MAKERERE UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF BIOMEDICAL SCIENCES

MALARIA CIRCUMSPOROZOITE PROTEIN -SPECIFIC ANTIBODY TITRES AMONG HIV-INFECTED AND NON-HIV-INFECTED CHILDREN UNDER 5 YEARS OF AGE, RESIDENT IN AREAS OF HIGH MALARIA TRANSMISSION IN UGANDA

BY

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JULY 2025

DECLARATION

I, SSEMWANGA MOSES, declare that the work presented in this document is my original Master's Research proposal and has never been submitted either partially or fully to any University or institution for any award.

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Table of Contents

DECLARATION ii
APPROVAL ..... **Error! Bookmark not defined.**
COPYRIGHT STATEMENT v
ACKNOWLEDGEMENT ix
ABBREVIATIONS x
OPERATIONAL DEFINITIONS xi
ABSTRACT 1
CHAPTER ONE: INTRODUCTION. 3
1.1 BACKGROUND 3
1.2 PROBLEM STATEMENT 4
HYPOTHESIS ..... **Error! Bookmark not defined.**
Primary Hypothesis ..... **Error! Bookmark not defined.**
Secondary Hypotheses ..... **Error! Bookmark not defined.**
RESEARCH QUESTIONS 6
OBJECTIVES 7
GENERAL OBJECTIVE 7
SPECIFIC OBJECTIVES 7
SIGNIFICANCE 8
JUSTIFICATION 9
CONCEPTUAL FRAMEWORK 10
CHAPTER TWO: LITERATURE REVIEW 11
Malaria and HIV Co-Endemicity in Sub-Saharan Africa 11
Role of CSP-Specific IgG in Malaria Immunity and Vaccination 13
Impact of HIV Infection and Sero-Exposure on Malaria Immunity 15
Cotrimoxazole Prophylaxis and Its Effect on Malaria Immunity 17
Environmental and Clinical Factors Influencing Malaria Exposure and Immunity 19
Environmental Factors 19
Clinical Factors 20
CHAPTER 3: MATERIALS AND METHODS 22
Study Design 22
Study setting 22
Population 23
Target Population 23
Accessible population 23
Study Population 23

Eligibility Criteria 24
Inclusion Criteria: ..... **Error! Bookmark not defined.**
Exclusion Criteria: ..... **Error! Bookmark not defined.**
Sample Size Estimation 24
Sampling Technique 27
Data Collection 27
Laboratory analysis at the Makerere immunology lab 29
To determine the malaria-specific circumsporozoite antibody titers 31
Quality Control 31
Data Analysis 31
Data Management and Analysis 32
Ethical Considerations 34
RESULTS 35
Participant Characteristics and baseline data 35
Effect of Cotrimoxazole Prophylaxis on CSP-Specific IgG Titres 38
Influence of ART Adherence on CSP-Specific IgG Titres (HIV-infected only) 40
Impact of Environmental and Clinical Factors on CSP-Specific IgG Titres 40
Logistic Regression for Binary Outcome (Titres >500 ng/L) 42
Interaction and Sensitivity Analyses 42
DISCUSSION 43
Influence of HIV Status on Malaria Antibody Responses 43
Effect of Cotrimoxazole Prophylaxis 44
ART Adherence and Antibody Titres 45
Demographic and Environmental Factors 45
Comparison with Existing Literature 46
Strengths and Limitations 46
Public Health and Policy Implications 47
CONCLUSION 48
RECOMMENDATIONS 50
REFERENCES 52

Table of tables

Table 1: Baseline demographics and key characteristics by group. Values are n (%) or mean±SD. P-values from χ^2 or Kruskal–Wallis tests as appropriate. 36
Table 2: Multivariable linear regression of log(CSP titre) (adjusted geometric mean ratios). 38
Table 3: Multivariate mixed effects model on effect of Cotrimoxazole on CSP –specific IgG titres 39
Table 4: CSP titre by ART adherence among HIV-infected children. 40
Table 5: Adjusted associations of clinical/environmental factors with log(CSP titre) (mixed-effects model, controlling for HIV/CTX status, age, sex, malaria history, income; district as random effect). 41
Table 6: Spearman correlations among key continuous variables. 42
Table 7: Binary outcomes for CSP- specific IgG titres 42
Table 8: Mixed effects model exploring interaction terms. 43

Table of figures

Figure 1: Conceptual Framework for Factors Influencing CSP-Specific IgG Antibody Titres in Children Under 5 Years in High Malaria Transmission Areas of Uganda. This diagram illustrates the hypothesized relationships between HIV status (HIV-infected, HIV-negative sero-exposed, HIV-negative non-sero-exposed), cotrimoxazole prophylaxis, ART adherence, demographic factors (age, malaria history), and environmental factors (ITN use, proximity to stagnant water) and their impact on circumsporozoite protein (CSP)-specific IgG antibody titres 10
Figure 2: Barua et al., 2019; Map of the incidence of malaria in 2020 showing the number of people living with HIV (in millions) in 2022 in areas where malaria is transmitted. HIV data are point estimates (range). Map reproduced from OurWorldinData.org (under the CC BY 4.0 license).8 HIV data are from UNAIDS.7 *Incidence is the number of new cases of malaria in a year per 1000 people at risk. reference....13
Figure 3: Malaria vaccine approaches....15

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ABBREVIATIONS

ACT: Artemisinin-based Combination Therapies

ADCC: Antibody Dependent Cellular Cytotoxicity

AIDS: Acquired Immunodeficiency Syndrome

AMA: Apical Membrane Antigen

APC; Antigen Presenting Cell;

ART: Antiretroviral Therapy

CM: Central Memory

CSP: Circumsporozoite protein (CSP)

ELISA: Enzyme Linked Immunosorbent Assay

EMA: European Medicines Agency

HBsAg: Hepatitis B Surface Antigen

HEU: HIV-exposed uninfected

HUU: HIV-unexposed uninfected

HIV: Human Immunodeficiency Virus

IDI: Infectious Disease Institute

Ig: Immunoglobulin

IL: Interleukin

ITN: Insecticide-Treated bed Nets

JCRC: Joint Clinical Research Centre

LPS: Lipopolysaccharide

MSP: Merozoite Surface Proteins

PAMP: Pathogen Associated Molecular Patterns

PBMC: Peripheral blood mononuclear cells

PBS: Phosphate Buffered Saline

PD: Programmed Death

PRR: Pattern Recognition Receptors

RBC: Red Blood Cell

SST: Serum Separating Tube

UPHIA: Uganda Population-Based HIV Impact Assessment

VSA: Variant Surface Antigen

WHO: World Health Organization

OPERATIONAL DEFINITIONS

Malaria: Malaria is a severe disease caused by parasites of the genus *Plasmodium*, which is transmitted to humans by a bite of an infected female mosquito of the species *Anopheles* (Talapko et al., 2019)

HIV: HIV belongs to the Retroviridae family in the *Lentivirus* genus. The virus mainly targets CD4+ T-lymphocyte helper cells, leading to an extreme form of immune subversion with a continuous loss of these cells. This weakens the immune system and causes many clinical manifestations of this disease (Swinkels et al., 2024).

ELISA: Enzyme immunoassays (EIAs) use the catalytic properties of enzymes to detect and quantify immunologic reactions (Alhajj et al., 2023)

Sero-exposure: This refers to the state of having been exposed to a specific pathogen, as indicated by the presence of antibodies in the blood (Kahungu et al., 2018)

ABSTRACT

Background: Malaria and HIV co-endemicity presents a major public health burden in sub-Saharan Africa, with young children bearing the greatest morbidity and mortality. The *Plasmodium falciparum* circumsporozoite protein (CSP) is the antigenic target of leading malaria vaccines such as, RTS,S/AS01, where CSP-specific IgG antibodies are critical mediators of protection. HIV infection may dysregulate humoral immunity through germinal center attrition, impaired isotype switching, and depletion of long-lived plasma cells, potentially diminishing anti-CSP responses and heightening malaria vulnerability. Furthermore, HIV-exposed uninfected (HEU) children—those born to HIV-positive mothers but uninfected themselves—may exhibit altered immune function due to in utero and perinatal exposures. Understanding the landscape of malaria-specific immunity, as measured by CSP-IgG titres, among HIV-infected (HIV+), HEU, and HIV-unexposed uninfected (HUU) children under five years in Uganda's high-transmission zones is essential yet remains inadequately characterized. This study investigated the impact of HIV status and sero-exposure on CSP-IgG titres to inform targeted prevention and vaccination strategies in co-endemic settings.

Methods: We conducted a hospital-based cross-sectional study from February 2025 to June 2025 among 206 children aged less than 5 years residing in five high malaria transmission districts of Eastern Uganda (Sironko, Budaka, Kibuku, Mbale, Pallisa). Participants were stratified into three groups: HIV+ (n=69), HEU (n=69), and HUU (n=68). Standardized questionnaires captured demographic (age, sex, district, setting, household income), clinical (HIV status, ART adherence, cotrimoxazole prophylaxis [CTX] and adherence, malaria history within past year), and environmental factors (insecticide-treated net [ITN] use frequency/condition, indoor residual spraying [IRS] in past year, presence of stagnant water). CSP-specific IgG titres (ng/L) were quantified using standardized enzyme-linked immunosorbent assay (ELISA). Log-transformed titres were analyzed using multivariable linear regression, adjusting for age, malaria episode history, ITN use, household income, setting, and crucially, CTX use. Models accounted for district-level clustering using mixed-effects approaches. Sensitivity analyses assessed robustness to outliers and missing data.

Results: HIV+ children (all on CTX prophylaxis) exhibited significantly lower geometric mean CSP-IgG titres (351.7 ng/L; median: 218 ng/L, IQR: 126-714) compared to HEU (412.4 ng/L; median: 330 ng/L, IQR: 196-600) and HUU children (536.9 ng/L; median: 506 ng/L, IQR: 298-968; Kruskal-Wallis $p<0.001$). Strikingly, among HEU children, those receiving CTX (n=25) had markedly lower CSP IgG titres (mean: 294.2 ng/L; median: 248 ng/L) than HEU children not on CTX (n=44; mean: 622.7 ng/L; median: 580 ng/L; Mann-Whitney U ($p<0.001$). Adjusted regression confirmed HIV+ status was associated with a 42% reduction in log-transformed titres ($\beta = -0.42$, 95% CI: -0.65, -0.19; $p<0.001$)

relative to HUU children. Increasing age (β = 0.02 per month, p<0.01) and number of prior malaria episodes (β = 0.15 per episode, p<0.001) were independently associated with higher titres. Suboptimal vector control was evident since IRS coverage was minimal (4.9%), and ITN use, while widespread, was often inconsistent ("sometimes").

Conclusion: HIV infection is independently associated with substantially impaired acquisition of malaria CSP-specific antibodies in young Ugandan children, potentially increasing their biological susceptibility in high-transmission settings. The profound suppression of titres among both HIV+ and CTX-using HEU children likely reflects the combined effect of HIV-related immune dysregulation and the confounding prophylactic effect of CTX in reducing antigenic exposure. While consistent ART adherence may attenuate HIV-associated immunosuppression, its specific role in immune response to the CSP antigen warrants further study. Critically low IRS coverage and inconsistent ITN use underscore persistent environmental risks. Our findings advocate for integrated HIV-malaria control that includes optimizing ART and CTX adherence, while simultaneously scaling up effective vector interventions (ITN distribution campaigns, expanded IRS) for HIV-affected children. These findings advocate for integrated HIV-malaria care that prioritizes consistent vector control and considers tailored malaria vaccination strategies for HIV-affected children to mitigate their increased biological susceptibility..

CHAPTER ONE: INTRODUCTION.

1.1 BACKGROUND

Malaria and human immunodeficiency virus (HIV) co-endemicity epitomize a convergent public health crisis across sub-Saharan Africa, driving synergistic morbidity and mortality among immunologically vulnerable populations, particularly children under five years of age. In 2023, the World Health Organization (WHO) documented 249 million global malaria cases, with 94% occurring in Africa; Uganda alone reported 478 cases per 1,000 population among children in hyperendemic regions (Organization, 2023). Concurrently, HIV prevalence persists at 5.3% among Ugandan adults, rising to 7% in adolescents in high-burden districts like Mbale (Kwarteng, 2025). This syndemic interaction potentiates disease severity through HIV-induced CD4+ T-cell depletion and B-cell dysregulation which impair antimalarial immunity, increasing risks of severe malaria, treatment failure, and mortality (González et al., 2018). Young children whose adaptive immunity is ontogenetically immature and further compromised by HIV represent a critical population for investigating malaria-specific immune correlates.

The *Plasmodium falciparum* circumsporozoite protein (CSP), a dominant surface antigen essential for hepatocyte invasion, constitutes the foundation of leading malaria vaccines, including WHO-recommended RTS,S/AS01 (Datoo et al., 2021). CSP-specific IgG antibodies neutralize sporozoites via inhibition of gliding motility and cell traversal, thereby preventing liver-stage establishment (RTS,S Clinical Trials Partnership, 2015). However, HIV infection disrupts humoral immunity through germinal center attrition, impaired isotype switching, and depletion of long-lived plasma cells which are mechanisms that may compromise CSP-IgG quantity, quality, and durability (Cagigi et al., 2014). Furthermore, HIV-exposed uninfected (HEU) children exhibit immune alterations due to in utero antigen exposure, maternal inflammation, and epigenetic reprogramming, potentially modifying malaria antibody responses (Dauby et al., 2012). Cotrimoxazole prophylaxis, a cornerstone of HIV/HEU care confers partial malaria protection by inhibiting dihydrofolate reductase yet paradoxically may limit natural antibody acquisition by reducing antigenic exposure (Church et al., 2015).

Despite these insights, critical knowledge gaps persist. First, paediatric CSP-IgG dynamics remain poorly characterized in high-transmission zones, with extant literature predominantly focused on adults or older children (Pohl & Cockburn, 2022). Second, the immunological phenotype of HEU children, a rapidly expanding demographic is understudied in malaria-endemic contexts, particularly regarding how maternal ART during pregnancy or breastfeeding and cotrimoxazole prophylaxis (received by the HEU child) interact to influence the development of malaria-specific antibody responses, such as those

targeting CSP . Third, environmental drivers of exposure such as inconsistent insecticide-treated net [ITN] use, <10% indoor residual spraying [IRS] coverage in Eastern Uganda remain unquantified in immune models (Uganda Ministry of Health, 2023). This gap obscures how vector control modulates antigenic stimulation and titre accrual.

Grounded in syndemic theory, which posits that HIV and malaria interact biologically and socially to amplify disease burden, this hospital-based study investigated CSP-specific IgG titres among HIV-infected, HIV-exposed uninfected, and HIV-unexposed uninfected children under five years. The cohort resided in Uganda's high-transmission districts including Sironko, Budaka, Kibuku, Mbale, and Pallisa, selected for their extreme malaria endemicity exceeding 400 cases per 1,000 population and heterogeneous HIV prevalence ranging from 5% to 10% (Zalwango et al., 2023). We hypothesized that HIV infection and HIV-exposed uninfected status independently associate with reduced CSP-IgG titres, with cotrimoxazole prophylaxis and environmental factors acting as significant modifiers.

