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# *Schistosoma mansoni* Infection: A Major Contributor of Reduced Effective T Helper Responses against *Plasmodium falciparum* and *Schistosoma mansoni* Co-Infection in *ex vivo*: A Cross-Sectional Study to Assess Th1, Th2 & Th17 Immune Responses

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## Abstract

**Background:** Parasitic worms evade immune responses, and interactions between diseases can cause altered immunologic outcomes compared to what usually occurs with single infections. These interactions may influence vaccine and chemotherapeutic efficacy. *Schistosoma mansoni* and *Plasmodium falciparum* are co-endemic in Uganda and are the leading parasitic causes of public health problems across sub-Saharan Africa. **Objectives:** The overall aim was therefore, to elucidate the impact of *S. mansoni* infection on protective T helper immune responses on *P. falciparum* and *S. mansoni* co-infection. **Methodology:** This study evaluated the T helper immune responses in individuals with independent *S. mansoni* infection, independent *P. falciparum* infection, co-infection and non-infection in school attending children in a co-endemic area along Lake Victoria shores, Uganda. Immune responses were categorized into Th1, Th2, and Th17 based on unique cytokine(s) produced by the T helper subpopulation in *ex vivo*. Kato Katz thick smears and circulating cathodic antigen tests were performed for *S. mansoni* screening, whereas thick and thin blood smear techniques were performed for *P. falciparum* screening. **Results:** We observed an up regulated Th1 T helper subpopulation in independent *P. falciparum* infections compared to the uninfected group. Suboptimal T helper immune responses were detected in independent *S. mansoni*

infections characterized by significantly down regulated Th1 (Z = −1.425, p = 0.0313) response in comparison to the non-infected group. Suboptimal T helper immune responses were also recorded in the co-infected individuals characterized by significantly down regulated Th1 (Z = −3.260, p = 0.0273) and Th2 (Z = −1.180, p = 0.0078) responses compared to independent *P. falciparum*. **Conclusions:** *S. mansoni* infection is a major contributor of a reduced effective T helper immune response against *P. falciparum* in *P. falciparum* and *S. mansoni* co-infection.

## Keywords

*Plasmodium falciparum*, *Schistosoma mansoni*, Peripheral Blood Mononuclear Cells (PBMCs), T Helper Immune Response, *Ex Vivo*

## 1. Introduction

*Schistosoma mansoni* is one of the causative agents of intestinal schistosomiasis and *Plasmodium falciparum* is the major causative agent of malaria. They are parasitic infections causing leading public health problems in sub-Saharan Africa, mostly affecting children [1] [2] [3]. *P. falciparum* and *S. mansoni* infections initiate a T helper immune response [4] [5], which links the innate and adaptive immune responses against the parasites. The T helper human immune response to infections can be categorized into Th1, Th2 and Th17. They are identified based on the unique cytokine/s produced by the T helper cells, INF-$\gamma$, IL-4 and IL-17A respectively for Th1, Th2 and Th17 [6]. Parasitic worms evade immune responses [7]-[14] and interactions between diseases can cause altered immunologic outcomes compared to what usually occurs with single infections [15]. These differential outcomes may influence vaccine and chemotherapeutic efficacy [16] [17]. Co-infection with *P. falciparum* and *S. mansoni* is common and may therefore, alter immune responses that could lead to suboptimal chemotherapy and/or vaccine efficacy for one or both of these diseases [18] [19] [20] [21]. The overall aim was therefore, to elucidate the impact of *S. mansoni* infection on protective T helper immune responses in *P. falciparum* and *S. mansoni* co-infection. This will help provide evidence to evaluate chemotherapy and vaccine efficacy to these diseases especially in co-infection situations.

There are several studies which have looked at the separate immune responses of these diseases, indicating that in malaria infection, a Th1 response is initiated [22] [23]. Other studies have shown that the traditional Th1 immune response appears to be suboptimal, indicating pathways such as combined Th$\alpha\beta$ and Th17 immune responses are associated with malaria [4] [5] [24] [25]. In *S. mansoni* infections, there have been reports of both Th1 and Th2 immune responses, with a trend towards a Th1 response during the acute infection phase and Th2 in the chronic phase [26] [27] [28] [29]. The adult worms stimulate a Th1 immune response and their eggs stimulate a Th2 immune response [30]-[37]. These im-

mune responses inhibit each other resulting in down regulation of both [38] [39]. This means that the co-infection of *P. falciparum* and *S. mansoni* could result in interplay of Th1 and Th2 subpopulations of T helper immune responses and potential inhibitory effects.

Independent *P. falciparum* and *S. mansoni* infections elicit distinct T helper immune responses. We hypothesize that during *P. falciparum* and *S. mansoni* co-infection, these immune responses maybe antagonistic to each other and may result in down regulation of protective responses with subsequent suboptimal immunity to the disease(s). This was determined by performing the *ex vivo* Th1, Th2 and Th17 phenotyping on the peripheral blood mononuclear cells (PBMCs) of school attending children in a co-endemic area.

## 2. Materials and Methods

### 2.1. Study Setting

The study was conducted in two primary schools (p/s) in Mayuge District; Bwondha p/s and Kaluuba p/s. The District is endemic for both *P. falciparum* and *S. mansoni* with prevalence of 51% and 28.1% respectively, a co-infection prevalence of 26% [40]. Mayuge District is bordered by Iganga District to the north, Bugiri District to the northeast, Namayingo District to the east, Jinja District to the west and the Republic of Tanzania to the south. The coordinates of the district are: 00˚20'N, 33˚30'E in the eastern region of Uganda.

