Research Square

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Transcriptome profiling reveals genes associated with inflammation and fibrosis among 10 - 15-year-old children with Schistosoma mansoni and Plasmodium falciparum coinfection along the Albert Nile in Uganda

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Abstract

Background

Malaria and schistosomiasis are significant parasitic diseases in Uganda and coinfections with the two are not uncommon in areas endemic to both parasites. The aim of this study was to determine the effect of *P. falciparum* and *S. mansoni* coinfection on the gene expression in peripheral blood of school age children aged between 10–15 years.

Methods

A cross sectional study of children aged 10–15 years, was conducted in selected sites along the Albert Nile in Pakwach District in northwest Uganda. Quantitative PCR (qPCR) was used to test for *S. mansoni* and *P. falciparum* infection. Furthermore samples that were sequenced using Illumina NovaSeq S4 and the reads aligned to the GRCh38 human genome were matched with those with *S. mansoni* and *P. falciparum* qPCR results. Differential gene expression analysis was done using DESeq2.

Results

Of the 210 study particpants, 76.2% (160/210) were *P. falciparum* positive, 91% (191/210) were *S. mansoni* positive and 150 (71%) had coinfection with both *P. falciparum* and *S. mansoni*, which was slightly fewer coinfections than expected by chance (Fisher exact test p-value of 0.02). RNAseq data was obtained for 33 participants of which 17 had *P. falciparum* and *S. mansoni* coinfection, 4 *S. mansoni* infection only, 1 had *P. falciparum* infection only while 11 were uninfected. Principal component analysis revealed clustering of gene expression by gender and infection status when *S. mansoni* and *P. falciparum* coinfected children were compared with uninfected children. We observed 15 DEGs of which 2 (CEPT1 and RETREG1) were downregulated and 13 (GAS6, DEXI, PALMD, SAMD15 AC138028.4, GFOD1-AS1, AC034102.6, AC005153.1, AC020914.1, AC017028.2, AC244502.3, AC013486.1, AC106760.1) upregulated when *S. mansoni* and *P. falciparum* coinfected children were compared with uninfected children. The differentially expressed genes are associated with inflammation and fibrosis and also included regulatory long noncoding RNA.

Conclusions

By molecular detection, this study observed a high prevalence of *P. falciparum* among the school age children (10–15 years) living in the *S. mansoni* endemic hotspots along the Albert-Nile region of Pakwach district, northwestern Uganda. The study shows differential expression of genes associated with inflammation and fibrosis among coinfected when compared to the uninfected children.

Author Summary

Schistosomiasis and malaria are important parasitic diseases endemic in Uganda. The predominant schistosomiasis causing species in Uganda is *Schistosoma mansoni* while that for malaria is

Plasmodium falciparum. Coinfections occur in areas with high endemicity of these two parasites. *S. mansoni* coinfection has previously been shown to alter the clinical presentation of malaria. In this study, we used quantitative PCR (qPCR) to determine presence of *P. falciparum* infection among *S. mansoni* infected children. We observed high *P. falciparum* infection intensities in areas closer to the Albert Nile compared to those farther away. We recorded 71.4% coinfections with *P. falciparum* and *S. mansoni* using qPCR for the two parasites in children between 10 -15 years living along the Albert Nile. We also report expression of genes associated with inflammation and fibrosis among children with *P. falciparum* and *S. mansoni* coinfection.

Background

Schistosomiasis and malaria are important parasitic diseases that cause morbidity and mortality worldwide [1–3]. Schistosomiasis is widespread in tropical and sub-tropical regions with over 78 countries reporting transmission [4]. The World Health Organization (WHO) estimated that 236.6 million people required treatment for schistosomiasis in 2019 [5]. Additionally, WHO estimated 249 million malaria cases in the endemic areas [3]. Malaria and schistosomiasis coinfection has been reported in areas with high occurrence of both infections [6–8]. Additionally stunting among children has been shown in *S. mansoni* as well as *P. falciparum*[9] infection. Coinfection of malaria and schistosomiasis is particularly common in sub Saharan Africa (SSA) [10].

Despite control measures such as use of mosquito nets or Indoor Residual Spraying (IRS), Uganda has the third highest malaria incidence after Nigeria and the Democratic Republic of Congo [3] in Africa. In Uganda, malaria is predominantly caused by *Plasmodium falciparum* and infections are transmitted throughout the year [11]. High prevalence of malaria has been reported around Lake Albert [12]. A recent study showed a reduction in stunting following treatment of malaria infection[13].

Uganda is also endemic for schistosomiasis with varying prevalence in different localities. Our recent survey showed high prevalence of schistosomiasis caused by *S. mansoni* in children of school age from villages along the Albert Nile with high levels of stunting which were not associated with schistosomiasis [14]. This adds to previous findings on high prevalence of *S. mansoni* by Lake Albert [15, 16].

S. mansoni coinfections with *Plasmodium* species are known to influence clinical presentation of malaria[8, 17, 18] and may also alter the immune response to infection and affect response to treatment and vaccination [19]. Individuals with *P. falciparum / S. mansoni* coinfections are reportedly more prone to frequent and severe malaria [20]. Whether *P. falciparum* and *S. mansoni* coinfections affects the expression profile of genes in children is unknown. This study aimed to determine the effect of *P. falciparum* and *S. mansoni* coinfections on the gene expression in peripheral blood of school age children aged between 10–15 years.

Methods

Ethics statement

The study protocol was reviewed by the institutional review board of the Ministry of Health, Vector Control Division Research and Ethics Committee (Reference No. VCDREC106) and approved by the Uganda National Council for Science and Technology (Reference No. UNCST HS 118). The study was conducted with guidance from the district health officials, including the selection and training of the village health teams that were involved in the mobilisation and recruitment of the children (10–15 years) into the study. The objectives, potential risks and benefits of the study were explained to the parents/guardians, who signed written informed consent. The same was later explained to the school age children in English and Alur dialect before they provided written assent for participation in the study. If a child was observed to have *S. mansoni* eggs in their stool, they were offered free treatment, which consisted of praziquantel at a dosage of 40mg/kg administered by trained Ministry of Health personnel, assisted by district health workers. Point of care cathodic circulating antigen (POC-CCA) and *P. falciparum* results were not used as an indication for treatment.

