A SYSTEMATIC REVIEW OF THE IMMUNOGENICITY, SAFETY AND EFFICACY OF THE RTS,S/AS01 MALARIA VACCINE

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A research report submitted to the Faculty of Health sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Science in Medicine (Vaccinology)

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Johannesburg, 2025

DECLARATION

I, Darlington Chukwuma Ugwu declare that this Research Report is my own, unaided work. It is being submitted for the Degree of Master of Science in Medicine (Vaccinology) at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University

Darlington Chukwuma Ugwu

25th day of **March 2025** at the University of the Witwatersrand, Johannesburg, South Africa

DEDICATION

I would like to dedicate this work to the memory of my beloved mother, Ugwu Grace Ngozi, whose love, resilience and encouragement continue to inspire me daily.

Though she is no longer here to witness this achievement, her influence and values have been the guiding light in my life and work. Her belief in the power of education and her dedication to her family laid the foundation for my journey.

ABSTRACT

Malaria remains a major global health challenge, with *Plasmodium falciparum* causing high morbidity and mortality, especially in sub-Saharan Africa. The RTS,S/AS01 vaccine, endorsed by the WHO, represents a significant advancement in malaria prevention, though questions persist regarding its long-term efficacy, immune durability and safety across populations. To address these gaps, this systematic review and meta-analysis evaluated available evidence on the immunogenicity, efficacy and safety of RTS,S/AS01.

Following PRISMA 2020 guidelines and registered in PROSPERO (CRD42023374259), studies published between 2012 and 2024 were identified through PubMed, Cochrane Library, Scopus and ClinicalTrials.gov. Risk of bias was evaluated using ROB-2 and ROBINS-I tools. Data were synthesized using fixed- and random-effects models, with subgroup and heterogeneity analyses.

From 12,427 records, 51 studies were included qualitatively, and 12 in the meta-analysis. Immunogenicity was higher in children aged 5-17 months (GMT 621 EU/mL) than in infants (GMT 209 EU/mL), with stronger responses in Phase II trials. Booster doses enhanced antibody levels initially, but titres declined over time. Combining RTS,S/AS01 with seasonal malaria chemoprevention (SMC) improved immune responses, with regional variation. Safety analysis showed no significant difference in adverse events between vaccinated and control groups. Slight increases were noted in children aged 5-17 months and after booster doses but were not statistically significant. RTS,S/AS01 combined with SMC significantly reduced adverse events. Vaccine efficacy was highest in children aged 5-17 months (log OR -0.42), with improved protection from booster doses (log OR -0.34) and the RTS,S-SMC combination (log OR -0.92). Regional heterogeneity was observed, with moderate reductions in malaria cases in Ghana, Kenya, Malawi, Mozambique and Tanzania.

RTS,S/AS01 offers moderate, time-limited protection with an acceptable safety profile. These findings support targeted vaccination in children aged 5-17 months and integration with existing interventions like SMC.

Keywords: RTS,S/AS01, malaria vaccine, immunogenicity, efficacy, safety, systematic review, meta-analysis

ACKNOWLEDGEMENTS

Sincerest thanks to my supervisors, Prof. Robyn van Zyl and Dr. Sarentha Chetty, for their constant and patient guidance, encouragement and constructive feedback. Their expertise and insights greatly enriched the quality of this research project and have been instrumental to its success. I would also like to extend my thanks to Dr. Clare Cutland, Kay Roberts, Sanele Mdada and Members of Graduate Studies Committee, Prof. Nigel Crowther and Kgomotso Tlala for their input and advice**.**

Special thanks to my family and friends, whose support and encouragement have been instrumental throughout this journey.

Finally, I am deeply thankful to Lilian Ramaphoko, Lerato Makhalemele and Dr. Yah Clarence who participated in this study and contributed their valuable time and insights. Their input was crucial to the success of this project, and I hope the findings will contribute positively to public health interventions globally.

Thank you all for helping me bring this research to fruition

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ABBREVIATIONS

ACT	-	Artemisinin-based Combination Therapies
ALIVE	-	African Leadership in Vaccinology Expertise
AS01	-	Adjuvant System 01
CI	-	Confidence Intervals
CSP	-	Circumsporozoite Protein
EMA	-	European Medicines Agency
EPI	-	Expanded Programme on Immunization
et al.	-	and others
EU/mL	-	ELISA Units per Millilitre
GMT	-	Geometric Mean Titre
GSK	-	GlaxoSmithKline
H^2	-	Variance across studies exceeds what is expected by chance
HbSAg	-	Hepatitis B Surface Antigen
I^2	-	Percentage of variation across studies caused by true heterogeneity
LMICs	-	Low- and Middle-Income Countries
Log ORs	-	Logarithmic odds ratios
MeSH	-	Medical Subject Headings
NANP	-	Asparagine-Alanine-Asparagine-Proline Repeats
PICO	-	Population, Intervention, Comparison, Outcome
PRISMA	-	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
P-value	-	Probability Value
Q	-	Measures total heterogeneity across studies
RCTs	-	Randomized Controlled Trials
REML	-	Restricted Maximum Likelihood
ROB-2	-	Risk of Bias 2 Tool
ROBINS-I	-	Risk of Bias in Non-randomized Studies - of Interventions
RTS,S/AS01	-	Recombinant Protein Malaria Vaccine
SMC	-	Seasonal Malaria Chemoprevention
WHO	-	World Health Organization
Z-score	-	Measures how far a value is from the mean in standard deviations
θ	-	Pooled effect size
θi	-	Effect size estimate for the i-th study

DEFINITION OF TERMS

Adjuvant System 01 (AS01)	-	A proprietary adjuvant used to enhance the immune response of the RTS,S malaria vaccine
Antigen	-	A substance that induces an immune response, often a component of a pathogen or vaccine
Artemisinin-based Combination Therapies (ACTs)	-	Frontline treatment for uncomplicated malaria
Booster Dose	-	An additional vaccine dose given after the primary series to maintain immunity
Circumsporozoite Protein (CSP)	-	The main antigenic component of the RTS,S malaria vaccine
Efficacy	-	The ability of a vaccine to prevent disease under ideal and controlled conditions
Expanded Programme on Immunization (EPI)	-	A global initiative to increase immunization coverage for children
Global Alliance for Vaccines and Immunization (GAVI)	-	A partnership promoting immunization in low-income countries
GlaxoSmithKline (GSK)	-	The pharmaceutical company that developed RTS,S
Immunogenicity	-	The ability of a vaccine to induce an immune response
Indoor Residual Spraying	-	A malaria prevention strategy involving insecticide application indoors
Insecticide-Treated Nets	-	Nets treated with insecticide to prevent mosquito bites
Intermittent Preventive Treatment in Pregnancy	-	Use of antimalarial drugs to prevent malaria in pregnant women
Low- and Middle-Income Countries (LMICs)	-	Countries classified by the World Bank based on income levels
Observational Study	-	A study that observes outcomes without intervention
Placebo	-	A substance with no therapeutic effect used as a control in testing vaccines.

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)	-	Guidelines for systematic reviews.
Randomized Controlled Trial (RCT)	-	A study design that randomly assigns participants to experimental or control groups
Risk of Bias 2 (ROB-2)	-	A tool to assess the risk of bias in randomized trials
Risk of Bias in Non-Randomized Studies- of Interventions (ROBINS-I)	-	A tool to assess bias in observational studies
RTS,S/AS01	-	The first malaria vaccine approved for widespread use in children
Seasonal Malaria Chemoprevention (SMC)	-	Administration of antimalarial drugs during high malaria transmission seasons
Systematic Review	-	A structured analysis of existing research on a specific topic
World Health Organization (WHO)	-	A United Nations agency promoting global health initiatives
Zotero	-	A reference management software used in systematic reviews

CHAPTER ONE – INTRODUCTION

1.1 BACKGROUND

Malaria remains a major global health challenge, with the highest burden concentrated in sub-Saharan Africa and Southeast Asia, despite decades of control efforts (WHO, 2024c). Since the launch of the global malaria eradication programme in 1955, the World Health Organization (WHO) has implemented strategies centred on strengthening primary health care, early diagnosis, prompt treatment, and prevention through vector control (Talapko *et al*., 2019). Over the years, these interventions have achieved important gains, yet malaria continues to contribute substantially to morbidity and mortality. The complexity of the parasite's biology, life cycle, and genetic diversity, alongside its ability to evade immune responses, have complicated eradication efforts (El-Moamly & El- Sweify, 2023).

Vaccines have emerged as a critical addition to existing control measures. Since the first attempts at vaccine development in the 1960s, progress has been slow, largely due to the parasite's complexity. A major breakthrough came with the RTS,S/AS01 vaccine, the first malaria vaccine to receive WHO recommendation for routine use in children living in areas of moderate to high *Plasmodium falciparum* transmission (Björkman *et al.,* 2023).

1.1.1 Malaria and its Impact

Malaria is caused primarily by *Plasmodium falciparum*, which is transmitted through the bite of infected female *Anopheles* mosquitoes. This parasite is responsible for over 99% of malaria-related deaths globally (Varo *et al.,* 2020). In 2023, approximately 263 million malaria cases were recorded globally, with 94% of the cases concentrated in Africa (WHO, 2024c). Children below the age of five carry the heaviest load of severe illness, constituting 67% of total global deaths (Varo *et al.,* 2020).

The disease has a disproportionate impact on vulnerable populations such as pregnant women and young children (Al-Awadhi *et al.,* 2021; Laurens, 2018). Beyond health outcomes, malaria exerts a substantial socio-economic toll. For individuals, costs include treatment expenses, lost workdays, school absenteeism, and burial costs (WHO, 2023). Governments face significant expenditures for health services, drug procurement and distribution, and vector

control programmes, while also experiencing broader economic losses due to reduced productivity (Umumararungu *et al.,* 2023). In 2020, the annual funding requirement for malaria conttrol and elimination was set at $6.8 billion, with projections increasing to $9.3 billion by 2025 and reaching $10.3 billion by 2030 (WHO, 2023). The global funding for malaria control and elimination in 2023 was US$4.0 billion across 90 countries. The WHO African Region was allocated 75% of the funding because of the high malaria burden in the region (WHO, 2024c). Furthermore, the 2023 report on malaria by WHO revealed that the estimated global malaria cases in 2022 surpassed the levels recorded before the COVID-19 pandemic in 2019. The report emphasizes various challenges to the global response to malaria, with climate change emerging as a notable threat (Venkatesan, 2024). The 2024 World Malaria Report also indicated that in 2023, approximately 597 000 deaths worldwide were linked to malaria, leading to a mortality rate of 13.7 deaths per 100,000 population at risk. Notably, the African Region remains the most affected by malaria-related deaths, accounting for 95% of estimated deaths worldwide (WHO, 2024c). Nigeria (26%), the Democratic Republic of the Congo (13%), Uganda (5%), Ethiopia (4%), and Mozambique (4%) were the five countries with the highest estimated number of malaria cases in 2023 (WHO, 2024c).

1.1.2 Need for Malaria Vaccines

Malaria vaccines are very important in public health, especially in countries where malaria is endemic. Studies have shown that children and pregnant women face an increased risk of severe outcomes from malaria. In pregnant women, malaria infections can lead to adverse outcomes such as miscarriages, low birth weight and maternal anaemia. Severe malaria in children can progress rapidly, resulting in complications like cerebral malaria, respiratory distress, and death *(*Al-Awadhi *et al.,* 2021; Laurens, 2018; Park *et al.,* 2020).

The rise of drug-resistant strains of the *P. falciparum* is a huge problem for controlling and treating malaria. Some strains are becoming resistant to commonly used antimalarial drugs like artemisinin- based combination therapies (ACTs). The emergence of drug-resistant strains of the *P. falciparum* presents a major concern to malaria control and treatment efforts. Resistance to commonly used antimalarial drugs like ACTs including reported cases of cytotoxicity and multiple organ failure associated with the use of the antimalarial drugs (Balaji *et al.,* 2020), highlights the pressing need for alternative strategies of which vaccines represent a potential option (Umumararungu *et al.,* 2023). Moreover, although strategies such as the use of bed nets

treated with insecticides and indoor spraying have been successful in decreasing malaria transmission, the effectiveness of these measures has been affected by the increasing resistance of mosquitoes to insecticides. Thus, vaccines offer an additional layer of protection by directly targeting the parasite within the human host. As a cost-effective intervention, malaria vaccination would play a very important role in effectively reducing the global malaria burden and thus enhance efforts to eradicate the disease globally especially, in sub-Saharan Africa, where they are needed the most.

1.1.3 Development of RTS,S/AS01 Malaria Vaccine

Scientific progress has opened avenues for developing malaria vaccines, such as the RTS,S/AS01 vaccine, which is commercially known as Mosquirix®. The RTS,S malaria vaccine stands as the most advanced contender in the fight against human malaria. The journey began in the early 1980s with its initial conception and design (Casares *et al.,* 2010), resulting in its approval and recommendation in 2021 (Egbewande, 2022). Throughout this period, the RTS,S vaccine has successfully navigated numerous challenges, including the complexities of design, trial setbacks, limited resources and logistical constraints (Egbewande, 2022). The RTS,S malaria vaccine is the result of approximately 30 years of research, developed through a collaboration between GlaxoSmithKline (GSK) and the PATH Malaria Vaccine Initiative (Cohen *et al.,* 2010; Keating, 2020). The first successful human trial, which demonstrated protection against *Plasmodium falciparum* sporozoite infection, was conducted in 1996 at the Walter Reed Army Institute of Research using the RTS,S vaccine developed by GSK (Egbewande, 2022; Stoute *et al.,* 1997). The vaccine was designated as RTS,S due to its formulation, which involved the fusion of genes from the repeat ('R') and T-cell epitope ('T') regions of the pre-erythrocytic circumsporozoite protein (CSP) of the *P. falciparum* malaria parasite with a surface antigen ('S') derived from the hepatitis B virus. This was subsequently combined with additional hepatitis B surface antigen (HBsAg) to enhance purification, thereby accounting for the additional "S" in its name (Egbewande, 2022). The AS01 adjuvant system of this vaccine was formulated at GSK Biologicals by mixing a concentrated liposome bulk intermediate with a formulation buffer (PO_4/NaCl) and then adding QS-21 liquid bulk (Egbewande, 2022).

RTS,S/AS01 is provided as a lyophilized preparation and administered through intramuscular

injection. It is stored and administered in a manner consistent with other paediatric vaccines, which facilitates its distribution even in countries with limited economic infrastructure (Mumtaz *et al.,* 2023). The WHO has endorsed a four-dose regimen of RTS,S/AS01 for use in regions of Africa with moderate to high malaria transmission. They recommended administration of a 4-dose schedule in children as early as 5 months of age in order to reduce malaria burden and cases. Countries may choose to administer this first dose before the age of 5 months due to operational considerations, to increase impact or coverage. The shortest gap between doses is four weeks; however, to achieve long-term protection, the fourth dose should be administered six to eighteen months following the third (WHO, 2024a). Furthermore, RTS,S/AS01 may be co-administered with other vaccines as part of a national immunization program (Mumtaz *et al.,* 2023; Praet *et al.,* 2022; WHO, 2024a).

The development of RTS,S/AS01 has involved multiple clinical trials to assess its safety and efficacy in different populations, particularly in malaria-endemic regions. One significant study, discussed by Praet *et al*. (2022), highlights that RTS,S/AS01 was evaluated in Phase III trials, where it demonstrated a 39% reduction in clinical malaria cases and 29% of severe malaria cases over a four- year follow-up period in children aged 5 months or older. Furthermore, the vaccine was associated with a decrease in overall hospitalizations, including those due to severe anaemia and the need for blood transfusions. Although the vaccine exhibited a generally favourable safety profile, it was noted to have greater reactogenicity than control vaccines, with most side effects being mild to moderate and transient in nature.

Similarly, in a cluster-randomized trial conducted in Ghana, Kenya and Malawi, involving 158 geographical clusters to evaluate the real-world implementation of the RTS,S/AS01E malaria vaccine, the study demonstrated high vaccine coverage rates, no evidence of previous safety signals and significant reductions in severe malaria and child mortality (Asante *et al.,* 2024). The vaccine was administered to children in two phases: an early introduction group and a delayed introduction group. The primary outcomes were vaccine feasibility (coverage), safety (incidence of meningitis, cerebral malaria, and mortality, particularly among girls), and impact (reduction in severe malaria and all- cause mortality). The findings of the study were promising. The RTS,S vaccine demonstrated a significant impact on reducing the burden of malaria, with a 32% reduction in hospital admissions due to severe malaria and a 9% reduction in all-cause mortality among children who received three doses of the vaccine (Asante *et al.,* 2024). Importantly, the study found no evidence of increased risks for meningitis, cerebral malaria, or mortality among girls, addressing the concerns raised in earlier trials. The vaccine was

effectively integrated into the existing immunization programs, achieving substantial coverage rates and reinforcing its potential as a critical tool in the fight against malaria (Asante *et al.,* 2024).

In 2021, the WHO recommended the use of the RTS,S/AS01 malaria vaccine as part of malaria prevention strategies for children from age 5 months in regions with moderate to high *P. falciparum* malaria transmission, especially in sub-Saharan Africa, due to its strong safety profile and high impact (Björkman *et al.,* 2023; The Lancet, 2021).

As of February 2024, Cameroon and Burkina Faso have integrated the RTS,S/AS01 malaria vaccine into their routine immunization programs, following approval from Gavi, the Vaccine Alliance, for malaria vaccine roll-out support across 20 African countries (Adepoju, 2024; Amani *et al.,* 2024). Cameroon launched the four-dose RTS,S/AS01 vaccine on January 22, 2024, targeting 249,133 children in 42 high-risk health districts across all 10 regions, while Burkina Faso began rolling out the vaccine on February 5, 2024, in 27 prioritized districts across seven regions, aiming to vaccinate 248,986 children (Amani *et al.,* 2024).

1.2 Research Problem

The RTS, S/AS01 malaria vaccine represents a milestone in malaria prevention. While research indicates that the vaccine has proven to provide protection against clinical and severe malaria among African children (The RTS,S Clinical Trials Partnership, 2011), the variability in vaccine efficacy and immunogenicity across different age groups raises critical questions about the factors influencing the age-dependent protections (Laurens, 2018; White *et al.,* 2015). For instance, the levels of RTS,S/AS01-induced anti-circumsporozoite antibody titres were found to be higher in children aged 5-17 months compared to those aged 6-12 weeks. It has also been reported that the efficacy of RTS,S decreases to 3% after 5 years (White *et al.,* 2015). Varying efficacy and immunogenicity values from different studies stand the chance of impacting clinical decisions.

However, despite the initial success and the positive endorsement from the WHO in 2021 (Björkman *et al.,* 2023; The Lancet, 2021), there remain critical gaps in understanding the full scope of the vaccine's efficacy, immunogenicity and safety across diverse populations and under real-world conditions.

In the only systematic review currently available on the safety of the RTS,S/AS01 vaccine,

Yihunie *et al.,* (2023) focused primarily on data from randomized controlled trials (RCTs) to assess the safety of the RTS,S/AS01 vaccine. While their review offers important insights into the vaccine's safety within the controlled settings of clinical trials, it does not fully capture the complexities and variabilities of vaccine performance in routine immunization settings. This is particularly important as countries begin to integrate RTS,S/AS01 into their national immunization programs, where real-world factors such as healthcare infrastructure, population diversity and varying levels of malaria transmission intensity can influence vaccine outcomes (Adepoju, 2024; Amani *et al.,* 2024; Laurens, 2018; Varo *et al.,* 2020).

Moreover, the real-world implementation of the RTS,S/AS01 vaccine introduces new variables that were not fully explored in clinical trials. These include the potential for unreported or rare adverse events, differences in vaccine efficacy among various demographic groups and the impact of co-administration with other routine vaccines (Asante *et al.,* 2024; Praet *et al.,* 2022). Observational studies, which were not included in the Yihunie *et al.* (2023) systematic review, are crucial for capturing these real-world outcomes, as they reflect the performance of RTS,S/AS01 vaccine in a broader population over a longer period and under less controlled conditions.

Given the limitations of previous reviews that have focused exclusively on RCTs, there is a pressing need for a comprehensive systematic review that includes both RCTs and observational studies. This approach will provide a more complete picture of the RTS,S/AS01 vaccine's effectiveness and safety, particularly in the context of its integration into routine immunization programs in countries where malaria is endemic.

1.3. Statement of Purpose

The purpose of this systematic review was to comprehensively evaluate and analyse the immunogenicity, efficacy and safety of the RTS,S/AS01 malaria vaccine. This study aimed to synthesize existing literature from both RCTs and observational studies, particularly those conducted after the vaccine's integration into routine immunization programs based on predefined criteria in order to provide a thorough understanding of the vaccine's performance in terms of immunological response, effectiveness in preventing malaria infections and its safety profile. Through a careful examination of peer-reviewed articles, clinical trial reports, and epidemiological studies, this review intended to identify patterns, trends, and variations in the immunogenicity of the RTS,S/AS01 vaccine across diverse populations. Furthermore, it seeks

to evaluate the vaccine's efficacy in preventing malaria infections, considering factors such as age groups, geographic locations, and malaria transmission intensities. In addition to focusing on immunogenicity and efficacy, this systematic review critically evaluated the safety profile of the RTS,S/AS01 malaria vaccine. It investigated reported adverse events, potential side effects, and the overall safety considerations associated with the vaccine, considering both short-term and long-term implications.

The findings from this study will not only inform healthcare professionals, policymakers, and researchers but also guide future research directions and public health interventions related to malaria prevention.

1.4. Research Questions

1.4.1 Primary Research Question

What is the overall immunogenicity, safety, and efficacy of the RTS,S/AS01 malaria vaccine across diverse populations, based on evidence from phase I–IV clinical trials?

1.4.2 Secondary Research Questions

- What is the immunogenicity profile of RTS,S/AS01 in different age groups and regions?
- What is the safety profile of RTS,S/AS01, including common and rare adverse events in different age groups, phase of trials and regions??
- What is the efficacy/effectiveness of RTS,S/AS01 in preventing malaria infections in different age groups, phase of trials and regions?
- Does adding booster doses or combining RTS,S/AS01 with seasonal malaria chemoprevention enhance the protective efficacy of the vaccine in children?

1.5 Aim of the Study

Given that the RTS,S/AS01 malaria vaccine is currently in phase IV trials and substantial data from phase I-III clinical trials being available, there is a critical need for a comprehensive review of the evidence on its efficacy, immunogenicity and safety. The aim of this study was to conduct a comprehensive systematic review that evaluated the safety, efficacy and immunogenicity of the RTS,S/AS01 malaria vaccine. In contrast to previous reviews that have

predominantly focused on safety data from RCTs, this study integrated findings from both RCTs and observational studies. Special attention was given to studies conducted after the vaccine's introduction into routine immunization programs, in order to capture real-world outcomes and provide a more complete understanding of the vaccine's performance across different populations and settings.