A large proportion of the district surface area is open water of Lake Victoria, estimated to represent 77% of the total surface area in the district. This plays a major role in the existence of both *P. falciparum* and *S. mansoni* transmission as their respective vectors; *Anopheles* mosquitoes and *Biomphalaria* snails breed in the water body. The district has a population of over 479,000 people [41] and the main economic activities include; fishing, subsistence agriculture and bee keeping for production of honey.

### 2.2. Study Design and Participants

This was a cross-sectional study, with 120 participants aged 8 - 17 years selected from the two primary schools, with 60 children per-school. Recruitment was performed using stratified and systematic random sampling techniques to obtain gender balance and representative participants respectively. Participants were screened for *P. falciparum*, *S. mansoni*, other haemoparasites and other intestinal worms. Those who were found with other haemoparasites and intestinal worms were excluded, whereas those with no infection, *P. falciparum* only infection, *S. mansoni* only infection and *P. falciparum* and *S. mansoni* co-infection only were included in the study. The sample size calculation indicated that a minimum sample size of 40 was required as [5] with significance level at $\alpha = 0.05$ and power of 80%. After screening the 120 eligible participants, 40 individuals were enrolled to perform T helper phenotyping from their PBMCs. PBMCs were isolated from whole blood of the included participants and *ex vivo* Th1/Th2/ Th17 phenotyping was performed. The final enrolled individuals included; *Plasmodium falci-*

*parum* alone (n = 9), *Schistosoma mansoni* alone (n = 12), co-infection (n = 13) and no infection (n = 6).

## 2.3. Data Collection Methods

### 2.3.1. Sample Collection

Stool, urine and capillary blood samples were collected from the participants for screening the infections, and venous blood sample collected in Acid Citrate Dextrose (ACD) tubes for PBMCs isolation for included participants after screening as described [42].

### 2.3.2. Laboratory Procedures

1) *Schistosoma mansoni* screening methods

a) *Kato Katz technique*

The Kato Katz thick smear technique was used to screen for *S. mansoni*, other intestinal worms, and to determine *S. mansoni* infection intensity. A section of the stool sample was passed through a 250 μm sieve and placed onto the Kato Katz template, of size 41.7 mg, on a glass slide. A cellophane cover slip soaked in 50% glycerol methyl green was placed on the sample and spread to make smears. The smears were examined under a compound microscope with 10× objective to look for eggs of the worms. The infection intensity (eggs per gram (e.p.g)) was obtained by multiplying the egg count in a 41.7 mg smear by 24. Infections were categorized as light, moderate or high infection (1 - 99 e.p.g = light infection, 100 - 199 e.p.g = moderate infection and ≥200 e.p.g = high infection) as described previously [43] [44] [45].

b) *Circulating cathodic antigen cassette test* (*CCA*)

The CCA is a semi quantitative method of detecting an active *S. mansoni* infection, with antigens released by live adult parasites secreted in the host's urine. A drop of urine is placed in the circular well of the test cassette, followed by a drop of buffer and allowed to stand for 20 minutes to read the result. A positive CCA test result (a red band in the control and test windows) on randomly collected midstream urine indicates an active *S. mansoni* infection, whereas the negative CCA test was when the red band only formed in the control window [46].

2) *Plasmodium falciparum* screening methods

a) *Thin blood smears geimsa technique*

A drop of finger prick blood was put near one edge of a slide, the edge of clover slip brought to touch the blood at 45˚ angle and spread to make a thin smear with a mono layer of RBCs towards the tail. This was allowed to dry, the smears were fixed with absolute methanol, and the slides stained with 10% giemsa stain for 10 minutes. Slides were examined under a compound microscope with ×100 oil immersion objective lens. *P. falciparum* was identified by seeing the infected red blood cell (RBC) with the *Plasmodium* not changing shape, multiple infection of a RBC with *Plasmodium* and presence of double nuclear (chromatin dot) on *Plasmodium* as described previously [47] [48] [49].

b) *Thick blood smears technique*

A drop of finger prick blood was put at center of a slide and spread to make a thick smear. The slide was allowed to dry, stained with 10% giemsa stain for 10 minutes and examined under a compound microscope with ×100 oil immersion objective lens. The *P. falciparum* parasites were counted against 200WBCs and multiplied by factor 40 to get parasitaemia per 1 µl of blood, since in 1 µl of blood there is estimated 8000 WBCs as described previously [47] [48] [49].

3) T helper cells subpopulation phenotyping methods

a) *PBMCs isolation using Ficoll plaque separation technique and storage*

The PBMCs were isolated from citrated blood by gradient centrifugation over Ficoll-plaque™ plus media, manufactured by GE Healthcare Bio Sciences AB. The cells were washed twice with RPMI supplemented with 1% penicillin/ streptomycin, 1% L-glutamine and 1% hepes buffer. The cells were then counted, adjusted to the required concentration and cryopreserved in 10% dimethylsulfoxide (DMSO)/Foetal bovine serum (FBS) as described [50].

b) *PBMCs Thawing and counting*

The cryopreserved PBMCs were quickly thawed in a 37˚C water bath. The cells were washed twice with warm supplemented RPMI by centrifugation, re-suspended in R10 culture medium. We counted the cells by mixing tryptan blue and resuspended cells into 1:1 ratio, which was then loaded into a counting chamber of the haemocytometer and examined under a compound microscope to count viable cells. The PBMCs were added at a concentration of $1 \times 10^6$ cells/ml to a culture plate for *ex vivo* assay as described [50].