Study design and study sites

This was a cross-sectional study that was carried out between October and November 2020 in communities along the Albert Nile in Pakwach District in North Western Uganda. School aged Children (SAC) of 10–15 years with consent from the parent/guardian and the child assent were included in this study. Purposive sampling was done based on availability of resources. The selected sampling sites were in four (4) sub-counties of Pakwach district: Pakwach, Panyingoro, Panyimur and Alwi all in close vicinity to the Albert Nile. The study mobilized children of school age 10 -15years with the help of village health teams (VHTs). The collection sites were schools in Panyigoro, Kivuje, Nyakagei, Kayonga, Pamitu and Alwi within that sub-county or in the Dei Health Center. Pamitu and Alwi were farther (3 Km and 10 Km respectively) from the Albert Nile while Panyigoro, Kivuje, Nyakagei, Kayonga, and Dei collection sites were within 1 Km of the river.

Screening and sample collection

The point of care cathodic circulating antigen (POC-CCA) (Rapid Medical Diagnostics, Pretoria, South Africa, batch No. 191031120) was used in the field for screening for *S. mansoni* and classifying participants by infection intensity based on band intensity as Negative 0 (G1), trace (G2, G3), 1+ (G4,G5), 2+ (G6,G7), 3+ (G8, G9) or 4+ (G10) previously decribed by Mulindwa et al [14] and Namulondo et al [21] with modifications from Casacuberta-Partal et al. [22] to select and collect blood samples that were tested for *P. falciparum* and stool for *S. mansoni* by quantitative PCR (qPCR) as previously described [14, 21]. Following the interview, each selected participant was requested to provide stool in stool containers after consenting to be part of the study with both parent/guardian consent and the child's assent. Part of the stool was used for miscroscopic detect of *S. mansoni* eggs and ruling out other helminths by Kato Katz (KK) and the rest was frozen immediately in liquid nitrogen to be transported to the laboratory for molecular detection of helminths by qPCR in the laboratory. The enrolled participants additionally provided peripheral blood in EDTA tubes (BD Biosciences, US) for plasma separation in the field. Briefly,

to obtain the plasma, 4 ml of venous collected in an EDTA tube was centrifuged at 5000 rpm to separate the sample into packed cells and plasma. The plasma was temporarily stored in liquid nitrogen while in the field and transfered to -80^{0}C freezer in the central laboratory for quantification of schistosome infection intensity by the Circulating Anodic Antigen (CAA) test. The packed cells were lysed using Red Blood Cell (RBC) lysis solution (Qiagen) and the remaining nucleated cells stored in liquid nitrogen in which they were transprted to the laboratory for *P. falciparum* infection testing by qPCR.

Circulating Anodic Antigen (CAA) assay for S. mansoni infection

To further classify infection intensity, CAA levels were measured in plasma using the up-converting phosphor lateral flow-circulating anodic antigen (UCP-LF CAA) assay [23]. A standard curve was generated using human negative serum spiked with a known concentration of CAA standard (100,000 pg/ml) and diluted up to eight standard points, with two negative controls. Briefly, 50 µl of each of the plasma and standards was mixed with 50 µl of 4% trichloroacetic acid (TCA; Merck Life Science NV, the Netherlands),vortexed and incubated for 5 minutes at room temperature. Following centrifugation at 13000rpm for 5 minutes, 20µl of the supernatant was incubated in the wells containing 100 ng dry UCP particles (400 nm Y2O2S: Yb3+, Er3+) coated with mouse monoclonal anti-CAA antibodies hydrated with 100µl of high salt lateral flow buffer (HSLF: 200 mM Tris pH8, 270 mM NaCl, 0.5% (v/v) Tween-20, 1% (w/v) BSA) for 1 hour at 37^{0}C while shaking at 900 rpm. Pre-labelled CAA lateral flow strips were placed in the wells on the UCP plate and allowed to flow. The strips were left to dry overnight and quantified using the Labrox Upcon scanner (Labrox Oy, Finland) from which CAA concentrations were calculated in pg/ml. The test line signals (T; relative fluorescent units, peak area) were normalized to the flow control signals (FC) of the individual strips and the results were expressed as ratio values.

Plasmodium falciparum detection

To determine *P. falciparum* presence, DNA was extracted from whole blood pellets using the QIAamp DNA Blood Mini Kit (Catalogue number 51106, QIAGEN) following the manufacturer’s manual. The real-time PCR was performed with the ABI 7500 Fast Real-time machine and data processed using 7500 Fast Systems software version 1.5.1. A final volume of 25 µl containing 2 µl of DNA 1 µl of PhHV DNA (internal control) and 22 µl of PCR master mix made of HotStarTaq Master Mix (Catalogue Number 203446), primers, and probes was added (**Table S1**). A pool of DNA extracted from *P. falciparum* positive samples was used to set serially diluted standards tested alongside the samples on every plate run. The following cycling parameters were used; 95°C for 15 min; 95°C for 15 sec; 60°C for 30 sec; and 72°C for 300 sec, steps 3–5 repeated 50 times.

Helminth detection

Frozen stool samples were used for detecting helminths in stool by qPCR. Briefly, helminth DNA was extracted from stool using the Fast DNA Spin Kit for Feces (catalogue number 116570200, MP Biomedicals Germany GmbH) to determine (using multiplex real-time PCR) *Schistosoma mansoni* (*S. mansoni*), *Strongyloides stercoralis* (*S. stercoralis*) and *Necator americanus* (*N. americanus*) infections.

The real-time PCR was performed with the ABI 7500 Fast Real-time machine and data processed using 7500 Fast Systems software version 1.5.1. A final volume of 25 µl containing 2 µl of DNA 1 µl of PhHV DNA (internal control) and 22 µl of PCR master mix made of HotStarTaq Master Mix (Catalogue Number 203446), primers, and probes was added (**Table S1**). The following cycling parameters were used; 95°C for 15 min; 95°C for 15 sec; 60°C for 30 sec; and 72°C for 300 sec, steps 3–5 repeated 50 times.