1.6 Objectives of the Study

1.6.1 Primary objective

To evaluate the immunogenicity and efficacy the RTS,S/AS01 vaccine across diverse populations through a systematic review and meta-analysis.

1.6.2 Secondary objectives

To evaluate the safety of the RTS,S/AS01 vaccine across diverse populations.

1.7 Significance of Study

- In the global health landscape, a comprehensive understanding of the immunogenicity, efficacy, and safety of the RTS,S/AS01 vaccine will contribute to the broader efforts for malaria control and eradication.
- By incorporating both RCTs and observational studies, the review will offer a well-rounded perspective on the vaccine's safety, efficacy and immunogenicity. This holistic approach is essential for understanding the vaccine's true impact beyond the controlled conditions of clinical trials.
- The focus on studies conducted after the vaccine's introduction into routine immunization programs will help to identify and assess outcomes in real-world settings, where variables such as healthcare infrastructure, population diversity and co-administration with other vaccines come into play. This will provide valuable insights into the vaccine's practical effectiveness and safety.
- Previous reviews have primarily concentrated on safety aspects derived from RCTs, leaving gaps in the understanding of how the vaccine performs in broader contexts, including its efficacy and immunogenicity. This study will fill these gaps, thereby contributing to a more comprehensive body of knowledge

- The findings from this study will have direct implications for public health policy and malaria prevention strategies. By providing strong evidence on the vaccine's performance, the study can guide decision-makers in optimizing the use of the RTS,S/AS01 vaccine in different settings, potentially improving its overall impact on malaria control.
- Furthermore, this study will highlight areas where further research is needed, thus shaping the future research agenda in malaria vaccine development. This can lead to more targeted studies that address unresolved questions and challenges, ultimately supporting the goal of malaria eradication.

1.8 Scope of the Study

This research covered a thorough investigation of the RTS,S/AS01 malaria vaccine that was systematically reviewed and synthesized available evidence on immunogenicity, efficacy and safety of the RTS,S/AS01 malaria vaccine. The study included both RCTs and observational studies to provide a holistic understanding of the vaccine's performance across diverse populations and settings. The systematic analysis included a quantitative meta-analysis to identify patterns and variations in vaccine performance.

1.9 Summary

Chapter 1 of the report sets the stage for the systematic review and focusses on the RTS,S/AS01 malaria vaccine. The introduction included a background discussion, highlighting the global impact of malaria, a brief overview of the disease, establishing its significant burden and emphasizing the need for effective prevention strategies. The need for malaria vaccines, specifically the importance of vaccines in the context of malaria-endemic regions and vulnerable populations was discussed. The development of the RTS,S/AS01 malaria vaccine was introduced as a pivotal scientific advancement, setting the foundation for the study.

The research problem was outlined focusing on immunogenicity and efficacy of the RTS,S/AS,01 vaccine, and potential safety concerns, especially in clinical trials and real-world settings. The statement of purpose clearly articulated the intention of undertaking this study – a systematic review of the vaccine's immunogenicity, efficacy and safety. The research questions were formulated to guide the investigation, emphasizing immunogenicity of the

vaccine, effectiveness across diverse populations, safety considerations, and influencing factors.

The aim of the study was clearly stated, highlighting the need for a comprehensive review of evidence on the RTS,S/AS01 malaria vaccine. The objectives of the study were outlined, focusing on evaluating immunogenicity, efficacy, and safety across diverse populations. The significance of the study was discussed, emphasizing its contributions to malaria vaccine development and public health interventions. The scope of the study was defined, comprising a thorough examination of existing literature, clinical trials, and epidemiological studies.

Chapter 2 provided a comprehensive literature review addressing the research problem and objectives. **Chapter 3** outlined the entire methodology, from the initial formulation of the search strategy to the subsequent data analysis. **Chapter 4** presented the results of the study. Subsequently, **Chapter 5** described a detailed discussion of these results and a concluding section where the study's findings were summarized, and recommendations were documented.

CHAPTER TWO – LITERATURE REVIEW

2.1 Malaria Parasite and Epidemiology

Malaria occurs as a result of infection by protozoan parasites within the *Plasmodium* genus. Nearly all human infections of malaria are attributed to four species namely, *Plasmodium falciparum, P. vivax*, *P. malariae,* and *P. ovale. P. falciparum* is predominant in Africa causing the majority of infections and is the primary driver for severe illness and mortality. The transmission of malaria is facilitated by various species of the infected female *Anopheles* mosquitoes, each exhibiting distinct behaviours. This diversity plays a role in shaping the diverse epidemiological patterns observed worldwide for the disease (Greenwood *et al.,* 2005).

The geographical distribution of malaria is predominantly influenced by climatic variables such as temperature, humidity, and rainfall. Transmission of malaria occurs primarily in regions characterized by tropical and subtropical climates. This contributes to why Africa remains the hotspot for malaria transmission (Figure 2.1) (Endo *et al.,* 2017; Centre for Disease Control and Prevention, 2020). However, much of the epidemiological data comes from routine health facility reporting, which often underestimates community-level transmission, especially asymptomatic infections (Endo *et al*., 2017; Varo *et al*., 2020; Umumararungu *et al*., 2023). This raises questions about the accuracy of regional burden estimates used for vaccine impact modelling.

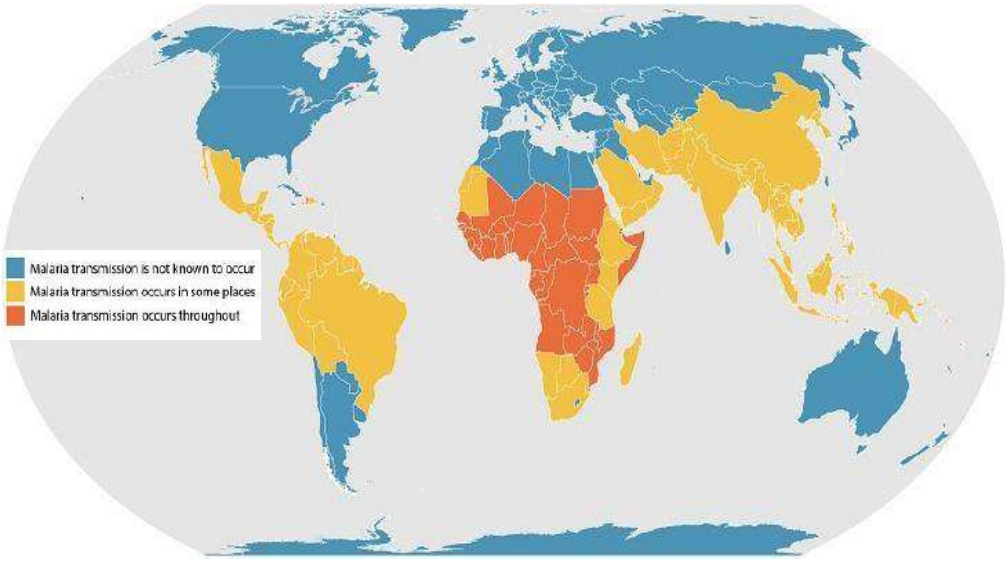


Figure 2.1: Regions susceptible to malaria transmission worldwide (Centers for Disease Control and Prevention, 2020).

Malaria affects mostly children and pregnant women. Across 33 countries with moderate to high malaria transmission in the WHO African Region, approximately 35.4 million pregnancies were reported. Of these, 12.7 million pregnancies (36%) experienced exposure to malaria during gestation. The prevalence of malaria exposure during pregnancy in 2022 peaked in West Africa (39.3%) and Central Africa (40.1%), while it was comparatively lower in the East and Southern Africa subregion (27.0%) (WHO, 2023). In 2023, an estimated 36 million pregnancies occurred in 33 moderate-to- high transmission countries within the WHO African Region, with 12.4 million (34%) affected by malaria (WHO, 2024c). In 2024, Egypt was declared malaria-free (Figure 2.2) (WHO, 2024b).

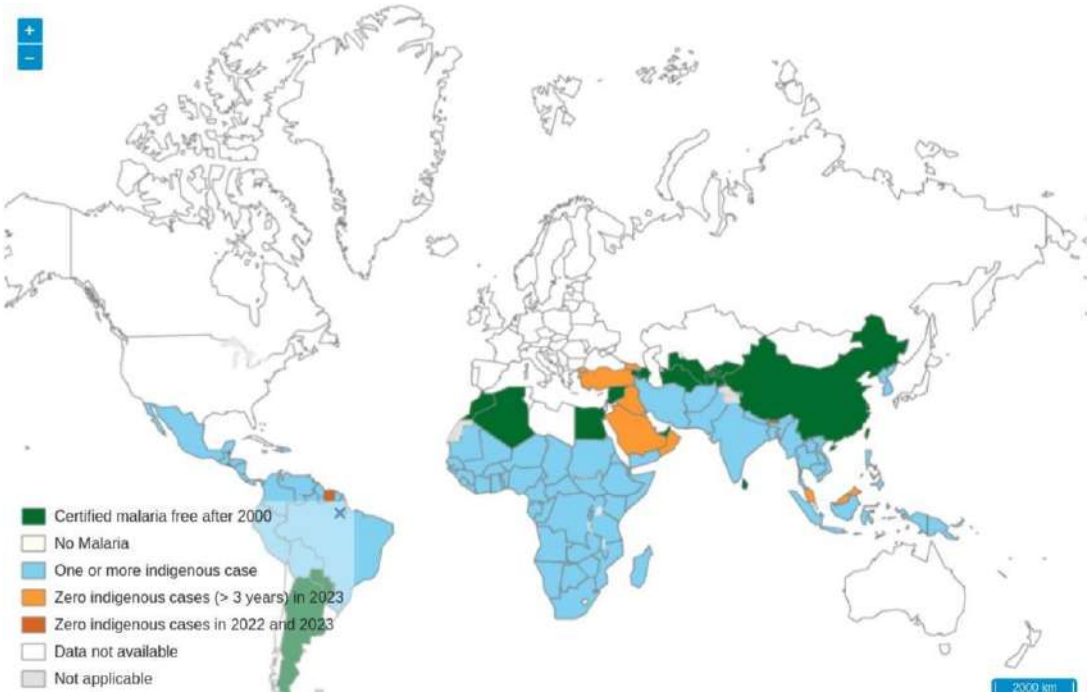


Figure 2.2: Status of countries with indigenous malaria cases in 2000 and their progress by 2023 (WHO, 2024b).

2.1.1 Life Cycle and Infection

The infection cycle of malaria begins with the bite of the infected female *Anopheles* mosquito during her blood meal from the host (Figure 2.3). During this process, the sporozoites, which are present in the mosquito's salivary gland, are released into the host. These sporozoites quickly migrate to the liver and infect hepatocytes. In the liver, the merozoites mature and are released into the bloodstream, and each merozoite proceeds to infect a fresh red blood cell

for replication. Within the red blood cell, merozoites undergo distinct phases, including rings, trophozoites, and schizonts. The mature schizont stage comprises of 16–24 merozoites, which exit the red blood cell through egress in order to initiate the infection of new red blood cells, and this marks the commencement of a new cycle (Figure 2.3). The stages within red blood cells hold clinical significance as they contribute to the manifestation of symptoms in the host throughout the course of a malaria infection (Balaji *et al.,* 2020). The common symptoms of malaria include headaches, nausea, dry cough, muscle aches and tiredness. A malaria infection that is not treated can lead to anaemia (Guggisberg *et al.,* 2014).

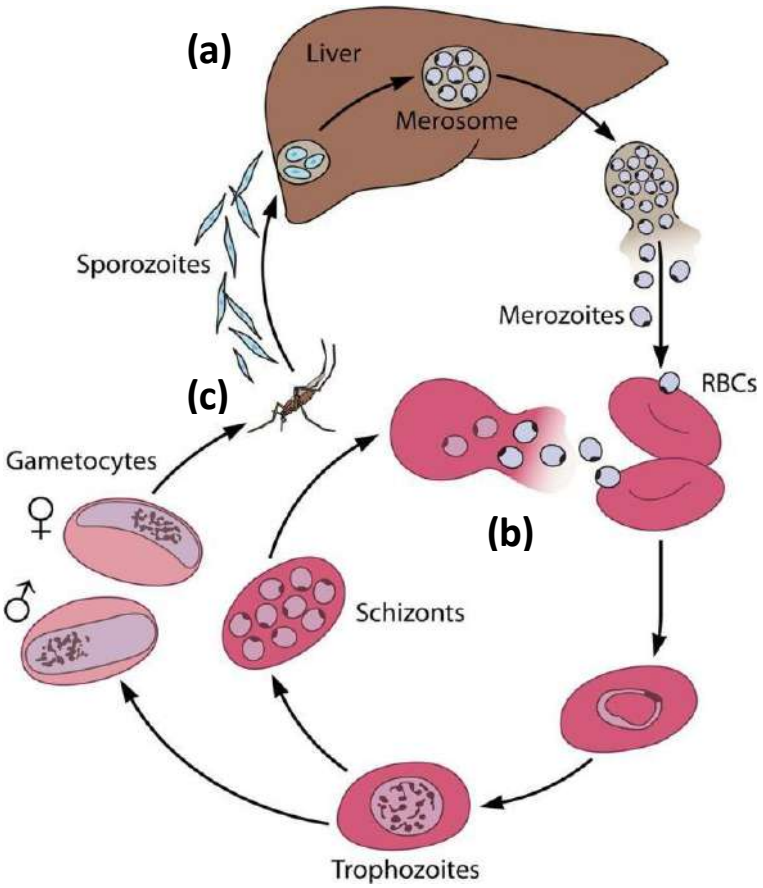


Figure 2.3: The life cycle of *Plasmodium falciparum* in its human host, showing three possible vaccine targets: (a) hepatic stages, (b) intra-erythrocytic malaria stages, (c) mosquito stages (Guggisberg *et al.,* 2014; Zheng *et al.,* 2019).

2.1.2 Socioeconomic Consequences

Malaria exerts serious economic consequences on the regions affected, especially in sub-Saharan Africa. The economic burden of malaria impacts both individuals and entire countries.

It was estimated that the global malaria spending, including government and out-of-pocket cost, was to be $4.3 billion (95% UI 4.2-4.4) in 2016, representing an 8.6% (95% UI 8.1-8.9) annual increase over malaria spending in 2000 (Andrade *et al.,* 2022). Available evidence indicates that the monthly household spending on malaria-related preventative measures ranges from $0.05 in rural Malawi to $2.10 in metropolitan Cameroon. Malaria also has an impact on labour productivity. The recurrent and debilitating nature of malaria frequently leads to absenteeism and reduced work capacity among those affected. This not only affects individual income but also reduces the overall economic output of a nation (Andrade *et al.,* 2022). For healthcare systems, the costs associated with malaria treatment, prevention, and control contribute to the overall burden, diverting resources that could be allocated to other critical health interventions. Furthermore, these consequences can extend beyond the immediate health sector to impact education. The disease often leads to school absenteeism among children, affecting their cognitive development and educational attainment. This, in turn, has long-term implications for workforce capabilities and economic growth.

2.2 Prevention and Control Strategies

Strategies for malaria prevention and control play a crucial role in mitigating the impact of this deadly disease, particularly in regions where it poses a significant public health threat. The approach to malaria control mostly includes preventive measures, early diagnosis and effective treatment.

Vector control stands out as a cornerstone in malaria prevention efforts. The use of insecticide-treated bed nets has proven to be highly effective in reducing mosquito bites, especially during the night when the *Anopheles* mosquitoes, responsible for malaria transmission, are most active. They are a cost-effective preventive measure (Ndiath, 2019; WHO, 2023). Manufacturers reported sales of 4.4 billion rapid diagnostic tests (RDTs) for malaria and 4.5 billion ACT treatment courses administered between 2010 and 2023. In 2023, national malaria programs distributed 345 million RDTs and 235 million ACTs, with the majority of these medications being distributed in sub-Saharan Africa.

Indoor residual spraying is another vector control strategy. This involves applying insecticides to the interior walls of houses. This method targets the mosquitoes that rest indoors after feeding. Indoor residual spraying has demonstrated effectiveness in reducing malaria transmission,

particularly in areas where mosquitoes have developed resistance to certain insecticides used in bed nets (Ndiath, 2019; WHO, 2023). Preventive treatment, which is also known as chemoprevention, is important in high-risk populations. Intermittent preventive treatment in pregnancy involves administering antimalarial drugs to pregnant women during routine prenatal care visits, thereby, protecting both the mother and the unborn child from the adverse effects of malaria. Seasonal malaria chemoprevention targets young children in areas with high seasonal malaria transmission, providing a course of antimalarial drugs during the peak transmission season (WHO, 2023). Early diagnosis and prompt treatment are critical components of malaria control. Rapid diagnostic tests enable quick and accurate diagnosis in remote settings where laboratory facilities may be limited. ACTs are the frontline treatments for uncomplicated malaria, ensuring effective and timely management of the disease (Umumararungu *et al.,* 2023; WHO, 2023).

Community engagement and education are essential for the success of malaria prevention and control initiatives. Empowering communities with knowledge about the importance of using preventive measures, seeking prompt treatment, and participating in control programs fosters a collective effort to combat the disease.

2.2.1 Barriers to Malaria Control Strategies

In the application of malaria control strategies, there are a number of barriers that hinder progress. One important concern is the unequal distribution of resources, which poses challenges to the accessibility of essential tools, including insecticide-treated bed nets, antimalarial drugs, and diagnostic facilities. This resource imbalance disproportionately affects regions with limited healthcare infrastructure and, therefore the burden of the disease. Inadequate healthcare infrastructure in malaria- endemic areas is a critical barrier to effective prevention and control. Owing to this, timely diagnosis and treatment are hindered by limited access to healthcare facilities, which can lead to delayed intervention. The shortage of skilled healthcare personnel further intensifies the problem of providing essential services.

The emergence of insecticide-resistant mosquitoes presents a significant problem to traditional vector control methods, such as indoor residual spraying (Ndiath, 2019)**.** The drawbacks associated with these strategies, such as the emergence of resistance, limited coverage in certain areas, high costs, and logistical challenges, highlight the need for effective complementary

approaches. Additionally, antimalarial drug use has also been linked to toxic effects and multiple organ failures in certain circumstances (Balaji *et al.,* 2020).

Among the various strategies for combating malaria, vaccination holds immense promise to make a huge difference in the fight against this deadly threat (Laurens, 2021; The Lancet, 2021). Vaccines offer a proactive and preventive approach to malaria control, targeting not only the parasites and mosquitoes but also potentially providing long-term protection to individuals and communities, thus offering a multifaceted advantage over other strategies (Laurens, 2021; The Lancet, 2021).

2.3 Vaccination in Infectious Disease Control

Vaccination remains a key component in the fight against infectious diseases. It serves as a significant preventive measure against several life-threatening pathogens (Kayser & Ramzan, 2024). Furthermore, it can also reduce the incidence, severity, and mortality associated with infectious diseases.

The primary objective of vaccination is to stimulate the immune system of the body to recognize and mount a defence against specific pathogens. This immunization process involves introducing a harmless form of the pathogen or a piece of the pathogen, which is also called an antigen, into the body. This exposure prompts the immune system to produce an immune response, including the production of antibodies and the development of memory cells (Iwasaki & Omer, 2020). So, when the individual is later exposed to the actual pathogen, the immune system can mount a swift and effective response, preventing or mitigating the infection.

Vaccines have played a pivotal role in controlling infectious diseases that were once widespread and deadly. One of the most compelling success stories is the eradication of smallpox through a global vaccination campaign led by the WHO. This remarkable achievement serves as important evidence of the impact and effectiveness of vaccination on a global scale (Kayser & Ramzan, 2024). In addition to smallpox, vaccines have proven instrumental in controlling and preventing a range of infectious diseases, including measles, polio, diphtheria, pertussis, tetanus, influenza, hepatitis and more. These preventive measures have not only saved countless lives but also contributed to the overall well-being and productivity of populations worldwide.

The significance of vaccination extends beyond individual protection, fostering the concept of

herd immunity. When a sufficiently high proportion of a population is immune to a particular disease through vaccination, the spread of the pathogen is impeded, protecting even those who may not be eligible for or unable to receive vaccination. However, the success of vaccination programs is not without challenges. Vaccine hesitancy, driven by misinformation, mistrust, or complacency, poses a threat to achieving high vaccination coverage. Access to vaccines, particularly in resource-limited settings, remains a barrier, emphasizing the need for equitable distribution and outreach efforts (Grant *et al.,* 2022; Sallam *et al., 2*024).

2.3.1 Malaria Vaccines

The history of malaria vaccines demonstrates the persistent efforts of the global scientific community to fight one of the most prevalent and deadly infectious diseases. The quest for a malaria vaccine started in the 1930s when researchers embarked on pioneering studies involving inactivated or attenuated whole parasites. While laying the foundation for future endeavours, these early attempts faced formidable challenges in achieving consistent and strong protection (Ouattara & Laurens, 2015).

In subsequent decades, the focus shifted towards subunit vaccines targeting specific components of the *Plasmodium* parasite. Among the first candidates was the SPf66 vaccine in the 1980s, a synthetic peptide vaccine that has antigens from the blood stages of malaria coupled together with an antigen from the sporozoite stage. Despite early promise, SPf66 fell short of providing the high-level, long- lasting protection essential for an effective malaria vaccine (Graves & Gelband, 2006).

Significant progress in the malaria vaccine development was reported in 1967, with the use of irradiated sporozoites to vaccinate mice, which resulted in sterile immunity against the disease (Tsoumani *et al.,* 2023). While initial clinical trials demonstrated encouraging results, challenges in achieving sustained efficacy and overcoming the genetic diversity of the parasite hindered its progression. A wave of research explored alternative antigens and vaccine formulations, including DNA-based vaccines, viral vectors, and innovative approaches aimed at eliciting a more comprehensive immune response (Ghattas *et al.*, 2021; Hasson *et al.,* 2015).

Amid these endeavours, the RTS,S/AS01 vaccine marked a ground breaking advancement in the malaria vaccine landscape. Developed by GlaxoSmithKline in collaboration with the PATH Malaria Vaccine Initiative, RTS,S/AS01 became the first malaria vaccine to receive a positive scientific opinion from the European Medicines Agency in 2015 (Keating, 2020). The vaccine

primarily targets the CSP (Laurens, 2018) and is designed for use in children in malaria-endemic regions, with a particular focus on *P. falciparum.*

2.3.1.1 Types of Malaria Vaccines and How they Work.

Several potential malaria vaccine candidates targeting various stages of the malaria parasite's life cycle, such as the pre-erythrocyte stage, the blood stage, and the mosquito stage (Figure 2.3 points (a), (b) and (c) in life cycle), are at various stages of clinical development (Kurtovic *et al.,* 2021b).