c) *Ex vivo* Th1/Th2/Th17 *Phenotyping using BD Human* Th1/Th2/Th17 *Phenotyping kit* (*Ref:* 560751)

i) *Stimulation of the PBMCs*

The rested PBMCs were put in culture media of R10 (87% RPMI, 10% FBS, 1% pen/step, 1% hepes buffer and 1% L. glutamine) on culture plate, 0.7 µl of BD GolgiStop™ Protein Transport Inhibitor (Monensin) was added per ml of the PBMCs and mixed well. This prevented protein (cytokine) secretion from the golgi apparatus, by interacting with the golgi transmembrane $Na^{2+}/H^{+}$ transport. Then 0.05 µl of 1mg/ml PMA (Phorbol ester) [Sigma P8139] was added into 1ml of the PBMCs in the culture media to give the concentration of the PMA 50 ng/ml. 1 µl of 1 mg/ml Ionomycin (Calcium Ionophore) [Sigma 10,634] was also added into 1ml of the PBMCs in the culture media giving the concentration of the Ionomycin 1 µg/ml. This was mixed well and incubated at 37˚C for 5 hours. These enhanced the activation of protein kinase C to induce T helper cells to produce cytokines. Hence cytokines produced by T helper cells were prevented from leaving the golgi apparatus resulting in a build up [6].

ii) *Fixing the PBMCs*

The stimulated PBMCs were thoroughly suspended with 1ml of cold BD Cytofix™ Fixation buffer and then incubated for 20 minutes at room temperature (RT). They were then centrifuged at 1500 rpm for 10 minutes at RT to remove the fixation buffer; this was followed by the addition of stain buffer to wash

through using centrifugation at 1500 rpm for 10 minutes at RT twice. This preserved the markers like the cytokines and cluster of differentiation [6].

iii) *Permeabilizing the fixed PBMCs*

The 10× BD perm/Wash™ buffer was diluted in distilled water to make 1 × solution prior to use. The PBMCs were suspended in 1ml of 1 × BD perm/Wash™ buffer, incubated at RT for 15 minutes, then centrifuged at 1500 rpm for 10 minutes at RT and the supernatant removed. This perforated the PBMCs for stain penetration [6].

iv) *Staining the PBMCs with the cocktail*

Thoroughly suspended fixed/permeabilized PBMCs in each tube, with 50 µl of 1 × BD perm/Wash™ buffer to enhance stain penetration. Added 20 µl/tube of cocktail stain of fluorescent antibodies-specific for Human IL-17A PE (clone: N49-653), Human IFN-GMA FITC (clone: B27) and Human IL-4 APC (clone: MP4-25D2) for intracellular staining, and Human CD4 perCP-Cy5.5 (clone: SK3) for surface staining. The PBMCs were then incubated at RT for 30 minutes in the dark to prevent degradation of light sensitive fluorescent stains, and then washed twice with 1ml of 1 × BD perm/Wash™ buffer by centrifuging at 1500 rpm for 10 minutes at RT. The stained PBMCs were suspended in stain buffer prior to flow-cytometric analysis, to categorize the T helper cells into Th1, Th2 and Th17 [6].

v) *Flow Cytometric Analysis*

The stimulated, fixed, permeabilized and stained PBMCs were detected using BD FACSDiva version 6.12 Software on a Becton Dickinson (BD) FACSCalibur flow cytometer, where 100,000 cell events were acquired for each sample and the data analysed using Flow Jo version 10 Software (**Figure 1**).