Identification of DEGs

To identify differentially expressed genes (DEGs), RNA was extracted, purified, sequenced and the reads aligned as described previously [21]. Differentially expressed genes between phenotypes were identified using DESeq2[24]. Analysis of read counts for each gene considered *P. falciparum / S. mansoni* coinfection the independent variable with age and sex as covariates. Principal component analysis was done using PCA Explorer to determine the clustering of the genes [25]. Genes were considere significantly if they had adjusted p-value < 0.05, Log2 (FC) > 1.0 for up-regulated genes and Log2 (FC) $<$ -0.8 for down regulated genes. Pairwisw analysis for differeential expression of genes was done for 1) 1) *P. falciparum* and *S. mansoni* coinfected vs uninfected children, 2) *P. falciparum and S. mansoni* coinfected vs *S. mansoni* only infected children and 3) *S. mansoni* only vs uninfected including sex and age as covariates.

Statistical analysis

Data analysis was done in R software version 4.22. For all the parasites tested, qPCR cycle threshold values (Ct-values) above 40 were classified as negative. PCR Ct-values are inversely proportional to infection intensity i.e; individuals with low Ct-value had higher infection intensity and those with high Ct-values had low infection intensity. CAA concentrations > 30 pg/mL were classified as positive; negative (CAA < 30 pg/mL). Additionally, CAA levels from 30–1000 pg/mL were classified as low infection intensity and CAA > 1000 pg/mL as high infection intensity as adapted from Corstjens et al [26] and with advice from the assay development team at the Leiden University Medical Centre, The Netherlands (Leiden Diagnostic Research Group | LUMC). Pearson's correlation test and linear regression analysis were used to determine correlation between *P. falciparum* infection and height by age z-scores. The Fisher exact test was done to establish whether the coinfections were more or fewer than expected by chance. DESeq2 was used to identify the differentially expressed genes (DEGs) for each pair. 1) *P. falciparum* and *S. mansoni* coinfected vs uninfected children, 2) *P. falciparum and S. mansoni* coinfected vs *S. mansoni* only infected children and 3) *S. mansoni* only vs uninfected including sex and age as covariates.

Results

A total of 210 children had samples matched with stool and CAA results of which 98 were female with mean age 11 and 112 were males with mean age 12. **(Table 1).** From the total study participants (210), 76.2% (160 /210) were *P. falciparum* positive whereas 91% (191/210) were *S. mansoni* positive as detected by quantitative PCR (qPCR). Of the *P. falciparum* positive participants 42.5% (68/160) were

female and 57.5% (92/160) were male. The difference in *P. falciparum* prevalence in males and females was significant with a Chi-squared p-value of 0.04 (**Figure 1A &B**). Of the *S. mansoni* positive, 46.6% (89/191) were female and 53.4% (102/191) were male but the differences in prevalence were not significant. Nyakagei village had the highest *P. falciparum* 92% (69/75) and *S. mansoni* 95% (71/75) infections. Alwi village, which is farther from the river, had the least infections with both *P. falciparum* 8% (1/12) and *S. mansoni* 33% (4/12) (**Figure 2)**.

Parasitic coinfections

Of the 210 children tested, 150 (71.4%) had coinfections with *P. falciparum* and *S. mansoni* with slightly less coinfections than expected by chance (Fisher exact test p-value of 0.02). Additionally, 41(19.5%) had only *S. mansoni* infection, 10 (4.8%) had only *P. falciparum* infection and 9 (4.3%) had neither infection (**Table 2**).

Association between *P. falciparum* infection and *S. mansoni* infection

There was a significant negative association (p-value = 7.3e-10, r^2 = -0.17) between *P. falciparum* infection and *S. mansoni* CAA **(Figure 3A)** and a significant positive correlation (p-value = 2.9e-06, r^2 = 0.10) between *P. falciparum* and *S. mansoni* infection intensity by qPCR (**Figure 3B**). A multivariate linear regression further showed an association between *P. falciparum* as well as *S. mansoni* with age, sex and sites (**Table 3**).

Distribution of *P. falciparum* and *S. mansoni* infection intensity by location

Alwi, which was furthest from the Albert Nile had the lowest *P. falciparum* and *S. mansoni* prevalence and infection intensities measured by the mean qPCR Ct-values **(Figure 2)**. Areas closer to the Albert Nile [14] had a higher infection intensity of both *P. falciparum* and *S. mansoni* compared to those further away. Nyakagei village had infections with highest intensity for *P. falciparum* as well as *S. mansoni* based on the mean Ct-value, followed sequentially by Kayonga, Dei, Kivuje and Panyigoro villages.

Gene expression differences between the *S. mansoni* and *P. falciparum* infected children and the uninfected infection

Similar to the previous findings, PCA analysis showed a separation of gene expression by gender (**Figure 4**). The number of differentially expressed genes for each comparison was summarised in **Table 4.** We identified 15 significant differentially expressed genes when coinfected children children (number of samples) were compared to the uninfected children (number of samples) of which 13 (87%) were upregulated and 2 (13%) were downregulated **(Figure 5).** Only 2 genes were significantly differentially expressed in the comparison of coinfected children to those with *S. mansoni* only as well as that of *S. mansoni* infected children and the uninfected (Table 4). The genes of the coinfected vs uninfected comparison included 6 protein coding genes (GAS6, DEXI, CEPT1, PALMD,

SAMD15 and RETREG1) and 9 long noncoding RNA (AC138028.4, GFOD1-AS1, AC034102.6, AC005153.1, AC020914.1, AC017028.2, AC244502.3, AC013486.1, AC106760.1) (**Table 5)**.

Expression of genes associated with *S. mansoni* infection

To identify differentially expressed gene in individuals with *S. mansoni* only, we conducted a comparison between children with *S. mansoni* infection alone children (number of samples) and the uninfected children (number of samples). We identified significant downregulation of 2 DEGs (AC092821.3 and PKDREJ) (**Table 5)**. We further compared *S. mansoni* infected with coinfected children and identified downregulation of (AC092821.3 and AC018761.2) (**Table 5)**.

Discussion

Parasitic coinfections have been reported in several studies, as recently reviewed [8], among which are *P. falciparum* and *S. mansoni* coinfection [6–8]. In this study, we present findings of a cross-sectional transcriptome analysis to determine the effect of *P. falciparum* and *S. mansoni* coinfections on the gene expression in peripheral blood of school age children aged between 10 - 15 years. Our data shows an association between real-time PCR and CAA as tests for *S. mansoni* detection. Our model however, showed only a weak correlation between the two methods of detection of *S. mansoni* with an r^2 value -0.27. The negative correlation is due to the fact that lower qPCR Ct-values are indicative of high infection intensity contrary to CAA for which higher values indicate high infection intensity.