(a) Pre-erythrocytic Vaccines

The aim of this vaccine strategy is to stimulate a humoral immune response that can effectively destroy the sporozoites, hence impeding its ability to invade the hepatocyte (Figure. 2.3 (a)) (Orenstein *et al.,* 2024). RTS,S is the most advanced pre-erythrocytic malaria vaccine currently being rolled out in several countries (Duffy & Patrick Gorres, 2020). Other pre-erythrocytic malaria vaccines include R21/Matrix-M, which is recently being endorsed by WHO to be used in endemic regions (WHO, 2024a)

(b) Blood Stage Vaccines

Merozoites, which are the stage of the parasite that erupts from hepatocytes and enters the bloodstream. The merozoites then invade and lyse red blood cells (RBCs), breaking them down in seconds. the maturation process occurs within cells that lack the expression of major histocompatibility complex components, which suggests that once inside RBCs, the parasite develops further (Figure 2.3 (b)). Consequently, the vaccine approach has been characterised as antibody-dependent, which is achieved either by the inactivation of merozoites during their limited exposure to the external environment or by specifically targeting malarial antigens expressed on the surface of erythrocytes to induce cellular inhibition mediated by antibodies (Orenstein *et al.,* 2024).

(c) Mosquito Stage Vaccines

Vaccines targeting mosquito stages rely on the mosquito ingesting the antibody and complement alongside the parasite during a blood meal (Figure 2.3 (c)). During the maturation of the parasite within the mosquito, these antigens are exposed to antibodies, neutralizing the

sexual stages (Orenstein *et al.,* 2024). Pfs230 is a mosquito stage antigen-based vaccine currently undergoing phase 2 clinical trials in endemic countries (Kurtovic *et al.,* 2021b)

2.3.2 RTS,S/AS01 Malaria Vaccine

RTS,S/AS01 is a recombinant protein candidate malaria vaccine that targets the CSP of *P. falciparum*, expressed by the malaria parasite during the pre-erythrocytic stage and is formulated with the AS01 adjuvant (Zheng *et al.,* 2019). The vaccine is designed to target the sporozoite phase of the malaria parasite development cycle, preventing infection of the liver, which is normally where the parasite matures, reproduces, re-enters the circulation, and infects red blood cells (The Lancet, 2021).

Although pilot studies undertaken in Ghana, Kenya and Malawi demonstrated feasibility, variations in efficacy were reported across regions with differing transmission intensities. For instance, higher efficacy was observed in moderate transmission settings compared to holoendemic areas, where reinfection pressure may blunt vaccine-induced protection (Varo *et al*., 2020). Such geographic variation has critical implications for targeting vaccine rollout (Laurens, 2018; Varo *et al.,* 2020). Following the administration of four doses, the vaccine has been shown to exhibit 27% efficacy against clinical malaria in children aged 6–12 weeks and 39% efficacy in children aged 5–17 months (Laurens, 2018). In 2015, the European Medicines Agency provided a favourable assessment of the vaccine (Laurens, 2021). Subsequently, on October 6, 2021 the WHO made an announcement endorsing the widespread use of the RTS,S/AS01 malaria vaccine for children residing in sub-Saharan Africa and other areas characterised with moderate-to-high *P. falciparum* transmission (The Lancet, 2021).

2.3.2.2 RTS, S/AS01 Malaria Vaccine Component

The RTS,S/AS01 malaria vaccine is the leading pre-erythrocytic subunit malaria vaccine. It is made up of a protein (RTS) from the NANP repeat and the C-terminal parts (R and T) (Figure 2.4) of the NF54 strain of *P. falciparum* CSP combined with the hepatitis B virus surface antigen (HBsAg; the S portion) (Tsoumani *et al.,* 2023). It is given together with an adjuvant based on liposomes (AS01), which is intended to boost immunity (Tsoumani *et al.,* 2023). The CSP is a key protein on the surface of the malaria parasite during its sporozoite stage, which is the initial phase in the parasite's life cycle and is transmitted to humans through mosquito bites (Figure 2.5).

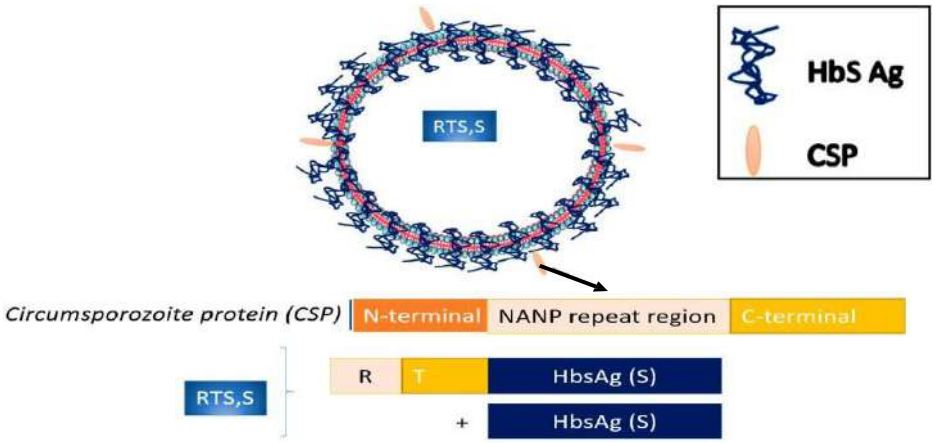


Figure 2.4: RTS,S CSP subunit vaccine structure (Tsoumani *et al.,* 2023).

2.3.1.3 Mechanism of Action of RTS,S/AS01

The mechanism of action of the RTS,S malaria vaccine involves a comprehensive strategy targeting the *P. falciparum* parasite by primarily focusing on the CSP and utilizing the AS01 adjuvant system (Ouattara & Laurens, 2015). The intricate life cycle of the parasite, particularly the role of the sporozoite in transitioning between the definitive mosquito host and the intermediate human host, forms the basis for the vaccine's design (Figure 2.5).

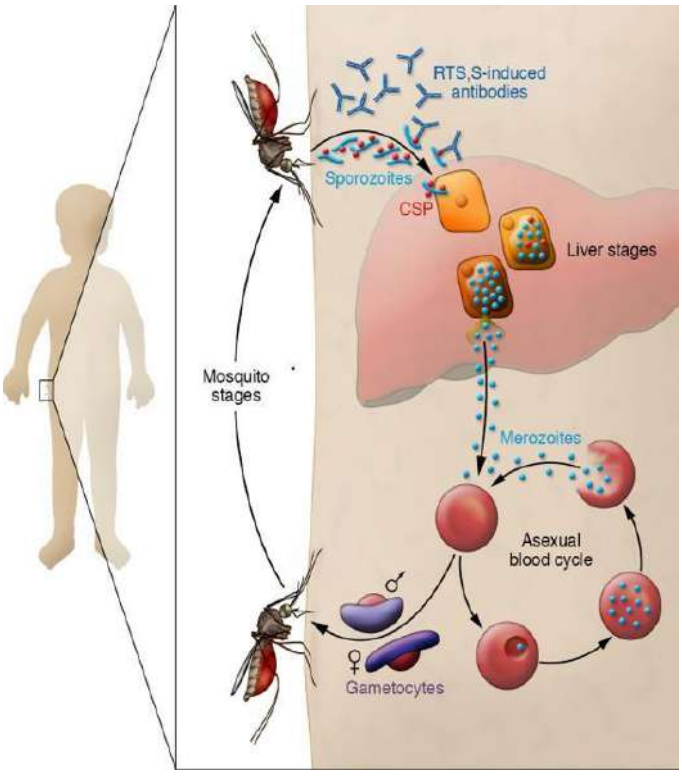


Figure 2.5: The effect of the RTS, S/AS01 malaria vaccine on malaria parasite transmission and infection (Zavala, 2022).

The design of the vaccine is based on the complex life cycle of the parasite as the sporozoites are transmitted between the final mosquito host and the intermediate human host (Figure 2.5) (Tsoumani *et al.,* 2023). Generally, when RTS,S is administered, it stimulates antibody production against CSP, which is expressed by *P. falciparum* sporozoites (Figure 2.5). When an unvaccinated person contracts malaria, the sporozoites enter the liver. In the liver, they pass through the hepatocytes and eventually develop into hepatic merozoites. CSP is expressed in the early liver stage (Figure 2.5); however, it is not expressed by liver merozoites (Sinnis & Zavala, 2012). Antibodies to CSP that are produced following the vaccination of RTS,S/AS01 malaria vaccine block the sporozoites, thereby preventing hepatocyte infection. Protection against a malaria infection and the development of severe malaria generated by RTS,S/AS01 decreases with time, and this is correlated with decreasing anti-CSP antibody levels. However, the immune responses generated by the vaccine do not affect the ability of *Plasmodium* gametocytes to infect mosquitoes. Therefore, even after vaccination, most children still carry malaria parasites, which will infect mosquitoes; as such the RTS,S/AS01 does not inhibit parasite transmission to the population (Zavala, 2022).

Molecularly, the circumsporozoite protein, a major surface protein of *Plasmodium* sporozoites, plays a critical role in parasite development within the mosquito and in the infection of the mammalian host. The C- terminus and the final 18 NANP repetitions make up the portion of the CSP, that is contained in the RTS,S/AS01 malaria vaccine (Figure 2.4) (Laurens, 2020). The self-assembly of monomers from the hepatitis B virus surface antigen (HBsAg) results in the formation of virus-like particles. Within the RTS,S vaccine, about 25% of these HBsAg monomers are genetically linked to the truncated CSP, serving as carriers for the protein component of the vaccine (Figure 2.6). The RTS,S vaccine incorporates a fragment of the CSP containing 189 amino acids from CSP (NF54 199-387aa). This fragment includes the final 18 NANP repeats and the C-terminus, excluding the GPI anchor addition sequence. Within this CSP fragment, three recognized T-cell epitopes are present. They are variable CD4+ T-cell epitope preceding the TSP-like domain (TH2R), a variable CD8+ T-cell epitope within the TSP-like domain (TH3R), and a conserved universal CD4+ T-cell epitope (CS.T3) located at the C-terminus (Kaslow & Biernaux, 2015). Despite the self-assembly of monomers from the Hepatitis B virus surface antigen (HBsAg), which results in the formation of virus-like particles, the adjuvant- free RTS,S exhibits low immunogenicity, and this makes it necessary to add an adjuvant in order to enhance the strength and duration of immune responses to CSP. The AS01 adjuvant in the RTSS,AS01 vaccine, contains two immunostimulants which are 3-

O-desacyl-4′-monophosphoryl lipid A and QS-21 adjuvant (Stimulon®) in a liposome formulation (Laurens, 2020). The mechanism of action of AS01 involves triggering the innate immune system and significantly activating antigen-presenting dendritic cells in the draining lymph nodes. Potential targets for protective antibodies have been identified in the CSP N-terminal region, which contains two functional domains that elicit immune responses (Kaslow & Biernaux, 2015).

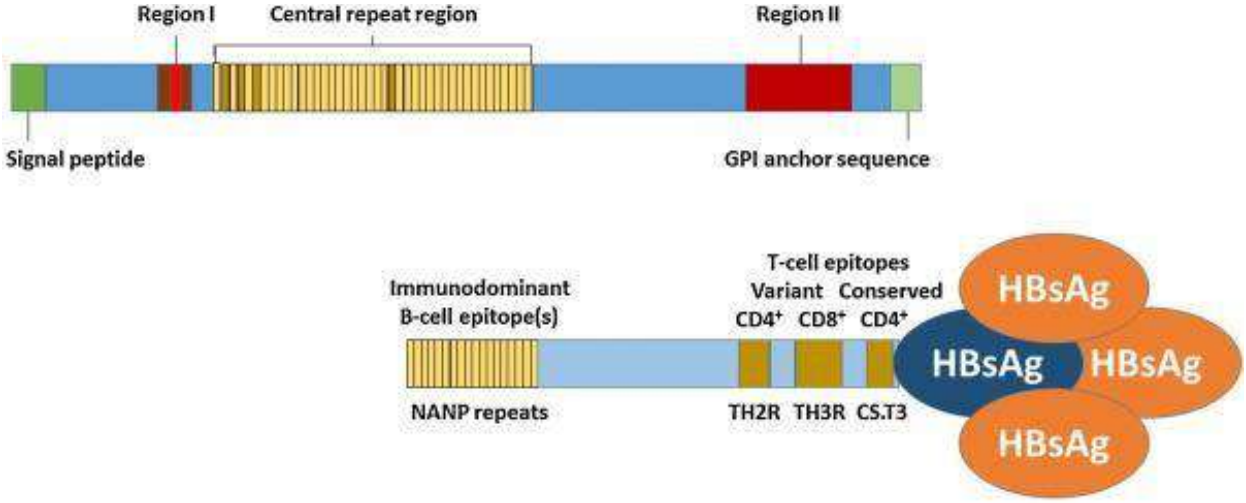


Figure 2.6: The CSP and RTS,S structures (Kaslow & Biernaux, 2015).

2.3.1.4 Efficacy and Immunogenicity Studies of RTS,S/AS01

The efficacy and immunogenicity studies of the RTS,S/AS01 malaria vaccine have provided valuable insights into the performance and the immune responses induced by this vaccine. The Phase III study of the RTS,S/AS01 was conducted in seven African countries between 2009 and 2014. While the primary goal was to assess vaccine efficacy over a year, the secondary objective was to determine the impact of the booster dose in the 20th month. Efficacy estimates varied substantially by age group, with protection nearly twice as high in children 5–17 months compared to infants (55.8% vs 31.3%). This suggested that immune system maturity may influence vaccine responsiveness. However, follow-up data showed waning immunity in both groups, raising concerns about the durability of protection, particularly in high-transmission settings.

In the extended follow-up analysis, the four-dose regimen of the vaccine exhibited efficacy at 25.9% in infants (6–12 weeks) and 36.3% over a median of 48 months in children (5–17 months). Over the same period, the three-dose regimen showed declining efficacy against clinical malaria in both age groups, with a slower decline observed in the four-dose group.

From this study, the efficacy of the 3- and 4-dose regimens against clinical malaria and severe malaria was shown to be lower in infants aged 6–12 weeks compared to children aged 5–17 months (Laurens, 2020). Most trials used clinical malaria incidence as the primary endpoint, which may underestimate efficacy in high-transmission settings where repeated exposures occur. Few studies evaluated severe malaria as a primary outcome, limiting comparability across trials and reducing the ability to assess the vaccine's impact on the most life-threatening forms of the disease.

Immunologically, the anti-circumsporozoite antibody titres elicited by RTS,S/AS01 were higher in children aged 5-17 months compared to 6-12 weeks (White *et al.,* 2015). The anti-circumsporozoite antibody titres that were against the NANP repeat region and are associated with increased titres and responses have been associated with a lower risk of clinical malaria, although no threshold for protection has been determined (Laurens, 2020). Additionally, following the administration of RTS,S/AS01, CD4+ T cell responses increased, which may be related to protection (Laurens, 2020). The Phase III clinical trial reported that the RTS,S/AS01 vaccine-induced immunogenicity was higher in older children than in infants. Anti-circumsporozoite antibody increased to 318.2 EU/mL in children aged 5-17 months after 1 month of the booster dose administration and 34.2 EU/mL in children that did not receive the boost (Laurens, 2020). Anti-CSP antibody titres were described as a surrogate of protection for the duration and magnitude of efficacy of RTS,S/AS01 malaria vaccine (White *et al.,* 2015).

2.3.1.5 Safety Studies of RTS,S/AS01

The reported Phase III clinical trials of RTS,S/AS01 included comprehensive safety monitoring, involving thousands of participants across malaria-endemic regions (Kaslow & Biernaux, 2015). The vaccine demonstrated a generally favourable safety profile (El-Moamly & El-Sweify, 2023), with most adverse events being mild and transient in nature. Common side effects included pain at the injection site, fever and mild local reactions, reflecting the expected immune response elicited by the vaccine (Yihunie *et al.,* 2023).

2.3.1.6 Regulatory Approval

In June 2014, GlaxoSmithKline submitted a regulatory application to the European Medicines Agency (EMA), marking the first step in the policy and regulatory process. The quality, safety,

and efficacy of the RTS,S malaria vaccine were assessed by the EMA through an evaluation process conducted under the Article 58 protocol (Kaslow & Biernaux, 2015). EMA carried out the assessment in collaboration with the WHO and non-European Union Regulators. The European Scientific Opinion was released in July 2015 by the Committee for Medicinal Products for Human Use, adopting a positive opinion and recommending the granting of a marketing authorization for the active immunization of children aged 6 weeks to 17 months against hepatitis B and malaria caused by *P. falciparum* (Kaslow & Biernaux, 2015).

In July 2022, RTS,S/AS01 was prequalified by the WHO (WHO, 2024d) and has been included for review by GAVI for Vaccine Investment Strategy (Kaslow & Biernaux, 2015). In January 2024, the Cameroon launched RTS,S/AS01 as a component of their routine immunization programme (Nalova Akua, 2024).

CHAPTER THREE – METHODS AND MATERIALS

3.1 Study Design

This study follows a systematic review and meta-analysis design to assess the immunogenicity, efficacy and safety of the RTS,S/AS01 malaria vaccine. The study adhered to the guidelines outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement to ensure a structured and transparent review process. The protocol for this systematic review was registered with the International Prospective Register of Systematic Reviews (PROSPERO), registration number [CRD42023374259], on 12 February 2023 and was last updated on 16 June 2024. The deviations from the protocol were documented and justified.

Ethical clearance for this study was waived by the Human Research Ethics Committee (Non-Medical) of the University of the Witwatersrand, Johannesburg, as documented in waiver letter number HRECNMW24/03/06 (Appendix C).

3.2 Search Strategy and Information Sources

In consultation with the review team, a comprehensive search strategy for this study was carefully designed to identify and synthesize relevant data on the immunogenicity, safety and efficacy of the RTS,S/AS01 malaria vaccine. The approach was structured to align with the rigorous standards of systematic reviews in order to ensure that all relevant evidence was captured and analysed.

A comprehensive literature search was conducted across several major databases including PubMed, Cochrane, Clinical Trials.gov and Scopus. The search aimed to identify studies that evaluated the vaccine's immunogenicity, efficacy and safety. Studies were carefully selected using a combination of Medical Subject Headings (MeSH) and free-text terms such as "vaccine," "immunogenicity," "efficacy," "effectiveness," "safety," "randomized controlled trial," "clinical trial," and "observational study." Boolean operators were employed to refine the search and ensure that all relevant studies were included.

The Population, Intervention, Comparison, Outcome (PICO) framework (Table 3.1) was employed as a structured tool to systematically analyse the quantitative evidence gathered in response to the research question.

Table 3.1: PICO framework applied to assess the eligibility of studies relevant to the research question.

Criteria	Determinants
P – Population	Malaria-infected and uninfected individuals, including males and females of all ages, across any country, race and gender.
I – Intervention	RTS,S/AS01 vaccine for the prevention of malaria infection.
C – Control	Placebo or conventional methods of malaria chemoprevention
O – Outcome	Primary outcome - To evaluate the immunogenicity and efficacy of the RTS, S/AS01 vaccine in the control of malaria infection. Secondary outcome - To evaluate the safety of the RTS, S/AS01 vaccine.
S – Study design	Randomized controlled trials, single or double blinded randomized trials, and observational studies.

3.3 Study Selection

Search terms, as outlined in Appendix A, were entered into several key databases, including Cochrane, PubMed, ClinicalTrials.gov and Scopus on 1 July 2024. This process adhered strictly to the predefined search strategy detailed in Appendix A. The search was conducted to retrieve relevant literature published between 1 January 2012 and 30 June 2024.

The search terms were carefully selected from the titles and abstracts of relevant articles, as well as from index terms describing these articles. These terms were then systematically applied across the databases to ensure comprehensive coverage of the literature. The results, including the number of records retrieved from each database and the subsequent deduplication process are summarized in (Appendix A).

Records retrieved by the search strategy was imported into Zotero V20 for reference management and deduplication, leveraging its capability to identify and remove duplicate records from the dataset. Following this, the unique records were exported from Zotero and uploaded into an Excel tool. During the initial screening phase, two independent reviewers examined the titles and abstracts of the articles, categorizing each record as "yes," "maybe," or "no" based on predefined inclusion and exclusion criteria (Table 3.2). Discrepancies between the reviewers were highlighted within the Excel tool and any disagreements were resolved

through discussion, with a third reviewer intervening when necessary to reach a consensus. Records classified as "yes" or "maybe" were advanced to the full-text screening stage.

In the full-text review, the same two reviewers assessed the articles in detail against the inclusion criteria. Disagreements during this phase were similarly resolved by involving a third reviewer if consensus could not be reached. The reference lists of all studies selected for inclusion were screened for additional relevant studies.

The entire selection process, including the number of records screened, assessed for eligibility and included in the final review, was documented using an adapted PRISMA flow diagram (Figure 4.1) (Moher et al., 2009). This diagram also detailed the reasons for excluding studies at each stage, ensuring transparency in the study selection process (Figure 4.1).

Table 3.2: Inclusion and Exclusion Criteria.

Inclusion Criteria	Exclusion Criteria
Evidence pertaining to the safety, efficacy, immunogenicity, antibody response, immune cell response including adverse events and long-term safety data	Studies involving non-human subjects including animal and *in-vitro* investigations
Evidence gathered from randomized controlled trials, including single- or double-blinded trials, as well as observational studies	Studies involving patients who are HIV positive and co-infected with malaria.
Studies published in English between 1 January 2012, and 30 June 2024, from any country, race, age and gender.	Conference abstracts, editorials, reviews, bulletins and any RCTs or observational studies that focused on malaria vaccines other than RTS,S/AS01.
	Studies based on implementation and the socio-economic impact of the vaccine.

3.4 Study Quality Evaluation

The quality assessment of the studies included in this review was conducted using comprehensive and standardized tools to ensure the evaluation of potential biases. For the RCTs, the Cochrane Risk of Bias tool version 2 (ROB-2) was employed. This tool provided a

structured framework to assess biases across several domains such as the randomization process, deviations from intended interventions, missing outcome data, measurement of outcomes and selection of the reported results. Each of the forty-four RCTs was carefully analysed within these domains, leading to an overall classification of the risk of bias as "Low Risk," "Some Concerns," or "High Risk." (Figure 4.2). This classification facilitated a clear understanding of the extent to which bias might have influenced the study findings. Furthermore, the risk of bias assessment for cluster-randomized trials was conducted using the RoB 2 tool for cluster-randomized trials (Figure 4.3).

For the observational studies, the Risk of Bias in Non-randomized Studies of Interventions (ROBINS- I) tool was employed. This tool was specifically chosen because of its ability to assess biases inherent in non-randomized studies, covering domains such as confounding, participant selection, intervention classification, deviations from intended interventions, missing data, outcome measurement and selection of reported results. The seven observational studies were carefully evaluated using these criteria and each study's overall risk of bias was classified as "Low Risk," "Some Concerns," or "High Risk.