## 2.4. Quality Control

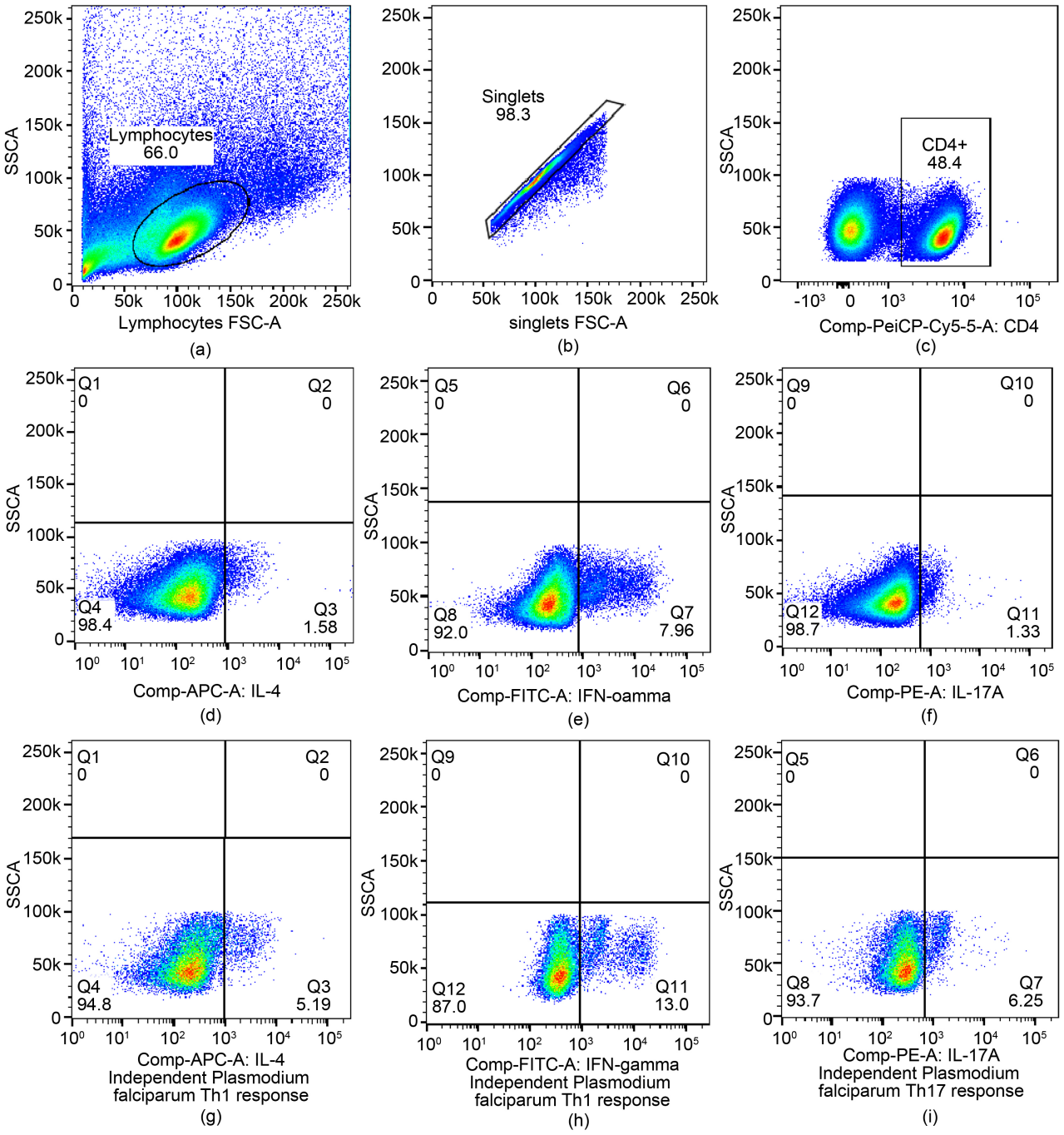
Two smears were made for each participant to diagnose *P. falciparum* and *S. mansoni* to increase accuracy of detection of the parasites from the samples and two technicians were used to examine the samples independently. Optimization and calibration for the flow cytometry machine was performed prior to running the samples.

## 2.5. Data Handling and Statistical Analysis

The data were collected on the pro-former forms transferred in Excel software and some collected on the computer attached to the flow cytometry machine, analyzed with flow Jo software version 10 and Graphpad prism software. The test for significance of association between infection groups and the particular T helper immune response ($p < 0.05$) was determined using Wilcoxon Signed Rank test to show that the median differences in T helper immune responses in the groups were not zero and Mann Whitney U test to determine whether the CD4+ cells population medians of the infection groups differ. F-tests were used to compare variances of parasitaemia/intensity in infection groups.

## 2.6. Ethical Consideration

The ethical issues concerning children include lack of capacity to make informed decision, vulnerable to harm and injustice by researchers. In the light of these, the study sought clearance from the Institution Review Board of School of Biomedical Sciences, Makerere University. Permission was sought from school administration where the study took place. The informed consent obtained from parent/guardian on behalf of participant(s) by availing them with local language translated informed consent form to read through, and allowed to ask question on what was not clear to obtain answer to enhance understanding of the study to