Our findings recorded 76.2% *P. falciparum* and 91% *S. mansoni* infections among the 210 children tested. The frequency of *P. falciparum* infections was higher in males compared to females with Chi-squared p-value of 0.04. This may be attributed to a variety of factors including lifestyle as previously shown [11, 28, 29].

Our study area has a high prevalence of *S. mansoni* [14] as well as high *P. falciparum* transmission [30]. High *P. falciparum* infection intensities were observed in areas closer to the Albert Nile than those further away which may indicate high vector infestation in proximity to the Nile. Coinfection of *P. falciparum* and *S. mansoni* is common in areas where both parasites are endemic. In this study, we recorded 71.4% coinfections with *P. falciparum* and *S. mansoni* using PCR for the two parasites. Previous findings in Ethiopia showed that individuals with high *S. mansoni* intensity had high *P. falciparum* intensity [17] contrary to previous findings among pre-school children in Uganda [31]. Our data showed a weak correlation between *P. falciparum* and *S. mansoni* infection intensity in children between 10 -15 years living along the Albert Nile with an absolute r^2 0.1 – 0.17 by Pearson's correlation coefficient. In Ethiopia, the 6-10 age group was reportedly more prone to severe malaria if coinfected with *S. mansoni* [17]. Whether the intensity is due to shared risk of proximity to the Albert Nile or pathogen factors within the parasites was not established in our study. Coinfections have been shown to alter susceptibility to pathogens hence altering the immune activity [32] and further affecting diagnosis which may lead to prolonged disease [8].

Coinfections have been shown to affect susceptibility to pathogens hence altering the immune activity [32] and further affecting diagnosis which may lead to prolonged disease [8]. This study also presented data on gene expression in peripheral blood of the *P. falciparum* and *S. mansoni* coinfected children. Gene expression was compared between all coinfected vs uninfected, *S. mansoni* infected vs uninfected, and coinfected vs *mansoni* infected. There was upregulation of 13 genes and downregulation of 2 genes when coinfected children were compared to the uninfected. Of note, most of the upregulated protein coding genes play a role in inflammation and fibrosis. The Growth arrest specific 6 (GAS6), plays an important role in inflammation and fibrosis [33] and also important in the immune response to malaria infection [34]. The DEXI gene is a candidate gene for type 1 IFN signalling pathway [35]. PALMD is involved in NF-kB mediated inflammation [36, 37]. Reticulophagy regulator 1 (RETREG1) involved in regulating Endoplasmic reticulum (ER) autophagy and inflammation [38] and choline/ethanolamine phosphotransferase 1 (CEPT1) were downregulated. Additionally, some long noncoding RNA (lncRNA) were upregulated (AC138028.4, GFOD1-AS1, AC034102.6, AC005153.1, AC020914.1, AC017028.2, AC244502.3, AC013486.1, AC106760.1).

Animal studies have shown that coinfection with *S. mansoni* is associated with an increase in *Plasmodium* parasitemia but reduced *S. mansoni* induced pathology [39]. Recent studies have reported increased risk of anaemia in individuals with both malaria and *S. mansoni* [6]. In our previous study, we observed high levels of stunting amongst participants but this had no association with *S. mansoni* infection. We also observed no correlation between *P. falciparum* infection and stunting contrary to previous studies [40].

Limitations of the study

Further analysis of pathways was not possible owing to the few differentially expressed genes. Validation of expressed genes by an additional laboratory method (e.g. qPCR or other method) would have been an added advantage.

Conclusions

In conclusion, our study shows a high prevalence of *P. falciparum* infection among 10–15 year old *S. mansoni* infected children living along the lake Albert with concurrent infections.. Furthermore, the data shows expression of genes associated with inflammation and fibrosis among children with *P. falciparum* and *S. mansoni* coinfection.

Declarations

Ethics approval and consent to participate

The study protocol was reviewed by the institutional review board of the Ministry of Health, Vector Control Division Research and Ethics Committee (Reference No. VCDREC106) and approved by the

Uganda National Council for Science and Technology (Reference No. UNCST HS 118). The study was conducted with guidance from the district health officials, including the selection and training of the village health teams that were involved in the mobilisation and recruitment of the children (10 -15 years) into the study. The objectives, potential risks and benefits of the study were explained to the parents/guardians, who signed written informed consent. The same was later explained to the school age children in English and Alur dialect before they provided written assent for participation in the study. If a child was observed to have *S. mansoni* eggs in their stool, they were offered free treatment, which consisted of praziquantel at a dosage of 40mg/kg administered by trained Ministry of Health personnel, assisted by district health workers. Point of care cathodic circulating antigen (POC-CCA) and *P. falciparum* results were not used as an indication for treatment. The processes and methods used were according to the relevant national and international standards and regulations.

Consent for publication

Not applicable.

Availability of data and material

The phenotype and RNA sequence data have been deposited in the European Genome-Phenome Archive with accession number EGAS00001007173, and can be accessed via the link https://ega-archive.org/search/EGAS00001007173.

Competing interests

The authors declare no conflict of interests.

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Authors' contributions

JN: Conceptualization, Methodology, Investigation, Administration, Formal analysis, Writing – Original Draft preparation; **OAN:** Formal analysis, Writing – review & editing; **MPK**: review & editing; **PN**: Formal analysis, Writing – review & editing; **JN:** Writing – review & editing**, AE:** Resources Writing – review & editing; **ME:** Writing – review & editing; **JK:** Writing – review & editing; **BN:** review & editing; **SB**: review & editing; **CMM:** review & editing; **HN:** Investigation, Supervision, Formal analysis, Writing – review & editing; **RT:** supervision,review & editing; **EM:** Investigation, Supervision, Resources, Writing – review & editing; **JM**: Conceptualization, Methodology, Investigation, Supervision, Formal analysis, Writing – review & editing.

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Tables

Table 1: Summary of parasitic infections by gender**.** Of the 210 individuals tested, 160(76%) were infected by *P. falciparum*, 191(91%) with *S. mansoni*. There were very few *N. americanus* and *S. stercolaris* infections among these children.

	Female	Male	Total	%positivity
Total	98	112	210	-
Mean Age	11.82	12.17	12.01429	-
Mean HAZ	-1.758980	-2.390179	-2.095619	-
P. falciparum	68	92	160	76%
S. mansoni	89	102	191	91%

Table 2: A summary of *P. falciparum* and *S. mansoni* infections by PCR (negative, mono and coinfections): There were 71% (150/210) concurrent infections with both *P. falciparum* and *S. mansoni* which was slightly less than the expected 69% (145.5/210) by chance (p-value = 0.02).