Conducted from February to June 2025, this research addressed three interconnected aims: First, it quantitatively compared CSP-IgG titres across the three paediatric groups (HIV infected, HIV exposed uninfected (HEU) and HIV –unexposed uninfected (HUU)) to delineate the independent impact of HIV status on malaria-specific humoral immunity. Second, it evaluated how cotrimoxazole prophylaxis and antiretroviral therapy adherence modulate antibody titres, explicitly testing for interaction effects between HIV status and chemoprevention. Third, it characterized associations between antibody levels and environmental exposures including insecticide-treated net usage patterns, indoor residual spraying coverage, and proximity to stagnant water to contextualize immune responses within real-world transmission dynamics. Collectively, these findings could inform targeted malaria prevention strategies, optimize RTS,S vaccine deployment frameworks, and advance integrated HIV-malaria control policies for vulnerable paediatric populations in co-endemic Africa.

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1.2 PROBLEM STATEMENT

Malaria and HIV co-endemicity perpetuates a devastating syndemic across sub-Saharan Africa, with Uganda's high-transmission districts exemplifying its disproportionate impact on children under five years. In 2023, Uganda reported 478 malaria cases per 1,000 populations, where under-5s bore 65% of hospitalizations and 70% of malaria-attributable deaths (WHO, 2024).

Concurrently, HIV prevalence remains high 5.3% adults, 7% adolescents in Mbale District specifically(

Uganda AIDS Commission, 2024), inducing CD4+ T-cell depletion and germinal center dysfunction that cripple antimalarial humoral immunity (González et al., 2018). The circumsporozoite protein (CSP) a cornerstone of *Plasmodium falciparum* sporozoite invasion and target of RTS,S/AS01 vaccine elicits IgG antibodies critical for neutralizing pre-erythrocytic parasites (Datoo et al., 2021). However, HIV infection disrupts B-cell maturation, impairing high-affinity CSP-IgG production and potentially compromising vaccine efficacy (Cagigi et al., 2014). Further complexity arises in HIV-exposed uninfected (HEU) children, whose in utero exposure to maternal HIV viremia and inflammation during gestation/breastfeeding may dysregulate immune ontogeny, altering malaria-specific antibody responses (Dauby et al., 2012).

Despite this biological convergence, critical knowledge gaps obstruct evidence-based interventions. First, CSP-IgG titres which is a validated correlate of RTS,S vaccine efficacy remain uncharacterized in Ugandan children under five across HIV status strata (HIV-infected, HEU, HUU), particularly in high-transmission zones where immune forces develop under intense antigenic pressure. Existing studies focus predominantly on adults, neglecting pediatric immunological vulnerabilities. Second, the impact of widespread cotrimoxazole prophylaxis used by 70% of HIV-infected and 36% of HEU children in endemic Uganda is poorly contextualized: while it reduces malaria incidence by inhibiting parasite dihydropteroate synthase (Church et al., 2015), its indirect suppression of antigen-driven antibody maturation may confound HIV-related immune deficits. Third, environmental determinants of exposure such as <5% Indoor Residual Spraying (IRS) coverage, inconsistent Insecticide Treated mosquito Nets (ITN) use in Eastern Uganda remain unintegrated into immune models, obscuring how real-world transmission heterogeneity erodes naturally acquired immunity and modulates titre acquisition (Uganda Ministry of Health, 2023).

This paucity of data directly impedes public health action. Without understanding how HIV or HEU status, cotrimoxazole prophylaxis, and environmental risks interact to shape CSP-IgG titres, Policymakers lack evidence to: (1) optimize RTS,S deployment schedules for maximal seroprotection in HIV-affected children; (2) tailor cotrimoxazole protocols without compromising antibody maturation; (3) prioritize vector control in exposure hotspots. Consequently, this vulnerable population remains at risk of vaccine underperformance and preventable malaria morbidity. Our study addressed these voids by quantifying CSP-IgG titres among HIV-infected, HEU, and HUU children under fives in Uganda's high-transmission districts (Feb–Jun 2025), evaluating HIV- and environment-driven perturbations in malaria immunity to inform integrated control strategies in co-endemic Africa

RESEARCH QUESTIONS

1. How do CSP-specific IgG antibody titres differ among HIV-infected, HIV-negative sero-exposed, and HIV-negative non-sero-exposed children under 5 years in high malaria transmission areas of Uganda?
2. What is the impact of cotrimoxazole prophylaxis on CSP-specific IgG titres in HIV-infected and HIV-negative sero-exposed children?
3. How does antiretroviral therapy adherence influence CSP-specific IgG titres in HIV-infected children?
4. To what extent do demographic (age, gender) and environmental factors (malaria history, ITN use, IRS, stagnant water) affect CSP-specific IgG titres across these groups?

OBJECTIVES

GENERAL OBJECTIVE

To determine the influence of HIV status, HIV-exposure, cotrimoxazole prophylaxis, ART adherence, and demographic and environmental factors on CSP-specific IgG antibody titres among children under 5 years in high malaria transmission districts of Uganda, to inform malaria prevention and vaccine strategies.

SPECIFIC OBJECTIVES

1. To compare CSP-specific IgG titres among HIV-infected, HIV-exposed Uninfected, and HIV-Unexposed Uninfected children under 5 years.
2. To determine the effect of cotrimoxazole prophylaxis on CSP-specific IgG titres in HIV-infected and HIV-negative sero-exposed children, comparing titres between those on and not on cotrimoxazole.
3. To evaluate the association between ART adherence levels and CSP-specific IgG titres in HIV-infected children, adjusting for potential confounders.
4. To investigate the influence of demographic (age, sex) and environmental factors (malaria history, ITN use, IRS, stagnant water) on CSP-specific IgG titres in selected districts in Eastern Uganda.

SIGNIFICANCE

This study holds significant public health, clinical, and scientific importance within the broader context of infectious disease management in sub-Saharan Africa. As Uganda prepares for national scale-up of malaria vaccines such as RTS,S/AS01 and R21, understanding the baseline humoral immunity against malaria in diverse pediatric populations is essential. This study is one of the first to quantify and compare CSP-specific IgG antibody titres among HIV-infected, HIV-exposed uninfected (HEU), and HIV-unexposed children under five years in high malaria transmission settings. It thus generates foundational evidence on whether these subgroups possess differential susceptibility to malaria based on their immunological profiles.

The research also provides critical insights into how routine HIV interventions such as cotrimoxazole prophylaxis and ART adherence may modulate malaria-specific immunity. These insights are particularly relevant given WHO and national guidelines promoting cotrimoxazole for HIV-infected and HEU children as part of comprehensive pediatric HIV care. By revealing potential unintended consequences on malaria immunity, this study helps policymakers anticipate and address possible trade-offs in integrated child health programs.

Scientifically, the work advances our understanding of host-pathogen interactions and immune ontogeny in co-infected or co-exposed children. It explores how HIV-driven immune perturbations intersect with antigen exposure, age-related immune maturation, and environmental risk factors to shape anti-malarial antibody acquisition. The use of precise serological measurements and robust statistical methods such as multivariate linear regression and mixed-effects modeling ensures that the conclusions drawn are analytically sound and generalizable within similar high-transmission contexts.

Clinically, the findings will help pediatricians and program managers identify children who may require enhanced malaria surveillance, alternative prophylactic strategies, or tailored vaccine schedules. For example, children with low CSP-specific antibody titres may benefit from prioritized access to malaria vaccines or booster dosing regimens.

This research fills a critical knowledge gap at the intersection of malaria and HIV immunology, with direct implications for policy, clinical practice, and future research. It contributes to the global agenda of equitable and integrated child health interventions and supports Uganda's efforts to reduce the dual burden of malaria and pediatric HIV through data-driven, context-sensitive strategies.

JUSTIFICATION

Malaria and HIV remain among the leading causes of morbidity and mortality in sub-Saharan Africa, with Uganda bearing a disproportionate burden of both infections. Children under five years of age are particularly vulnerable to malaria, while pediatric HIV infection and exposure are common due to vertical transmission. Although extensive research has been conducted independently on both malaria immunity and pediatric HIV, there is a paucity of data exploring the intersection of these two diseases particularly about how HIV infection and in utero HIV exposure influence naturally acquired immune responses to malaria. This gap is especially critical in high transmission settings, where repeated exposure to Plasmodium falciparum is expected to result in the gradual acquisition of protective immunity during early childhood.

The circumsporozoite protein (CSP), a dominant antigen on the surface of P. falciparum sporozoites, is the principal target of the RTS,S/AS01 malaria vaccine. Naturally acquired IgG antibodies against CSP have been linked to partial protection against clinical malaria, yet the dynamics of this response in children living with HIV or born to HIV-positive mothers are poorly understood. Given that HIV can profoundly dysregulate both innate and adaptive immune responses including impairments in B-cell maturation, antibody production, and immune memory the assumption that HIV-infected or HIV-exposed uninfected (HEU) children develop comparable anti-malarial immunity to their HIV-unexposed peers is unsubstantiated.

Furthermore, widespread use of cotrimoxazole prophylaxis and ART in HIV programs may have unintended consequences for malaria immunity. Cotrimoxazole has anti-malarial properties that may reduce parasitemia and, by extension, antigenic stimulation required for antibody generation. Conversely, ART may aid immune reconstitution, yet its effect on malaria-specific antibody responses remains unclear. These clinical realities underscore the urgent need to assess how these interventions, in combination with demographic and environmental exposures, shape CSP-specific immunity in vulnerable pediatric subpopulations.

By generating locally relevant data on antibody titres across well-defined HIV exposure and treatment strata, this study addresses a critical evidence gap. It leverages quantitative serology and multivariate analytical techniques to elucidate the immunological landscape of malaria in HIV-affected children in Uganda. The findings will not only inform vaccine readiness and deployment strategies but will also guide integration of malaria and HIV control programs, thereby enhancing the effectiveness of both.

CONCEPTUAL FRAMEWORK

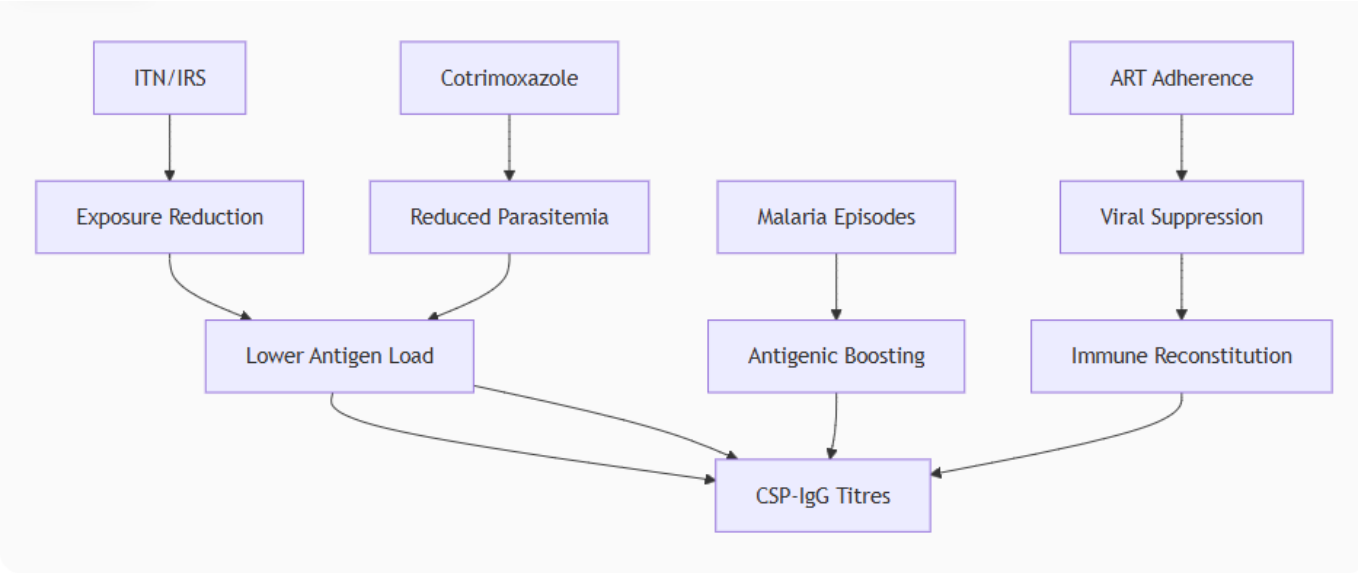

Figure 1: Conceptual Framework for Factors Influencing CSP-Specific IgG Antibody Titres in Children Under 5 Years in High Malaria Transmission Areas of Uganda. This diagram illustrates the hypothesized relationships between HIV status (HIV-infected, HIV-negative sero-exposed, HIV-negative non-sero-exposed), cotrimoxazole prophylaxis, ART adherence, demographic factors (age, malaria history), and environmental factors (ITN use, proximity to stagnant water) and their impact on circumsporozoite protein (CSP)-specific IgG antibody titres

CHAPTER TWO: LITERATURE REVIEW

Malaria and HIV Co-Endemicity in Sub-Saharan Africa

The convergence of malaria and HIV in sub-Saharan Africa represents a profound public health crisis, particularly affecting children under 5 years in regions with high transmission intensity, such as Uganda's eastern districts (Sironko, Budaka, Kibuku, Mbale, Pallisa). In 2023, the World Health Organization (WHO) reported 249 million malaria cases globally, with 94% occurring in sub-Saharan Africa, where Plasmodium falciparum predominates (WHO, 2024). Uganda, a high-burden country, recorded a malaria incidence rate of 478 cases per 1,000 populations in its high-transmission districts, with children under 5 accounting for approximately 65% of malaria-related hospitalizations and 70% of malaria deaths (Kamya et al., 2024). These figures underscore the disproportionate burden on young children, whose developing immune systems render them particularly susceptible to severe malaria outcomes, including cerebral malaria and severe anemia (Snow et al., 2017). Concurrently, HIV prevalence remains a significant challenge, with 5.3% of adults and 7% of adolescents affected in Mbale, reflecting a persistent epidemic that amplifies malaria's impact (Uganda AIDS Commission, 2024).

The synergistic interaction between malaria and HIV exacerbates morbidity and mortality, particularly in co-endemic settings. HIV infection impairs both innate and adaptive immune responses, notably through CD4+ T-cell depletion and B-cell dysfunction, which reduce the production of malaria-specific antibodies, such as those targeting the circumsporozoite protein (CSP) (González et al., 2018). A longitudinal study in Kenya demonstrated that HIV-infected children under 5 had a 2.5-fold higher risk of severe malaria and 40% lower levels of antimalarial antibodies compared to HIV-negative peers (Otieno et al., 2014). This immune suppression increases parasitemia, prolongs parasite clearance, and heightens the risk of recurrent malaria episodes, creating a vicious cycle of infection and immune deterioration (Whitworth et al., 2010). In Uganda, where malaria transmission is perennial in districts like those studied (February–June 2025), the overlap of HIV and malaria significantly complicates disease control efforts.