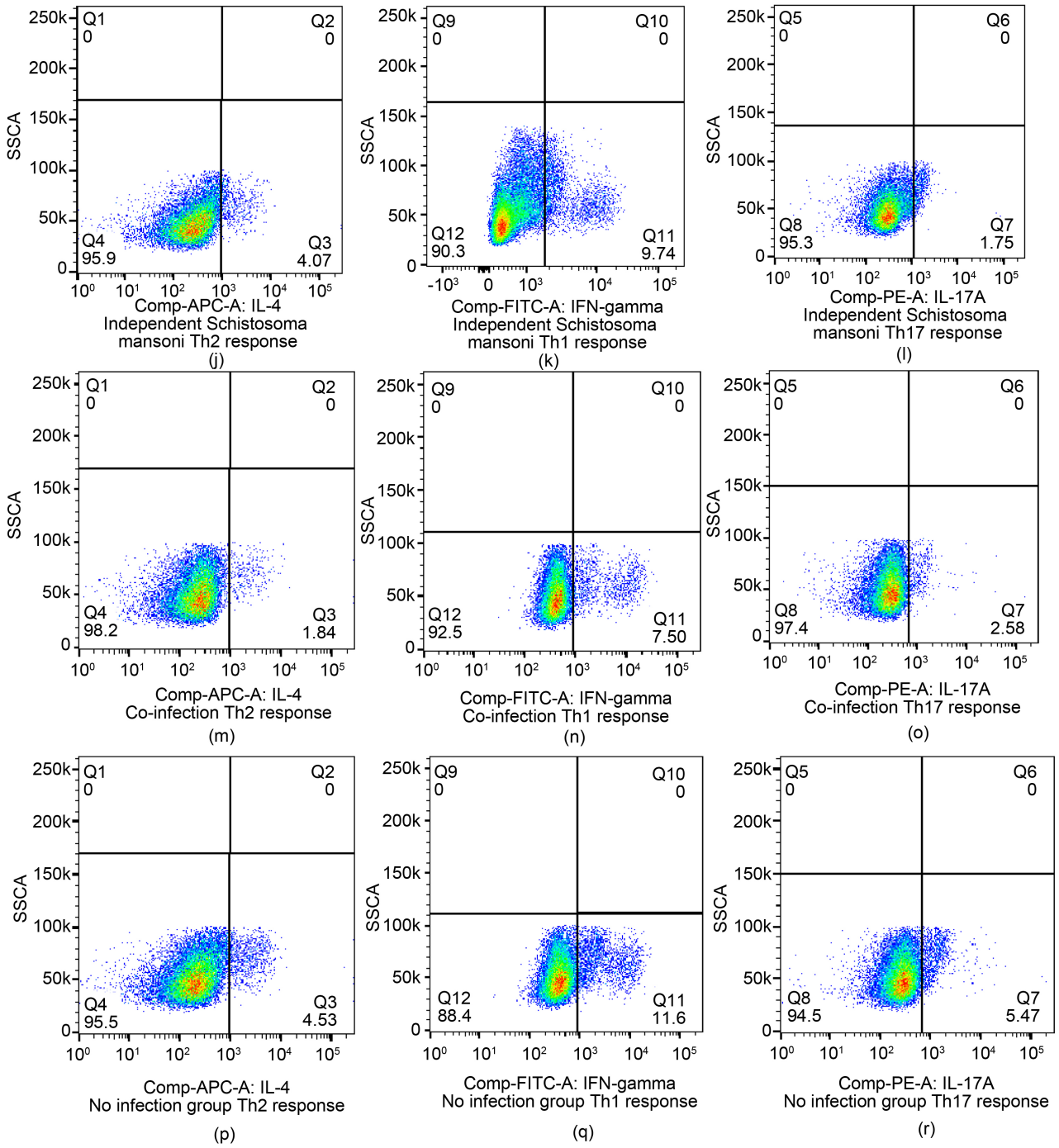



**Figure 1.** Flow Jo analysis was performed by gating the lymphocytes population (a), from which the singlets population gated (b), followed by the gating of CD4+ cells population (c), then the CD4+ cells producing IL-4 (Th2) were gated (d), CD4+ cells producing IFN-$\gamma$ (Th1) were gated (e) and CD4+ cells producing IL-17A (Th17) were gated (f), the independent *P. falciparum* infections stimulate proportion of CD4+ cells producing IL-4 (Th2) (g), INF-$\gamma$ (Th1) (h), and IL-17A (Th17) (i), the independent *S. mansoni* infections stimulate proportion of CD4+ cells producing IL-4 (Th2) (j), INF-$\gamma$ (Th1) (k), and IL-17A (Th17) (l), the co-infection infection stimulate proportion of CD4+ cells producing IL-4 (Th2) (m), INF-$\gamma$ (Th1) (n), and IL-17A (Th17) (o), and the un infected also stimulated proportion of CD4+ cells producing IL-4 (Th2) (p), IFN-$\gamma$ (Th1) (q), and IL-17A (Th17) (r).

allow the child to participate or not to allow. Those children for whom the informed consent obtained from their parent/guardian also assented before data

was collected. The pupils were assembled and the participants selected according to the sampling techniques, then the participants briefed on what to do to obtain sample (stool, urine and blood) from them by technically competent personnel. After the study the results were disseminated to the administration to enable those who are found infected to receive treatment in the nearby heath center or community drug distributor.

## 3. Results

### 3.1. The Characteristics of the Study Participants, Burden and Immune Responses of *P. falciparum* and *S. mansoni* Infections in the Population

Of the 40 participants included for *ex vivo* T helper phenotyping from their PBMCs, there was a significant difference in the burden of *S. mansoni* infection between the co-infected group (with moderate to high infections) compared to *S. mansoni* only infections (with lower infection intensities) ($F(11, 11) = 7.898$, $p = 0.0019$). Using thick blood geimsa smear technique results, the burden of *P. falciparum* was higher in independent *P. falciparum* infected individuals than in the *P. falciparum* and *S. mansoni* co-infected individuals ($F(8, 12) = 3.841$, $p = 0.0362$) (Table 1). The CD4+ cell population was suppressed ($U = 41$, $p = 0.0454$) in independent *S. mansoni* compared to the co-infection (Figure 2).

### 3.2. The T Helper Cell Subpopulations Involved in Independent *P. falciparum* and *S. mansoni* Infections

The Th1 immune response was significantly down regulated in independent *S. mansoni* ($Z = -1.425$, $p = 0.0313$) and up regulated in independent *P. falciparum* (Figure 3(a)). Both the Th2 (Figure 3(b)) and Th17 (Figure 3(c)) immune responses were down regulated in independent *P. falciparum* and *S. mansoni* infections albeit not significantly.

Table 1. The infections intensity/parasitaemia among school going children a long lake victoria shores, mayuge district. N = 40.

| Categories | | Arithmetic Mean | | | |
|---|---|---|---|---|---|
| | | Intensity/Parasitaemia | | | |
| | | *S. mansoni alone* | *P. falciparum alone* | Co-infection | |
| | | Kato katz (e.p.g) | Thick smear (mps/µl) | Kato Katz (e.p.g) | Thick smear (mps/µl) |
| Included participants (n = 40) | Male n = 20 | 220 (n = 6) | 2680 (n = 7) | 451 (n = 5) | 832 (n = 5) |
| | Female n = 20 | 76 (n = 6) | 3160 (n = 2) | 435 (n = 8) | 1195 (n = 8) |

e.p.g means number of eggs in gram of stool sample and mps/µl means number of malaria parasites in microliter of blood.