Exact values				**Expected values**	
Pathogen		***S. mansoni***			
P. falciparum		Negative	Positive	Negative	Positive
	Negative	9 (4.3%)	41 (19.5%)	4.5	45.5
	Positive	10 (4.8%)	150 (71.4%)	14.5	145.5

Table 3: Linear regression model *P. falciparum* PCR vs *S. mansoni* CAA

	Estimate	Std. Error	t value	pvalue	Odds Ratio
log(CAA)	-0.52	0.16	-3.351	0.000961 ***	0.59
Age	0.42	0.23	1.848	0.06 .	1.52
Sex	-1.47	0.73	-2.013	0.04 *	0.23
Alwi	5.64	1.99	2.831	0.005 **	282
Dei	2.54	1.19	2.130	0.03 *	12.6
Kayonga	0.46	1.09	0.424	0.67	1.58
Kivuje	2.96	1.15	2.561	0.01 *	19.2
Panyigoro	2.27	1.28	1.774	0.07	9.69

Significance. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1, Residual standard error: 5.135 on 201 degrees of freedom (DF), Multiple R-squared: 0.2564, Adjusted R-squared: 0.2268, F-statistic: 8.663 on 8 and 201 DF, p-value: 3.881e-10

Table 4: Summary of number of significant upregulated and downregulated DEGs in the different comparisons

Pair (total observations)	*Details*	*DEGs*	*Up regulated*	*Down regulated*
Coinfected vs uninfected (n=28)	Coinfected: 17 Uninfected: 11	15	13(87%)	2(13%)
S. mansoni only vs uninfected (n=15)	*S. mansoni*: 4 Uninfected: 11	2	0	2 (100%)
Coinfected vs S. mansoni only	Coinfected: 17 *S. mansoni*: 4	2	2 (100%)	0

Table 5: Differential expression of genes with different comparisons

***S.mansoni* and *P. falciparum* coinfected vs uninfected**

GeneName	pvalue	padj	log2Fold Change	Details
Growth arrest specific 6 (GAS6)	7.56E-07	0.005	1.01	• **Genetype:** Protein coding • **Description:** growth arrest specific 6 • **Functions:** • Regulation of cell growth and survival, cell adhesion and migration • Cytokine signaling during natural killer cell development, platelet activation and regulation of thrombotic responses • Immunosuppression • Binding molecule of TAM receptors in malaria
DEXI (Dexi Homolog)	9.35E-06	0.01	0.807	• **Genetype:** Protein coding • **Description:** Dexi homolog • **Functions:** • Associated with immunoglobulin A deficiency 1 and selective immunoglobulin deficiency disease • Unknown function - activated by immune suppressors • Over expression leads to increase of STAT1 and proinflammatory chemokines
CEPT1	1.98E-05	0.02	-0.58	• **Genetype:** Protein coding • **Description:** choline/ethanolamine phosphotransferase 1 • **Functions:** • Biosynthesis of phosphatidylethanolamine from CDP-choline and CDP-ethanolamine • Protein- dependent phospholipid transport to the lumen surface
PALMD (palmdelphin)	2.86E-05	0.03	1.21	• **Genetype:** Protein coding • **Description:** palmdelphin • **Functions:** • Regulation of cell shape • NF-κB-mediated inflammation • Glycolysis

SAMD15	6.68E-05	0.04	0.77	**Genetype:** Protein coding **Description:** sterile alpha motif domain containing 15 **Functions:** - Protection against chronic pain
RETREG1	4.28E-05	0.03	-0.71	**Genetype:** Protein coding **Description:** reticulophagy regulator 1 **Functions:** - Regulate autophagy
AC138028.4	3.09E-06	0.01	1	**Genetype:** lncRNA **Description:** novel transcript, antisense to FAM38A **Functions:** - Cell proliferation in cancer - Regulates FAM38A
GFOD1-AS1	9.50E-06	0.01	1.12	**Genetype:** lncRNA **Description:** GFOD1 antisense RNA 1
AC034102.6	1.37E-05	0.02	0.73	**Genetype:** lncRNA **Description:** novel transcript, antisense to ZC3H10 and ESYT1
AC005153.1	3.24E-05	0.03	1.59	**Genetype:** lncRNA **Description:** novel transcript, antisense to GRB10
AC020914.1	4.67E-05	0.03	1.15	**Genetype:** lncRNA **Description:** novel transcript, antisense to SIGLEC6
AC017028.2	6.55E-05	0.04	0.86	**Genetype:** lncRNA **Description:** novel transcript, antisense to HDAC4
AC244502.3	7.36E-05	0.04	1.03	**Genetype:** lncRNA **Description:** novel transcript
AC013486.1	7.88E-05	0.04	0.82	**Genetype:** lncRNA

GeneName	pvalue	padj	log2Fold Change	Details
				• **Description:** novel transcript, antisense to PML
AC106760.1	8.78E-05	0.04	0.95	• **Genetype:** lncRNA • **Description:** novel pseudogene
***S. mansoni* only vs uninfected**				
GeneName	**pvalue**	**padj**	**log2Fold Change**	**Details**
AC092821.3	5.49E-08	0.0005	-6.16	• **Genetype**: processed_pseudogene • **Description**: RAN binding protein (RANBP) pseudogene
PKDREJ	2.92E-07	0.0015	-1.91	• **Genetype:** protein_coding • **Description:** polycystin family receptor for egg jelly
***S. mansoni* only vs coinfected**				
GeneName	**pvalue**	**padj**	**log2Fold Change**	**Details**
AC092821.3	4.01E-08	0.0002	-6.41	• **Genetype**: processed_pseudogene • **Description**: RAN binding protein (RANBP) pseudogene
AC018761.2	2.94E-08	0.0002	-2.22	• **Genetype**: lncRNA • **Description:** novel transcript, antisense to PRDX2