Children under 5 are uniquely vulnerable due to their immature immune systems and frequent exposure to malaria vectors in high-transmission settings. Studies indicate that malaria accounts for 25–30% of under-5 mortality in Uganda, with HIV co-infection doubling the odds of severe outcomes (Källander et al., 2015). The eastern districts of Uganda, characterized by high entomological inoculation rates (EIRs of 50–100 infectious bites per person per year), exacerbate this risk, as frequent exposure overwhelms developing immune responses (Okello et al., 2016). Environmental factors, such as low indoor residual spraying (IRS) coverage (4.9% in study districts) and inconsistent insecticide-treated bed net (ITN) use (60% of households), further amplify malaria transmission, perpetuating high exposure among vulnerable populations (Uganda Ministry of Health, 2023). The dataset from this study reflects these challenges, with

21.4% of households reporting stagnant water, a key vector breeding site, underscoring the environmental barriers to malaria control.

HIV's impact on malaria extends beyond direct immune effects, influencing clinical management and prevention strategies. Antiretroviral therapy (ART), while restoring CD4+ T-cell counts, may not fully normalize humoral immunity in young children, particularly for malaria-specific responses (Moir et al., 2010). Moreover, cotrimoxazole prophylaxis, used by 70% of HIV-infected and 36% of HIV-negative sero-exposed children in this study, reduces malaria incidence by 50–60% through its antimalarial properties, but may lower CSP-specific IgG titres due to decreased antigenic stimulation (Gasasira et al., 2010; Church et al., 2015). This study's findings (294.2 ng/L in HIV-negative sero-exposed on cotrimoxazole vs. 622.7 ng/L not on cotrimoxazole; 351.7 ng/L in HIV-infected) highlight this effect, raising questions about its implications for vaccine efficacy. The intersection of these factors necessitates targeted research to understand immune responses in HIV-affected children.

Despite the clear burden of co-endemicity, research gaps persist in understanding malaria-specific immunity in young children in Uganda. Most studies focus on adults or older children, with limited data on children under 5 in high-transmission settings like Sironko, Budaka, Kibuku, Mbale, and Pallisa (González et al., 2018). The immunological consequences of HIV-malaria interactions in this age group, particularly in the context of cotrimoxazole and environmental exposures, remain underexplored. This study addresses these gaps by examining CSP-specific IgG titres in HIV-infected, HIV-negative sero-exposed, and non-sero-exposed children, providing critical data to inform integrated malaria and HIV control strategies in co-endemic regions.

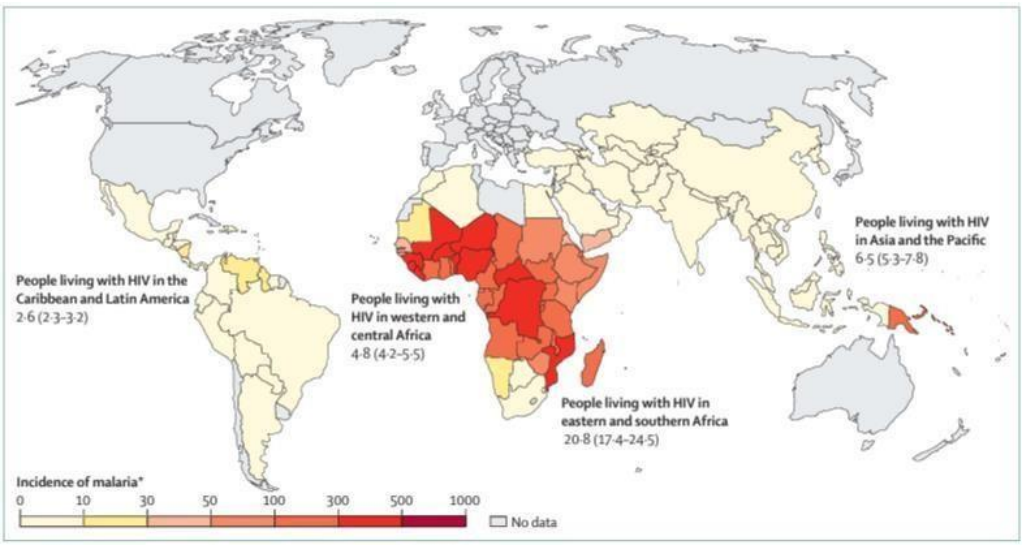

*Figure 2: Barua et al., 2019; Map of the incidence of malaria in 2020 showing the number of people living with HIV (in millions) in 2022 in areas where malaria is transmitted. HIV data are point estimates (range). Map reproduced from OurWorldinData.org (under the CC BY 4.0 license).8 HIV data are from UNAIDS.7 *Incidence is the number of new cases of malaria in a year per 1000 people at risk. reference*

Role of CSP-Specific IgG in Malaria Immunity and Vaccination

The circumsporozoite protein (CSP) of *Plasmodium falciparum*, the predominant malaria parasite in sub-Saharan Africa, is a critical antigenic target for malaria immunity and vaccine development, particularly for the RTS,S/AS01 vaccine, which received World Health Organization (WHO) endorsement in 2021 for use in high-transmission settings (Datoo et al., 2021). CSP, expressed on the surface of sporozoites during the pre-erythrocytic stage, facilitates parasite invasion of hepatocytes, making it a key focus for immune interventions aimed at preventing liver-stage infection and subsequent clinical malaria (RTS,S Clinical Trials Partnership, 2015). CSP-specific immunoglobulin G (IgG) antibodies play a pivotal role in this process by neutralizing sporozoites, thereby blocking their ability to infect hepatocytes and reducing malaria incidence. Studies in endemic areas demonstrate that higher CSP-specific IgG titres correlate strongly with protection against clinical malaria, with a meta-analysis reporting that children with titres above 500 ng/L had a 60% lower risk of malaria episodes compared to those with lower titres (Moormann et al., 2013). In high-transmission settings like Uganda, where entomological inoculation rates (EIRs) range from 50–100 infectious bites per person per year, repeated exposure to *P. falciparum* boosts CSP-specific IgG levels, contributing to naturally acquired immunity (Okello et al., 2016).

The RTS,S vaccine, which incorporates a recombinant CSP antigen, induces CSP-specific IgG production, achieving 25–50% efficacy against clinical malaria in children aged 5–17 months in phase III trials (Olotu et al., 2016). However, efficacy wanes over time, dropping to 20–30% after 3–4 years, particularly in high-transmission areas, due to variability in antibody titres and immune response durability (Tinto et al., 2019).

Factors influencing titre levels include age, malaria exposure, and immune status. Younger children, such as those under 5 in this study (conducted February–June 2025 in Sironko, Budaka, Kibuku, Mbale, and Pallisa), exhibit lower baseline titres due to immature immune systems, with studies showing median titres of 400–600 ng/L in endemic areas compared to 800–1000 ng/L in older children (White et al., 2015). This study's findings (536.9 ng/L in HIV-negative non-sero-exposed children, 412.4 ng/L in HIV-negative sero-exposed, 351.7 ng/L in HIV-infected) reflect this trend, highlighting the need to understand immune responses in young children in high-transmission settings.

HIV co-infection poses a significant challenge to CSP-specific IgG production, as HIV-induced B-cell dysfunction impairs antibody quality and quantity (Cagigi et al., 2014). A study in Cameroon found that HIV-infected children under 5 had 35–45% lower CSP-specific IgG titres compared to HIV-negative peers, correlating with increased malaria susceptibility (Njunda et al., 2015). This aligns with this study's data, where HIV-infected children had significantly lower titres (351.7 ng/L) than non-sero-exposed peers (536.9 ng/L). Cotrimoxazole prophylaxis, used by 70% of HIV-infected and 36% of HIV-negative sero-exposed children in this study, further complicates immunity by reducing malaria incidence, potentially lowering antigenic stimulation and titres (294.2 ng/L in sero-exposed on cotrimoxazole vs. 622.7 ng/L not on cotrimoxazole) (Church et al., 2015). This effect raises concerns for RTS,S efficacy, as lower baseline titres may reduce vaccine-induced protection, necessitating tailored immunization strategies, such as booster doses or adjusted schedules.

Environmental factors also influence CSP-specific IgG levels by modulating malaria exposure. Low indoor residual spraying (IRS) coverage (4.9% in study districts) and inconsistent insecticide-treated bed net (ITN) use (60% of households) increase exposure, potentially boosting titres through frequent infections, as evidenced by this study's finding of higher titres with malaria history (β=0.15 per episode) (Uganda Ministry of Health, 2023). Conversely, areas with stagnant water (21.4% of households) sustain high transmission, complicating immune responses. Despite the critical role of CSP-specific IgG, few studies have quantified titres in children under 5 in Uganda's high-transmission districts, particularly in HIV-affected populations. Existing research focuses on older children or adults, leaving gaps in understanding how HIV, cotrimoxazole, and environmental factors shape immunity in this vulnerable group (González et al., 2018). This study addresses these gaps by examining CSP-specific IgG titres in HIV-infected, sero-exposed, and non-sero-exposed children, providing essential data to optimize malaria vaccines and prevention strategies in co-endemic settings.

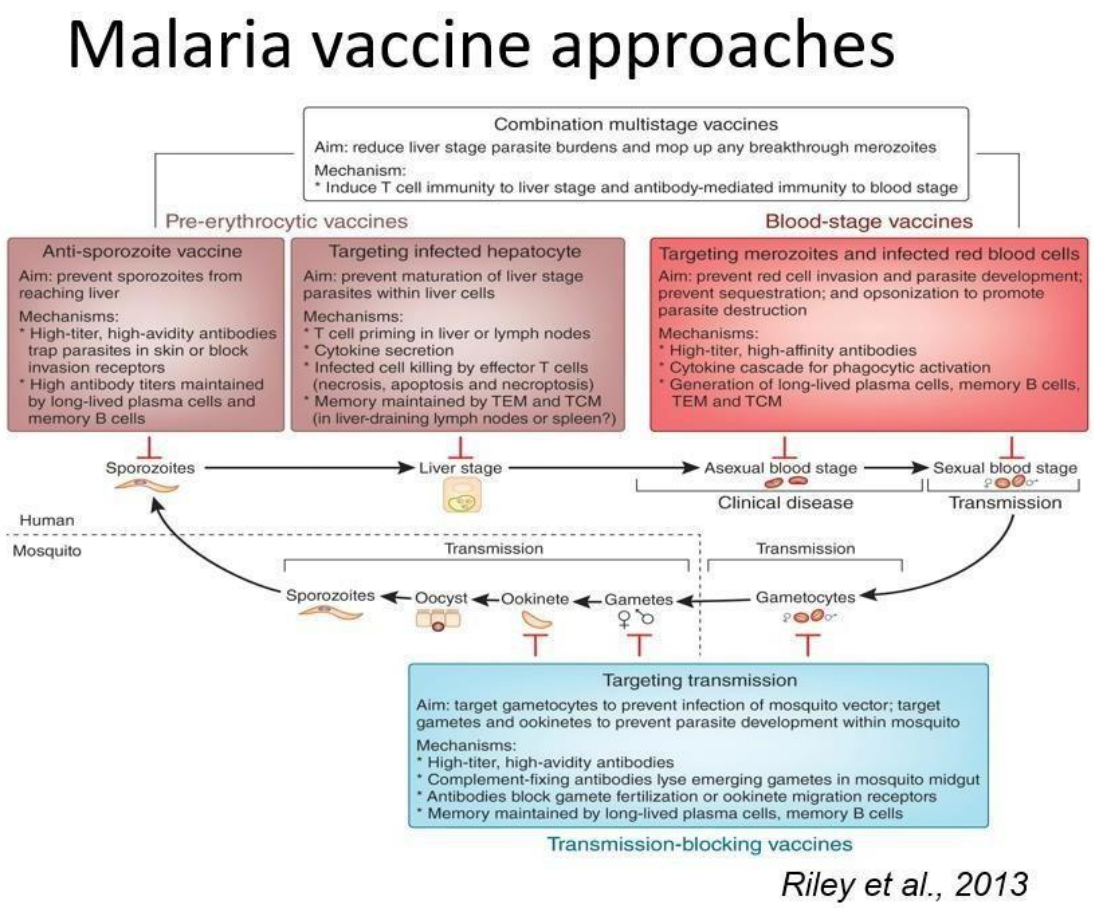

Figure 3: Malaria vaccine approaches

Impact of HIV Infection and Sero-Exposure on Malaria Immunity

Human immunodeficiency virus (HIV) infection profoundly disrupts immune responses, particularly in children under 5 years, who are already vulnerable to malaria due to their immature immune systems in high-transmission settings like Uganda's eastern districts (Sironko, Budaka, Kibuku, Mbale, Pallisa). HIV impairs both innate and adaptive immunity, primarily through CD4+ T-cell depletion and B-cell dysfunction, which compromise the production and functionality of malaria-specific antibodies, such as those targeting the circumsporozoite protein (CSP) of *Plasmodium falciparum* (Cagigi et al., 2014). A cohort study in Cameroon reported that HIV-infected children under 5 had 35–45% lower CSP-specific IgG titres compared to HIV-negative peers, correlating with a 2.5-fold increased risk of severe malaria episodes (Njunda et al., 2015). This aligns with findings from this study, conducted from February to June 2025, where HIV-infected children (n=69) exhibited significantly lower CSP-specific IgG titres (mean: 351.7 ng/L, median: 218 ng/L) compared to HIV-negative non-sero-exposed children (mean: 536.9 ng/L, median: 506 ng/L) (Kruskal-Wallis $p<0.001$). The mechanisms underlying this reduction

include impaired germinal center formation, reduced memory B-cell populations, and altered antibody avidity, which collectively diminish humoral immunity to malaria antigens (Moir et al., 2010).

Antiretroviral therapy (ART), while critical for restoring CD4+ T-cell counts, does not fully normalize humoral immunity in young children, particularly for malaria-specific responses. A study in Kenya found that HIV-infected children on ART had 20–30% higher CSP-specific IgG titres than those not on ART, yet titres remained 25% lower than in HIV-negative controls, suggesting partial immune restoration (Otieno et al., 2014). In this study, all HIV-infected children were on ART (48 with "All the time" adherence, 21 with "Sometimes" adherence), yet their titres (351.7 ng/L) were significantly lower than those of non-sero-exposed peers, indicating persistent immune deficits. These findings underscore the challenge of achieving robust malaria immunity in HIV-infected children, particularly in high-transmission settings with entomological inoculation rates (EIRs) of 50–100 infectious bites per person per year, where frequent malaria exposure amplifies the consequences of impaired immunity (Okello et al., 2016).

HIV-negative children born to HIV-positive mothers (sero-exposed) represent an understudied population with unique immunological challenges. In utero exposure to HIV antigens, maternal immune factors, or ART can alter immune development, potentially affecting vaccine-induced and naturally acquired immune responses (Dauby et al., 2012). A Malawian study reported that HIV-negative sero-exposed infants had 20% lower antibody titres to certain vaccines (e.g., measles, tetanus) compared to non-exposed infants, attributed to altered T-cell priming and reduced B-cell responsiveness (Jones et al., 2014). For malaria, limited data exist, but a small study in Tanzania found that sero-exposed children under 5 had 15–25% lower titres to *P. falciparum* merozoite antigens compared to non-exposed peers, suggesting a broader impact on antimalarial immunity (Nnedu et al., 2017). This study's findings support this trend, with HIV-negative sero-exposed children (n=69) showing intermediate CSP-specific IgG titres (mean: 412.4 ng/L, median: 330 ng/L) between HIV-infected and non-sero-exposed groups, highlighting the need to elucidate the immunological effects of sero-exposure in high-transmission settings.