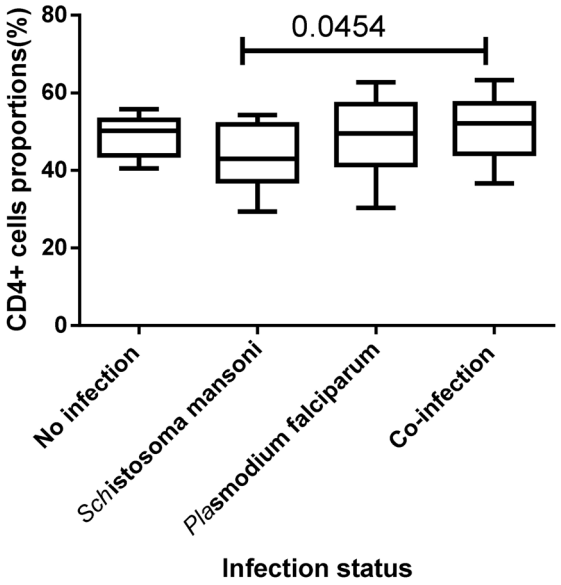


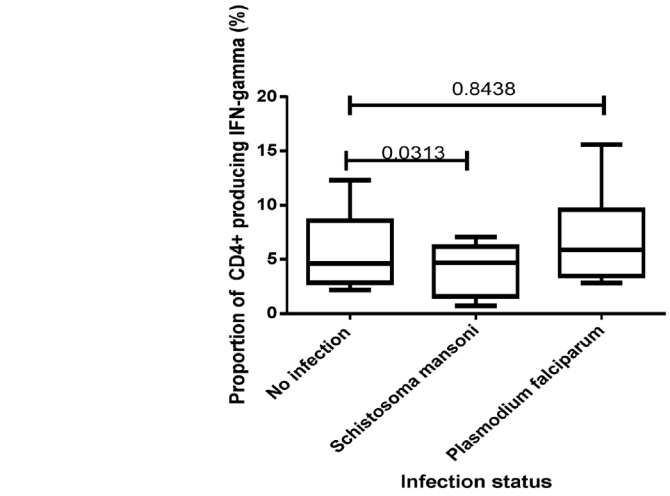

**Figure 2.** The CD4+ cells proportion among school going children along Lake Victoria shores, Mayuge District. The *ex vivo* T helper phenotyping using the human Th1/ Th2/Th17 phenotyping kit through utilizing specific signature marker cytokines and surface marker; IFN-γ, IL-4, IL-17A and CD4 respectively. The PBMCs were non specifically stimulated using PMA/Ionomycin after PBMCs treated with protein transport inhibitor to enhance cytokine production and build up in golgi apparatus, fixed, permeabilized, intracellular stained with fluorescent attached monoclonal antibodies of IFN-γ, IL-4 and IL-17A, surface stained with fluorescent attached monoclonal antibody of CD4 and flow cytometric analysis done. The CD4+ cell proportion was significantly suppressed in independent *S. mansoni* (U = 41, p = 0.0454) compared to co-infection (Figure 2).

### 3.3. The T Helper Cell Subpopulation(s) Involved in *P. falciparum* and *S. mansoni* Co-Infection

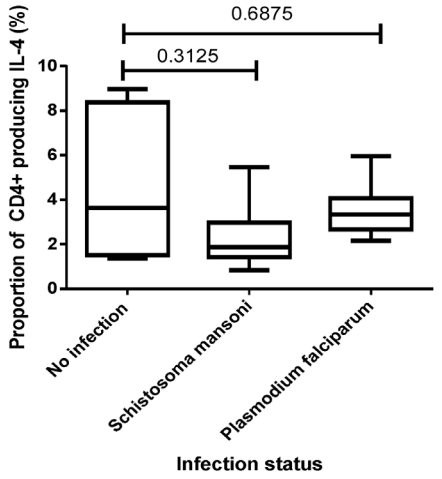
There were significant down regulated Th1 (Z = −3.260, p = 0.0273) and Th2 (Z = −1.180, p = 0.0078) responses, and non-significantly down regulated Th17 responses in co-infection compared to independent *P. falciparum* (Figures 4(a)-(c)). And non-significant down regulated Th2 and Th17 immune responses in co-infection compared to independent *S. mansoni* (Figure 4(b) & Figure 4(c)).

## 4. Discussion

This study demonstrates that *S. mansoni* infection is associated with a significantly reduced effective T helper response against the *P. falciparum* in a *P. falciparum* and *S. mansoni* co-infection. We found in independent *S. mansoni* infection, there were significantly down regulated Th1 (Z = −1.425, p = 0.0313), slightly down regulated Th2 response which is effective response against *S.*




(a)

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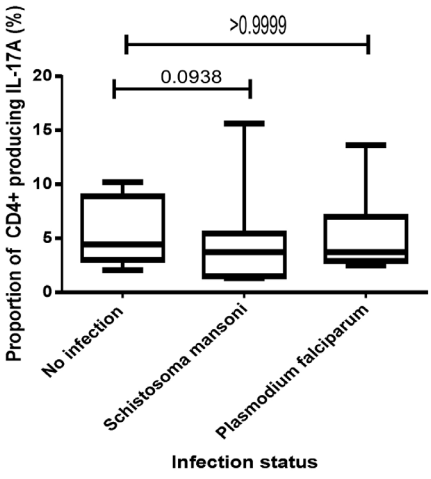


(c)

**Figure 3.** The proportions of Th1, Th2 and Th17 during independent infections, compared to no infection group among school going children along Lake Victoria shores, Mayuge District. The *ex vivo* T helper phenotyping using the human Th1/Th2/Th17 phenotyping kit through utilizing specific signature marker cytokines and surface marker; IFN-*γ*, IL-4, IL-17A and CD4 respectively. The PBMCs were non specifically stimulated using PMA/Ionomycin after PBMCs treated with protein transport inhibitor to enhance cytokine production and build up in golgi apparatus, fixed, permeabilized, intracellular stained with fluorescent attached monoclonal antibodies of IFN-*γ*, IL-4 and IL-17A, surface stained with fluorescent attached monoclonal antibody of CD4 and flow cytometric analysis done. There was significantly down regulated Th1 immune response in independent *S. mansoni* ($Z = -1.425$, $p = 0.0313$) compared to no infection group (**Figure 3(a)**).