3.5 Study Data Extraction

The data extraction process was carried out systematically using a pre-determined and standardized Microsoft Excel template (version 16.45). This template was designed to ensure consistency and accuracy in collecting relevant information from each included study. Data extraction was conducted in alignment with the study objectives and focused on capturing essential details. Upon completion, all extracted data were reviewed to ensure completeness and quality.

Data from the studies were extracted under several categories (Table 4.1). First, study characteristics were documented, including the names of the authors, the study design and the demographics of participants, such as their age and the countries in which the studies were conducted (Table 4.1). These details provided a foundational understanding of the studies and their contexts. Intervention details were also carefully extracted. This included information on the vaccine type, vaccine regimen/strategy, dosage and vaccination schedule. The outcomes were categorized into immunogenicity, safety and efficacy. Finally, limitations identified in each study were documented to provide context and highlight potential biases or challenges within the studies (Table 4.1).

Furthermore, in order to facilitate a meta-analysis, relevant quantitative data were extracted and categorized into efficacy, immunogenicity and safety domains, which allowed for subgroup analyses based on study design, age groups and outcome measures. For efficacy, extracted variables included study design, vaccine regimen/strategy, country, age group and the number of malaria cases recorded in both the vaccine and control arms (Appendix B). The efficacy dataset included studies that were undertaken in several countries to evaluate vaccine efficacy or effectiveness in various age groups, including newborns (6-12 weeks) and children (5-17 months) (Appendix B).

The geometric mean titres of anti-CSP antibodies across multiple investigations, as well as their corresponding confidence intervals, were the primary focus of the extracted data for immunogenicity (Table 4.2). Study designs, vaccination regimens/strategies, age categories and countries were among the additional data that was extracted. However, a significant limitation was the absence of standard deviation data for geometric mean titres (GMTs) in all included studies, which restricted the possibility of statistical analysis. Finally, for safety, the data on adverse events of interest, including suspected clinical meningitis, convulsions, fever, and deaths from the included studies were primarily extracted (Appendix C). The dataset includes the number of occurrences of each adverse event in both the vaccine and control arms which was stratified by study designs, vaccine regimen/strategy and methods, age groups and countries (Appendix C).

3.6 Data Analysis

The data analysis process was systematically conducted to ensure accuracy, reliability and strength in evaluating vaccine efficacy and safety. It began with data cleaning and preparation in Excel to ensure consistency and accuracy, followed by effect size estimation using odds ratios in fixed and random-effects models based on heterogeneity. Heterogeneity assessment was performed using Cochran's Q and I^2, with subgroup analyses to explore variability. Meta-analysis was visualized through forest plots, while sensitivity analysis utilized Galbraith plots to identify outliers and assess study influence. The publication bias assessment was conducted using funnel plots and Harbord regression to detect small-study effects.

3.6.1 Data Cleaning and Preparation

All datasets from the included studies were reviewed for completeness, consistency and accuracy before analysis. Microsoft Office 365 (Excel) was used for preliminary data cleaning, including the removal of duplicates, handling of missing values and ensuring that data formatting was uniform across all the studies included for the quantitative analysis.

3.6.2 Effect Size Estimation

The outcomes of interest were vaccine efficacy and safety, and both were expressed as log-transformed odds ratios (ORs) with 95% confidence intervals (CIs). The efficacy analysis was conducted using the restricted maximum likelihood (REML) method in a random-effects model, as a result of the significant heterogeneity observed across studies ($I^2 > 96\%$). In contrast, a fixed-effects model was used for safety analysis due to the minimal heterogeneity ($I^2 < 35\%$). The selection of the model was influenced by heterogeneity assessments to guarantee that the analytical approaches were suitable for each outcome.

Vaccine efficacy was measured in terms of malaria case reduction, stratified by age group and vaccine regimen, including vaccine regimen/strategy, study design and country. Safety outcomes were assessed for reported adverse events, stratified by age group and vaccine regimen, including vaccine regimen/strategy, study design and country, with effect sizes estimated using log ORs to maintain consistency with the efficacy analysis.

Immunogenicity was identified as an outcome of interest. However, a meta-analysis of GMTs was not performed because most included studies reported GMT values with confidence intervals only, without providing the standard deviations or standard errors required for quantitative pooling. Furthermore, considerable heterogeneity was observed in the immunogenicity assays used (e.g., ELISA vs. neutralization assays), making statistical harmonisation unreliable. To maintain methodological accuracy and avoid potentially misleading estimates, immunogenicity results were summarised descriptively. Similar approaches have been adopted in related meta-analyses when variance data were unavailable or assay methods were heterogeneous (Afolabi *et al.,* 2021; Yin *et al.,* 2024)

3.6.3 Assessment of Heterogeneity

Heterogeneity across studies was assessed using Cochran's Q test and the I^2 statistic. An I^2 statistic ($I^2 > 96\%$) indicated substantial heterogeneity, warranting the use of a random-effects model for efficacy analysis, while lower heterogeneity ($I^2 < 35\%$) justified the use of a fixed-effects model for safety analysis (Figures 4.4 & 4.13). To explore potential sources of variability, subgroup analyses were conducted based on different age groups, vaccine regimen, including vaccine regimen/strategy, study design and country for the efficacy and safety outcomes (Figures 4.5, 4.6, 4.7, 4.8, 4.14, 4.15, 4.16, 4.17, 4.18).

3.6.4 Statistical Modelling and Sensitivity Analysis

Meta-analyses were performed using both fixed-effects and random-effects models, as illustrated in the forest plots (Figures 4.4 & 4.13). Subgroup analyses were carried out to assess potential effect modifications by age groups, vaccine regimen, including vaccine regimen/strategy, study design and country. Sensitivity analyses were performed using Galbraith plots to identify outliers and examine the influence of individual studies on the pooled estimates (Figures 4.12 & 14.22).

3.6.5 Publication Bias Assessment

Potential publication bias was assessed through funnel plots and contour-enhanced funnel plots to visually inspect asymmetry (Figures 4.9, 4.10, 4.19 & 4.20). The Harbord regression test for small-study effects was performed to statistically evaluate the presence of publication bias (Figure 14.21).

CHAPTER FOUR – RESULTS

4.1 Search Results

A total of 12,427 publications was screened for efficacy, immunogenicity and safety of the RTS,S/AS01 malaria vaccine. These records were identified from PubMed (n = 11,090), Cochrane (n = 736), ClinicalTrials.gov (n = 27), and Scopus (n = 574). After removing 3,875 duplicates, 8,552 records remained for the title and abstract screening. Of these, 8,300 studies were excluded for reasons such as in vitro studies, reviews, editorials and commentaries. This left 252 full-text articles that were assessed for eligibility (Figure 4.1).

Following the full-text review, 201 studies were excluded due to reasons such as lack of relevant outcome data, studies involving patients who are HIV positive and co-infected with malaria, studies that are based on implementation and the socio-economic impact of the vaccine, leaving a total of 51 studies that were included in the final review. From these, 12 studies were also incorporated into the quantitative synthesis, providing numerical data relevant to the research question (Figure 4.1).

All included studies were written in English. Among the 12 RCTs included in the meta-analysis, 6 were double-blind and 6 open-label trials (Figure 4.1).

4.1.1 Summary of Search Query Outcomes and Article Retrieval

The search was conducted in PubMed, Cochrane Library, ClinicalTrials.gov and Scopus, incorporating both Medical Subject Headings (MeSH) terms and keyword variations to maximize retrieval of relevant literature. The search comprised of four main queries, which were designed to maximize the retrieval of studies that were related to the RTS,S/AS01 malaria vaccine, with an emphasis on immunogenicity, efficacy and safety (Appendix A).

The first query was conducted using a MeSH term-based approach, with a specific focus RTS,S/AS01. This approach resulted in the retrieval of 1,788 articles from PubMed, 101 articles from Cochrane Library, 27 articles from ClinicalTrials.gov and 338 articles from Scopus. The second query broadened the scope by incorporating keyword variations and Boolean operators, and this yielded 9,231 records from PubMed, 534 from Cochrane and 204 from Scopus (Appendix A).

In the third query, the results were further refined to include safety, efficacy, risks and benefits.

This query retrieved 30 articles from PubMed, 57 from Cochrane Library and 26 from Scopus (Appendix A).

Finally, a comprehensive combination search integrating immunogenicity, immune response, antibody involvement and safety/efficacy metrics yielded 41 articles from PubMed, 44 from Cochrane and 6 from Scopus (Appendix A).

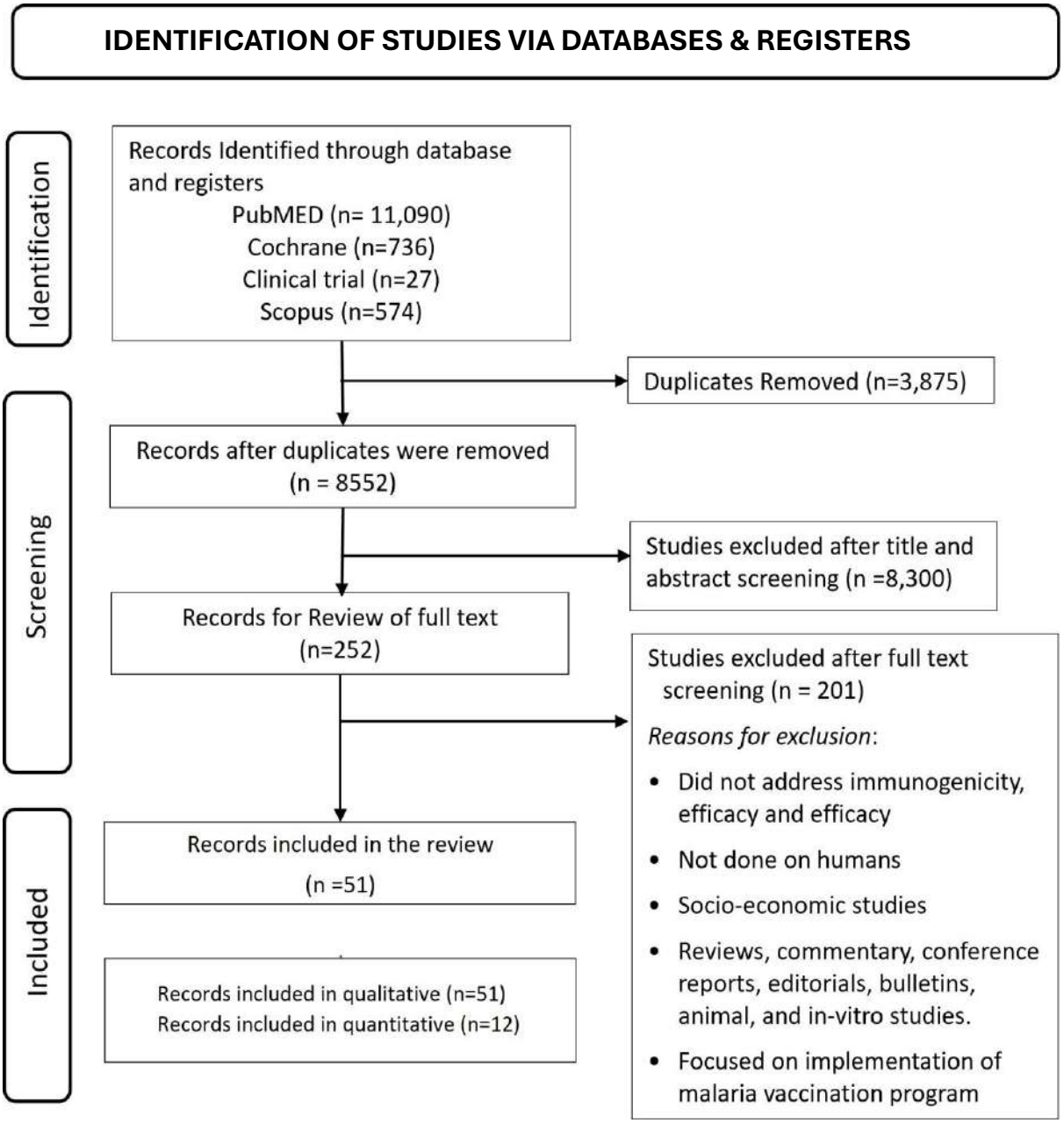


Figure 4.1: The PRISMA 2020 flow diagram of the study selection to evaluate the immunogenicity, efficacy and safety of RTS,S/AS01 malaria vaccine. Adapted from (Page *et al.,* 2021; Yihunie *et al.,* 2023).

4.2 Characteristics of Included Studies

The studies that were included in this systematic review was a combination of Phase I/II (n=1) (Kurtovic *et al.,* 2021a), Phase II (n=24) and Phase III (n=21) RCTs, case-control studies (n=4) (Dobaño *et al.,* 2019c; Moncunill *et al.,* 2017a; Olotu *et al.,* 2014; Ubillos *et al.,* 2018) and observational studies (n=1) (Asante *et al.,* 2024) (Table 4.1). These studies were conducted in 13 countries, with 10 of them being in Africa (Burkina Faso, Ghana, Kenya, Tanzania, Mozambique, Gabon, Malawi, Nigeria and Mali), as well as in malaria-naive populations (Belgium, United States, and Thailand) (Table 4.1). The age categories of participants in the studies were diverse, with trials directed toward infants (6–12 weeks), children (5–17 months) and adults (18–50 years). Forty-six of the studies followed RCT designs. Four of the studies also included case-control or nested study designs within larger trials to investigate specific immunological markers. The age groups varied, with studies including infants (6–12 weeks) evaluating the earliest immunization schedules, children (5–17 months) as the primary population targeted for malaria vaccination, older children (1–4 years) in some trials to assess prolonged vaccine effects and adults (18–50 years) in malaria-naive populations for controlled human malaria infection studies. All 51studies investigated RTS,S/AS01 with different dosing regimens and strategies. Most trials administered three primary doses at months 0, 1 and 2. Some studies included an additional booster dose at 18 or 20 months to assess long-term protection. Variations such as fractional doses and seasonal malaria vaccination strategies were also assessed (Table 4.1).

All of the studies reported either on vaccine efficacy, safety and immunogenicity as primary outcomes, with additional assessments of malaria risk factors. Protection against clinical malaria varied across studies, with reported efficacy ranging from 25% to 55.8% in children and 31.3% to 36.3% in infants over 12–48 months. Twenty three of the trials found about 30–70% reduction in malaria cases with RTS,S/AS01. Seventeen trials revealed that vaccine efficacy declined after 18 months, but a booster dose partially restored immunity.

Thirty of the trials induced strong anti-CSP antibody responses, which were associated with protection against malaria. $CD4^+$ T-cell responses, cytokine secretion and memory B-cell activity were commonly assessed. Higher immunogenicity was noted in children older than in infants, possibly due to maternal immunity interference in younger infants. Seven studies showed that IgG1 and IgG3 responses were associated with protection, while 1 study mentioned that IgG4 responses were linked with higher malaria risk. Three studies mentioned

that fractional dosing regimens showed comparable immunogenicity to full-dose regimens (Table 4.1).

The vaccine was generally well tolerated, with mild-to-moderate injection site pain, fever and irritability. Nine of the trials reported cases of meningitis and febrile seizures. Seven studies evaluated alternative strategies such as seasonal malaria chemoprevention with RTS,S/AS01, delayed fractional doses. The limitations identified in the studies include loss to follow-up in long-term studies, possibility of maternal immunity interference in infants which can potentially affect vaccine responses. Some studies relied on passive case detection, which may have underestimated malaria incidence. There was also variability in malaria transmission intensity across study sites which could have influenced observed vaccine efficacy. Nine studies included malaria-naïve populations (Belgium, USA, Thailand), limiting direct applicability to endemic settings (Table 4.1).

Table 4.1: Information from the included studies in the systematic review on the immunogenicity, safety and efficacy of the RTS,S/AS01 Malaria Vaccine.

S/N	Authors	Study Design	Age Month/day	Country	Type of Vaccine	Dosage	Vaccination Schedule	Outcomes	Limitations
1	Horowitz *et al.*, 2012	Phase IIb, randomized controlled trial	Children aged 5–17 months	Tanzania	RTS,S/AS01E	Three doses	Three primary doses at 0, 1 and 2 months	**Immunogenicity:** RTS,S/AS01E vaccination induced stronger T cell responses to HBsAg and CSP, including increased NK cell activation and IL-2 secretion, correlating with NK cell CD69 expression. NK cells contributed to the IFN-g response post-vaccination	The study analysed 185 out of 447 children due to sample issues. This can lead to a risk of bias.
2	Ndungu *et al*., 2012	Phase IIb randomized controlled trial	Children aged 5–17 months	Kenya	RTS,S/AS01E	Three doses	Administered at 0, 1, 2 months	**Immunogenicity:** RTS,S/AS01E induced CSP-specific CD4+ T cell responses, significantly reducing the risk of clinical malaria. Synergistic effects between CD4+ T cells and anti-CSP antibodies provided enhanced protection. It induced strong and durable immunogenicity, particularly IFNγ−IL2+TNF+ T cell responses and high anti-CSP antibody titres.	The study assessed polyfunctional T cell responses, but it did not evaluate how long these responses persisted beyond 12 months or their role in long-term protection.
3	The RTS,S Clinical Trials Partnership, 2012*	Phase III, randomized controlled trial	Infants aged 6–12 weeks	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	RTS,S/AS01	Full dose (0.5 mL)	Administered at 0, 1, and 2 months	**Immunogenicity:** 99.7% developed anti-circumsporozoite antibodies post-vaccination with a geometric mean titre of 209 EU/mL. **Safety:** Serious adverse events were similar in both vaccine and control groups. **Efficacy:** Vaccine demonstrated 31.3% efficacy against clinical malaria and 36.6% efficacy against severe malaria in infants 6–12 weeks of age over 12 months.	Although the study was double- blinded, the use of a non-malaria comparator vaccine (meningococcal serogroup C vaccine) could mean that some differences in adverse events and immune responses might not be completely attributable to RTS,S/AS01.

4	Olotu *et al.*, 2013*	Phase IIb randomized controlled trial	Children aged 5–17 months	Kenya	RTS,S/AS01E	Three doses	0, 1 and 2 months	**Immunogenicity:** It induced strong initial anti-circumsporozoite antibody responses, which waned over time but remained higher than the control. Vaccine efficacy was greater in low-exposure areas. **Safety:** Adverse events were minimal with no significant safety concerns reported **Efficacy:** vaccine provided 29.9% efficacy against the first malaria episode, declining to −0.4% by year four.	
5	Leroux-Roels *et al.,* 2014	Phase II, randomized clinical trial	18–45 years (malaria naïve adults)	Belgium	RTS,S/AS01, RTS,S/AS02	Three doses	0, 1, 2 months	**Immunogenicity:** RTS,S/AS01 elicited stronger immune responses than RTS,S/AS02 and non-adjuvanted RTS,S, with significantly higher anti-CS antibody levels and robust CD4+ T-cell activity. No CD8+ responses. Both adjuvanted vaccines exhibited acceptable safety profiles, with reactogenicity being slightly higher for RTS,S/AS01	All participants were malaria-naive Caucasians, which may not reflect the diverse populations affected by malaria
6	Olotu *et al.,* 2014	Case-control study nested within a Phase IIb randomized controlled trial	5–17 months age	Kenya	RTS,S/AS01E	3 doses	0, 1 and 2 months	**Immunogenicity:** Vaccine elicited high titres of anti- circumsporozoite (anti-CS) antibodies with a geometric mean titre of 732.1 EU/mL one month post-vaccination, declining over time. Avidity indices of antibodies remained stable over 12 months post-vaccination. Higher avidity was observed in children with greater malaria exposure.	No baseline samples for avidity in infants before vaccination.
7	The RTS,S Clinical Trials Partnership, 2014*	Phase III, randomized controlled trial	Infants aged 6–12 weeks and children aged 5–17 months	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	RTS,S/AS01	Full dose (0.5 mL)	0, 1, 2 months, with an 18-month booster for one group	**Immunogenicity:** The vaccine elicited strong immune responses post-vaccination, with high anti-CSP antibody titres observed. However, waning efficacy over time was noted. **Safety:** an increase in cases of meningitis and seizures were reported as safety concerns. **Efficacy:** RTS,S/AS01 prevented many cases of clinical and severe malaria, with vaccine efficacy (VE) of 46% in children and 27% in infants against clinical malaria. VE declined over time.	

8	Umeh *et al.*, 2014	Phase III, randomized controlled trial	Children aged 5–17 months	Nigeria	RTS,S/AS01	Three doses	0, 1 and 2 months	**Immunogenicity:** Vaccine induced consistent and strong anti-CS antibody responses across commercial-scale lots, with non-inferiority to the pilot-scale lot. All children achieved 100% seropositivity for anti-CS and seroprotective anti-HBs antibodies	
9	White *et al.*, 2014	Phase II, clinical trials	Infants (≤3 months), Children (>3 months and <5 years), Adults (>18 years)	Gambia, Kenya, Mozambique, Tanzania, Gabon, Ghana	RTS,S/AS01	2 or 3 doses depending on trial	0,1,2 months or 0,1,7 months depending on trial	**Immunogenicity:** Immunogenicity was higher in children (GMT: 621 ELISA units/mL) than infants (209 ELISA units/mL), with antibodies decaying biphasically. **Safety:** The vaccine was well-tolerated, with mild to moderate adverse events and no significant safety concerns reported.	Maternal immunity likely affected infant responses but was not deeply analysed.
								Efficacy: Vaccine showed 55.8% efficacy in children (5– 17 months) and 31.3% in infants (6–12 weeks), with efficacy waning over time.	
10	Ajua *et al., 2015*	Phase IIb, randomised controlled trial	Infants: 6–12 weeks	Gabon, Ghana, Tanzania	RTS,S/AS01E	Three doses in two schedules: 0-1-2 months or 0-1-7 months	schedule 1: 0, 1, 2 months; Schedule 2: 0, 1, 7 months	**Efficacy:** A 54% risk reduction of getting malaria was observed in those participants with a significant change in antibody avidity between the second and third doses. A 77% risk reduction was noted with an increase in IgG concentration. The study did not find a strong association between IgG avidity and vaccine efficacy after the third dose	
11	RTS,S Clinical Trials Partnership, 2015*	Phase III, randomized, controlled trial	Infants aged 6–12 weeks and children aged 5–17 months .	Burkina Faso, Gabon, Ghana, Kenya Malawi, Mozambique, Tanzania	RTS,S/AS01	Three primary doses were administered at months 0, 1, and 2. A booster dose was given at month 20 for participants in the R3R group	R3R Group received 3 doses of RTS,S/AS01 at months 0, 1, 2, plus a booster dose at month 20. R3C Group received 3 doses of RTS,S/AS01 at months 0, 1, 2, and a comparator vaccine at month 20.	**Immunogenicity:** The vaccine elicited significant anti- CSP antibody responses which were boosted further by the fourth dose, although antibody levels declined over time. **Safety:** The safety profile was acceptable, with similar serious adverse events across groups, except for higher meningitis cases in vaccinated children. Seizure incidence remained low. **Efficacy:** The vaccine demonstrated a moderate efficacy. It reduced clinical malaria cases by 36.3% in children (5– 17 months) and 25.9% in infants (6–12 weeks) over 4 years with a booster dose enhancing protection. Severe malaria episodes were reduced by 32.2% in children.	