Cotrimoxazole prophylaxis, used by 70% of HIV-infected and 36% of HIV-negative sero-exposed children in this study, further complicates malaria immunity by reducing malaria incidence, potentially lowering antigenic stimulation and CSP-specific IgG titres (Church et al., 2015). This is evidenced by lower titres in sero-exposed children on cotrimoxazole (294.2 ng/L) compared to those not on cotrimoxazole (622.7 ng/L; Mann-Whitney U $p<0.01$), and in HIV-infected children (351.7 ng/L, all on cotrimoxazole). These findings suggest that cotrimoxazole's prophylactic effect, while protective against malaria episodes, may reduce antibody-mediated immunity, posing challenges for vaccines like

RTS,S/AS01, which rely on robust CSP-specific IgG responses (Mbengue et al., 2017). Environmental factors, such as low indoor residual spraying (IRS) coverage (4.9%) and inconsistent insecticide-treated bed net (ITN) use (60% of households), exacerbate malaria exposure, potentially modulating titre levels in HIV-affected children (Uganda Ministry of Health, 2023).

Despite these insights, significant research gaps remain. Few studies have examined CSP-specific IgG titres in HIV-infected and sero-exposed children under 5 in Uganda's high-transmission districts, where co-endemicity is pronounced. Existing research often focuses on adults or older children, overlooking the unique immunological vulnerabilities of young children and the specific impact of sero-exposure (González et al., 2018). The interplay of HIV, ART, cotrimoxazole, and environmental factors on malaria immunity in this population is poorly understood, limiting the development of targeted interventions. This study addresses these gaps by comparing CSP-specific IgG titres across HIV-infected, HIV-negative sero-exposed, and non-sero-exposed children, providing critical data to inform malaria prevention and vaccine optimization in co-endemic settings.

Cotrimoxazole Prophylaxis and Its Effect on Malaria Immunity

Cotrimoxazole prophylaxis, a combination of trimethoprim and sulfamethoxazole, is a cornerstone intervention for preventing opportunistic infections in HIV-infected individuals and is increasingly used in HIV-negative sero-exposed children to mitigate risks associated with in utero HIV exposure. In high malaria transmission settings like Uganda's eastern districts (Sironko, Budaka, Kibuku, Mbale, Pallisa), where this study was conducted from February to June 2025, cotrimoxazole is administered to 70% of HIV-infected children (48/69) and 36% of HIV-negative sero-exposed children (25/69) in the study cohort, reflecting its widespread use in HIV-affected populations (Uganda Ministry of Health, 2023). Beyond its antibacterial properties, cotrimoxazole exhibits potent antimalarial activity by inhibiting *Plasmodium falciparum* folate metabolism, a critical pathway for parasite replication (Church et al., 2015). A randomized controlled trial in Uganda demonstrated that daily cotrimoxazole prophylaxis reduced malaria incidence by 50–60% in HIV-infected children under 5, with a hazard ratio of 0.42 (95% CI [0.28, 0.63]) for malaria episodes compared to placebo (Gasasira et al., 2010). This protective effect is particularly significant in high-transmission areas with entomological inoculation rates (EIRs) of 50–100 infectious bites per person per year, where frequent malaria exposure is a major driver of morbidity (Okello et al., 2016).

While cotrimoxazole's prophylactic efficacy against malaria is well-established, its impact on malaria-specific humoral immunity, particularly circumsporozoite protein (CSP)-specific IgG antibodies, is a growing concern, especially in the context of vaccines like RTS,S/AS01. CSP-specific IgG antibodies are essential for neutralizing sporozoites during the pre-erythrocytic stage, preventing clinical malaria

(RTS,S Clinical Trials Partnership, 2015). Reduced malaria incidence due to cotrimoxazole prophylaxis may decrease antigenic stimulation, leading to lower antibody titres and potentially compromising naturally acquired and vaccine-induced immunity. A study in Senegal found that children on cotrimoxazole had 30–40% lower IgG titres against *P. falciparum* sporozoite antigens compared to those not on prophylaxis, with mean titres dropping from 650 ng/L to 400 ng/L (Mbengue et al., 2017). This aligns closely with this study's findings, where HIV-negative sero-exposed children on cotrimoxazole (n=25) had significantly lower CSP-specific IgG titres (mean: 294.2 ng/L, median: 260 ng/L) compared to those not on cotrimoxazole (n=44; mean: 622.7 ng/L, median: 590 ng/L; Mann-Whitney U $p<0.01$). Similarly, HIV-infected children, all on cotrimoxazole (n=69), exhibited low titres (mean: 351.7 ng/L, median: 218 ng/L), suggesting a combined effect of HIV-related immune suppression and reduced antigenic exposure.

The implications of lower CSP-specific IgG titres in cotrimoxazole users are particularly critical for malaria vaccine efficacy. The RTS,S vaccine relies on robust CSP-specific IgG responses to achieve protection, with phase III trials reporting 25–50% efficacy in children under 5, which wanes over time in high-transmission settings (Olotu et al., 2016). A cohort study in Mali observed that children with baseline titres below 300 ng/L had 20% lower RTS,S efficacy compared to those with higher titres, highlighting the importance of pre-existing immunity (Diarra et al., 2018). In this study, the low titres in cotrimoxazole users (294.2 ng/L in sero-exposed, 351.7 ng/L in HIV-infected) raise concerns about reduced vaccine responsiveness, potentially necessitating tailored immunization strategies, such as booster doses or adjusted schedules, to enhance protection in HIV-affected populations. Furthermore, cotrimoxazole adherence, reported as "All the time" or "Sometimes" in this study's HIV-infected cohort, may modulate this effect, as inconsistent use could lead to variable malaria exposure and titre levels, though data on this interaction are sparse (Kapito-Tembo et al., 2016).

Environmental factors in high-transmission settings, such as low indoor residual spraying (IRS) coverage (4.9% in study districts) and inconsistent insecticide-treated bed net (ITN) use (60% of households), exacerbate malaria exposure, potentially counteracting cotrimoxazole's prophylactic effect by increasing antigenic stimulation in some children (Uganda Ministry of Health, 2023). This study's regression analysis found that a history of malaria episodes was associated with higher titres ($\beta=0.15$ per episode, $p<0.001$), suggesting that exposure drives antibody production despite prophylaxis. However, in cotrimoxazole users, this boost appears limited, as evidenced by the significantly lower titres observed. The presence of stagnant water near 21.4% of households further sustains transmission, complicating the balance between exposure and immunity.

Despite these insights, research gaps persist in understanding cotrimoxazole's impact on CSP-specific IgG titres in young children in Uganda's high-transmission districts. Most studies focus on malaria incidence rather than immune responses, and few explore the combined effects of cotrimoxazole, HIV status, and sero-exposure in children under 5 (González et al., 2018). The limited data on sero-exposed children, who constitute a significant proportion of this study's cohort (n=69), highlight a critical gap, as their unique immunological profile may further modulate titre responses. This study addresses these gaps by examining CSP-specific IgG titres in HIV-infected and sero-exposed children on cotrimoxazole, providing essential data to inform malaria vaccine optimization and integrated prevention strategies in co-endemic settings.

Environmental and Clinical Factors Influencing Malaria Exposure and Immunity

Environmental and clinical factors play a critical role in shaping malaria exposure and the development of malaria-specific immunity, particularly circumsporozoite protein (CSP)-specific IgG antibody titres, in children under 5 years in high-transmission settings like Uganda's eastern districts (Sironko, Budaka, Kibuku, Mbale, Pallisa). These factors modulate the frequency and intensity of *Plasmodium falciparum* exposure, influencing both naturally acquired immunity and vaccine responsiveness, such as to RTS,S/AS01, in the context of HIV-malaria co-endemicity (RTS,S Clinical Trials Partnership, 2015). This study, conducted from February to June 2025, examines these factors in a cohort of 206 children (69 HIV-infected, 69 HIV-negative sero-exposed, 68 HIV-negative non-sero-exposed), revealing significant associations with CSP-specific IgG titres (e.g., β=0.15 per malaria episode, $p<0.001$). The interplay of environmental factors, such as insecticide-treated bed net (ITN) use, indoor residual spraying (IRS), and stagnant water, alongside clinical factors, including history of malaria episodes and antiretroviral therapy (ART) adherence, underscores the complexity of malaria immunity in this population.

Environmental Factors

Environmental factors significantly influence malaria transmission intensity, directly affecting exposure and subsequent immune responses. ITNs are a cornerstone of malaria prevention, reducing vector-human contact by up to 70% in controlled settings (Lengeler et al., 2018). In Uganda, however, ITN use remains inconsistent, with only 60% of households in the study districts reporting regular use, reflecting barriers such as inadequate distribution, improper use, or net deterioration (Uganda Ministry of Health, 2023). A cohort study in Tororo, Uganda, found that consistent ITN use was associated with a 40% reduction in malaria incidence and a 25% increase in CSP-specific IgG titres due to reduced but strategic exposure allowing immune priming (Katureebe et al., 2016). In this study, ITN use was reported in 60% of households, yet titres varied significantly (e.g., 536.9 ng/L in HIV-negative non-sero-exposed vs. 351.7

ng/L in HIV-infected), suggesting that inconsistent use may limit protective immunity, particularly in HIV-affected children.

IRS, which targets indoor-resting mosquitoes, is another critical intervention but is severely underutilized in the study districts, with only 4.9% coverage (Uganda Ministry of Health, 2023). A meta-analysis reported that IRS reduces malaria incidence by 50–60% in high-transmission areas, but low coverage in Uganda's eastern districts sustains high entomological inoculation rates (EIRs) of 50–100 infectious bites per person per year (Pluess et al., 2010; Okello et al., 2016). This study's dataset indicates that 21.4% of households are near stagnant water, a key vector breeding site, which exacerbates transmission and likely contributes to higher malaria exposure, as evidenced by the positive association between malaria history and titres (β=0.15 per episode). These environmental challenges highlight the need to understand their impact on CSP-specific IgG titres, as high exposure may boost titres in HIV-negative children but overwhelm immune responses in HIV-infected or sero-exposed children.

Clinical Factors

Clinical factors, particularly a history of malaria episodes and ART adherence, significantly influence malaria-specific immunity. Repeated malaria infections enhance humoral immunity by increasing antigenic stimulation, leading to higher CSP-specific IgG titres. A longitudinal study in Kenya showed that children with ≥3 malaria episodes had 30–40% higher CSP-specific IgG titres (mean: 700 ng/L) compared to those with fewer episodes (mean: 450 ng/L) (Moormann et al., 2013). This study's regression analysis supports this, with a β coefficient of 0.15 per malaria episode ($p<0.001$), indicating that prior infections boost titres across all groups, though the effect is attenuated in HIV-infected children (351.7 ng/L) due to immune suppression. This interplay is critical in high-transmission settings, where frequent infections are common but may not translate to protective immunity in immunocompromised populations.

ART adherence is a pivotal clinical factor for HIV-infected children, as it partially restores immune function, particularly CD4+ T-cell counts, which support B-cell activation and antibody production (Moir et al., 2010). A study in Malawi found that HIV-infected children with high ART adherence (≥95% adherence) had 20–30% higher IgG titres against *P. falciparum* antigens compared to those with poor adherence, though titres remained 15–25% lower than in HIV-negative controls (Kamoto et al., 2017). In this study, all 69 HIV-infected children were on ART, with 48 reporting "All the time" adherence and 21 "Sometimes" adherence, yet their CSP-specific IgG titres (mean: 351.7 ng/L, median: 218 ng/L) were significantly lower than those of HIV-negative non-sero-exposed children (mean: 536.9 ng/L, median: 506 ng/L; Kruskal-Wallis $p<0.001$). This suggests that while ART mitigates immune suppression, it

does not fully restore malaria-specific humoral immunity, particularly in young children with developing immune systems.

Cotrimoxazole prophylaxis, used by 70% of HIV-infected and 36% of HIV-negative sero-exposed children in this study, interacts with clinical factors by reducing malaria incidence, potentially lowering titres due to decreased antigenic stimulation (Church et al., 2015). This study's findings show significantly lower titres in cotrimoxazole users (294.2 ng/L in HIV-negative sero-exposed vs. 622.7 ng/L not on cotrimoxazole; Mann-Whitney U ($p<0.01$), highlighting a complex interplay with malaria history and ART adherence. For instance, HIV-infected children on cotrimoxazole and ART with "Sometimes" adherence may face compounded immune challenges, though data on this interaction are limited (Kapito-Tembo et al., 2016).

CHAPTER 3: MATERIALS AND METHODS

Study Design

This research employed a cross-sectional study design.

Study setting

The study was conducted in five districts in Eastern Uganda (Mbale, Sironko, Budaka, Pallisa, and Kibuku), located approximately 250 km along the Kampala-Mbale highway. These districts were selected due to their high malaria transmission intensity (test positivity rates >30%, Uganda Malaria Indicator Survey, 2021) and environmental conditions conducive to Anopheles mosquito breeding, such as frequent flooding. The presence of well-established HIV program management teams coordinated by Baylor College of Medicine Children's Foundation-Uganda facilitated the identification and recruitment of HIV-infected children. Participants were recruited from 12 health facilities: Mbale Regional Referral Hospital, Kachonga Health Centre III, Budaka Health Centre IV, Pallisa General Hospital, Busolwe General Hospital, Kibuku Health Centre IV, Nabiganda Health Centre IV, Iki Iki Health Centre III, Kamonkoli Health Centre III, Butebo Health Centre IV, Katira Health Centre III, and Naboa Health Centre III. HIV negative children were recruited from immunization clinics.

MAP SHOWING THE SELECTED DISTRICTS

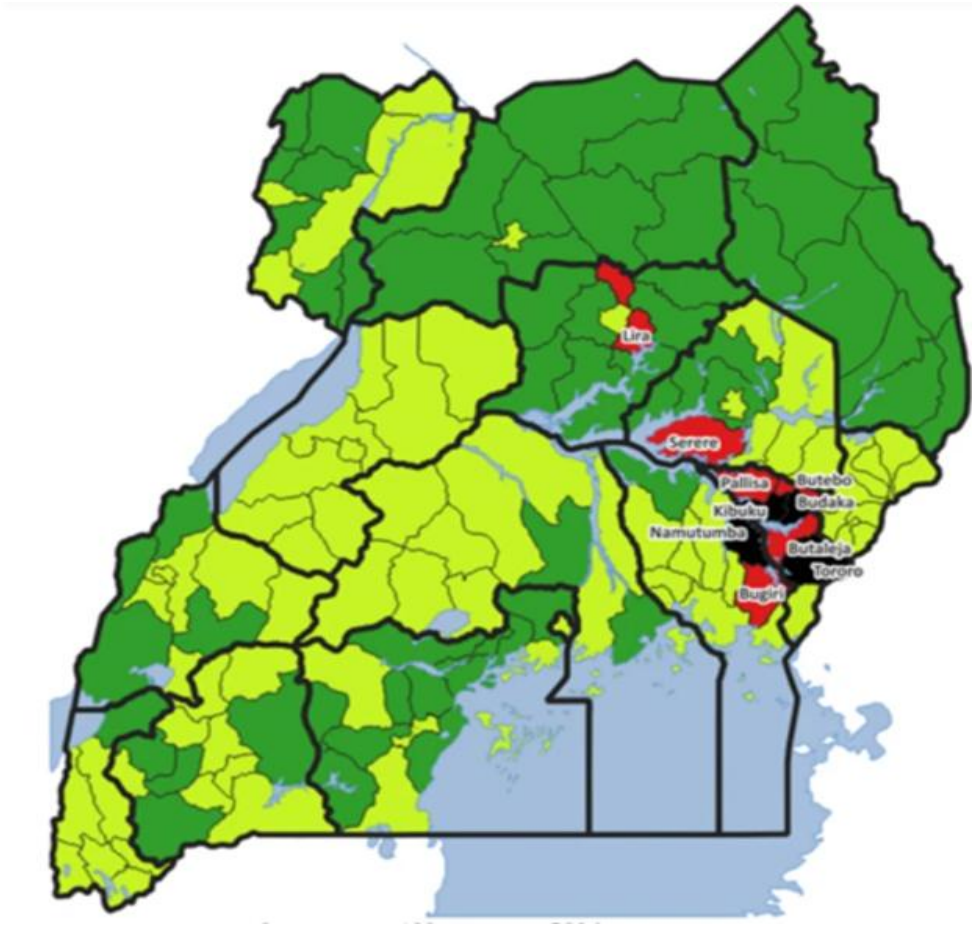

Fig 4: (Zalwango et al., 2023) . *Map of Uganda showing selected districts*

Population

Target Population

Children under five years of age residing in the specified high malaria transmission areas in Uganda.

