C5	0.01	0.42	2.56	X	X

Table 31*:* Results of experiment 2: distribution of *P.berghei* density over time.
This table shows the variation of *P.berghei* density monitored after the infection at different time point during the experiment 2 in the experimental group (HIPSE) and the control group (PBS).

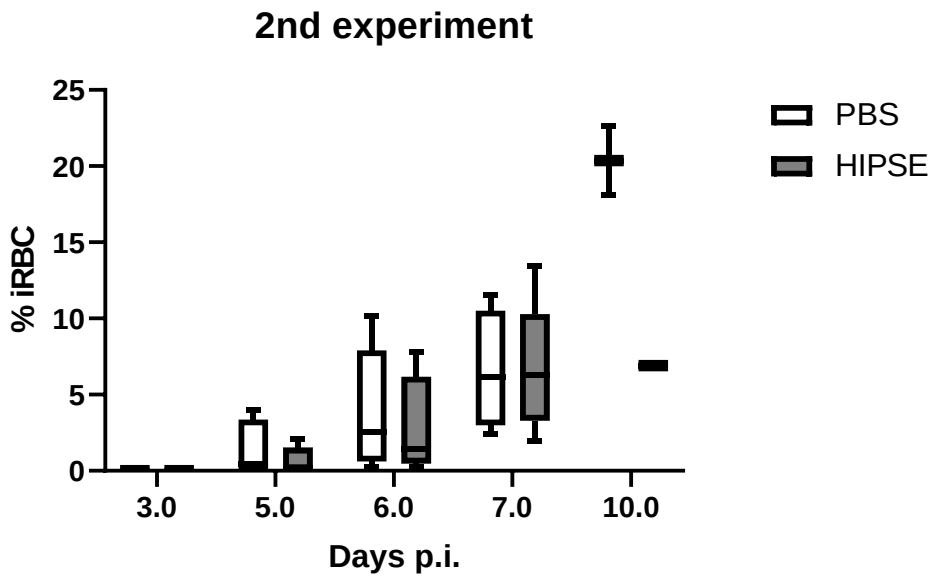

Figure 49: Boxplot of *P.berghei* parasitaemia (%iRBC) in HIPSE and PBS groups over time.
This figure shows boxplots indicating the distribution of *P.berghei* parasitaemia (%iRBC) at days 3, 5, 6, 7 and 10 post infection (days p.i). The horizontal black line indicates the 50% percentile, the blank box (PBS) and the grey box (HIPSE) indicates the 25%-75% percentile, the lower whisk indicates the 5% and the upper whisk the 95% percentile respectively.

Results of experiment 2 showed lower parasite density in treated mice versus controls at all time points except day 7 p.i., and slower growth of parasite density, with statistically significant differences (p-value=0.0048). However, when in order to compare data with those of the first experiment, analysis was repeated including only data from the same time points (3, 5, 7 days p.i.) no differences were observed (p-value=0.8033). Mice started to die on day 7 in the PBS group and at day 10 in the HIPSE group.

These preliminary results suggest an impact of HIPSE treatment in reducing *P. berghei* parasite density in mice, providing some evidence of a role of this antigen in modulating anti-malarial immune responses. However, it is not clear whether more significant effects are obtained when treatment is repeated during the course of malaria infection, and experiments must be repeated in order to draw any solid conclusion.

CHAPTER 5. CONCLUSIONS

The general aim of this thesis was to investigate the impact of *Schistosoma haematobium* infection on immunity to malaria.

An observational study *in natura* has been conducted in human populations living in rural villages from Burkina Faso, who had been previously shown to have different susceptibility to *P. falciparum* malaria, and where *S. haematobium* is also endemic.

Indirect (anti-SEA and anti-SWAP IgM and IgG antibodies) and direct (Circulating Anodic Antigen and circulating DNA) markers of *S. haematobium* infection were measured in plasma samples collected at baseline of a repeated cross-sectional malariological study.

The frequency of seropositive individuals for the different markers was anti-SWAP IgM=45.1%, anti-SWAP IgG=55.0%, anti-SEA IgM=72.4%, anti-SEA IgG=70.2%, *S. haematobium* CAA=28.3%, *S. haematobium* cDNA=26.0%. As expected, the seroprevalence of specific antibodies was much higher than that of either *S. haematobium* circulating antigen or DNA; the half-life of antibodies is indeed expected to be longer and therefore to indicate past exposure to the parasite and not only a current infection. The seroprevalence of anti-SEA antibodies was higher than that of anti-SWAP antibodies, while the seroprevalences of *S. haematobium* circulating antigen and DNA were very similar to each other.

In the multivariate logistic regression analysis of demographic factors that might affect the prevalence of *S. haematobium* infection, all markers showed a clear increase in seroprevalence from children 1-4 years old to 5-9 years old, with a maximum in the age group 10-19 years old and a decrease at older ages. No clear differences were observed between sexes, with some markers (anti-SWAP IgG, anti-SEA IgG, cDNA) showing a lower seroprevalence in females compared to males, and other markers (anti-SWAP IgM, anti-SEA IgM, CAA) showing comparable seroprevalences. Similarly, no clear differences were observed between villages, with some markers (anti-SWAP IgG, anti-SEA IgG, CAA) showing lower seroprevalence in Barkoundouba compared to Barkoumbilen, and other markers (anti-SWAP IgM, anti-SEA IgM, cDNA) showing

comparable seroprevalences. Finally, direct markers of infection as well as specific IgG antibodies did not show differences in prevalence between ethnicities, while specific IgM antibodies showed higher prevalence in Fulani compared to Non-Fulani, which could be interpreted as an indication of higher exposure to the parasite but comparable prevalence of infection.