12	Olotu *et al., 2016*	Phase IIb randomized controlled trial	Children aged 5–17 months	Kenya, Tanzania	RTS,S/AS01	Three doses	0, 1 and 2 months	**Efficacy:** Vaccine had 35.9% efficacy in year one, declining to 2.5% by year four with negative efficacy in high-exposure groups by year five. Immunogenicity was initially strong but waned over time, aligning with reduced protection	High loss to follow-up
13	Regules *et al.*, 2016	Phase IIa, randomized, controlled trial	Adults aged 18–50 years	United States	RTS,S/AS01B	Delayed fractional dose	0, 1, 7 months with fractional third and fourth dose	**Efficacy:** Delayed fractional third dose of the RTS,S/AS01 malaria vaccine improved efficacy (86.7% vs. 62.5% for the standard schedule), antibody avidity and immune response quality in malaria-naïve adults. While protection waned over time without a booster, administering a fractional booster dose sustained high efficacy.	Participants were malaria-naive adults
14	Chaudhury *et a*l., 2017	Phase IIa, controlled trial	Malaria-naive adults	United States	RTS,S/AS01	Delayed fractional dose regimen: 0, 1, 7 months with 20% dose at 7 months	Standard (0, 1, 2 months) vs. delayed (0, 1, 7 months, 20% dose at 7 months)	**Efficacy:** The delayed fractional dose regimen of the RTS,S/AS01 malaria vaccine improved efficacy to 86.7% (vs. 63% for the standard regimen) by enhancing antibody avidity, IgG4 responses and somatic hypermutation in CSP-specific B cells. It reduced CSP-mediated opsonophagocytic activity which associated with protection.	
15	Moncunill, *et al.*, 2017a	Phase III, randomized controlled trial	Children aged 5–17 months	Mozambique Tanzania	RTS,S/AS01E	Three doses	Administered at 0, 1, 2 months	**Immunogenicity:** Vaccine demonstrated efficacy by inducing strong CSP- and HBsAg-specific T cell responses with increased IL-2, TNF-α and CD154 expression. It elicited memory T cells (central and effector) and polyfunctional responses. Immunogenicity was higher for HBsAg due to prior hepatitis B exposure.	
16	Moncunill, *et al.*, 2017b	Matched case-control study nested within phase III trial	Infants (6–12 weeks) and children (5–17 months)	Tanzania, Gabon, Mozambique	RTS,S/AS01E	3 doses	Administered at months 0, 1 and 2	**Efficacy:** Vaccine showed moderate efficacy (56% in children, 31% in infants). TH1 cytokines (IFN-γ, IL-15, GM-CSF) were associated with protection, while TH2 markers (IL-5, RANTES) increased malaria risk, particularly in infants with immature immune systems. Infants exhibited lower immunogenicity compared to children. Adjuvants enhancing TH1 responses and reducing TH2 skew may improve efficacy, especially in younger populations.	Baseline (pre- vaccination) data for infants were not collected, which limits the ability to analyse changes over time in immune responses

17	Ubillos *et al.,* 2018	Case-control study within phase III clinical trial	Infants (6–12 weeks) and children (5–17 months)	Ghana, Mozambique	RTS,S/AS01E	3 doses	Administered at months 0, 1 and 2	**Immunogenicity:** Higher IgG1 and IgG3 responses to CSP and HBsAg were associated with protection, while non-cytophilic IgG2 and IgG4 were linked to increased risk. Cytophilic IgG1 and IgG3 antibodies to CSP and HBsAg were associated with protection against malaria. IgG2 and IgG4 responses and high baseline malaria exposure were associated with increased malaria risk.	The study only examined antibody responses, not other immune components.
18	Witte *et al.*, 2018	Phase II, randomized, controlled trial	1 to 7 days of age at enrolment	Malawi	RTS,S/AS01E	Varied regimens, including 3 doses at different intervals (e.g., 6, 10, and 14 weeks; ≤7 days, 10, 14 weeks; 10, 14, 26 weeks)	Seven regimens, including schedules starting from ≤7 days of age to 9 months	**Immunogenicity:** RTS,S/AS01E improved anti-CS antibody responses, especially when initiated at 10 weeks. Significantly higher anti-CS antibody concentrations were observed with regimens starting after 6 weeks. **Safety:** Fever was more frequent post-RTS,S/AS01E vaccination, but adverse events were generally mild to moderate and well-tolerated across groups.	No efficacy endpoint was measured.
19	Dobaño, *et al.,* 2019b	Phase III, randomized trial	Infants (6–12 weeks) and children (5–17 months)	Ghana, Burkina Faso, Tanzania	RTS,S/AS01E	Three doses	Administered at 0, 1, 2 months	**Immunogenicity:** Immunogenicity results showed significant increases in IgG concentrations post- vaccination with higher responses in children than infants. IgG avidity, especially to CSP's C-terminal domain, strongly correlated with efficacy. Higher concentrations and avidities of IgG to NANP and C-term circumsporozoite protein were significantly associated with vaccine efficacy.	
								Efficacy: Vaccine demonstrated partial efficacy, reducing clinical malaria incidence by 40% in children and 27% in infants within 12 months.	

20	Dobaño, *et* al., 2019a	Phase III, randomized trial	Infants aged 6–12 weeks and children aged 5–17 months)	Ghana, Mozambique	RTS,S/AS01E	Three doses	0, 1 and 2 months	**Immunogenicity:** IgG1 and IgG3 levels were predominantly associated with protection against malaria while IgG2 was linked with higher malaria risk. RTS,S vaccination significantly altered antibody levels, especially boosting IgG3 responses. IgG4, though generally lower, was unexpectedly associated with protection in some cases	Reliance on passive case detection in this study might have influenced the results.
21	Dobaño *et al.*, 2019c	Case-control study nested within phase III clinical trial	Infants aged 6–12 weeks and children aged 5–17 months	Ghana, Mozambique	RTS,S/AS01E	3 doses	3 doses at months 0, 1 and 2	**Immunogenicity:** Vaccine reduced antibodies to exposure markers like AMA1 and increased protective IgG responses to antigens like SSP2/TRAP and MSP5, correlating with reduced malaria risk. Efficacy varied by antigen response, with protective antibodies linked to lower infection risk. Immunogenicity showed enhanced immune profiles against specific malaria targets.	
22	Guerra Mendoza *et al., 2*019*	Phase III, randomized controlled trial	Infants aged 6–12 weeks and children aged 5–17 months	Burkina Faso, Ghana, Gabon, Kenya, Tanzania, Malawi, Mozambique	RTS,S/AS01 malaria vaccine	4 doses	3 primary doses at months 0, 1, 2, with a fourth dose at month 20 for the R3R group	This study demonstrated RTS,S/AS01 malaria vaccine efficacy of 36.3% in children (5–17 months) and 25.9% in infants (6–12 weeks) against clinical malaria. It induced significant immunogenicity with measurable antibody responses. The vaccine also elicited T-cell responses. The safety profile include increased risks of febrile convulsions, meningitis and cerebral malaria were noted in children. Mortality was higher among vaccinated girls compared to control	Site variability
23	Kurtovic *et al.,* 2019	Phase IIb, randomized controlled trial	Children aged 1–4 years	Mozambique	RTS,S/AS02A	Three doses	Administered at 0, 1 and 2 month	**Immunogenicity:** Immunogenicity was lower in older children and those with higher malaria exposure, suggesting malaria transmission intensity impacts vaccine performance **Efficacy:** Vaccine demonstrated moderate efficacy (approx. 30% protection initially) with rapidly waning immune responses over time. It induced strong anti-CSP IgG1 and IgG3 responses, enabling complement fixation though these functional antibodies decayed quickly (half-life approx. 5.2 months).	

24	Ndungu *et al*., 2019	Phase IIb, randomized controlled trial	Children aged 5–17 months	Kenya	RTS,S/AS01	Three doses	0, 1, 2 months	**Immunogenicity:** Vaccine reduced antibody levels to three merozoite antigens (AMA1, MSP1, EBA175), suggesting a delay in natural immunity acquisition but no consistent correlation with malaria protection was observed. Vaccine efficacy declined over time with	Only four merozoite antigens (AMA1, MSP1, EBA175 and MSP3) were analysed,
								antibodies acting as exposure markers rather than protective immunity indicators.	potentially missing broader immune responses relevant to malaria immunity.
25	Tinto Halidou *et al.,* 2019*	Phase III, randomized controlled trial	Infants aged 6–12 weeks and children aged 5–17 months	Burkina Faso, Kenya, Tanzania	RTS,S/AS01 malaria vaccine	4 doses	Three doses at months 0, 1, 2, with an optional booster dose at month 20 for extended protection	**Immunogenicity:** Immunogenicity waned over time, with antibodies remaining above baseline. **Safety:** The vaccine was generally safe, with no vaccine-related severe adverse events, though a few meningitis and cerebral malaria cases were reported without significant imbalance across groups **Efficacy:** The RTS,S/AS01 vaccine showed modest efficacy against severe malaria: 36.7% (four-dose) and 10.1% (three-dose) in older children and 31.0% (four- dose) and 34.2% (three-dose) in younger infants.	The malaria case definitions was adjusted for retrospective data inclusion which may have affected specificity
26	Asante *et al.,* 2020	Phase III, randomized controlled trial	Infants aged 6–12 month	Ghana	RTS,S/AS01	Three doses at 6, 7.5 and 9 months of age	RTS,S co-administered and RTS,S alone groups received RTS,S/AS01 vaccines at 6, 7.5, and 9 months; control group received YF and MR vaccines at 9 months	**Immunogenicity**: RTS,S/AS01 elicited strong immune responses with 99.5% seropositivity for anti-CS antibodies post-third dose. Co-administration with yellow fever and measles-rubella vaccines did not impair immunogenicity of other vaccines.	
27	Bell *et al.,* 2020	Phase III, randomized trial	Infants aged 6–12 weeks and children aged 5–17 months	Malawi	RTS,S/AS01	Three doses of RTS,S/AS01, with a fourth booster dose	3 primary doses administered at 0, 1, and 2 months, with a fourth dose at 18 months	**Efficacy:** Increased vegetation cover was associated with higher vaccine efficacy in the first 18 months but lower efficacy after 18 months if no booster dose was given. The fourth dose did not significantly impact efficacy related to vegetation cover. Vaccine efficacy varied with local environmental factors like vegetation cover.	Passive case detection was used which may introduce bias and the use of outdated vegetation data.

28	Moon *et al.,* 2020	Phase IIa, randomized controlled trial	Adults aged 18–55 years	USA	RTS,S/AS01E and RTS,S/AS01B	Three doses	0, 1, 7 months with fractional final doses	**Immunogenicity:** All 3-dose groups induced strong antibody responses, with higher concentrations than the 2- dose group. All regimens were immunogenic and well tolerated. **Efficacy**: 3-dose RTS,S/AS01 regimens provided significant protection against *Plasmodium falciparum* parasitaemia with vaccine efficacy ranging from 55% to 76%, compared to 29% for 2-dose regimens.	The study was done in malaria-naive adult population which may limit the generalizability of the findings to malaria-endemic regions.
29	Pallikkuth *et al., 2*020	Phase II, Controlled Human Malaria Infection (CHMI)	Adults	United States	RTS,S/AS01	Delayed fractional dose (third dose reduced)	0, 1 and 7 months	**Immunogenicity:** The DFD regimen preserved and enhanced functional immune responses, including CSP- specific memory B cells and IL-21-secreting pTfh cells. **Efficacy:** The Delayed Fractional Dose (DFD) regimen of the RTS,S/AS01 malaria vaccine achieved superior protection (86.7% efficacy) compared to the Standard Dose regimen (62.5%).	All participants were malaria-naive adults, generalizability to malaria-endemic areas is limited
30	Sánchez *et al.,* 2020	Phase III clinical trial	Children and infants	Mozambique	RTS,S/AS01E	Full dose (0.5 mL per dose of the vaccine)	0, 1, 2 months and a booster at month 20	**Immunogenicity:** Booster dose increased IgG, IgG1, IgG3 and IgG4 levels, with lower peak responses compared to primary immunization. Children exhibited stronger immune responses than infants.	
31	Suscovich *et al.,* 2020	Phase II, Controlled Human Malaria Infection (CHMI)	Adults	United States	RTS,S/AS01	Full dose (0.5 mL)	0, 1 and 2 months	**Immunogenicity:** Protection was linked to functional antibody responses such as antibody-mediated phagocytosis and Fc gamma receptor binding, rather than antibody concentration alone.	The study focused on malaria-naive adults and a single parasite strain, limiting generalizability to field settings
32	Valéa *et al*., 2020*	Phase III randomized controlled trial	Infants aged 8–12 weeks	Burkina Faso, Ghana	RTS,S/AS01E	Full dose (0.5 mL)	Administered at 0, 1 and 2 months	**Efficacy:** Vaccine demonstrated effective long-term immunogenicity, with strong anti-HBs and anti-CS antibody responses persisting for 48 months. A strong booster response confirmed effective hepatitis B priming	Small sample size for long-term conclusions, limited geographical scope to two countries

33	Von Seidlein *et al.,* 2020	Phase II, randomized controlled trial	Adults (18–55 years)	Thailand	RTS,S/AS01	Single, double, and fractional doses	0, 1, 2 months	**Immunogenicity**: The vaccine elicited strong immune responses, with all participants seroconverting to key circumsporozoite protein (CSP) antigens. 100% seroconversion to PfCSP (NANP)6 repeat region and PfCSP full length (N + C-Terminal) one month after the last vaccination. More than 90% seroconverted to the PfCSP C-Term antigen. Antibody levels peaked at month 2 or 3 and declined by month 6. Participants in the three- dose standard regimen had the highest antibody levels at month 6. Adverse events were mostly mild and evenly distributed, with no serious vaccine-related adverse events observed.	Exclusion of placebo control limits certain safety attributions.
34	Chandramohan *et al.,* 2021*	Phase III, randomized, controlled trial	Children aged 5–17 month	Burkina Faso, Mali	RTS,S/AS01E	Three doses of RTS,S/AS01E with boosters	Vaccination at 0, 1, 2 months, boosters at 12 and 24 months	**Immunogenicity:** RTS,S/AS01E was immunogenic, inducing CSP-specific antibodies. **Safety:** Safety was favourable, with five febrile seizures (all resolved) and no new safety concerns including meningitis	The study was limited by the possibility of bias, as 14% of children did not attend the first vaccination visit.
								Efficacy: Combination of RTS,S/AS01E and chemoprevention reduced the incidence of clinical malaria by 62.8% and severe malaria by 70.5% compared to chemoprevention alone.	
35	Kurtovi *et al.,* 2021a	Phase I/IIa, controlled trial	Healthy adults (malaria-naive)	United States	RTS,S/AS01	Three doses	0, 1 and 2 months	**Immunogenicity:** Vaccine induced higher functional antibodies associated with reduced parasitaemia risk and delayed malaria onset post-CHMI. Immunogenicity was robust with strong IgG responses especially after the second dose, and functional activity targeting CSP regions. High IgG-to-IgM ratios were protective. **Safety:** Adverse events were mainly mild to moderate local and systemic reactions.	Findings in malaria-naïve adults may not translate to endemic populations with prior exposure

36	Moon *et al.,* 2021	Phase IIa, randomized, controlled trial	Healthy adults	USA	RTS,S/AS01E	Fractional dose (1/5th dose volume)	Primary doses at months 0, 1 and 7, fractional booster at month 12	**Immunogenicity:** It induced strong anti-CS antibody responses, higher in previously protected participants and was well-tolerated. **Safety:** Common adverse events were mild, including injection site pain, fatigue and headache, with no vaccination-related serious adverse events **Efficacy:** Booster showed 53% efficacy against *P. falciparum,* with delayed parasitaemia onset (14.6 days in vaccinated vs. 11.6 days in controls).	Small sample size
37	Mugo *et al.*, 2021	Phase IIb, randomized controlled trial	Children aged 5–17 months	Kenya	RTS,S/AS01	Three doses	0, 1, 2 months	**Efficacy:** Vaccine demonstrated sustained immunogenicity over seven years, with high initial anti-CSP IgG levels waning rapidly within 6.5 months followed by slower decay. IgG1 and IgG3 dominated early responses, transitioning to less functional IgG2. Persistent IgM levels and elevated antibodies compared to controls were observed	Small sample size for subgroup analyses.
38	Suau *et al.,* 2021	Phase III clinical trial	Children aged 5–17 months	Mozambique, Ghana	RTS,S/AS01E	0.5 mL per dose	0, 1, 2 months	**Immunogenicity**: IgA levels increased significantly post- vaccination compared to the comparator group **Efficacy:** RTS,S induced significant IgA responses against CSP and other non-vaccine *P. falciparum* antigens, such as AMA1 and MSP3.	Small sample size
39	Bell *et al.,* 2022	Phase III, randomized trial	Children aged 5–17 months	Ghana, Malawi, Gabon	RTS,S/AS01	Three and four doses regimen	0, 1 and 2 months	**Immunogenicity:** In high-transmission areas, unvaccinated children developed natural immunity faster than vaccinated children who lost their vaccine protection. **Safety:** vaccine was safe, with no significant safety concerns reported in the study. **Efficacy:** Three-dose regimen showed waning efficacy over time, with negative efficacy in high-transmission settings after 4 years. The four-dose regimen prevented the rebound effect but still experienced waning immunity	Malaria cases were mostly detected through passive surveillance, meaning only symptomatic cases seeking treatment were recorded. This may have led to underestimation of transmission intensity, especially in children with limited healthcare access.
								Immunogenicity: Vaccine induced high initial immunogenicity with common mild injection site	

40	Cairns *et al.,* 2022	Phase III, randomized trial	Children aged 5–17 months	Burkina Faso, Mali	RTS,S/AS01E	Three doses + two seasonal boosters	Doses given in April, May, and June, with two additional booster doses in June of the subsequent years	reactions and transient fever as adverse events **Efficacy:** The combination of RTS,S/AS01E and Seasonal Malaria Chemoprevention (SMC) provided over 60% efficacy in preventing clinical malaria in the first 6 months. The protective efficacy of the combined intervention was higher than either intervention alone. Protection declined over time, particularly after 6 months and was not sustained beyond the high-transmission season	The trial was limited by the lack of a group receiving no intervention
41	Feng *et al.*, 2022	Phase IIb, randomized control	Children aged 1–4 year	Mozambique	RTS,S/AS02	Three doses	0, 1, 2 months	**Immunogenicity:** RTS,S vaccination induced functional antibodies that interacted with Fcγ-receptors and promoted cellular functions like phagocytosis. However, the magnitude of these responses varied with faster decay within the first 6 months post-vaccination. Neutrophil phagocytosis persisted longer than FcγR-binding responses. Malaria exposure reduced vaccine-induced antibody levels.	
42	Macià *et al.*, 2022	Phase III, randomized clinical trial	Infants aged (6–12 weeks) and children (5–17 months)	Mozambique, Gabon, Tanzania, Ghana, Kenya, Burkina Faso	RTS,S/AS01E	Three doses + booster	Administered at 0, 1, 2 months, with a booster at month 20	**Immunogenicity:** It induced strong anti-CSP IgG responses and unexpected off-target IgG reactivity which correlated with additional protection **Efficacy:** Vaccine demonstrated moderate efficacy with higher vaccine efficacy in children (55.8%) compared to infants (31.3%) over one year, though declining over time.	
43	Moncunill *et al.,* 2022	Matched case-control study nested within phase III clinical trial	Infants aged (6–12 weeks) and children (5–17 months)	Tanzania, Gabon, Mozambique	RTS,S/AS01	3 doses	3 doses at months 0, 1 and 2	**Immunogenicity:** Strong T-cell responses, with upregulated Blood Transcriptional Modules for T cells and CSP-specific CD4+ responses at month 3. Upregulation of T-cell responses and downregulation of B-cell and monocyte responses post-vaccination. Baseline dendritic and monocyte signatures associated with malaria risk. Adverse events reported were minimal and comparable to the control group.	Lack of baseline data in infants
44	Samuels *et al.,* 2022	Phase IIb, randomized controlled trial	Children aged 5–17 months	Ghana, Kenya	RTS,S/AS01E	Full dose (0.5 mL per dose of the vaccine)	Administered at 0, 1, 2 months, with varying regimens for	**Immunogenicity:** Fractional-dose regimens were immunogenic but not superior to full-dose schedules.	The study focused on children aged 5–17 months excludes insights into

						and fractional doses (0.1m L)	delayed and fractional dosing	**Safety:** Safety was favourable across groups, with most adverse events mild or moderate. **Efficacy:** Vaccine efficacy ranged from 35% to 54%, with substantial cases of malaria averted.	efficacy and safety in younger or older populations. Furthermore, fractional-dose regimens did not demonstrate superior vaccine efficacy compared to the standard full- dose regimens. which limits conclusions on alternative dosing strategies
45	Sagara *et al.,* 2022	Phase III, randomized controlled trial	Children aged 5–17 months	Burkina Faso, Mali	RTS,S/AS01E	3 doses + 2 seasonal boosters	0, 1, 2 months + boosters in June 2018 and 2019	**Immunogenicity:** RTS,S/AS01E vaccination induced strong anti-circumsporozoite antibody responses, though booster responses diminished with successive doses. Children with higher antibody titres post-vaccination had significantly lower malaria incidence. Combining RTS,S/AS01E with seasonal malaria chemoprevention was superior to either intervention alone in reducing uncomplicated malaria, severe cases and deaths.	
46	Bell *et al.*, 2023	Phase III, randomized trial	Children aged 5–17 months	Ghana, Malawi, Gabon	RTS,S/AS01	Three primary doses	0, 1, 2 months	**Immunogenicity:** It induced strong anti-CSP IgG antibody responses, with higher levels in high-transmission areas, though site-specific factors influenced this. **Safety:** Adverse events were mild to moderate, consistent with prior trials including fever, injection site reactions and transient systemic symptoms. **Efficacy:** The vaccine showed moderate efficacy against the first malaria case and was unaffected by parasitaemia during vaccination or background malaria incidence. Efficacy waned over time, supporting its use in high- transmission areas.	Reliance on passive surveillance was used and this may lead to under-detection of malaria cases during the vaccination period.