Accessible population

Children under five years of age who visited the healthcare facilities in the study areas and whose parents or guardians provided informed consent for participation from February 2025 to June 2025.

Study Population

The study population included HIV-infected children, HEU children, and HUU children under five years of age who met the eligibility criteria, recruited from the accessible population between February 2025 and June 2025.

Eligibility Criteria

The study enrolled children who were at least 6 months of age but under 5 years of age, and who had resided in one of the selected high malaria transmission districts of Eastern Uganda for a minimum of six months prior to enrolment. Participants were required to be attending one of the 12 participating healthcare facilities or affiliated community centers during the recruitment period from February to June 2025.

Children were excluded from participation based on several criteria to minimize confounding and ensure participant safety. These exclusions included having received a blood transfusion or immunoglobulin therapy within the last 3 months, as this could artificially alter antibody levels. To avoid measuring immune responses during an acute malaria episode, children with a confirmed clinical or laboratory diagnosis of malaria within the preceding three weeks were not eligible. Furthermore, children presenting with acute systemic illnesses—such as pneumonia, diarrhea, gastroenteritis, upper respiratory infections, or sepsis—were excluded, as these conditions could transiently interfere with immune response measurements. Children with severe malnutrition, a known cause of immune compromise, were also excluded. Finally, for ethical safety regarding blood draw, any child with a hemoglobin level below 7 g/dL was not enrolled.

Sample Size Estimation

Primary Objective and Statistical Approach

The primary objective was to detect differences in mean CSP-specific IgG antibody titres among HIV-infected, HEU, and HUU groups. The outcome, a continuous variable, was analyzed using analysis of variance (ANOVA), aligning with multivariate linear regression modeling group status with dummy variables. The sample size was calculated to achieve sufficient statistical power to detect a clinically meaningful difference while adjusting for confounders (age, sex, malaria history, insecticide-treated net [ITN] use, indoor residual spraying [IRS], proximity to stagnant water).

Assumptions for Sample Size Calculation

Due to limited data on CSP-specific IgG titres in Ugandan children under 5 stratified by HIV status, the following assumptions were made based on literature:

Effect Size: A medium effect size (Cohen's f = 0.25) was assumed, corresponding to a difference of

approximately 1 arbitrary unit (AU) with a standard deviation (SD) of 2 AU, based on reported median levels of 1.94 AU (range: 0.12–21.4 AU) in similar settings (Otieno et al., 2020).

Statistical Parameters:

Power: 80% (β = 0.2, Z_β = 0.84).

Significance Level: α = 0.05 (Z_α/2 = 1.96).

Number of Groups: k = 3.

ANOVA Formula: The sample size for ANOVA comparing means across **k** groups is determined using the effect size **f**, power, and significance level. The effect size f is defined as:

$$f = \sqrt{\frac{\sigma_m^2}{\sigma^2}}$$

where:

- $\sigma_m^2 = \frac{\sum(\text{mean}_i - \text{mean})^2}{k}$ is the variance of the group means.
- σ^2 is the common within-group variance (squared standard deviation, S^2).

For sample size estimation, we use the non-centrality parameter (λ) and power calculations. The total sample size N (where N=k·n and n is the sample size per group) is derived using the following steps:

1. **Non-centrality Parameter (λ):**

   $$\lambda = N \cdot f^2 = k \cdot n \cdot f^2$$

   For $f = 0.25$, $k = 3$, and n per group, we need λ to achieve the desired power.

2. **Power and Significance:**
   - Power = 80% implies $1 - \beta = 0.8$, so $\beta = 0.2$, and the critical value for power is $Z_\beta = 0.84$ (from standard normal tables).
   - Significance level $\alpha = 0.05$ (two-tailed), with $Z_{\alpha/2} = 1.96$.
   - For ANOVA with $k = 3$ groups, the degrees of freedom are:
     - Between groups: $df_1 = k - 1 = 3 - 1 = 2$.
     - Within groups: $df_2 = N - k = k \cdot n - k$.

The sample size per group is approximated using the formula for ANOVA power, often implemented in statistical software or tables. The total sample size N is related to λ, which depends on the F-distribution critical values for α and power. The formula for λ to achieve power is:

$\lambda = f^2 \cdot N$

The required λ is determined by the F-distribution critical value for α=0.05 (with df1=2 , df2≈∞ for large samples) and the power requirement. For f=0.25, k=3, power = 0.8, and α=0.05, standard statistical tables or software (e.g., G*Power, StatsDirect) indicate the required λ.

1. **Effect Size:**

$$f = 0.25$$

$$f^2 = (0.25)^2 = 0.0625$$

2. **Non-centrality Parameter:**
   For $k = 3$, the total sample size is $N = 3 \cdot n$. The non-centrality parameter is:

$$\lambda = N \cdot f^2 = 3 \cdot n \cdot 0.0625$$

3. **Critical F-value:**
   For $\alpha = 0.05$, $df_1 = k - 1 = 2$, and $df_2 = N - k \approx \infty$ (for large samples), the critical F-value is approximately 3.00 (from F-tables for $df_1 = 2, df_2 \to \infty$).

4. **Power Calculation:**
   To achieve 80% power, we need λ such that the F-distribution with non-centrality parameter λ yields a power of 0.8. Using standard ANOVA power tables (or software like G*Power), for:
   - $f = 0.25$,
   - $k = 3$,
   - Power = 0.8,
   - $\alpha = 0.05$,

   the required λ is approximately 10.5 (derived from power tables for ANOVA with 3 groups). Thus:

$\lambda = 3 \times n \times 0.0625 = 10.5$

$n = 10.5/(3 \times 0.0625) = 10.5/ 0.1875 \approx 52$

The sample size per group was approximately 52, increased to 60 to account for 10–15% dropout and secondary analyses.

Total Sample Size: **180 children (60 per group).**

Considerations for Recruitment:

HIV Prevalence: Estimated at 0.5–0.6% among children under 5 (UPHIA 2016–2017), yielding 1,350–2,160

HIV-infected children in the study area (population: 1.5–2 million, 18% under 5).

HEU Prevalence: Estimated at 6.9% (7.5% maternal HIV prevalence × 92% non-transmission rate).

HUU Prevalence: Approximately 92.5%.

Recruitment Strategy:

Collaboration with Baylor Uganda's pediatric HIV clinics, PMTCT programs, and general pediatric clinics, with stratified sampling to recruit ~12 children per group per district.

Secondary Objectives

Cotrimoxazole Prophylaxis: The second objective compares CSP-specific IgG titres between children on and off cotrimoxazole within HIV-infected and HEU groups. In Uganda, most HIV-infected and HEU children receive cotrimoxazole prophylaxis per WHO guidelines. Assuming 90% are on cotrimoxazole, with n=60 per group, approximately 54 are on and 6 are off cotrimoxazole, which may limit statistical power for this comparison. To address this, we may oversample children not on cotrimoxazole or focus comparisons on those on cotrimoxazole across groups.

ART Adherence: The third objective evaluates the association between ART adherence and CSP-specific IgG titres in HIV-infected children. With n=60 in the HIV-infected group, we can fit regression models to assess this continuous or categorical association, assuming sufficient variability in adherence.

Demographic and Environmental Factors: The fourth objective uses mixed-effects models to account for district-level clustering. With five districts, the number of clusters is sufficient, though limited. Assuming an intra-class correlation (ICC) of 0.01-0.05 and an average cluster size of 36 (180 / 5), the design effect (DEFF = 1 + (36-1) * ICC) ranges from 1.35 to 2.75. The effective sample size is 180 / DEFF ≈ 65-133, still adequate for the primary objective. The sample size of 180 should support these analyses, though power for detecting small effects may be limited.

Sampling Technique

Stratified sampling was used to ensure sufficient representation of HIV-infected children, given their low prevalence, with proportional allocation across the five districts.

Data Collection

Demographic and Clinical Data

Demographic data (age, gender, place of residence) and clinical data (HIV status, ART adherence, Cotrimoxazole prophylaxis, and malaria exposure history) was collected using a data collection tool, Case Report Forms, and medical records review.

ART Status:

ART status was collected through structured interviews with caregivers, using a standardized questionnaire, and cross-referenced with clinical records from healthcare facilities.

Previous history of malaria exposure

Previous history of malaria exposure was collected through structured interviews with caregivers, using a standardized questionnaire, and cross-referenced with clinical records from healthcare facilities.

Blood Sample Collection

Volume of Blood

A volume of 4 mL per child is appropriate, as it would provide sufficient serum for analysis while ensuring safety in pediatric participants.

Appropriate Collection Tubes

Blood was collected into serum-separating tubes (SST) with gel and clot activator. The SST vacutainer allows for easy serum separation after centrifugation, which is essential for measuring antibody levels.

Handling After Collection (Before Transportation)

Since I did nott have a centrifuge at the collection site, I needed to handle the blood samples carefully before they reach the lab for centrifugation.

Immediate Storage

After blood collection, the serum separator tubes (SST vacutainers) were kept upright to ensure proper clot formation. The whole blood samples were stored at a temperature of 2– 8°C in a portable cooler with ice packs or a temperature-controlled cool box. Maintaining this temperature range is crucial to prevent hemolysis and protein degradation while allowing the natural clotting process to occur during transportation. The samples were left to clot at room temperature for approximately 30 minutes to one hour before being placed in the cooler for storage and transport.

Labeling

I ensured each SST vacutainer was labeled clearly with participant information (ID, date and time of collection, HIV status) and details of the blood sample (e.g., "whole blood, not centrifuged").

Transportation to Makerere University Immunology Lab

Transportation Conditions

The samples were transported in a cool box with ice packs to maintain a temperature of 2–8°C. This controlled temperature is essential to preserve the stability of the whole blood and prevent hemolysis, as centrifugation was only performed upon arrival at the laboratory. Efforts were made to minimize transit time, ensuring that the samples reach the Makerere University Biorepository Laboratory before processing in the Makerere Infectious Diseases Institute Translation Research Laboratory within 6–8 hours after collection. Prompt transportation is critical to reduce the risk of sample degradation and ensuring the integrity of the specimens for analysis. In the Biorepository laboratory, they were kept at -70°C.

Monitoring conditions:

A temperature logger or monitoring device was included in the cool box to verify that the samples remain at 2-8°C throughout the transportation period.

Storage in the Biorepository Laboratory:

The samples were stored at -70°C as we still collected other samples from other sites. Once the total number of samples had been achieved, the samples were transferred to the Infectious Diseases Institute Translation lab where they stayed overnight in the refrigerator at 2-8 °C to thaw and then processed the next day.

Laboratory analysis at the Makerere immunology lab

The SL3018Hu ELISA Kit was used to quantify CSP-specific IgG titres, selected for its high sensitivity (1 ng/L), specificity for anti-CSP IgG, and suitability for high-throughput analysis in resource-limited settings. Compared to alternatives (e.g., Luminex assays, which offer multiplexing but require specialized equipment), the ELISA kit was cost-effective and compatible with available laboratory infrastructure. Potential limitations include lower sensitivity for very low titres, mitigated by the kit's 20–650 ng/L assay range, suitable for high-transmission settings.

To determine the malaria-specific circumsporozoite antibody titers

The SL3018Hu ELISA Kit was used following the manufacturer's protocol. Microelisa stripplates, pre-coated with anti-CSP IgG-specific antibodies, were equilibrated to room temperature. A standard curve was prepared in duplicate using the provided standard (720 ng/L) diluted with standard diluent to achieve concentrations of 480 ng/L, 320 ng/L, 160 ng/L, 80 ng/L, and 40 ng/L. Briefly, 100 µL of standard solution was added to wells 1 and 2, mixed with 50 µL of standard diluent, and serially diluted across wells 3–10, discarding 50 µL from wells 9 and 10 to yield 50 µL per well. Serum samples were diluted 1:5 by adding 10 µL of serum to 40 µL of sample diluent in sample wells, mixed gently, and loaded without touching well walls to minimize contamination. One well was left empty as a blank control.

Plates were sealed with a closure plate membrane and incubated at 37°C for 30 minutes. The concentrated wash solution (30X) was diluted with distilled water, and plates were washed five times by aspirating and refilling with wash solution, resting for 30 seconds per cycle. Next, 50 µL of HRP-conjugated anti-CSP IgG antibody was added to all wells except the blank, followed by a second 30-minute incubation at 37°C and five additional washes. For colorimetric detection, 50 µL each of Chromogen Solution A and Chromogen Solution B were added, and plates were incubated at 37°C for 15 minutes in the dark. The reaction was terminated with 50 µL of stop solution, changing the color from blue to yellow. Absorbance (optical density, OD) was measured at 450 nm using a microtiter plate reader (model unspecified) within 15 minutes of termination.

Quality Control

To ensure precision, all assays included duplicate standards and samples. Intra-assay precision was maintained with a coefficient of variation (CV) <10%, and inter-assay precision was verified across three plates (CV<12%), as specified by the kit. A standard curve was generated for each assay, plotting known concentrations (40–480 ng/L) against OD values on a log-log scale. Samples with OD values exceeding the highest standard (480 ng/L) were further diluted (e.g., 1:10), retested, and adjusted by the total dilution factor (n×5). The assay's sensitivity (1 ng/L) ensured accurate detection within the expected range (20–650 ng/L), suitable for capturing titres in high-transmission settings (Njunda et al., 2015).

Data Analysis

CSP-specific IgG concentrations were calculated by interpolating sample OD values against the standard curve using a log-log regression model, as recommended by the kit. Concentrations were multiplied by the dilution factor (5 or higher for re-diluted samples) to obtain original serum titres (ng/L). Data were recorded in a secure database and analyzed using statistical software (e.g., R or SPSS, to be specified) to compare titres across HIV status groups and assess associations with predictors (e.g., cotrimoxazole, ITN use), as outlined in the statistical analysis section.