The diagnostic agreement between plasma biomarkers of infection was evaluated using Circulating Anodic Antigen as the refence, or gold standard, test, as it is a validated marker of *S. haematobium* infection.

In the comparison between cDNA and CAA, overall agreement was 85%, with cDNA showing a 70% sensitivity and a 90% specificity compared to CAA. The rate of false negatives decreases with CAA concentration suggesting a limit in analytical sensitivity that could be overcome by using larger volumes of plasma for DNA isolation and Real Time PCR for cDNA detection. The rate of false positives decreases if using a CAA concentration cutoff for positivity >0 pg/ml instead of 10 pg/ml, leading to a 73% sensitivity and 94% specificity. Given the specificity of *S. haematobium* primers and probes used in Real Time PCR, the use of a composite reference standard where a sample is considered positive when either one or both direct markers are detected can be envisaged.

In the comparison between *S. haematobium* specific antibodies and CAA testing, it was observed that distribution in antibody levels was higher among CAA positive subjects compared to CAA negative subjects, and increased with CAA concentration ranges, with anti-SEA IgG showing the strongest association. Anti-SEA IgG levels also showed the highest correlation with CAA concentration. Receiver Operating Characteristic (ROC) analysis was employed to formally quantify the accuracy of *S.haematobium*-specific antibody tests to discriminate between CAA- and CAA+ subjects, i.e. to diagnose CAA positivity or *S. haematobium* infection, and the best diagnostic performance (highest AUC value) was shown as expected by anti-SEA IgG. Using cutoff value of 0.242 log10(normalized OD) results in anti-SEA IgG test having 82% sensitivity and 82% specificity compared to CAA test.

The association between CAA, as a validated marker of *S. haematobium* infection, and the prospective risk of *P. falciparum* malaria was assessed in the study populations by multivariate

regression analysis adjusted for the effect of age, sex, village, ethnicity and haemoglobin genotype. It was observed that: CAA positivity and higher CAA level increases the odds of getting infected with *P. falciparum* at least once during the study period, but the association does not reach statistical significance; both CAA positivity and higher CAA level significantly increase the incidence rate of *P. falciparum* over time by about 20% ($p<0.005$); CAA positivity and higher CAA level increase *P. falciparum* mean parasite density over 5 measurements (statistically significant association for CAA level only). Taken together these observations suggest that infection with *S. haematobium*, or higher infection intensity with this trematode, increases susceptibility to *P. falciparum* malaria. These results are in line with those of a systematic review and meta-analysis indicating an association between *S.haematobium* infection and increased prevalence of *P. falciparum* asymptomatic malaria (Degarege et al., 2016).

An experimental study has been conducted *in vitro* to evaluate the immunomodulatory properties of *S. haematobium* candidate antigens when used as stimuli in cultured cells from human donors. Tetraspanins 2, 6 and 23 (TSP2, TSP6 and TSP23) have been characterized for their ability to induce Dendritic Cells (DCs) maturation and cytokines production as well as to induce T helper cells differentiation and cytokine gene expression, compared to unstimulated controls.

Results of flow cytometry show that the three tetraspanins were able to induce the expression of maturation markers HLA-DR, CD80 and CD86. Except for TSP23, they were also able to induce the expression of maturation marker CD83. In particular, TSP2 was able to induce a greater increase in expression, in a comparable way to LPS.

Results of Luminex assay show that the three tetraspanins were able to induce the production of inflammatory cytokines IL6, TNFalpha, IL12p70 and IL33, as well as of the Th2 cytokine IL13 and the regulatory cytokine IL10. In particular, TSP2 was able to induce a greater increase in cytokine production, in a comparable way to LPS.

Finally, results of RT quantitative PCR show that the supernatants of tetraspanin-stimulated DCs were able to induce changes in cytokine gene expression in T CD4+ cells, with different pattern depending on the tetraspanin. TSP2 was able to induce an increase in Th1 cytokine IFNgamma and regulatory cytokine IL10 gene expression (significant differences). TSP6 was able to induce an

increase in gene expression of Th2 cytokines IL4, IL5 and IL13 (significant differences for IL4 and IL13). To a smaller extent also TSP23 was able to induce an increase in gene expression of Th2 cytokines IL4, IL5 and IL13, as well as of the regulatory cytokine IL10 (non-significant differences, p-value <0.15). Such interesting results deserve confirmation in a larger number of donors.

A different experimental study has been conducted *in* vivo to evaluate the impact of exposure to the known *S. haematobium* immunomodulatory antigen HIPSE on the course of *P. berghei* infection in mice. Two experiments were conducted in CD1 mice where a treatment group was injected with HIPSE and a control group injected with PBS; both groups were then infected with *P.berghei* parasites expressing green fluorescent protein and the growth of parasites density was monitored by flow cytometry. Results of the two experiments show a slower growth of parasite density in HIPSE treated versus control mice, but with inconclusive statistical evidence. A second round of experiment including a dose-response component would be useful in demonstrating an impact of HIPSE treatment in reducing *P. berghei* parasite density in mice, and therefore a role of this antigen in modulating anti-malarial immune responses.

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