47	Juraska *et al.*, 2023	Phase IIb, randomized controlled trial	Children aged 5–17 month	Ghana, Kenya	RTS,S/AS01E	Three doses	Administered at 0, 1, 2 months	**Immunogenicity:** Immunogenicity was influenced by baseline infection and force of infection. Immunogenicity improved with baseline malaria infection (68% VE) and high force of infection (57% VE) which could be likely due to natural immune priming and antigen exposure enhancing vaccine-induced immune responses. **Efficacy:** Vaccine demonstrated vaccine efficacy of 25– 43% against new infections, with higher VE (68%) in those infected at baseline. It reduced the mean number of new infections from 4.1 in controls to 2.6–3.0 in recipients over 20 months.	
48	Asante *et al.,* 2024	Phase IV, cluster-randomised introductio n Study	Children aged 1–59 months	Ghana, Kenya, Malawi	RTS,S/AS01E	Four doses: three primary doses and one booster	Doses administered between 5–9 months with a fourth dose at around 2 years	**Immunogenicity:** High immunogenicity was evident with significant antibody responses and > 60% uptake of the primary three doses. **Safety**: Adverse events were minimal, with no excess cases of meningitis, cerebral malaria, or gender-specific mortality observed. **Efficacy:** Vaccine demonstrated a 32% reduction in severe malaria admissions and a 9% reduction in all-cause mortality among vaccinated children.	
49	Dicko *et al.,* 2024*	Phase III, randomized , controlled trial	Children aged 5–17 months	Burkina Faso, Mali	RTS,S/AS01E	Three doses + two seasonal booster doses	Administered at 0, 1, and 2 months with boosters in subsequent years	**Immunogenicity**: The vaccine showed sustained immunogenicity over five years. **Safety:** Safety outcomes were favourable, with minor adverse events and rare febrile convulsions. Ten possible meningitis cases were investigated, but cerebrospinal fluid examinations excluded all as true meningitis. **Efficacy**: Combining RTS,S/AS01E malaria vaccination with seasonal malaria chemoprevention significantly reduced clinical malaria incidence (57.7% efficacy vs. SMC alone and 59.0% vs. RTS,S alone) and severe outcomes like hospitalizations for severe malaria (66.8%) and anaemia (65.9%).	There was a dropout rate of 13.7% from the initial trial to the extension phase.

50	Juraska *et al.,* 2024	Phase IIb, randomized controlled trial	Children aged 5–17 months	Ghana, Kenya	RTS,S/AS01E	Different dosing regimens including full doses and fractional doses	0, 1, and either at 2, 14, 20, 26, 32, or 38 months depending on the group	**Immunogenicity:** Baseline infection appeared to enhance immunogenicity, likely through natural priming. **Safety:** Most adverse events were mild to moderate and included local reactions such as injection site pain, redness and swelling, as well as systemic reactions like fever, irritability, and fatigue. **Efficacy**: Vaccine showed 25–43% efficacy against first malaria infection over 12–20 months. Efficacy was higher in previously infected children (68%) compared to uninfected ones (37%). All regimens reduced infection rates, particularly polyclonal infections with no significant differences across dosing schedules.	
51	Westercamp *et al.,* 2024*	Phase IIb, randomized controlled trial	Children aged 5–17 months	Ghana, Kenya	RTS,S/AS01E	Full and fractional doses (0.1 mL or 0.5 mL)	Administered at 0, 1, 2 months with booster variations at months 14, 20, 26, or 32	**Immunogenicity:** Strong immune responses across regimens. Adverse events were mostly mild to moderate, including pain, fever, and irritability. **Safety:** Serious events like meningitis and seizures were rare. Severe malaria cases significantly decreased in RTS,S groups compared to control **Efficacy:** Vaccine efficacy ranged from 38% to 53%, with up to 2450 cases of clinical malaria averted per 1,000 children vaccinated. Fractional doses showed a comparable impact to full doses.	

Key: * Included in quantitative meta-analysis

4.3 Quality Assessment

4.3.1 Risk of Bias 2 (RoB 2) for Randomized Controlled Trials

The risk of bias assessment for randomized controlled trials included five domains, which include bias arising from the randomization process (D1), bias due to deviations from intended interventions (D2), bias due to missing outcome data (D3), bias in measurement of the outcome D4) and bias in selection of the reported result (D5). Of the 46 studies that were included, 18 (39.13%) exhibited a low risk of bias in all domains. 11 studies (23.91%) had some concerns in at least one domain, mainly in the areas of deviations from intended interventions (D2) and missing outcome data (D3). The remaining 17 studies (36.95%) were classified as high risk, with concerns particularly in randomization (D1) and selection of the reported result (D5) (Figure 4.2).

Unique ID	Study ID	Experimental	Comparator	Outcome	Weight	D1	D2	D3	D4	D5	Overall
01DC	RTS,S Clinical Trials	Intervention (RTS,S/AS01)	Control (C3C)	NA	1	+	+	+	+	+	+
02DC	Ajua et al, 2015	Intervention (RTS,S/AS01I	Control (EPI vaccines)	NA	1	+	+	+	!	!	!
03DC	Asante et al 2021	Intervention (RTS,S/AS01)	Control (YF & MR Vaccine)	NA	1	+	+	+	!	+	!
04DC	Benjon et al, 2013	Intervention (RTS,S/AS01I	Rabies Vaccine & Hepatitis	NA	1	+	+	+	+	+	+
05DC	Bell et al, 2020	Intervention (RTS,S/AS01)	Control Vaccine (C3)	NA	1	+	+	+	!	+	!
06DC	Bell et al, 2023	Intervention (RTS,S/AS01	Control vaccine (non-RTS,S	NA	1	+	+	+	+	+	+
07DC	Bell et al, 2023	RTS,S/AS01 vaccine	Control vaccine	NA	1	+	+	+	+	+	+
08DC	Cairns et al, 2022	Combined Intervention Gi	Comparator Interventions	NA	1	+	!	+	-	+	-
09DC	Chandramohan et a	RTS,S/AS01E malaria vacc	Seasonal malaria chemopr	NA	1	+	+	+	+	+	+
10DC	Chaudhury et al, 20	Delayed Fractional Dose F	Standard regimen (012M)	NA	1	!	!	+	-	-	-
11DC	Dicko et al, 2024	RTS,S/AS01E Alone Group	SMC Alone	NA	1	+	+	+	+	+	+
12DC	Dobano et al, 2019	RTS,S/AS01E	Control Vaccine	NA	1	+	+	+	-	+	-
13DC	Dobaño et al, 2019	RTS,S/AS01E	non-RTS,S vaccine	NA	1	+	+	+	+	!	!
14DC	Feng et al, 2022	RTS,S/AS02 malaria vaccir	Non-malaria vaccines	NA	1	!	+	+	+	!	!
15DC	Guerra Mendoza et	RTS,S/AS01 malaria vaccir	Non malaria control vaccin	NA	1	+	+	+	+	+	+
16DC	Halidou et al, 2019	RTS,S/AS01 vaccine	Control Vaccine (meningoc	NA	1	+	+	+	-	+	-
17DC	Horowitz et al, 201	RTS,S/AS01E malaria vacc	Control Vaccine(human dip	NA	1	+	+	+	+	+	+
18DC	Juraka et al, 2024	RTS,S/AS01E malaria vacc	Rabies control vaccine	NA	1	!	!	+	+	+	!
19DC	Juraska et al, 2023	RTS,S/AS01E malaria vacc	Rabies vaccine	NA	1	!	!	+	-	+	-
20DC	Kurtovic et al, 2020	RTS,S/AS01 malaria vaccir	Controlled human malaria	NA	1	!	!	+	+	+	!
21DC	Kurtovic et al, 201	RTS,S/AS02A malaria vacc	Non-malaria vaccine	NA	1	+	!	+	+	!	!
22DC	Leroux-Roels et al,	RTS,S malaria vaccine forr	RTS,S malaria vaccine recor	NA	1	+	+	+	+	+	+

Figure 4.2.a: Risk of bias assessment of each included randomized controlled trial.

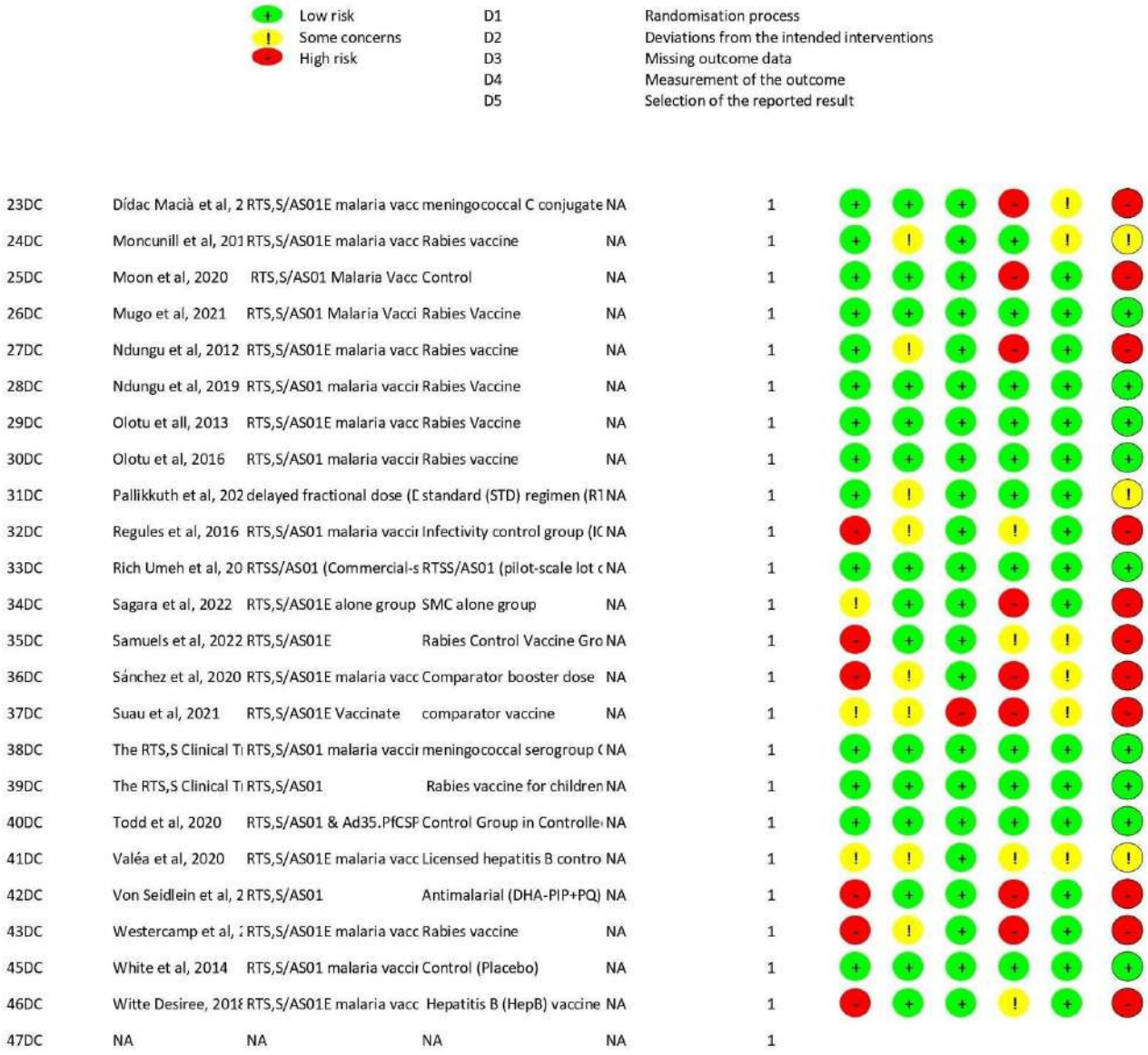


Figure 4.2b: Risk of bias assessment of each included randomized controlled trial.

4.3.2 Risk of Bias 2 (RoB 2) for Cluster-Randomized Trials

The risk of bias assessment for cluster-randomized trials was conducted using the RoB 2 tool for cluster- randomized trials, which includes six domains. The study Asante et al. (2024) was assessed for risk of bias, with an overall judgment of "some concerns". The primary sources of concern were identified in the randomization process (D1a) and selection of the reported result (D5). However, the study demonstrated a low risk of bias in other domains, such as deviations from intended interventions (D2), missing outcome data (D3), and measurement of the outcome (D4) (Figure 4.3).

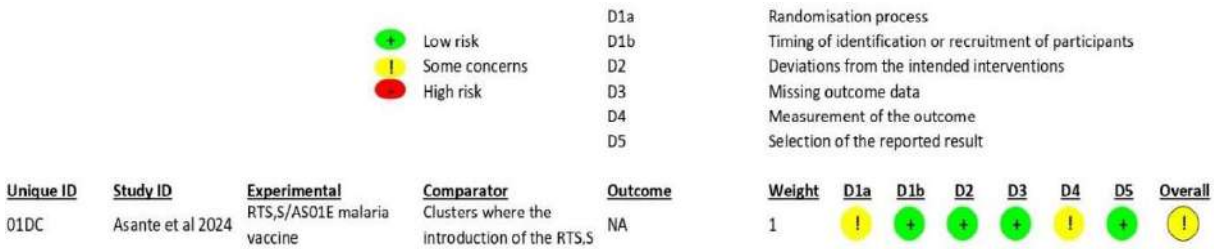


Figure 4.3: Risk of bias assessment of the included cluster-randomized trial

4.3.3 Risk of Bias in Non-Randomized Studies of Interventions (ROBINS-I)

The Risk of Bias in Non-Randomized Studies of Interventions assessment was conducted for the included non-randomized studies. The assessment covered seven domains such as bias due to confounding, selection of participants, classification of interventions, deviations from intended interventions, missing data, measurement of outcomes and selection of the reported result. For the four studies that were evaluated, the overall risk of bias judgement was found to be moderate. Potential confounding, selective reporting and measurement bias were the primary concerns.

4.4 Meta Analysis of Immunogenicity, Safety and Efficacy of RTS,S/AS01

4.4.1 Immunogenicity Outcomes of RTS,S/AS01

Although immunogenicity was a key outcome of interest, a meta-analysis of geometric mean titres (GMTs) was not performed due to the unavailability of standard deviations in the included studies. As standard deviations are critical for pooling estimates, their absence precluded reliable direct comparisons. To avoid introducing estimation errors, a descriptive synthesis of immunogenicity data was conducted, summarizing trends in immune responses across different age groups, vaccine regimens/strategies, study designs, and geographic regions.

The immunogenicity of the RTS,S/AS01 was measured as the GMTs of anti-CSP antibodies in different vaccine regimens, age groups, study phases and regions (Table 4.2).

A total of five studies evaluated the RTS,S/AS01 after 3 doses, while 2 studies focused on booster doses. One study assessed RTS,S in combination with SMC. Five studies focused on children aged 5–17 months, whereas two studies examined infants aged 6–12 weeks. Four were Phase III trials, while one was in Phase II trials (Table 4.2).

The Phase III trials were conducted in Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania, Nigeria and Mali, while the Phase II trials took place in Gambia,

Kenya, Mozambique, Tanzania, Gabon and Ghana (Table 4.2).

The GMT values varied significantly depending on the vaccine regimen. After three doses of RTS,S/AS01, the highest recorded GMT was 621 EU/mL (95% CI: 592–652) in a Phase II trial (White *et al.,* 2014). Other Phase III trials reported GMT values ranging from 34.2 EU/mL (95% CI: 30.5–38.3) (RTS,S Clinical Trials Partnership, 2015) to 383.5 EU/mL (95% CI: 326.0–451.1) (Sagara *et al.,* 2022). Studies conducted in Nigeria before the third dose had extremely low GMTs (0.2–0.3 EU/mL), which significantly increased after the third dose (up to 319.6 EU/mL, 95% CI: 268.9–379.8) (Table 4.2).

The only study evaluating RTS,S/AS01 combined with SMC observed a GMT of 368.9 EU/mL (95% CI: 317.7–428.4) (Sagara *et al.,* 2022)., suggesting an enhanced immune response compared to RTS,S/AS01alone (Table 4.2).

One month after the first booster among children aged 5–17 months, the GMT peaked at 318.2 EU/mL (95% CI: 295.1–343.0), but 12 months later, it declined sharply to 52.4 EU/mL (95% CI: 47.8–57.6) (RTS,S Clinical Trials Partnership, 2015). Similarly, one month after the first booster among infants aged 6–12 weeks, the GMT peaked at 169.9 EU/mL (95% CI: 153–187.7.0), but 12 months later, it declined also to 15.9 EU/mL (95% CI: 13.8–18.3) (RTS,S Clinical Trials Partnership, 2015), indicating immune waning over time. After the second booster, GMT values increased to 200.1 EU/mL (95% CI: 175.6–228.0) in Burkina Faso and 159.9 EU/mL (95% CI: 139.7–183.0) in Mali (Table 4.2).

Children aged 5–17 months showed higher immunogenicity than infants. The highest GMT in this group was 621 EU/mL (95% CI: 592–652) in a Phase II trial by White *et al.,* (White *et al.,* 2014) . Post-vaccination GMT values typically ranged from 200 to 400 EU/mL but declined without booster doses. In contrast, infants aged 6–12 weeks had lower GMT values. The highest GMT recorded in this age group was 209 EU/mL (95% CI: 197–222) in a Phase II trial. Some studies reported very low GMT values of 6.2 EU/mL (95% CI: 5.4–7.0) after three doses, and at 12 months post-booster, GMT declined to 15.9 EU/mL (95% CI: 13.8–18.3), reinforcing the need for stronger booster strategies (Table 4.2).

Phase II trials reported the highest GMT values, particularly 621 EU/mL (95% CI: 592–652) in a multi-country study. In contrast, Phase III trials showed greater variation in immune responses, with GMT values ranging between 34.2 EU/mL and 383.5 EU/mL. Booster doses were effective in temporarily enhancing immune responses, but immunity waned over time (Table 4.2).

Table 4.2: GMTs of anti-CSP antibodies for RTS,S/AS01 vaccination across vaccine regimens, age groups, study phases and geographic regions

Author	Vaccine Regimen/Strategy	Age group	Study Design/Phase	Country	GMT (EU/ml)			
3 DOSE GMTs DETAILS					**Baseline**	**95% CI**	**After 3 doses**	**95% CI**
RTS,S Clinical Trials Partnership, 2015	RTS,S	Children (5–17 months)	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	3.0	2.8–3.3	34.2	30.5–38.3
RTS,S Clinical Trials Partnership, 2012	RTS,S (After 3 Doses)	Children (5–17 months)	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	NI	NI	209	197–222
Umeh et al., 2014	RTS,S (Before 3 Doses)/Lot 1	Children (5–17 months)	RCT, Phase III	Nigeria	0.3	0.2–0.3	285.5	260.7–313.3
Sagara *et al.,* 2022	RTS,S (After 3 Dose)	Children (5–17 months)	RCT, Phase III	Mali	0.95	NI	383.5	326.0–451.1
Sagara *et al.,* 2022	RTS,S (After 3 Dose)	Children (5–17 months)	RCT, Phase III	Burkina Faso	2.59	2.27–2.91	349.7	264.1–463.0
White *et al.,*2014	RTS,S (After 3 Dose)	Children (5–17 months)	RCT, Phase II	Gambia, Kenya, Mozambique, Tanzania, Gabon, Ghana	2.59	2.27–2.91	621	592 to 652
RTS,S Clinical Trials Partnership, 2015	RTS,S (After 3 doses)	Infants (6–12 weeks)	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	2.4	2.2–2.6	6.2	5.4–7.0
White *et al.,*2014	RTS,S (After 3 doses)	Infants (6–12 weeks)	RCT, Phase II	The Gambia, Kenya, Mozambique, Tanzania, Gabon, Ghana	2.59	2.27–2.91	209	197 to 222

RTS,S Clinical Trials Partnership, 2015	12 months for Control Grp	Children (5–17 months)	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	3.0	2.8–3.3	19.3	17.2–21.8
RTS,S Clinical Trials Partnership, 2015	12 months for Control Grp	Infants (6–12 weeks)	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	2.4	2.2–2.6	3.7	3.3–4.2
Sagara *et al.,* 2022	RTS,S + SMC, After 3 doses	Children (5–17 months)	RCT, Phase III	Burkina Faso, Mali	0.95	NI	368.9	317.7–428.4
BOOSTER DOSE GMTs DETAILS					**Baseline**	**95% CI**	**After 3 doses**	**95% CI**
Sagara *et al.,* 2022	RTS,S + SMC, Before 1st Booster doses	Children (5–17 months)	RCT, Phase III	Burkina Faso, Mali	0.95	NI	42.4	37.1–48.5
Sagara *et al.,* 2022	RTS,S + SMC, After 1st Booster doses	Children (5–17 months)	RCT, Phase III	Burkina Faso, Mali	0.95	NI	257.5	234.5–282.8
Sagara *et al.,* 2022	RTS,S + SMC, Before 2nd Booster doses	Children (5–17 months)	RCT, Phase III	Burkina Faso, Mali	0.95	NI	44.4	39.2–50.1
Sagara *et al.,* 2022	RTS,S + SMC, After 2nd Booster doses	Children (5–17 months)	RCT, Phase III	Burkina Faso, Mali	NI	NI	177.4	161.4–195.0
RTS,S Clinical Trials Partnership, 2015	RTS,S (1 month After Booster)	Children (5–17 months)	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	3.0	2.8–3.3	318.2	295.1–343.0
Sagara *et al.,* 2022	RTS,S , Before first Booster	Children (5–17 months)	RCT, Phase III	Mali	0.95	NI	39	32.1–47.4
Sagara *et al.,* 2022	RTS,S , Before first Booster	Children (5–17 months)	RCT, Phase III	Burkina Faso	0.95	NI	46.5	38.6–56.0

Sagara *et al.,* 2022	RTS,S , After first Booster	Children (5–17 months)	RCT, Phase III	Mali	0.95	NI	258.6	226.3–295.6
Sagara *et al.,* 2022	RTS,S , After first Booster	Children (5–17 months)	RCT, Phase III	Burkina Faso	0.95	NI	256.3	224.5–292.5
Sagara *et al.,* 2022	RTS,S , Before second Booster	Children (5–17 months)	RCT, Phase III	Mali	0.95	NI	45	37.7–53.8
Sagara *et al.,* 2022	RTS,S , Before second Booster	Children (5–17 months)	RCT, Phase III	Burkina Faso	0.95	NI	43.6	36.9–51.6
Sagara *et al.,* 2022	RTS,S , After second Booster	Children (5–17 months)	RCT, Phase III	Mali	0.95	NI	159.9	139.7–183.0
Sagara *et al.,* 2022	RTS,S , After second Booster	Children (5–17 months)	RCT, Phase III	Burkina Faso	0.95	NI	200.1	175.6–228.0
RTS,S Clinical Trials Partnership, 2015	RTS,S (12 months After Booster)	Children (5–17 months)	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	3.0	2.8–3.3	52.4	47.8–57.6
RTS,S Clinical Trials Partnership, 2015	RTS,S (12 months After Booster)	Infants (6–12 weeks)	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	2.4	2.2–2.6	15.9	13.8–18.3
RTS,S Clinical Trials Partnership, 2015	RTS,S (1 month After Booster)	Infants (6–12 weeks)	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	2.4	2.2–2.6	169.9	153.8–187.7

Key: NI: Not Indicated

4.4.2 Safety Outcomes of RTS,S/AS01

The safety of the RTS,S/AS01 malaria vaccine was assessed by comparing the incidence of adverse events between the vaccine and control groups across several variables. The analysis was based on vaccine regimen, age group, study design, and country. Safety outcomes were evaluated using the odds ratios presented in a forest plot, which measured the relative occurrence of adverse events among vaccine groups compared to those in the control group.