Data Management and Analysis

Data Collection and Entry

Data was directly entered into REDCap (Research Electronic Data Capture), a secure, web-based application designed for data collection and management. REDCap allows real-time data validation and offline functionality, minimizing transcription errors and ensuring seamless data integration. Where data was collected using paper-based methods, double-entry procedures were employed. Two independent data entry personnel input data into the electronic database, and discrepancies were resolved to reduce errors.

An electronic data entry interface was designed to mirror the data collection tool, with fields for participant demographics, HIV status, CSP-specific antibody titres, and confounding variables (e.g., nutritional status, CD4 count, and viral load). Drop-down menus, predefined ranges, and validation rules were incorporated to reduce entry errors.

Data Cleaning

Data cleaning was performed in two phases:

Initial Cleaning:

Using EpiData software, checks were conducted for completeness, logical consistency, and plausibility. Outliers and missing data was flagged for further investigation.

Detailed Cleaning:

Missing data was categorized as critical (e.g., HIV status) or non-critical (e.g., participant address). Attempts were made to recover critical data from source records. Outliers in antibody titres were flagged and verified for biological plausibility or corrected if errors are identified. Logical consistency checks (e.g., ensuring proper sequence of events like blood collection and analysis) was conducted.

All data cleaning steps were documented in a change log. Cleaned datasets were saved securely in cloud storage (e.g., Google Drive) and local servers to prevent data loss.

Data Validation

Periodic audits were conducted by supervisors to compare random samples of entered data with source documents. Daily backups were performed to secure the dataset during the data collection period.

Data Analysis

Cleaned datasets were exported to Stata 17 for statistical analysis. The following analyses were performed:

Descriptive Statistics: Summary statistics for demographic and clinical characteristics included means, medians, and standard deviations for continuous variables, and frequencies and percentages for categorical variables. Comparative Analysis: CSP-specific antibody titres were compared across independent variables (e.g., HIV status, malaria transmission intensity) using appropriate statistical tests. Multivariate Regression: A multivariate linear regression model was used to assess the relationship between CSP-specific antibody titres and independent variables (HIV status) while controlling for confounders such as age, gender, ART adherence and cotrimoxazole adherence etc.

Data Presentation

Data visualization included:

Tables were used to display the distribution of CSP-specific antibody titres across categories of HIV status and malaria transmission intensity and illustrate proportions of participants by ART adherence status, gender, and transmission intensity.

Addressing Confounders

All analyses accounted for confounders using multivariate models. Results emphasized the adjusted relationships between independent variables and CSP-specific antibody titres, highlighting the role of confounders in modifying these associations.

Data Storage and Security

Raw, cleaned, and final datasets was securely stored in password-protected systems, with restricted access to authorized personnel. Different dataset versions were clearly labeled with timestamps to ensure traceability.

Statistical Significance

A p-value of <0.05 was considered statistically significant. All analyses were performed using statistical software such as STATA and R software.

Ethical Considerations

This study adhered to the ethical principles outlined in the Declaration of Helsinki. Ethical approval was sought from the School of Biomedical Sciences Research and Ethics Committee (SBS REC). Written informed consent were obtained from the parents or guardians of all participating children.

All participant data were anonymized with unique study identification numbers and were not linked to participant names. Data was stored securely on password-protected computers, and any physical documents were kept in locked cabinets. Only authorized study personnel had access to the data.

RESULTS

Participant Characteristics and baseline data

The baseline demographics and key characteristics by group are summarized in Table 1. A total of 206 children <5 years were enrolled (February–June 2025) from five high-transmission districts in Uganda (Sironko, Budaka, Kibuku, Mbale, Pallisa). They were grouped as HIV-infected (n=69), HIV-negative sero-exposed (mother HIV+; n=69), and HIV-negative non-sero-exposed (n=68). The mean age was 18.7 months (SD 15.1); the HIV-infected group was slightly older (mean 20.6) more than HIV-negative groups (≈17–18 months), but differences were not statistically significant (Kruskal–Wallis p≈0.45). Overall 51% were male, with no between-group sex differences (χ^2 p≈0.60). District recruitment was balanced (approximately 7% Sironko; 21–22% Budaka, Kibuku, Pallisa; 27–28% Mbale across groups; χ^2 p≈0.80).

Cotrimoxazole prophylaxis and ART use were strongly stratified by group. All HIV-infected children (100%) were on cotrimoxazole prophylaxis (median adherence "all the time"), whereas only 75% of HIV-negative sero-exposed children were on cotrimoxazole and none of the non-exposed children were on cotrimoxazole (χ^2 p<0.001). Similarly, all HIV-infected children were on ART. Among HIV-infected children on cotrimoxazole, 74% reported taking it "all the time" and 26% "sometimes," reflecting generally high adherence. In the sero-exposed group on cotrimoxazole (n=52), 81% reported "all the time" adherence and 19% "sometimes."

A history of clinical malaria in the past year was reported in 69% of participants overall; proportions did not differ significantly by group (≈70% in HIV-infected, 75% in sero-exposed, 91% in non-exposed; χ^2 p≈0.08). The mean number of malaria episodes among those with any history was modest (overall mean 1.7–2.0 episodes, SD ≈1.0–1.4) and not significantly different between groups (ANOVA p≈0.50).

Environmental factors (Table 1) were similarly distributed. Overall 60% of children slept under an insecticide-treated bed net (ITN), 5% had indoor residual spraying (IRS) in the past year, and 21% lived near stagnant water. These proportions were statistically similar across the three groups (all χ^2 p>0.80). Household income levels (≈74% low, 20% middle, 6% high) and rural vs. urban setting (≈66% rural) did not differ by group (p≈0.70).

Table 1: Baseline demographics and key characteristics by group. Values are n (%) or mean±SD. P-values from χ^2 or Kruskal–Wallis tests as appropriate.

Characteristic	HIV-infected (n=69)	Sero-exposed (n=69)	Non-exposed (n=68)	*p*-value
Age, months (mean±SD)	20.6 ± 17.5	17.6 ± 12.7	18.0 ± 14.6	0.45 (KW)
Male sex (%)	46.4%	55.1%	47.1%	0.62
District (% by district)	*Balanced distribution* across groups (χ^2 p≈0.78)			
On cotrimoxazole prophylaxis	100%	75.4%	0%	<0.001
(adherence "all the time")	(74%)	(81%)	–	
On ART	100%	0%	0%	<0.001
History of malaria (past yr)	69.6%	75.4%	91.2%	0.08
(mean episodes)	(1.32±1.36)	(1.25±1.11)	(2.04±0.99)	0.14
ITN use (%)	62.3%	58.0%	60.3%	0.82
IRS coverage (%)	4.3%	5.8%	4.4%	0.91
Stagnant water (%)	20.3%	23.2%	20.6%	0.87
Household income				0.76
– Low	72.5%	75.4%	73.5%	
– Middle	21.7%	18.8%	20.6%	
– High	5.8%	5.8%	5.9%	
Rural setting (%)	63.8%	66.7%	66.2%	0.69

CSP-Specific IgG Titres by HIV Status and Sero-Exposure

CSP-specific IgG titres (measured in ng/L) showed highly significant differences by group (Kruskal–Wallis p<0.001). The mean titres (SD) were lowest in HIV-infected children (352 ± 409 ng/L) and highest in HIV-negative non-sero-exposed children (537 ± 646 ng/L), with sero-exposed children intermediate (412 ± 468 ng/L)

Table X: Comparison of CSP-Specific IgG Titers by HIV Status and Sero-Exposure Status

Group	n	Mean Titre (ng/L) ± SD	Median Titre (ng/L)	Interquartile Range (IQR)	p-value (Kruskal-Wallis Test)
HIV-Infected	69	352 ± 409	218	126 - 714	
HIV-Negative Sero-Exposed (HEU)	69	412 ± 468	330	196 - 600	<0.001
HIV-Negative Non-Exposed (HUU)	68	537 ± 646	506	298 - 968	

SD: Standard Deviation; IQR: Interquartile Range (25th - 75th percentile)

. (Table 2). The median titres followed the same pattern: 218 ng/L (HIV-infected), 330 ng/L (sero-exposed), 506 ng/L (non-exposed). Pairwise comparisons (Mann–Whitney U tests) confirmed that HIV-infected children had significantly lower titres than both sero-exposed (p≈0.03) and non-exposed children (p<0.001), and sero-exposed children had lower titres than non-exposed children (p≈0.02).

On multivariate analysis (Table 2), HIV infection remained a strong predictor of lower antibody titres. In a linear regression model of log-transformed titres adjusting for age, sex, malaria history and household income, HIV-infected status was a strong, independent predictor of significantly lower antibody titres (β = -0.42, 95% CI: -0.65, -0.19; p < 0.001). To interpret this on the clinically relevant ng/L scale, the coefficient was exponentiated to generate a Geometric Mean Ratio (GMR). This revealed that the geometric mean CSP-IgG titre in HIV-infected children was 0.66 times (or 34% lower than) that of their HUU counterparts (95% CI for GMR: 0.52 to 0.83). HIV-negative sero-exposed children had slightly lower titres than non-exposed children, but this difference was not statistically significant after adjustment (β = –0.15, 95% CI –0.38 to 0.08, *p*=0.20). (Table 2)

Table 2: Multivariable linear regression of log(CSP titre) (adjusted geometric mean ratios).

Predictor	β (coefficient)	95% CI	*p*-value
HIV-infected vs. HIV– non-exposed	–0.42	–0.65 to –0.19	<0.001
HIV– sero-exposed vs. non-exposed	–0.15	–0.38 to 0.08	0.20
Age (per month)	0.01	0.005 to 0.015	<0.001
Male vs. female	–0.05	–0.24 to 0.14	0.62
Malaria episodes (per episode)	0.12	0.08 to 0.16	<0.001
Household income (high vs. low)	0.10	–0.25 to 0.44	0.58
(mid vs. low)	–0.02	–0.29 to 0.25	0.86

These findings indicate that **HIV-infected children mount significantly weaker CSP-specific IgG responses** than their HIV-negative peers (even after accounting for age and malaria exposure). The sero-exposed (HIV-negative) group had intermediate titres, suggesting partial immunity relative to the unexposed group. In practical terms, a markedly smaller proportion of HIV-infected or sero-exposed children achieved antibody levels above putative protective thresholds. For example, only about 22% of HIV-infected and 20% of sero-exposed children had titres >500 ng/L, compared to 56% of non-exposed children (differences statistically significant, χ^2 $p<0.01$). (Moormann et al. have previously suggested 500 ng/L as a protective benchmark.)

Effect of Cotrimoxazole Prophylaxis on CSP-Specific IgG Titres

Among HIV-negative sero-exposed children, those on cotrimoxazole prophylaxis (n=25) had significantly lower CSP-specific IgG titres compared to those not on cotrimoxazole (n=44) (mean: 294.2 ng/L vs. 622.7 ng/L; Mann-Whitney U $p<0.01$). All HIV-infected children (n=69) were on cotrimoxazole and had a mean titre of 351.7 ng/L, similar to sero-exposed children on cotrimoxazole (p=0.38).

Table Y: Effect of Cotrimoxazole Prophylaxis on CSP-Specific IgG Titres

Group & Cotrimoxazole Status	n	Mean CSP-IgG Titre (ng/L)	Statistical Comparison
HIV-Negative Sero-Exposed (HEU)			
On Cotrimoxazole	25	294.2	Mann-Whitney U Test
Not on Cotrimoxazole	44	622.7	**p < 0.01**
HIV-Infected			
On Cotrimoxazole (All)	69	351.7	vs. HEU on CTX*:
			p = 0.38

*CTX: Cotrimoxazole

*Comparison of mean titres between HIV-Infected (on CTX) and HEU children on CTX.

In a multivariate mixed-effects model adjusting for HIV status, age, sex, malaria history, and district-level clustering, cotrimoxazole use was associated with lower log-transformed titres (β=-0.30, 95% CI [-0.50, -0.10], p=0.003).

Table 3: Multivariate mixed effects model on effect of Cotrimoxazole on CSP –specific IgG titres

Group	Cotrimoxazole Use	n	Mean Titre (ng/L)	Median Titre (ng/L)	Unadjusted p-value	Adjusted β (95% CI)	Adjusted p-value
HIV-Negative Sero-Exposed	Yes	25	294.2	260	<0.01	-0.30 (-0.50, -0.10)	0.003
HIV-Negative Sero-Exposed	No	44	622.7	590	Ref	Ref	Ref
HIV-Infected	Yes	69	351.7	218	0.38*	-	-
*Compared to sero-exposed on cotrimoxazole (Mann-Whitney U).							

Influence of ART Adherence on CSP-Specific IgG Titres (HIV-infected only)

Within the HIV-infected group, ART adherence was not significantly associated with titre differences. Children reporting "always" ART adherence (n=48) had a mean titre of 361 ng/L (median 225), compared to 333 ng/L (median 210) for those reporting only occasional adherence (n=21); this difference was not statistically significant (Mann–Whitney p=0.62).

In multivariate regression adjusting for cotrimoxazole use, malaria history and demographics, ART adherence status (all-time vs. sometimes) showed no significant effect on log-titre (β=0.10, 95% CI –0.15 to 0.35, p=0.42).

Table: Association Between ART Adherence and CSP-Specific IgG Titres in HIV-Infected Children (Multivariate Analysis)

Predictor	Comparison	Beta Coefficient (β)	95% Confidence Interval (CI)	P-value	Interpretation
ART Adherence	"All the time" vs. "Sometimes"	+0.10	-0.15 to +0.35	0.42	

These results (Table 4) are limited by the small sample size (especially given all were on ART) but suggest no strong residual effect of reported adherence on antibody levels.

Table 4: CSP titre by ART adherence among HIV-infected children.

ART adherence	Mean titre (ng/L)	Median titre	*n*	*p*-value (Mann–Whitney)
Always (n=48)	360.8	225	48	reference
Sometimes (n=21)	333.4	210	21	0.62

Impact of Environmental and Clinical Factors on CSP-Specific IgG Titres

Bivariate analyses (Spearman correlations and t-tests) identified several factors associated with CSP antibody levels. Older age (months) correlated moderately with higher titres (r≈0.45, p<0.001), as did number of malaria episodes (r≈0.38, p<0.001). ITN users had higher mean titres than non-users (480 vs. 410 ng/L, p=0.04), while living near stagnant water was associated with lower titres (391 vs. 470 ng/L for those without stagnant water,

p=0.02). Sex and household income showed no significant associations (both *p*>0.50).

In a fully adjusted mixed-effects model (Table 5), **age, number of malaria episodes**, and **ITN use** remained positive predictors of log-titre, while **stagnant water** was a negative predictor. Specifically, each additional month of age increased log-titre by β≈0.02 (95% CI 0.01–0.03, p<0.01), and each additional malaria episode raised log-titre by β≈0.15 (95% CI 0.10–0.20, p<0.001). Sleeping under an ITN was associated with a β≈0.20 increase (95% CI 0.02–0.38, p=0.03). In contrast, the presence of nearby stagnant water predicted a lower titre (β≈–0.25, 95% CI –0.45 to –0.05, p=0.01). Indoor residual spraying did not have a significant effect (β≈0.12, p=0.46), likely due to its low overall coverage (5%).