Figures

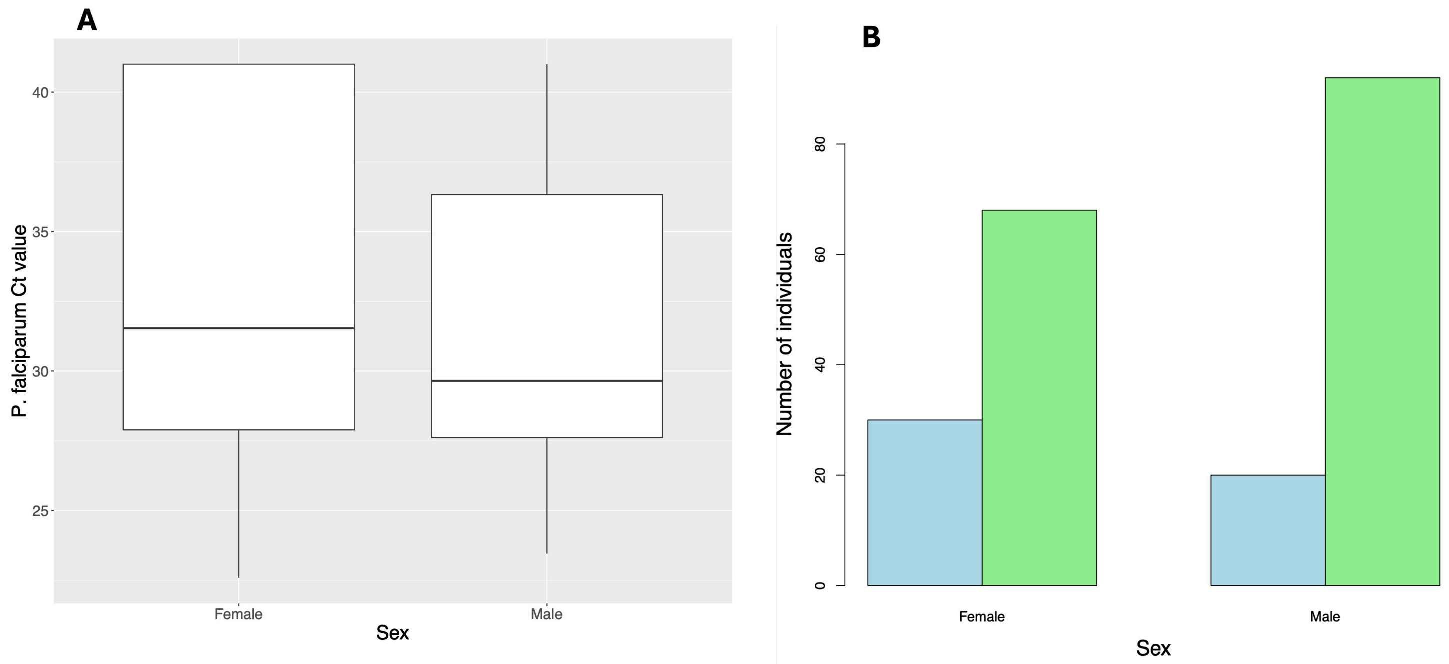

Figure 1

A.Boxplot showing distribution of *P. falciparum* infection among males and females. **B.** Bar graph showing the differences of *P. falciparum*infection between males and females.