4.4.2.1 Safety Outcome 1: Pooled log OR for adverse events among RTS,S/AS01 vaccine recipients compared with control groups in all included studies using a fixed-effects Mantel– Haenszel model

pooled log OR for adverse events among RTS,S/AS01 vaccine recipients compared with control groups was log OR –0.01 (95% CI –0.10 to 0.08) (Figure 4.4). Heterogeneity was moderate (I^2 = 33.47%). The test for overall effect (Z = -0.18, p = 0.86) suggested no significant difference in adverse event rates between the vaccine and control groups. Though some studies show variations in adverse event rates, overall, the findings suggest that the vaccine is well-tolerated.

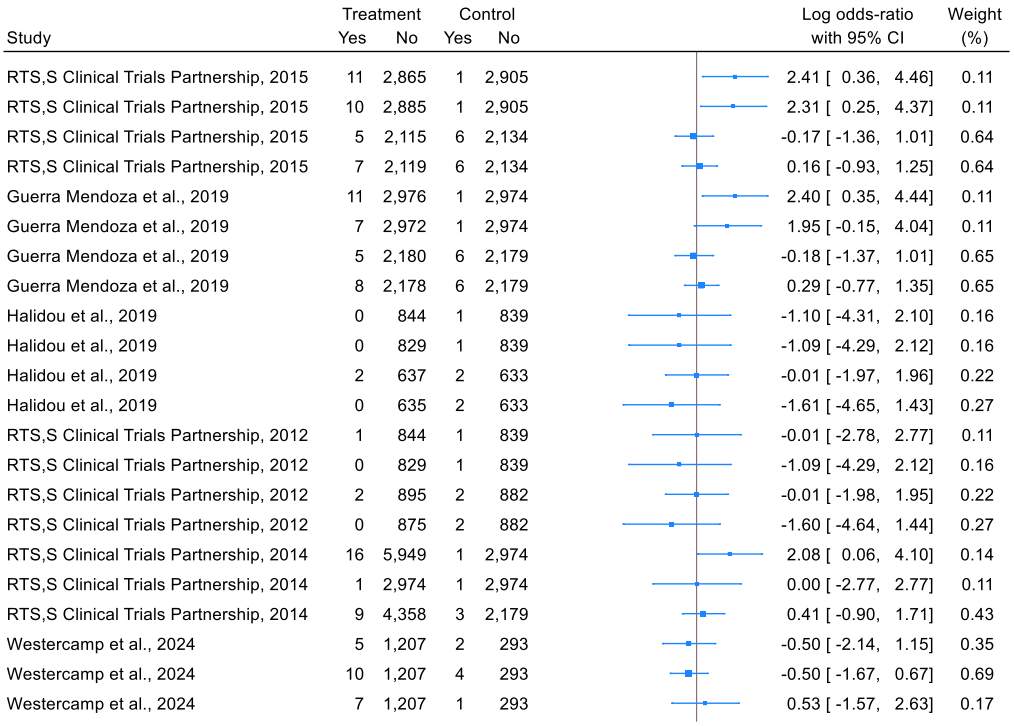


Figure 4.4: Forest plot of pooled log OR for adverse events among RTS,S/AS01 vaccine recipients compared with control groups in all included studies using a fixed-effects Mantel–Haenszel model

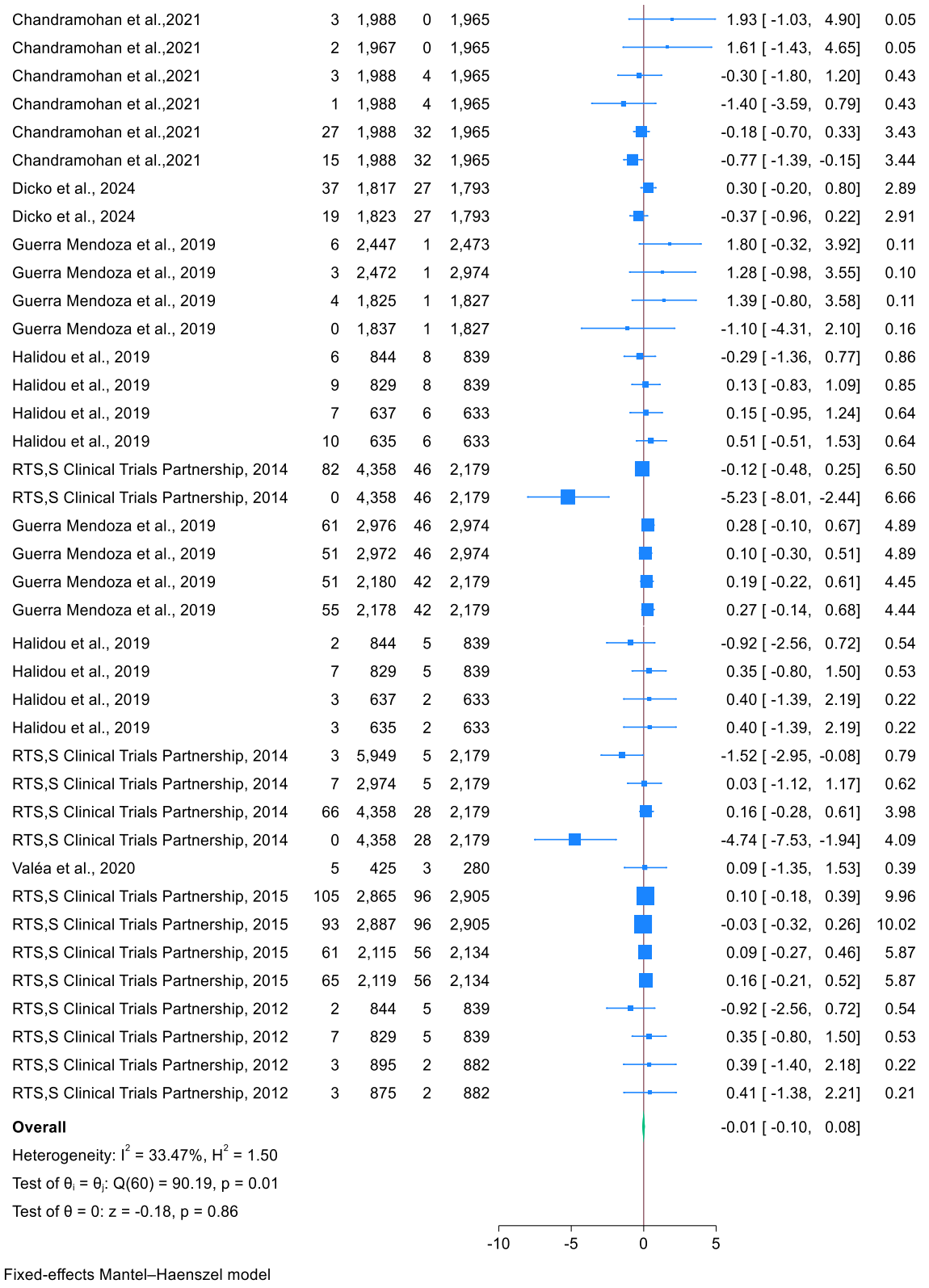


Figure 4.4 continued: Forest plot of pooled log OR for adverse events among RTS,S/AS01 vaccine recipients compared with control groups in all included studies using a fixed-effects Mantel–Haenszel model

4.4.2.2 Safety Outcome 2: Subgroup analysis of log OR for adverse events among RTS,S/AS01 vaccine recipients compared with control groups, stratified by age group, using a fixed-effects Mantel–Haenszel model

The log OR for adverse events among RTS,S/AS01 vaccine recipients compared with control groups were stratified by age group (Figure 4.5). In children aged 5–17 months, the pooled log of OR was 0.09 (95% CI: -0.03 to 0.21), with moderate heterogeneity ($I^2 = 37.10\%$). In infants aged 2–26 months, the pooled log of OR was 0.09 (95% CI: -1.35 to 1.53), with no heterogeneity ($I^2 = 0.00\%$). In infants aged 6– 12 weeks, the pooled log of OR was -0.13 (95% CI: -0.26 to 0.01), with moderate heterogeneity ($I^2 = 44.3\%$). The test for subgroup differences was not statistically significant (Q (2) = 5.33, p = 0.07), indicating no strong evidence of variation in adverse event risk across age groups.

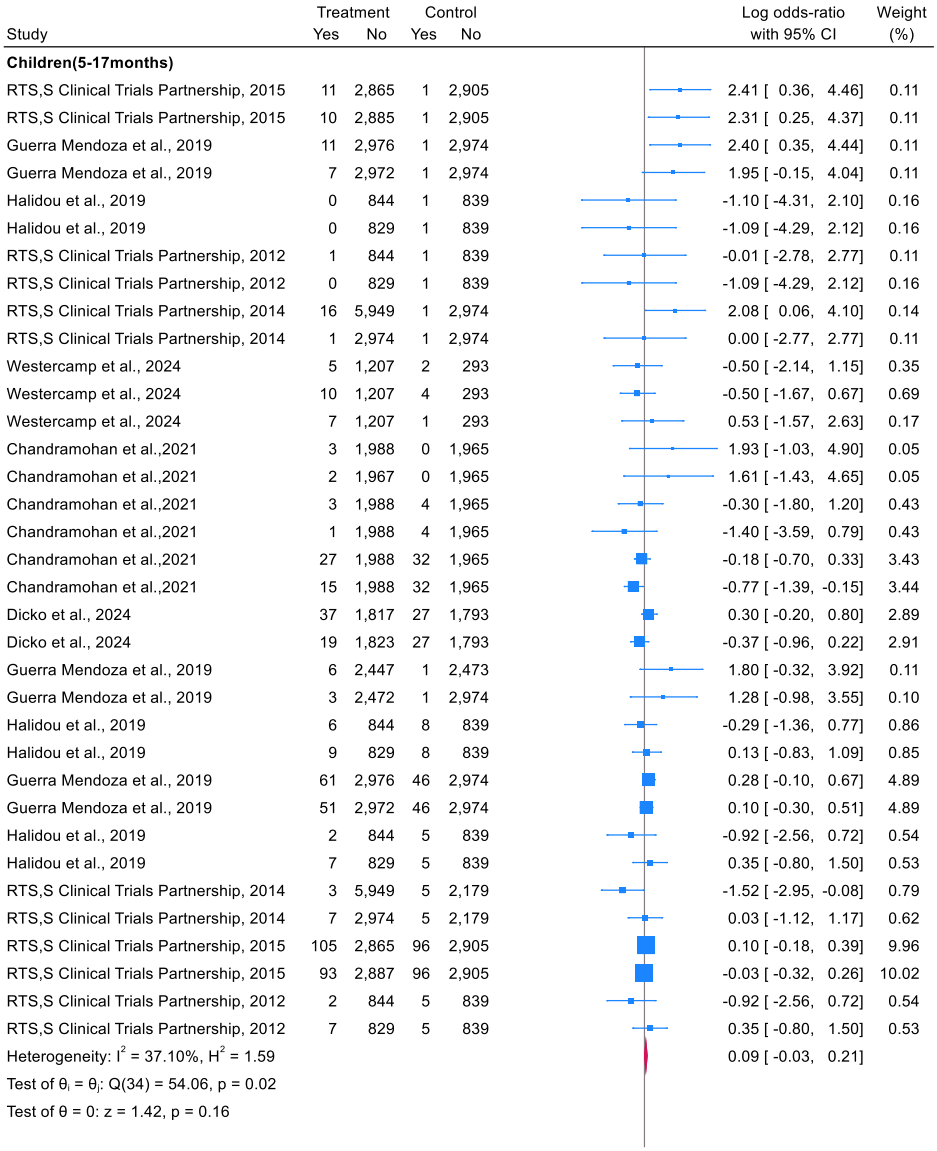


Figure 4.5: Forest plot of subgroup analysis of log OR for adverse events among RTS,S/AS01 vaccine recipients compared with control groups, stratified by age group, using a fixed-effects Mantel–Haenszel model

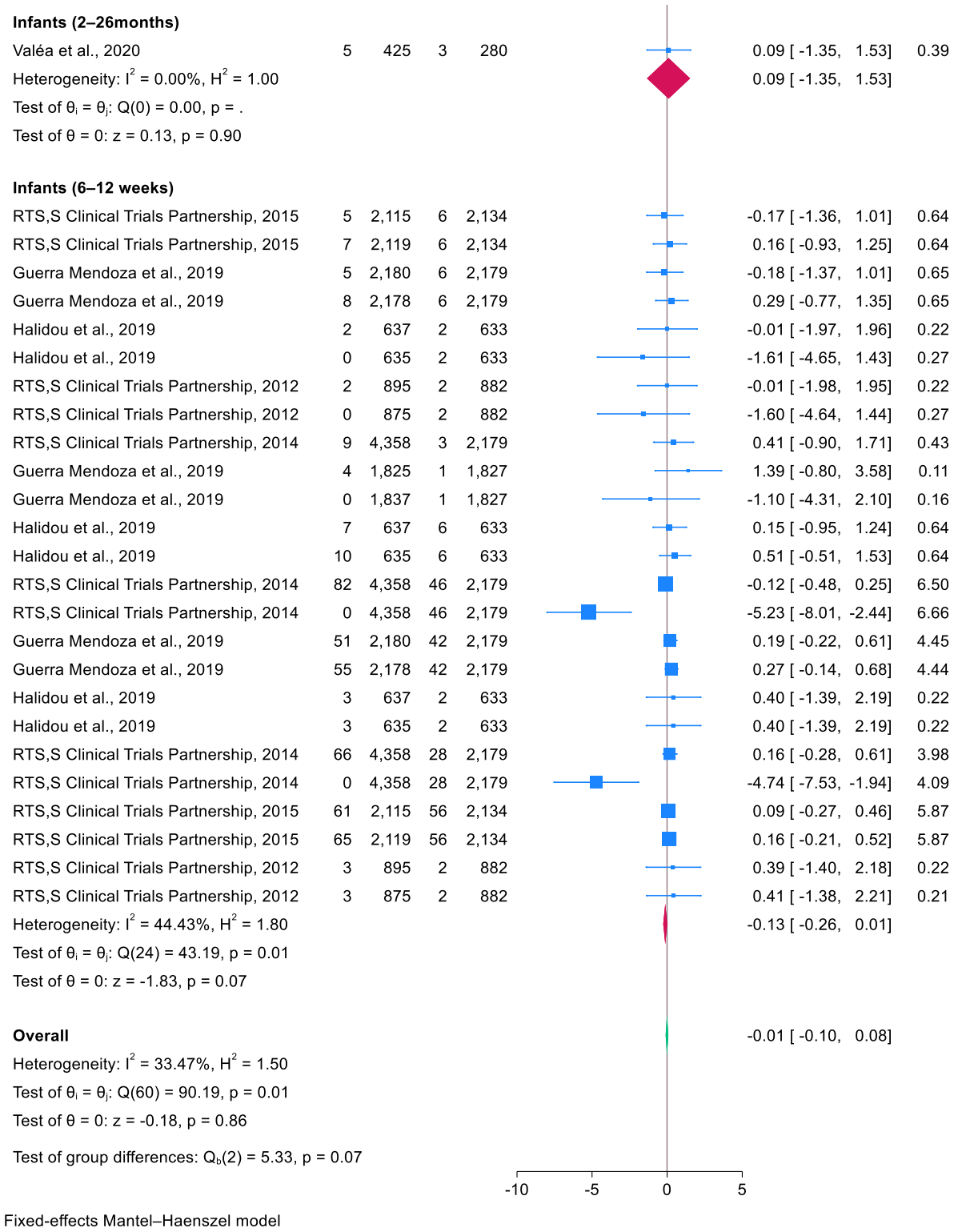


Figure 4.5 continued: Forest plot of subgroup analysis of log OR for adverse events among RTS,S/AS01 vaccine recipients compared with control groups, stratified by age group, using a fixed-effects Mantel–Haenszel model

4.4.2.3 Safety Outcome 3: Subgroup analysis of log OR for adverse events (convulsions, death and meningitis) among RTS,S/AS01 vaccine recipients compared with control groups, using a fixed-effects Mantel–Haenszel model

The log of OR for adverse events among RTS,S/AS01 vaccine recipients compared with control groups were analysed for convulsions, death, and meningitis (Figure 4.6). The pooled log of OR for convulsions was -0.39 (95% CI: -0.63 to -0.15), with substantial heterogeneity (I^2 = 62.24%), indicating a significantly lower risk in the vaccine group (p = 0.00). For death, the pooled log of OR was 0.01 (95% CI: -0.09 to 0.12), with moderate heterogeneity (I^2 = 28.98%), and no significant difference between groups (p = 0.81). The pooled log of OR for meningitis was 0.46 (95% CI: 0.14 to 0.79), with moderate heterogeneity (I^2 = 27.68%), suggesting a significantly higher risk in the vaccine group (p = 0.01). The test for subgroup differences was statistically significant (Q (2) = 18.02, p = 0.00), indicating variation in adverse event risk across outcomes.

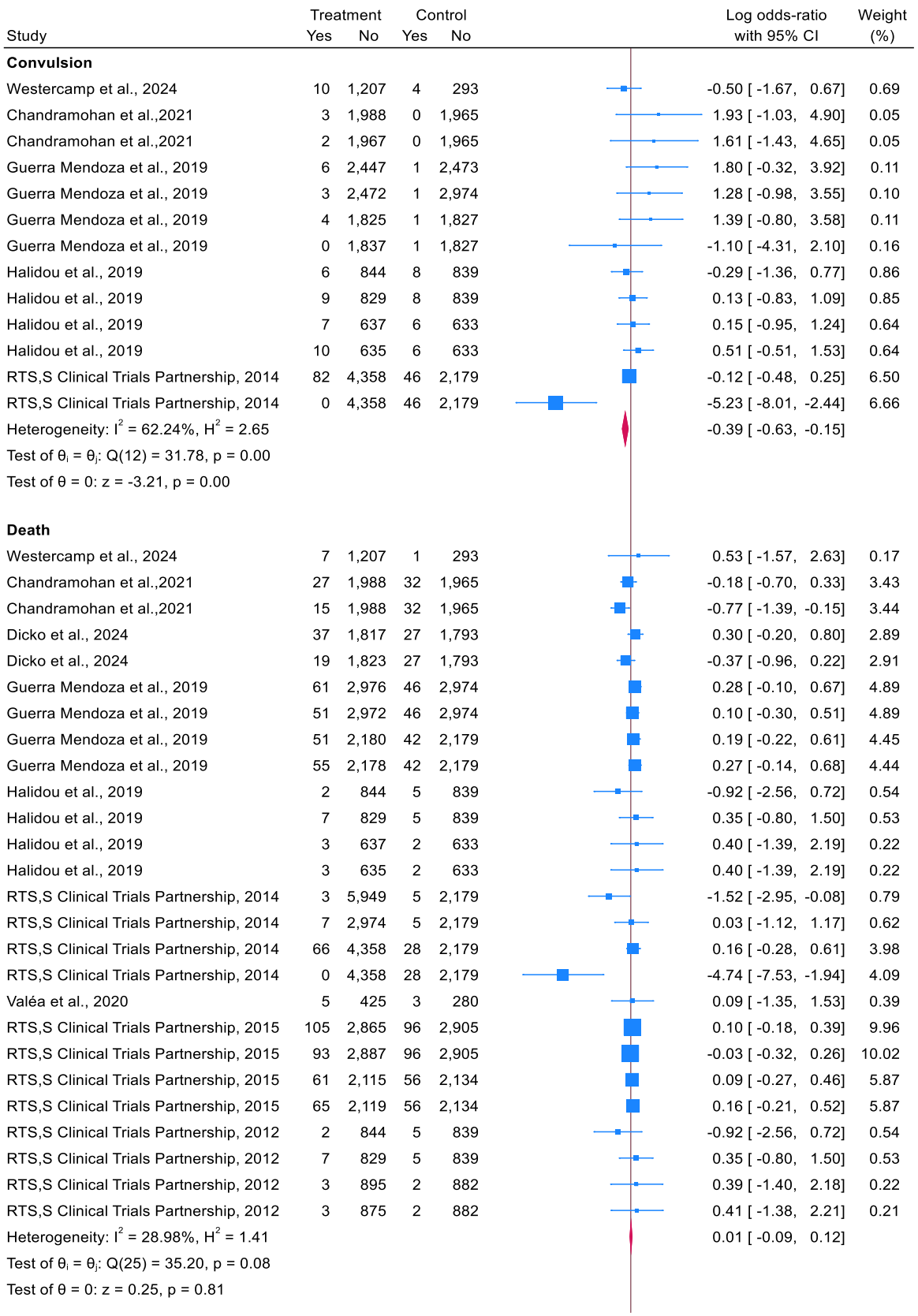


Figure 4.6: Forest plot of subgroup analysis of log OR for adverse events (convulsions, death, and meningitis) among RTS,S/AS01 vaccine recipients compared with control groups, using a fixed-effects Mantel–Haenszel model