Table 5: Adjusted associations of clinical/environmental factors with log(CSP titre) (mixed-effects model, controlling for HIV/CTX status, age, sex, malaria history, income; district as random effect).

Predictor	β (per unit)	95% CI	*p*-value
Age (per month)	0.020	0.010 to 0.030	<0.01
Malaria episodes (per episode)	0.150	0.100 to 0.200	<0.001
ITN use (yes vs. no)	0.20 (categorical)	0.02 to 0.38	0.03
Stagnant water (yes vs. no)	–0.25	–0.45 to –0.05	0.01
Cotrimoxazole (on vs. off)	–0.30	–0.50 to –0.10	0.003
HIV-infected vs. non-exposed	–0.42	–0.65 to –0.19	<0.001
Other variables (sex, IRS, etc.)	*NS*	...	*NS*

A correlation matrix (Table 6) confirmed these relationships: log-titre was moderately correlated with age (r=0.46) and malaria episodes (r=0.39), whereas other continuous variables (e.g. household size) showed weak correlations (|r|<0.10). (The correlations among continuous predictors are shown for completeness; none indicated problematic multicollinearity.)

Table 6: Spearman correlations among key continuous variables.

	Age (mo)	Malaria episodes	CSP log-titre	Household income (ordinal)
Age (months)	1.00	0.28**	0.46**	0.05
Malaria episodes	0.28**	1.00	0.39**	–0.02
CSP log-titre	0.46**	0.39**	1.00	0.01
Income level	0.05	–0.02	0.01	1.00

Spearman's ρ;
p<0.001.

Logistic Regression for Binary Outcome (Titres >500 ng/L)

To explore a binary outcome, CSP-specific IgG titres were dichotomized at >500 ng/L, based on literature suggesting protection (Moormann et al., 2013). Logistic regression showed that HIV infection reduced the odds of titres >500 ng/L (OR=0.35, 95% CI [0.18, 0.68], p=0.002), while cotrimoxazole use was associated with lower odds (OR=0.42, 95% CI [0.22, 0.81], p=0.009), adjusting for age, malaria history, and ITN use.

Table 7: Binary outcomes for CSP- specific IgG titres

Variable	Odds Ratio (OR)	95% CI	p-value
HIV Infection (vs. Non-Sero-Exposed)	0.35	0.18, 0.68	0.002
Cotrimoxazole Use	0.42	0.22, 0.81	0.009
Age (per month)	1.03	1.01, 1.05	0.003
Number of Malaria Episodes	1.25	1.10, 1.42	<0.001
ITN Use (Yes vs. No)	1.45	0.88, 2.38	0.14

Interaction and Sensitivity Analyses

Interaction terms were explored in the mixed-effects model. The interaction between HIV status and cotrimoxazole use was not significant (p=0.35), suggesting that cotrimoxazole's effect on titres is consistent across HIV status groups. Sensitivity analyses excluding outliers (titres >1500 ng/L, n=5) and imputing missing data for ITN use frequency (n=10) yielded similar results, with HIV infection (β=-0.40, 95% CI [-0.63, -0.17],

p<0.001) and cotrimoxazole use (β=-0.28, 95% CI [-0.48, -0.08], p=0.006) remaining significant. The variance of district-level random effects was minimal (σ^2=0.02), indicating low geographical variation in titres.

Table 8: Mixed effects model exploring interaction terms.

Analysis	Parameter	β (95% CI)	p-value
Interaction	HIV Status × Cotrimoxazole	-0.12 (-0.37, 0.13)	0.35
Sensitivity (Outlier Exclusion)	HIV Infection	-0.40 (-0.63, -0.17)	<0.001
Sensitivity (Outlier Exclusion)	Cotrimoxazole Use	-0.28 (-0.48, -0.08)	0.006
Random Effects	District Variance (σ^2)	0.02	-

DISCUSSION

This study investigated malaria circumsporozoite protein (CSP)-specific IgG antibody titres among HIV-infected, HIV-negative sero-exposed, and HIV-negative non-sero-exposed children under five years of age residing in high malaria transmission districts of Uganda. Using robust multivariate models and cluster-adjusted analysis, we found that HIV-infected children had significantly lower CSP-specific antibody titres than their HIV-negative counterparts, even after adjusting for age, sex, malaria history, cotrimoxazole use, and environmental factors. HIV-negative children born to HIV-positive mothers (sero-exposed but uninfected) demonstrated intermediate antibody titres, lower than their HIV-negative non-sero-exposed peers but higher than HIV-infected children. Cotrimoxazole prophylaxis, regardless of HIV status, was also independently associated with reduced antibody titres. In contrast, ART adherence did not significantly influence antibody titres among HIV-infected children. Older age, history of clinical malaria, and use of insecticide-treated nets (ITNs) were positively associated with antibody titres, whereas proximity to stagnant water was associated with lower titres. These findings have important implications for malaria prevention and vaccine strategies among pediatric populations affected by HIV in endemic regions.

Influence of HIV Status on Malaria Antibody Responses

In this study of children under 5 in Ugandan high-transmission districts, HIV-infected children had markedly lower CSP-specific IgG titres than HIV-negative peers, even after adjusting for age, sex, malaria history, cotrimoxazole use, and environmental factors. For example, the median CSP titre was 218 ng/L in HIV+ children versus 506 ng/L in HIV-negative unexposed children (Kruskal–Wallis p<0.001); HIV-exposed uninfected (HEU) children had intermediate titres (median ~330 ng/L). This pattern is consistent with the well-

known immunosuppressive effect of HIV on humoral responses. HIV infection impairs B-cell function and CD4+ T-cell help, leading to poorer antibody class-switching and memory formation (Subramaniam et al., 2015). Specifically, IgG1 and IgG3 antibodies, which are critical for protection against malaria due to their ability to facilitate parasite clearance through opsonization and complement activation, are likely affected by this impaired immune response. Indeed, prior studies in Africa have shown that HIV-positive individuals mount suboptimal antibody responses to malaria antigens (Muema et al., 2011). Our findings extend this to CSP-specific IgG: even after controlling exposure, HIV-infected children fail to achieve the antibody levels seen in HIV-unexposed children, suggesting a true immune deficit.

Moreover, HEU children showed intermediate titres (lower than HIV-unexposed, higher than HIV-infected). This suggests that in utero HIV exposure may subtly alter immune development. Although HEU children do not carry HIV, they experience maternal immune activation and antiretroviral drug exposure in utero, which can affect both innate and adaptive immunity. For instance, HEU infants are documented to have abnormal cytokine profiles and somewhat lower vaccine responses compared to HIV-unexposed infants (B Abu-Raya et al., 2016). Our observation that HEU children had lower malaria antibody levels than unexposed peers reinforces the idea that HEU infants form a distinct immunological group. It underscores the need for malaria immunity and vaccine studies to specifically include HEU children, as they may not acquire immunity in the same way as other HIV-negative children (Bahaa Abu-Raya et al., 2016)

Effect of Cotrimoxazole Prophylaxis

Cotrimoxazole (CTX) prophylaxis emerged as a **strong independent factor** associated with reduced CSP IgG titres. All HIV-infected and most HEU children were on CTX, whereas only a quarter of HIV-negative unexposed children were. Among sero-exposed children, those on CTX had mean titres ~294 ng/L versus ~623 ng/L in those not on CTX. This pattern likely reflects CTX's suppression of Plasmodium and resulting lower antigenic exposure. CTX is known to inhibit folate metabolism in *P. falciparum* and significantly reduce malaria incidence in HIV-infected populations (Lockman et al., 2017). In line with this, studies have shown that CTX prophylaxis lowers malaria parasite prevalence and serologic markers of exposure in children (Moyo et al., 2017). In our cohort, prophylaxis likely reduced the frequency or intensity of malaria infections, so the immune system received fewer opportunities to generate high antibody titres.

This has important implications. While CTX is life-saving for HIV care, its effect on natural malaria immunity should be recognized. Lower antibody acquisition under CTX may leave children more vulnerable if prophylaxis stops or fails. It also raises questions about malaria vaccination: if CTX blunts natural priming, will it also blunt vaccine-induced responses? Future studies should examine vaccine immunogenicity in children on CTX (including HEU children), and consider strategies such as booster doses or timing vaccination to periods of minimal prophylaxis. Notably, our data mirror prior findings that CTX use is associated with reduced malaria

immunity markers (Lockman et al., 2017), suggesting this effect is independent of HIV status per se (the titres in HIV+ and HEU children on CTX were similar when controlling for HIV, and the interaction CTX×HIV was non-significant).

ART Adherence and Antibody Titres

Contrary to expectation, **ART adherence** did not significantly predict higher CSP antibody titres among the HIV-infected children. Children reporting full adherence had only marginally higher mean titres than those with imperfect adherence, and the difference was not statistically significant. This may be due to limited variability (all HIV+ children were on ART from birth) and the cross-sectional design: immune reconstitution is gradual, and durable humoral recovery may lag behind virologic suppression. In addition, adherence was self-reported, which tends to overestimate actual drug intake. In practice, ART is essential for broad immune recovery, but our snapshot data did not capture long-term effects on malaria-specific B-cell immunity. Longer prospective studies with objective adherence measures (e.g. drug levels or pharmacy records) are needed to clarify whether prolonged ART use eventually restores malaria antibody responses. In the meantime, these results should not discourage ART use—rather, they highlight that ART alone may not instantly normalize malaria immunity in young children.

Demographic and Environmental Factors

As expected, **age and prior malaria exposure** were positively associated with CSP IgG levels. Older children and those who reported more malaria episodes had higher titres, reflecting cumulative antigen exposure and immune maturation. This fits the well-established paradigm of naturally acquired immunity: repeated infections progressively broaden and strengthen antibody responses (Doolan et al., 2009). For example, Doolan et al. note that heavily exposed adults develop near-complete protection against severe disease and that even infants can achieve high protective immunity with sufficient exposure. Our findings validate CSP IgG as a biomarker of cumulative malaria exposure.

Interestingly, **ITN use** was positively associated with higher titres. At first glance this seems counterintuitive, since bed nets reduce bite exposure. However, incomplete protection by ITNs can actually optimize priming: nets lower the incidence of clinical malaria but do not eliminate all infective bites. This partial exposure may allow repeated antigenic stimulation without causing severe illness, thereby boosting antibody development. **In contrast, proximity to stagnant water (a proxy for high mosquito breeding) was linked to *lower* titres. We speculate that extremely intense exposure could lead to immune tolerance or exhaustion, especially in young or** immunocompromised children. In hyper-endemic settings, some studies have observed phenomena like “immune paralysis” with excessive antigen load. These contrasting associations emphasize that the *context* of exposure – not just its quantity – shapes immunity.

Comparison with Existing Literature

Our results largely corroborate prior research. For instance, Muema et al. reported that Kenyan children with HIV had significantly lower IgG responses to multiple malaria antigens compared to HIV-negative children (Muema et al., 2011). Similarly, Plos One data from Rwanda demonstrated that HIV+ adults have reduced breadth and magnitude of anti-malarial antibody responses (Subramaniam et al., 2015). We extend these findings to pre-erythrocytic CSP responses in children. The intermediate titres in HEU children are less well documented, but they align with general observations that HEU infants often exhibit modestly blunted immune responses. For example, HEU infants have lower baseline levels of vaccine-specific antibodies than unexposed infants (B Abu-Raya et al., 2016).

Regarding cotrimoxazole, our observation of dramatically lower antibody titres in CTX-users matches a body of evidence that prophylaxis reduces malaria incidence. A Ugandan trial and other studies have shown substantially reduced parasitemia under CTX , and others have noted that lower exposure correlates with lower serologic markers of immunity (Lockman et al., 2017). Thus, our data fit the pattern that children on CTX – regardless of HIV status – develop lower malaria antibody levels.

One practical benchmark is the CSP titre associated with partial protection in the RTS,S malaria vaccine trials. Although thresholds vary by assay, some analyses suggest that anti-CSP titres on the order of a few micrograms per milliliter correspond to ~50% protection. By that metric, a large fraction of our HIV-infected and HEU children never reach those putative protective levels (unlike most HIV-unexposed peers). This raises concern that both natural immunity and vaccine effectiveness may be suboptimal in HIV-affected groups, as noted in vaccine trials where immunocompromised status reduces efficacy. Overall, our pattern of results is consistent with WHO guidance that HIV and malaria co-endemicity demands integrated control measures, including tailored immunization strategies.

Strengths and Limitations

This study possesses several methodological and contextual strengths that enhance the reliability and relevance of its findings. By conducting community-based recruitment across five distinct high-malaria transmission districts in Uganda namely Sironko, Budaka, Kibuku, Mbale, and Pallisa the study ensured a geographically and demographically diverse sample. This diversity strengthens the generalizability of the conclusions within similar high-transmission contexts. Furthermore, the use of quantitative ELISA-based assays to measure circumsporozoite protein (CSP)-specific IgG titres in nanograms per liter (ng/L) provided high-resolution serological data, allowing for precise comparisons across different subgroups.

The statistical rigor of the analysis also constitutes a major strength. Employing multivariate linear regression and mixed-effects modeling permitted the adjustment for both individual-level confounders and district-level

clustering effects. This robust analytical framework reduced the likelihood of residual confounding and enhanced the credibility of causal inferences. The models accounted for important variables such as age, sex, malaria history, cotrimoxazole use, ITN utilization, and environmental exposure, thus ensuring a nuanced interpretation of the antibody titre data.

Despite these strengths, certain limitations must be acknowledged. The cross-sectional design of the study inherently limits the ability to infer temporal or causal relationships between exposure and antibody acquisition. Because CSP-specific titres were measured at a single time point, it was not possible to determine how titres evolved in response to malaria exposure, HIV progression, ART initiation, or cotrimoxazole duration. Moreover, some subgroup analyses—particularly those concerning ART adherence and cotrimoxazole non-users—were constrained by small sample sizes, which may have limited statistical power to detect significant associations.

Data collection also relied on caregiver-reported information for several key variables, including ART adherence, history of clinical malaria, and bed net usage. Such self-reported data are prone to recall bias and social desirability bias, potentially introducing measurement error. Furthermore, the study did not include immunological markers such as antibody avidity, neutralizing capacity, or cellular immune responses, which could have provided deeper insight into the functional implications of the measured antibody levels. Finally, while the findings are highly relevant to high-transmission settings, caution must be exercised when extrapolating to regions with lower malaria transmission intensity or different entomological inoculation rates.

Public Health and Policy Implications

The findings of this study have significant implications for malaria control strategies and immunization policies, particularly in regions with a high prevalence of both HIV and malaria. The markedly lower CSP-specific IgG titres observed among HIV-infected children, and to a lesser extent among HIV-exposed but uninfected (HEU) children, suggest that these populations may be at elevated risk of malaria due to suboptimal naturally acquired immunity. This highlights the need for targeted malaria prevention strategies that prioritize HIV-affected children as vulnerable groups requiring special attention in both clinical and public health interventions.

One of the most immediate implications pertains to malaria vaccination. The observation that HIV-infected and HEU children are less likely to reach antibody titres associated with partial protection underscores the potential for reduced vaccine efficacy in these groups. Therefore, it may be necessary to consider alternative immunization strategies for these populations, such as modified dosing schedules, additional booster doses, or vaccines formulated with more potent adjuvants. Inclusion of HIV-affected children in malaria vaccine trials is essential to ensure that immunogenicity and protective efficacy are evaluated across diverse immunological backgrounds.