*mansoni* infections (reviewed in [51]) and non-significantly down regulated Th17 (**Figure 3**). In contrast, in independent *P. falciparum* infections there were up regulated Th1 response which is effective against malaria infection (reviewed in [52]), down regulated Th2 and Th17 (**Figure 3**) as compared to no infection group. Meanwhile in *P. falciparum* and *S. mansoni* co-infection there were significantly down regulated Th1 ($Z = -3.260$, $p = 0.0273$) and Th2 ($Z = -1.180$, $p = 0.0078$) which are supposedly effective responses against the individual infections

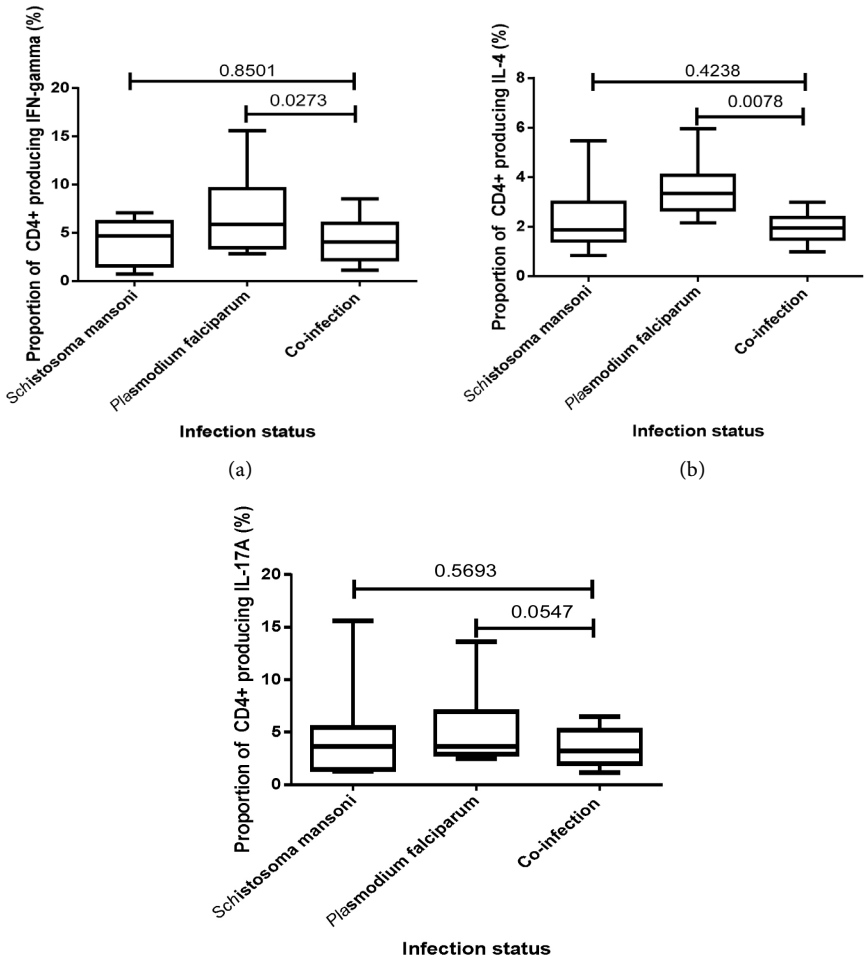


**Figure 4.** The proportions of Th1, Th2 and Th17 during co-infection, compared to independent infections among the school going children along Lake Victoria shores, Mayuge District. The *ex vivo* T helper phenotyping using the human Th1/Th2/Th17 phenotyping kit through utilizing specific signature marker cytokines and surface marker; IFN-*γ*, IL-4, IL-17A and CD4 respectively. The PBMCs were non specifically stimulated using PMA/Ionomycin after PBMCs treated with protein transport inhibitor to enhance cytokine production and build up in golgi apparatus, fixed, permeabilized, intracellular stained with fluorescent attached monoclonal antibodies of IFN-*γ*, IL-4 and IL-17A, surface stained with fluorescent attached monoclonal antibody of CD4 and flow cytometric analysis done. There were significantly down regulated Th1 ($Z = -3.260$, $p = 0.0273$) and Th2 ($Z = -1.180$, $p = 0.0078$) during co-infection, compared to the independent *P. falciparum* (**Figure 4(a)** & **Figure 4(b)**).

[51] [52], and down regulated Th17 (**Figure 4**) as compared to independent *P. falciparum* group.

The up regulated Th1 response in independent *P. falciparum* (**Figure 3**) supports previous studies [22] [23]. However, it contradicts another study which reported that *P. falciparum* evades the immune system by stimulating a Th17 and Th*αβ* bias over Th1, with these Th17 and Th*αβ* responses being ineffective against the parasite [5]. These differences may be explained by the different study methods, with the former study looking at gene expressions [5] in com-

parison to our study looking at protein expressions, as not all gene expression translates to protein expression. Furtherstudies [4] [24] [25] indicated that a Th1 immune response may not be predominant in *P. falciparum* as the parasite was not cleared as expected for predominant Th1 immune response. This has been evident in the current study, where the independent *P. falciparum* had higher parasitaemia than in the co-infection ($F(8, 12) = 3.841$, $p = 0.0362$) (**Table 1**).