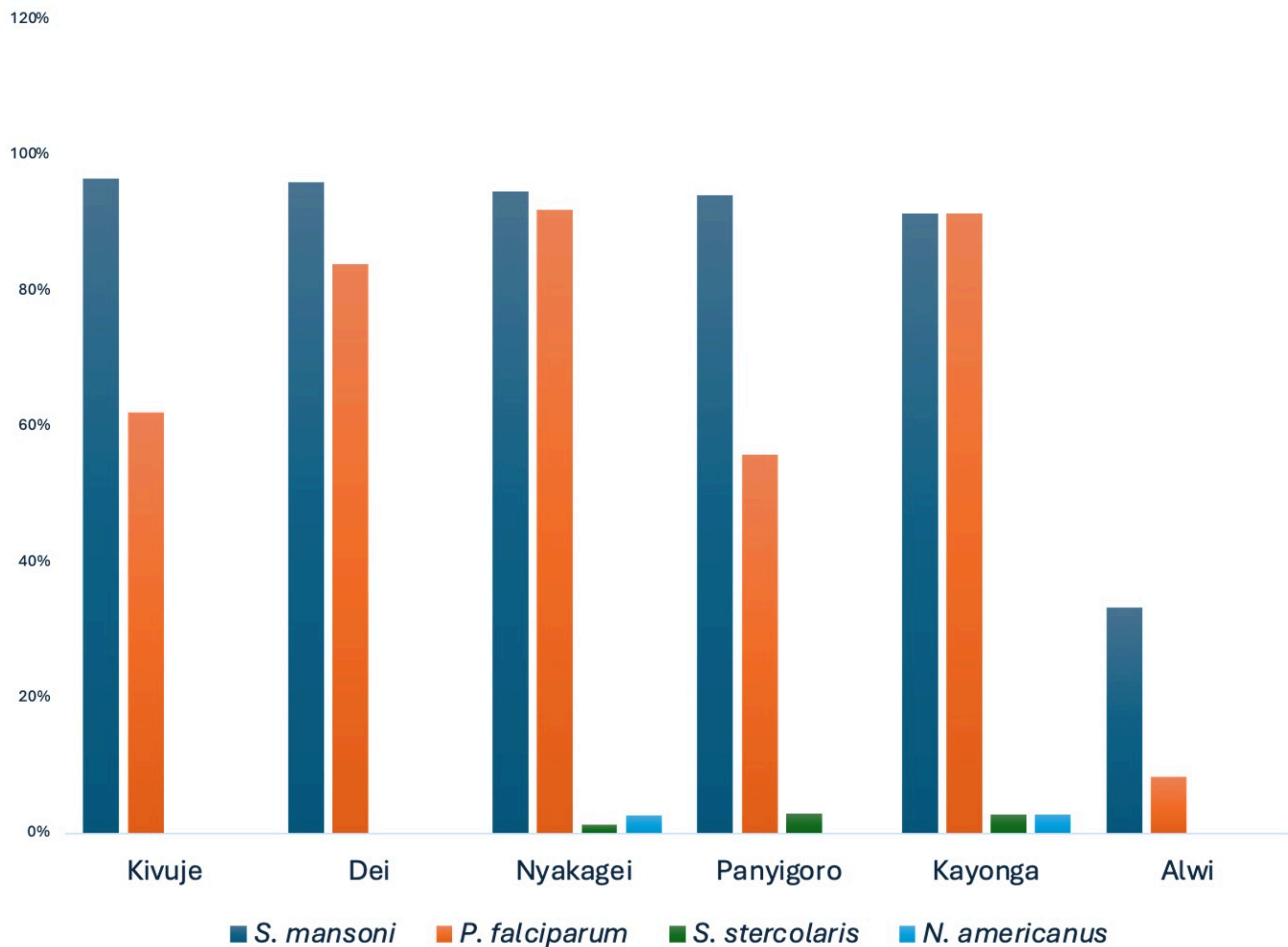

Figure 2

Graph showing the distribution of the different parasitic infections per site

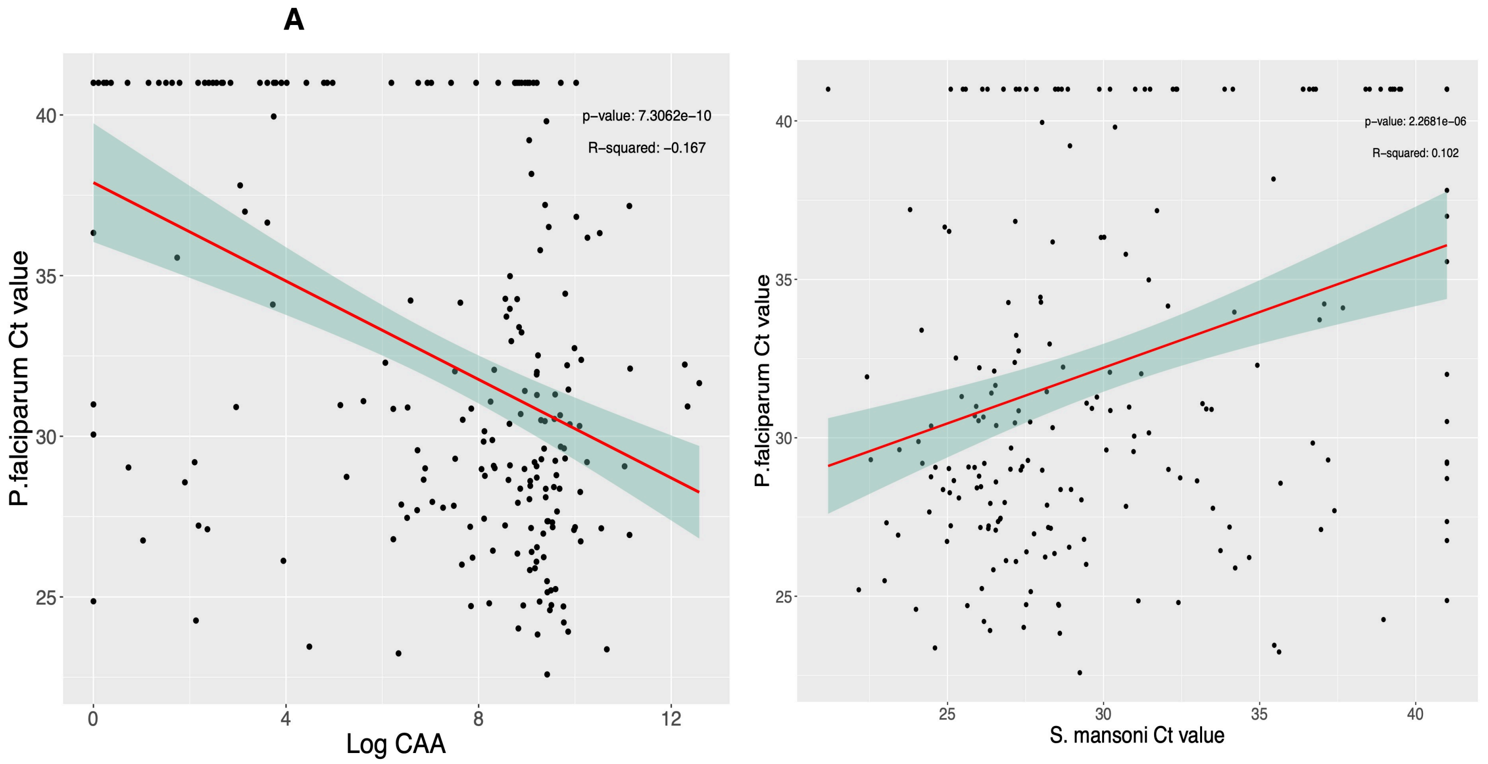

Figure 3

A. Correlation between *P. falciparum* infection intensity by PCR and *S. mansoni*infection intensity by CAA. **B.** Correlation between *P. falciparum*infection intensity by PCR and *S. mansoni* infection intensity by PCR