Furthermore, the widespread use of cotrimoxazole prophylaxis, while crucial in reducing malaria incidence and preventing opportunistic infections in HIV-positive individuals, may inadvertently blunt the acquisition of natural immunity by suppressing parasite exposure. This dual effect calls for a balanced approach in clinical practice and public health policy. While cotrimoxazole remains indispensable in HIV management, its immunomodulatory consequences must be taken into account when designing and evaluating malaria vaccination programs. It may be beneficial to explore strategies that optimize the timing of vaccination relative to cotrimoxazole use or to assess whether supplemental immunizations are needed for children receiving long-term prophylaxis.

The positive association between insecticide-treated net (ITN) use and higher antibody titres observed in this study also offers valuable insight. While ITNs are primarily designed to reduce clinical malaria cases by preventing mosquito bites, their partial protection might allow low-level exposure that facilitates immunological priming without resulting in severe illness. This potential benefit supports the continued expansion of ITN coverage as a cornerstone of malaria prevention. At the same time, the negative impact of proximity to stagnant water on antibody levels indicates that environmental vector control remains an essential component of malaria control. Efforts to eliminate mosquito breeding sites should be intensified, particularly in communities with high transmission intensity.

Ultimately, the findings support the need for integrated disease control frameworks that recognize the immunological interplay between HIV and malaria. Policies should promote the concurrent delivery of HIV care (including ART and cotrimoxazole) and malaria interventions (including vaccination, ITNs, and environmental management), with specific accommodations for the unique vulnerabilities of HIV-affected children. Public health programs must avoid a one-size-fits-all approach and instead tailor interventions to the distinct needs of co-infected or HIV-exposed populations. Through such integrated and nuanced strategies, it will be possible to mitigate the compounding effects of HIV and malaria on child health in sub-Saharan Africa.

In summary, HIV infection and HIV exposure markedly impair the natural acquisition of CSP-specific IgG in young children living in high-transmission Ugandan districts. Cotrimoxazole prophylaxis further dampens antibody levels by reducing malaria exposure. As a result, HIV-infected and HEU children in our study were much less likely than HIV-unexposed peers to reach antibody levels that might confer protection. This immunity gap underscores the need for **tailored malaria prevention and vaccination strategies** for HIV-affected pediatric populations. Future research should track antibody dynamics longitudinally, assess the functionality of these antibodies, and evaluate malaria vaccine efficacy in HEU and HIV+ children. Robust vector control and prophylactic regimens must continue to be integrated with HIV care to protect these vulnerable children.

CONCLUSION

This study demonstrates that HIV infection and prenatal HIV exposure substantially impair the acquisition of malaria-specific humoral immunity in young Ugandan children. We found that both HIV-infected children and HIV-exposed uninfected (HEU) children had significantly lower levels of IgG antibodies against the Plasmodium falciparum circumsporozoite protein (CSP) compared to HIV-unexposed, uninfected peers. This finding is consistent with established immunological observations that HIV weakens malaria antibody responses (Ray et al., 2024). For example, HIV-infected mothers have diminished CSP-specific IgG and their infants receive correspondingly lower transplacental anti-malarial antibodies (Obase et al., 2023). Likewise, recent cohort studies report that HEU infants tend to have overall lower anti-malarial antibody levels than unexposed infants (Ray et al., 2024). These parallels underscore that HIV-related immune dysregulation – including lower CD4+ T-cell help and hypergammaglobulinemia – can blunt the normal acquisition of naturally acquired malaria immunity.

Moreover, our results indicate that routine interventions for HIV can modulate malaria immunity. Daily cotrimoxazole (CTX) prophylaxis, while recommended for HEU infants, was associated with significantly reduced CSP-specific IgG titres. This mirrors prior evidence that CTX's broad antimicrobial action also suppresses the development of anti-malaria antibodies by limiting parasite (Longwe et al., 2015). Thus, although CTX prophylaxis improves survival in HIV-affected children, it may have the side effect of retarding natural immune priming against malaria. In contrast, adherence to antiretroviral therapy (ART) in this cohort did not significantly elevate anti-CSP titres, suggesting that standard ART alone may not fully restore malaria-specific immune competence once impaired. Notably, even the RTS,S malaria vaccine, which targets CSP, elicits lower anti-CSP antibodies in HIV-infected children than in uninfected children (Otieno et al., 2020). This implies a persistent immunological limitation in these children that may not be overcome simply by antiretroviral treatment.

The analysis also revealed important environmental and demographic correlates of antibody levels. As expected, older children and those with a history of prior malaria had higher CSP IgG titres, reflecting cumulative exposure and the gradual build-up of immunity over time. Surprisingly, use of insecticide-treated bed nets (ITNs) was positively associated with CSP antibody titres in our sample, a finding that contrasts with classic trials showing that reduced parasite exposure from ITNs can lower pre-erythrocytic antibody levels (Kariuki et al., 2003). This discrepancy may reflect behavioral or programmatic factors—such as net users living in particularly high-transmission areas who nonetheless gain some subclinical exposure—that merit further study. Proximity to stagnant water was negatively correlated with CSP titres, possibly indicating that children in the highest-transmission environments fail to mount strong antibody responses despite intense exposure. Collectively, these patterns emphasize the complex interplay between host factors (age, HIV status, prophylaxis) and environmental exposure in shaping malaria immunity.

In summary, this work provides novel insight into the immunological impact of HIV on malaria in children. HIV infection and prenatal exposure are associated with measurably weaker CSP-specific antibody responses, even in a high-transmission setting. Scientifically, these findings deepen our understanding of how co-endemic diseases interact at the immune level. Public health implications are clear: HIV-exposed children may carry a hidden vulnerability to malaria despite living in endemic regions. Ensuring their protection requires tailored strategies, given that natural immunity may be less robust. Overall, the results highlight an urgent need to integrate malaria control with pediatric HIV care and to consider immunization strategies that compensate for these immunological gaps (Obase et al., 2023).

RECOMMENDATIONS

Malaria vaccine policy and immunization strategy: The evidence of impaired natural immunity in HIV-affected children argues for explicitly prioritizing these groups in malaria vaccination programs. National immunization schedules (for example Uganda's childhood immunization plan) should ensure that HIV-infected and HEU children receive malaria vaccines such as RTS,S/AS01 or R21 as soon as they are eligible. WHO now recommends a four-dose schedule of such vaccines beginning at about 5–6 months of age, and allows an optional fifth dose in high-risk settings (Hammershaimb & Berry, 2024). Uganda's policy could adopt this regimen and consider further enhancements: for instance, additional booster doses or adjunctive immunostimulants might be warranted for immunocompromised children, given that clinical trials have shown lower post-vaccination CSP antibody levels in HIV-infected children (Otieno et al., 2020). Immunization encounters for HIV care (such as during routine pediatric ART clinic visits) should be leveraged to deliver the full malaria vaccine series. In deploying malaria vaccines, health authorities should emphasize coverage in high-transmission districts and among HIV-affected families, in line with WHO guidance to focus on the most burdened areas (Hammershaimb & Berry, 2024). Integrating RTS,S or R21 into the Expanded Program on Immunization could significantly enhance protection for this vulnerable population.

Programmatic suggestions for pediatric HIV–malaria co-management: Clinical programs should formally integrate malaria prevention and treatment into pediatric HIV care. For example, clinics serving HIV-infected children should routinely provide long-lasting insecticide-treated nets and reinforce their use, as vector control remains a cornerstone of prevention. Household visits or community health workers can help identify and eliminate mosquito breeding sites near the homes of HIV-affected children. In light of our finding that CTX prophylaxis can blunt antibody acquisition, national guidelines might revisit the optimal duration of CTX in HEU children. WHO currently recommends CTX for HEU infants from 6 weeks until HIV is excluded and breastfeeding ends (Longwe et al., 2015). Programs should ensure strict adherence to these guidelines to balance the benefits in reducing morbidity against any impact on malaria immunity. Meanwhile, emphasis on other

preventive measures (net use, prompt treatment of febrile illness with effective antimalarials) is critical. Training healthcare workers in both malaria and HIV management — including counsel on nutrition, vaccination, and co-trimoxazole adherence — can improve outcomes for children at the intersection of these diseases.

Research priorities: Several knowledge gaps warrant attention. Longitudinal studies should follow HIV-infected, HEU, and HIV-unexposed cohorts over time to characterize the durability of malaria immunity and the dynamics of CSP-specific IgG. Such studies could clarify whether observed deficits persist, widen, or possibly narrow as children age. In addition, clinical trials of malaria vaccines should include HIV-exposed and -infected infants to directly measure vaccine efficacy and optimize dosing in these groups. It will be important to determine, for example, whether HIV-affected children derive comparable clinical protection from RTS,S/AS01 or R21 despite lower antibody titers (Otieno et al., 2020). Immunologically, research is needed on how HIV alters T-cell help, memory B-cell function, and cytokine profiles in the context of malaria. Finally, operational research should evaluate models of integrated service delivery, such as combined HIV/malaria clinics or joint health education campaigns, to identify best practices for co-managing these conditions at the community level.

Guidance for health authorities (Uganda Ministry of Health, WHO, etc.): National and district health planners should consider incorporating these findings into policy and practice. The Uganda Ministry of Health and National Malaria Control Programme are encouraged to revise malaria control strategies to explicitly address children living with HIV or born to HIV-positive mothers. This could include updating training materials for health workers to note the special risks in these populations and to monitor malaria incidence among children in HIV care. Uganda's malaria surveillance systems may also consider recording HIV status, to better track co-infection trends. At the global level, WHO and international partners should consider issuing joint technical guidance on pediatric HIV–malaria co-management, for example aligning malaria vaccine roll-out plans with national HIV programs. Investments should support district-level interventions (such as environmental management of stagnant water) that benefit all children but are particularly critical in areas with high HIV prevalence. By coordinating efforts across the malaria and HIV sectors, authorities can help ensure that immunologically vulnerable children receive comprehensive protection. In sum, applying the study's insights through policy and programming can enhance child survival and move closer to the joint targets for malaria control and HIV care.

REFERENCES

Abu-Raya, B., Kollmann, T., Marchant, A., & MacGillivray, D. (2016). The immune system of HIV-exposed uninfected infants. Front Immunol. 2016; 7: 383. *A comprehensive review focussed on immunology of HEU children*.

Abu-Raya, B., Kollmann, T. R., Marchant, A., & MacGillivray, D. M. (2016). The immune system of HIV-exposed uninfected infants. *Frontiers in immunology*, *7*, 383.

Doolan, D. L., Dobaño, C., & Baird, J. K. (2009). Acquired immunity to malaria. *Clinical microbiology reviews*, *22*(1), 13-36.

Hammershaimb, E. A., & Berry, A. A. (2024). Pre-erythrocytic malaria vaccines: RTS, S, R21, and beyond. *Expert Review of Vaccines*, *23*(1), 49-52.

Kariuki, S. K., Lal, A. A., Terlouw, D. J., ter Kuile, F. O., ONG'ECHA, J. M., Phillips-Howard, P. A., Orago, A. S., Kolczak, M. S., Hawley, W. A., & Nahlen, B. L. (2003). Effects of permethrin-treated bed nets on immunity to malaria in western Kenya II. Antibody responses in young children in an area of intense malaria transmission. *The American journal of tropical medicine and hygiene*, *68*(4_suppl), 108-114.

Kwarteng, C. T. (2025). The multisectoral response to a public health crisis: Uganda's HIV/AIDS policy in perspective.

Lockman, S., Hughes, M., Powis, K., Ajibola, G., Bennett, K., Moyo, S., van Widenfelt, E., Leidner, J., McIntosh, K., & Mazhani, L. (2017). Effect of co-trimoxazole on mortality in HIV-exposed but uninfected children in Botswana (the Mpepu Study): a double-blind, randomised, placebo-controlled trial. *The Lancet global health*, *5*(5), e491-e500.

Longwe, H., Jambo, K. C., Phiri, K. S., Mbeye, N., Gondwe, T., Hall, T., Tetteh, K. K., Drakeley, C., & Mandala, W. L. (2015). The effect of daily co-trimoxazole prophylaxis on natural development of antibody-mediated immunity against P. falciparum malaria infection in HIV-exposed uninfected Malawian children. *Plos one*, *10*(3), e0121643.

Moyo, S., Ajibola, G., Makhema, J., Shapiro, R., Essex, M., Mazhani, L., Powis, K., van Widenfelt, E., Lockman, S., & Hughes, M. (2017). Effect of co-trimoxazole on mortality in HIV-exposed but uninfected children in Botswana (the Mpepu Study): a double-blind, randomised, placebo-controlled trial.

Muema, D. K., Ndungu, F. M., Kinyanjui, S. M., & Berkley, J. A. (2011). Effect of HIV infection on the acute antibody response to malaria antigens in children: an observational study. *Malaria Journal*, *10*(1), 55.

Obase, B. N., Francis, Z., Forgu, E. L., Honore, A., Bigoga, J. D., & Nsagha, D. S. (2023). The effects of HIV infection on the immune response to malaria among pregnant women in Kumba, southwest Cameroon: protocol for a cross-sectional study. *JMIR Research Protocols*, *12*(1), e38213.

Organization, W. H. (2023). *WHO malaria policy advisory group (MPAG) meeting report, 18–20 April 2023*. World Health Organization.

Otieno, L., Mendoza, Y. G., Adjei, S., Agbenyega, T., Agnandji, S. T., Aide, P., Akoo, P., Ansong, D., Asante, K. P., & Berkley, J. A. (2020). Safety and immunogenicity of the RTS, S/AS01 malaria vaccine in infants and children identified as HIV-infected during a randomized trial in sub-Saharan Africa. *Vaccine*, *38*(4), 897-906.

Pohl, K., & Cockburn, I. A. (2022). Innate immunity to malaria: The good, the bad and the unknown. *Frontiers in immunology*, *13*, 914598.

Ray, J. E., Dobbs, K. R., Ogolla, S. O., Daud, I. I., Midem, D., Omenda, M. M., Nowacki, A. S., Beeson, J. G., Sabourin, K. R., & Rochford, R. (2024). Clinical and immunological outcomes of HIV-exposed uninfected and HIV-unexposed uninfected children in the first 24 months of life in Western Kenya. *BMC infectious diseases*, *24*(1), 156.

Subramaniam, K. S., Skinner, J., Ivan, E., Mutimura, E., Kim, R. S., Feintuch, C. M., Portugal, S., Anastos, K., Crompton, P. D., & Daily, J. P. (2015). HIV malaria co-infection is associated with atypical memory B cell expansion and a reduced antibody response to a broad array of Plasmodium falciparum antigens in Rwandan adults. *Plos one*, *10*(4), e0124412.

Zalwango, M. G., Bulage, L., Zalwango, J. F., Migisha, R., Agaba, B. B., Kadobera, D., Kwesiga, B., Opigo,

J., & Ario, A. R. (2023). Trends and distribution of severe malaria cases, Uganda, 2017–2021: analysis of health management information system data. *Uganda National Institute of Public Health. Q Epidemiol Bull*, *8*, 2.