Meanwhile the independent *S. mansoni* infections with significantly down regulated Th1 responses ($Z = -1.425$, $p = 0.0313$) and non-significant down regulated Th2 and Th17 immune responses could be explained by the fact that the adult worm stimulating Th1 immune response and its eggs stimulate Th2 immune response [30]-[37]. These immune responses inhibit each other down regulating each response [38] [39]. This was supported by the overall suppression of CD4+ cells ($U = 41$, $p = 0.0454$) in independent *S. mansoni* (**Figure 2**). This may be explained by the high number of chronic schistosomiasis infections, demonstrated by positive CCA and Kato Katz tests indicating high worm numbers and high egg output. Hence the adult worms stimulate Th1 whereas the eggs stimulate Th2, the resultant effect is inhibitory [38] [39] thus suppressed CD4+. In addition several studies have reported that parasitic worms evade the immune system by deactivation of certain immune system cells that could be harmful to themselves and the host hence Th1, Th2 and Th17 down regulated [7]-[14].

Since *S. mansoni* suppresses Th1 immune response and the Th1 remains suppressed in the co-infection, this means that in a situation where malaria co-infection is preceded by the *S. mansoni* infection, the Th1 immune response is limited providing lower immune protection against *P. falciparum* infection [52]. This implies that a vaccine against *P. falciparum* (malaria vaccine) under development that is aimed at invoking a Th1 protective response may be less effective in an individual that is infected with *S. mansoni*. As *S. mansoni* and *P. falciparum* are frequently co-endemic with many individuals co-infected, this could have serious implications for the future success of any vaccines under development which would invoke a Th1 response [40]. This could be particularly important in high transmission areas where children as young as 6 months old are already infected with *S. mansoni* [53].

The reduced T helper immune responses observed in the co-infection seems to translate into worsening the burden of *S. mansoni* in the co-infected individuals with e.p.g ≥ 200 (high intensity) as opposed to independent *S. mansoni* with e.p.g < 200 (moderate intensity) (**Table 1**) ($F(11, 11) = 7.898$, $p = 0.0019$). This could be explained by the fact that the main T helper immune response believed to play major role in the protection for *S. mansoni* infection (Th2) was reduced further in co-infection. Whereas for the *P. falciparum* the co-infection seems to reduce the parasitaemia ($F(8, 12) = 3.841$, $p = 0.0362$) (**Table 1**) is not explained by our T helper response findings but supports previous findings [54]. This study indicated that co-infection with schistosome and *P. falciparum* is significantly associated with reduced risk of febrile malaria in long-term asymptomatic carriers of *P. falciparum* [54]. This indicates that co-infection could induce im-

munomodulatory mechanisms that protect against febrile malaria in co-endemic areas, supported by our lower parasitemia here. Their reduced T helper responses could be due to unbalanced regulation of the associated inflammatory response/cytokines by co-infection, which may have key impact on the acquired immune response [55] [56] [57] [58]. This has been demonstrated in our current study, where Th1 and Th2 were significantly down regulated in co-infection compared to independent *P. falciparum*.

The key anticipated limitations to our study were the infection with haemoparasites and intestinal worms other than *P. falciparum* and *S. mansoni* infections respectively, and insufficient sample size after applying the exclusion criteria. However we screened for these additional infections and participants with these parasites were excluded. This reduced bias in the samples due to these other parasitic infections but we did not exclude all other potential infectious agents or control for previous historical infections, which may still have affected the T helper immune responses which were due to *P. falciparum* and *S. mansoni* only. We believe however that these potential additional infections were likely to be low in prevalence and distributed randomly across the four infection groups. Despite excluding individuals with other infections the sample size at the screening stage was still higher than our estimated minimum sample size needed to detect differences between the participant groups.

This study provides important epidemiologic evidence that shows that there is the need to evaluate malaria vaccines under development in areas endemic to *S. mansoni* and malaria. Since *S. mansoni* infection suppresses the Th1 response which plays a key role in a malaria vaccine currently under development, particularly as there was no recovery response of immune suppression in co-infection group. *S. mansoni* infection could therefore reduce the efficacy of such a malaria vaccine under development. We suggest further research to look at the T regulatory cells and the memory cells involved in *P. falciparum* and *S. mansoni* co-infection in co-endemic areas to fully understand the influence of these infections on the T helper responses.

## 5. Conclusion

The study revealed that the *S. mansoni* infection is the major contributor of reduced effective T helper immune responses against *P. falciparum* in *P. falciparum* and *S. mansoni* co-infection.

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## Conflict of Interests

The authors declare that they have no conflict of interests.

## Author's Contribution

Candia Rowel was involved in study design, fund solicit, data collection, analyses, interpretation & drafting of the manuscript. Rose Nabatanzi was involved in data collection and analysis, and revising the manuscript. Joseph Olobo was involved in supervision, study design & revising the manuscript. Ann Auma contributed in supervision, study design & critical revising of the manuscript. Benon Asiimwe participated in supervision & critical revising of the manuscript. Olive Mbabazi was involved in data collection & critical revising of the manuscript. Alice Bayiyana was involved in data collection & critical revising of the manuscript. Annet Enzaru was involved in data collection and critical revising of the manuscript. And Edridah Tukahebwa was involved in supervision & critical revising of the manuscript.

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