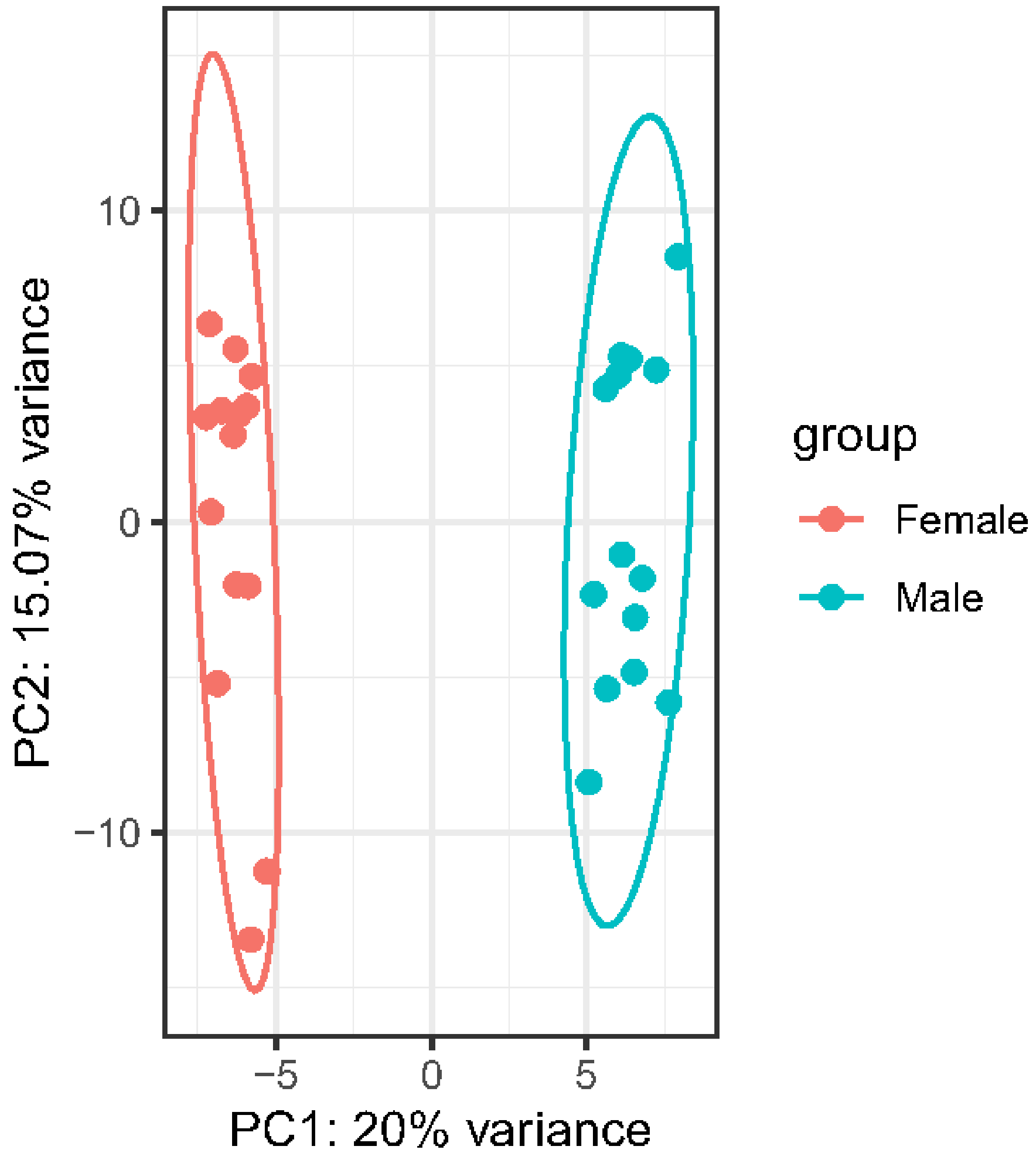

Figure 4

Principal component analysis showed clustering of differentially expressed genes by gender when *S. mansoni* and *P. falciparum* coinfected children were compared to uninfected.

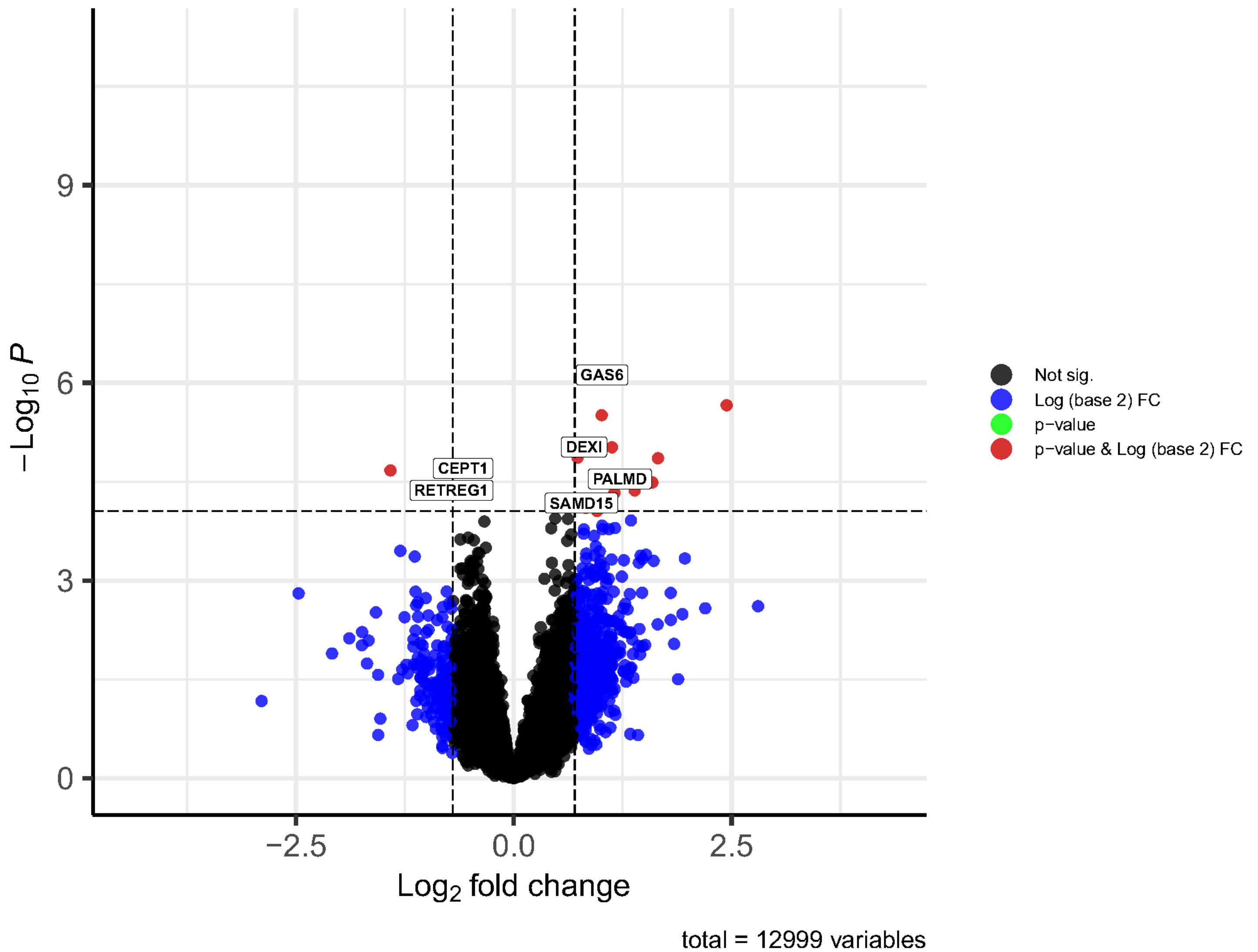

Figure 5

Volcano plot of differentially expressed genes between *P. falciparum* and *S. mansoni* coinfected and uninfected school age children

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarydata.docx