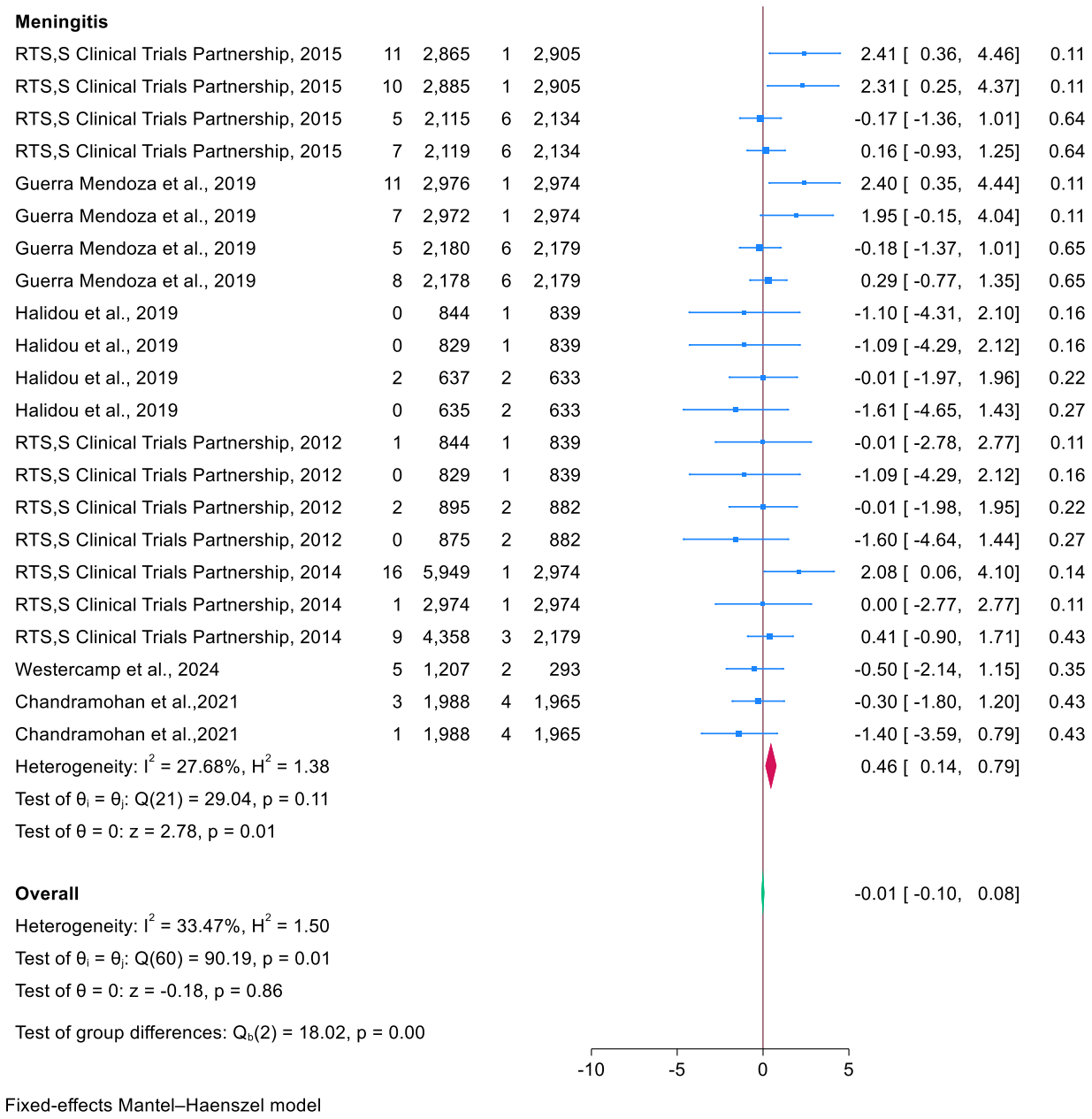


Figure 4.6 continued: Forest plot of subgroup analysis of log OR for adverse events (convulsions, death, and meningitis) among RTS,S/AS01 vaccine recipients compared with control groups, using a fixed-effects Mantel–Haenszel model

4.4.2.4 Safety Outcome 4: Subgroup analysis of log OR for adverse events among RTS,S/AS01 vaccine recipients compared with control groups, stratified by vaccine regimen/strategy (booster and chemoprevention status), using a fixed-effects Mantel–Haenszel model

The log of OR for adverse events among RTS,S/AS01 vaccine recipients compared with control groups were stratified by booster and chemoprevention status (Figure 4.7). For RTS,S/AS01 alone, the pooled log of OR was -0.10 (95% CI: -0.23 to 0.03), with moderate heterogeneity (I^2 = 41.68%), showing no significant difference between groups (p = 0.13). In the RTS,S/AS01 + booster group, the pooled log of log of OR was 0.15 (95% CI: 0.02 to 0.29), with moderate heterogeneity (I^2 = 19.43%), indicating a significantly higher risk of adverse events in the vaccine group (p = 0.03). In the RTS,S/AS01 + chemoprevention group, the pooled log of OR was -0.54 (95% CI: -0.95 to -0.13), with low heterogeneity (I^2 = 10.93%), suggesting a significantly lower risk of adverse events in the vaccine group (p = 0.01). The test for subgroup differences was statistically significant (Q (2) = 13.97, p = 0.00), indicating variation in adverse event risk across intervention groups.

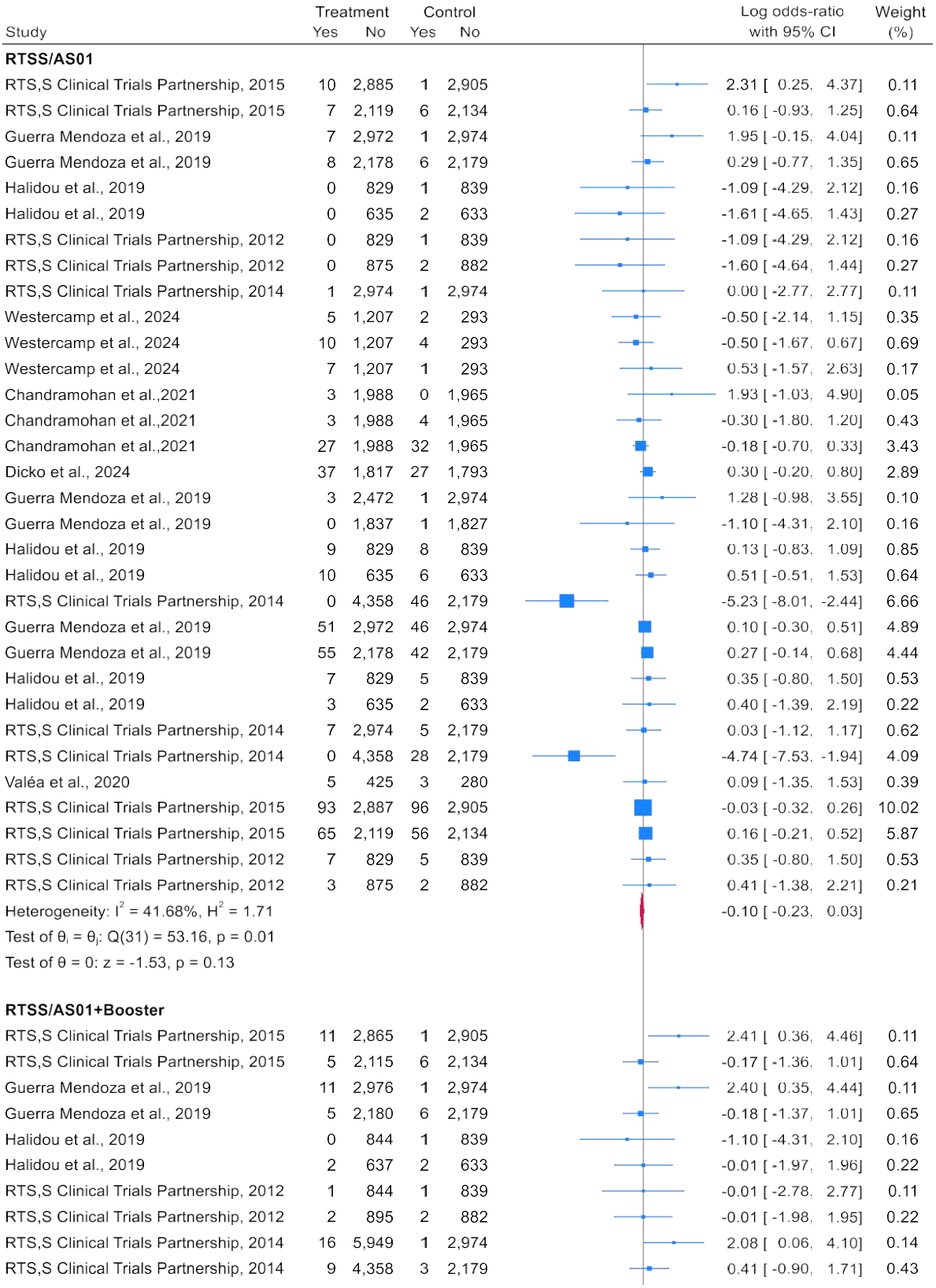


Figure 4.7: Forest plot of subgroup analysis of log OR for adverse events among RTS,S/AS01 vaccine recipients compared with control groups, stratified by vaccine regimen/strategy (booster and chemoprevention status), using a fixed-effects Mantel–Haenszel model

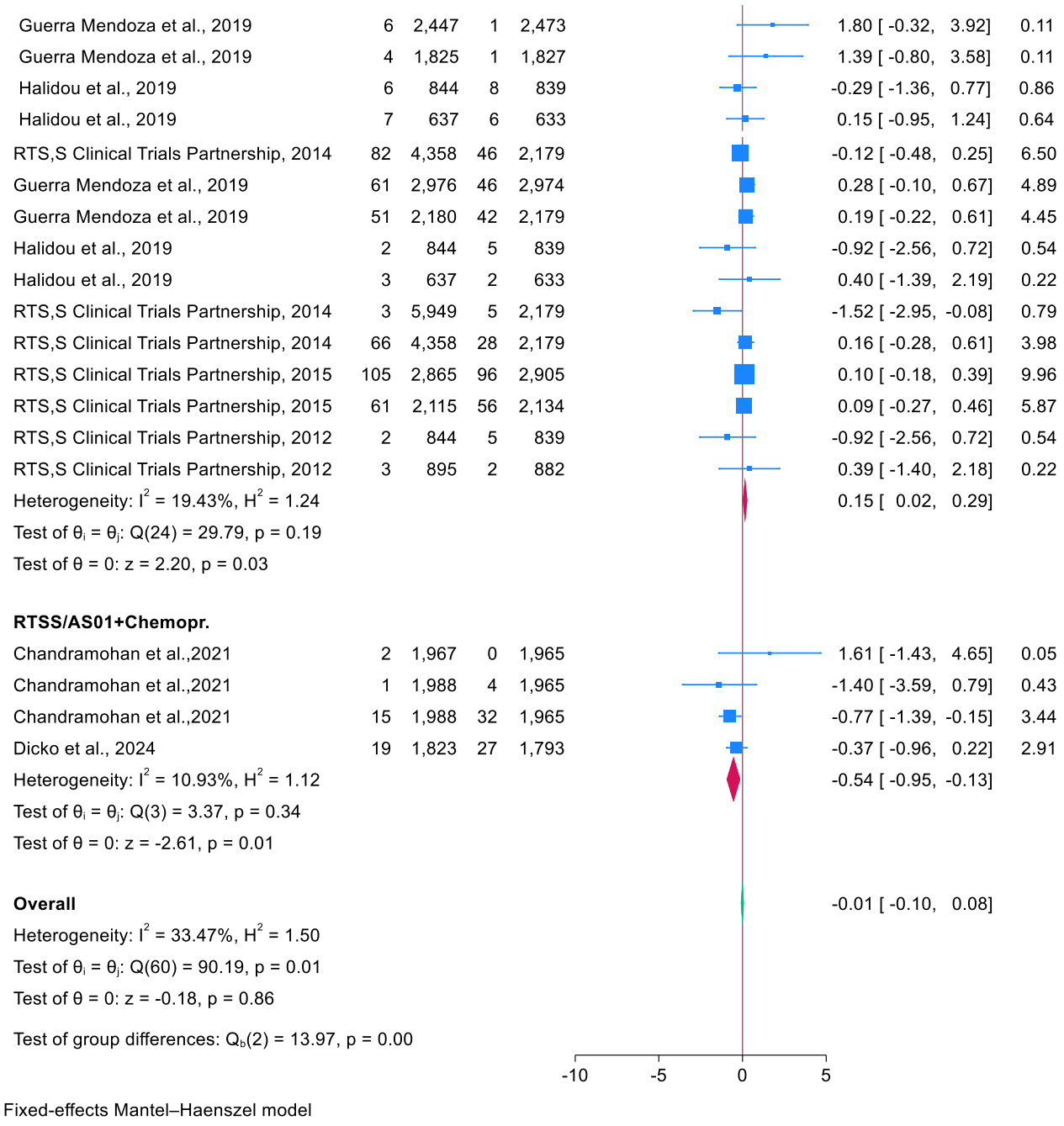


Figure 4.7 continued: Forest plot of subgroup analysis of log OR for adverse events among RTS,S/AS01 vaccine recipients compared with control groups, stratified by vaccine regimen/strategy (booster and chemoprevention status), using a fixed-effects Mantel–Haenszel model

4.4.2.5 Safety Outcome 5: Subgroup analysis of log OR for adverse events among RTS,S/AS01 vaccine recipients compared with control groups, stratified by study country, using a fixed-effects Mantel–Haenszel model

The log of OR for adverse events among RTS,S/AS01 vaccine recipients compared with control groups were stratified by study location (Figure 4.8). In Burkina Faso and Ghana, the pooled log of OR was 0.09 (95% CI: -1.35 to 1.53), with no heterogeneity (I^2 = 0.00%), showing no significant difference between groups (p = 0.90). In Burkina Faso and Mali, the pooled log of OR was -0.18 (95% CI: -0.43 to 0.08), with moderate heterogeneity (I^2 = 41.48%), also showing no significant difference (p = 0.10). In Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, and Tanzania, the pooled log of OR was -0.10 (95% CI: -0.23 to 0.02), with substantial heterogeneity (I^2 = 55.95%), indicating no significant difference (p = 0.00). In another dataset from Burkina Faso, Ghana, Gabon, Kenya, Tanzania, Malawi, and Mozambique, the pooled log of OR was 0.30 (95% CI: 0.12 to 0.49), with low heterogeneity (I^2 = 12.69%), showing a significant increase in adverse events among vaccine recipients (p = 0.00). In Burkina Faso, Kenya, and Tanzania, the pooled log of OR was 0.02 (95% CI: -0.37 to 0.41), with no heterogeneity (I^2 = 0.00%), showing no significant difference (p = 0.92). In Ghana and Kenya, the pooled log of OR was -0.27 (95% CI: -1.12 to 0.58), with no heterogeneity (I^2 = 0.00%), also showing no significant difference (p = 0.58). The test for subgroup differences was statistically significant (Q (5) = 14.99, p = 0.01), indicating variation in adverse event risk across study locations.

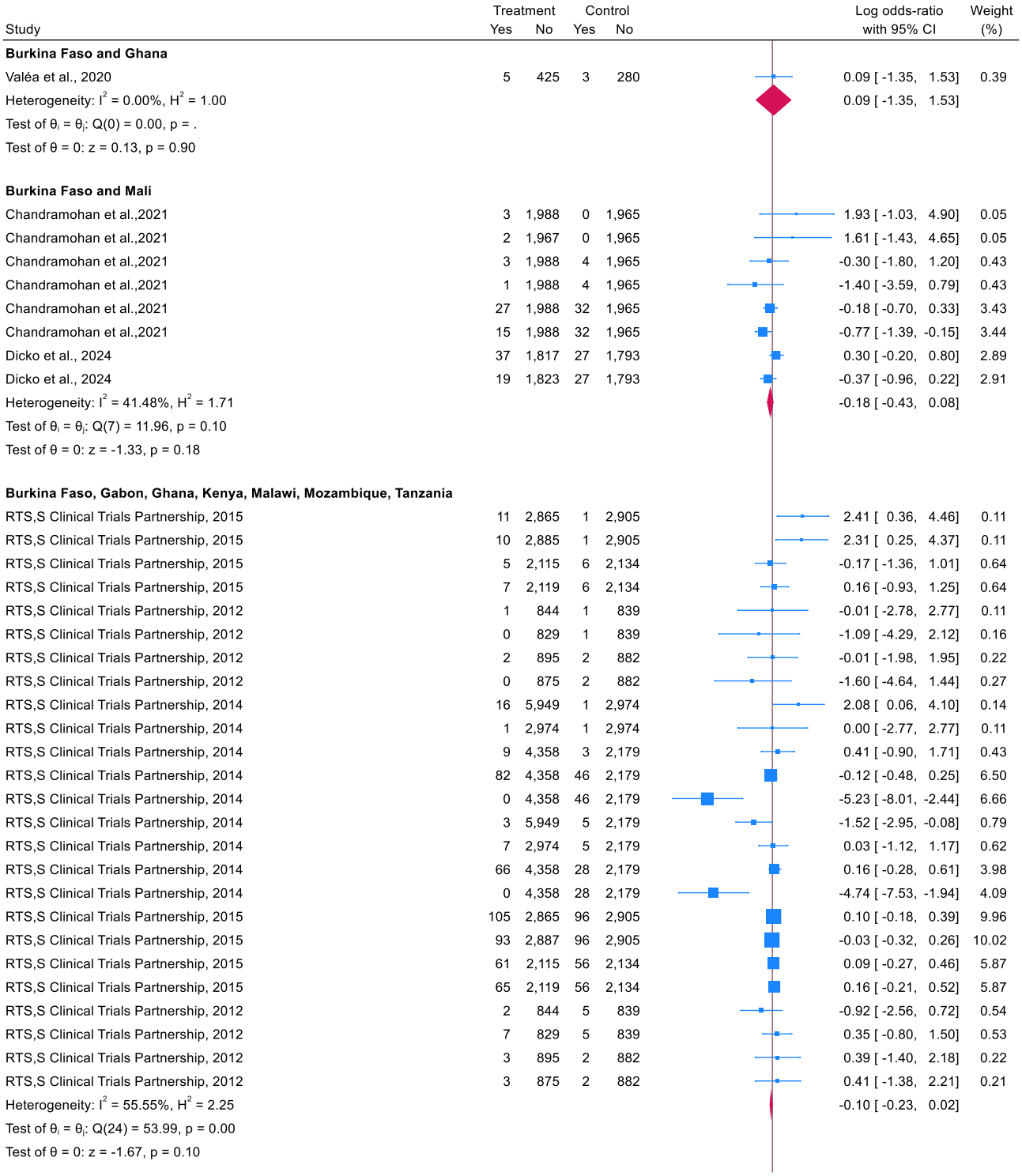


Figure 4.8: Forest plot of subgroup analysis of log OR for adverse events among RTS,S/AS01 vaccine recipients compared with control groups, stratified by study country, using a fixed-effects Mantel–Haenszel model

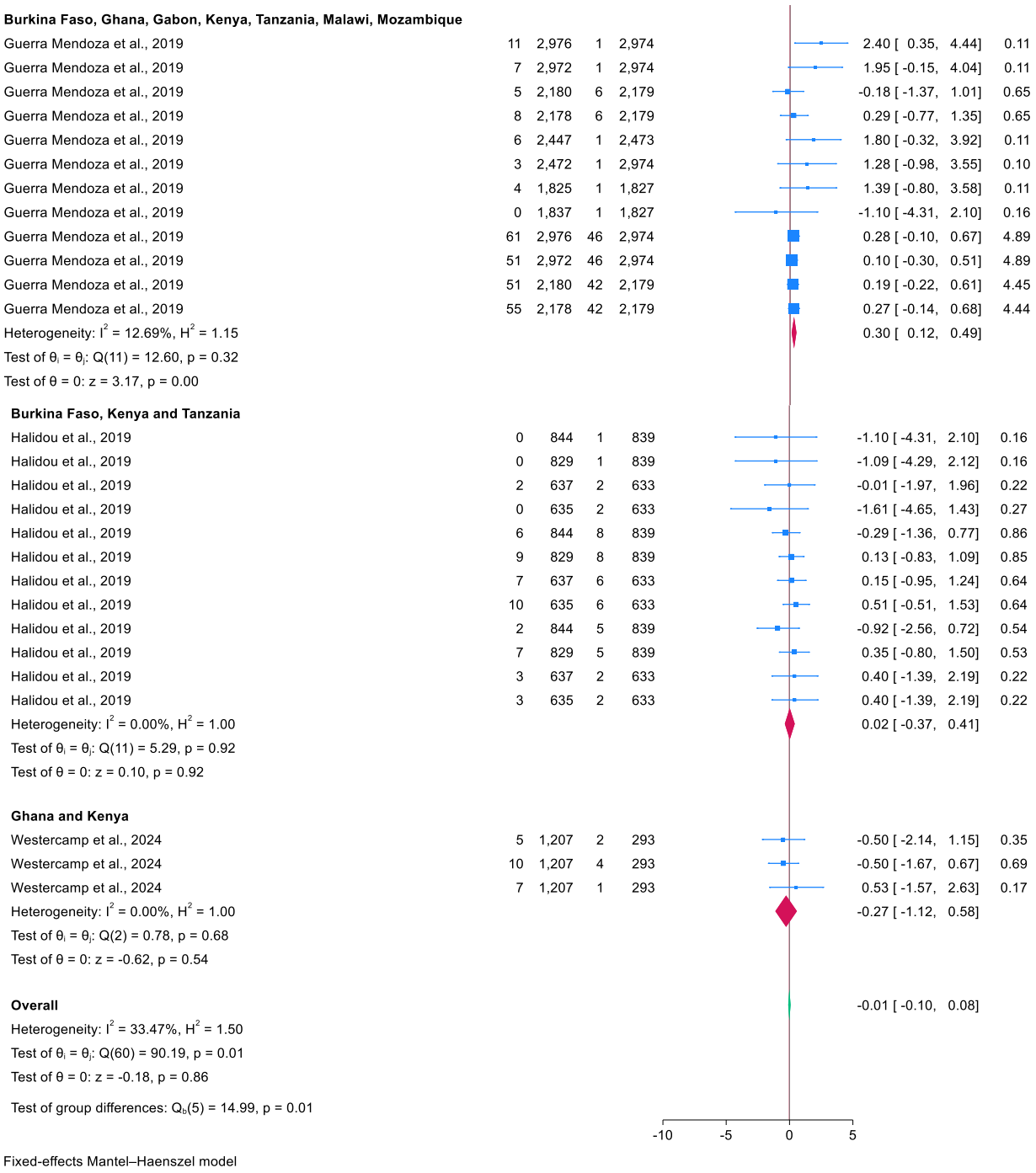


Figure 4.8 continued: Forest plot of subgroup analysis of log OR for adverse events among RTS,S/AS01 vaccine recipients compared with control groups, stratified by study country, using a fixed-effects Mantel–Haenszel model

4.4.2.6 Safety Outcome 6: Subgroup analysis of log OR for adverse events among RTS,S/AS01 vaccine recipients compared with control groups, stratified by study design/trial phase, using a fixed-effects Mantel–Haenszel model.

The log of OR for adverse events among RTS,S/AS01 vaccine recipients compared with control groups were stratified by trial phase (Figure 4.9). In RCT Phase III trials, the pooled log of OR was -0.01 (95% CI: -0.10 to 0.09), with moderate heterogeneity (I^2 = 35.76%), showing no significant difference between groups (p = 0.91). In RCT Phase IIb trials, the pooled log of OR was -0.27 (95% CI: -1.12 to 0.58), with no heterogeneity (I^2 = 0.00%), also showing no significant difference (p = 0.54). The test for subgroup differences was not statistically significant (Q(1) = 0.36, p = 0.55), indicating no strong evidence of variation in adverse event risk between trial phases.

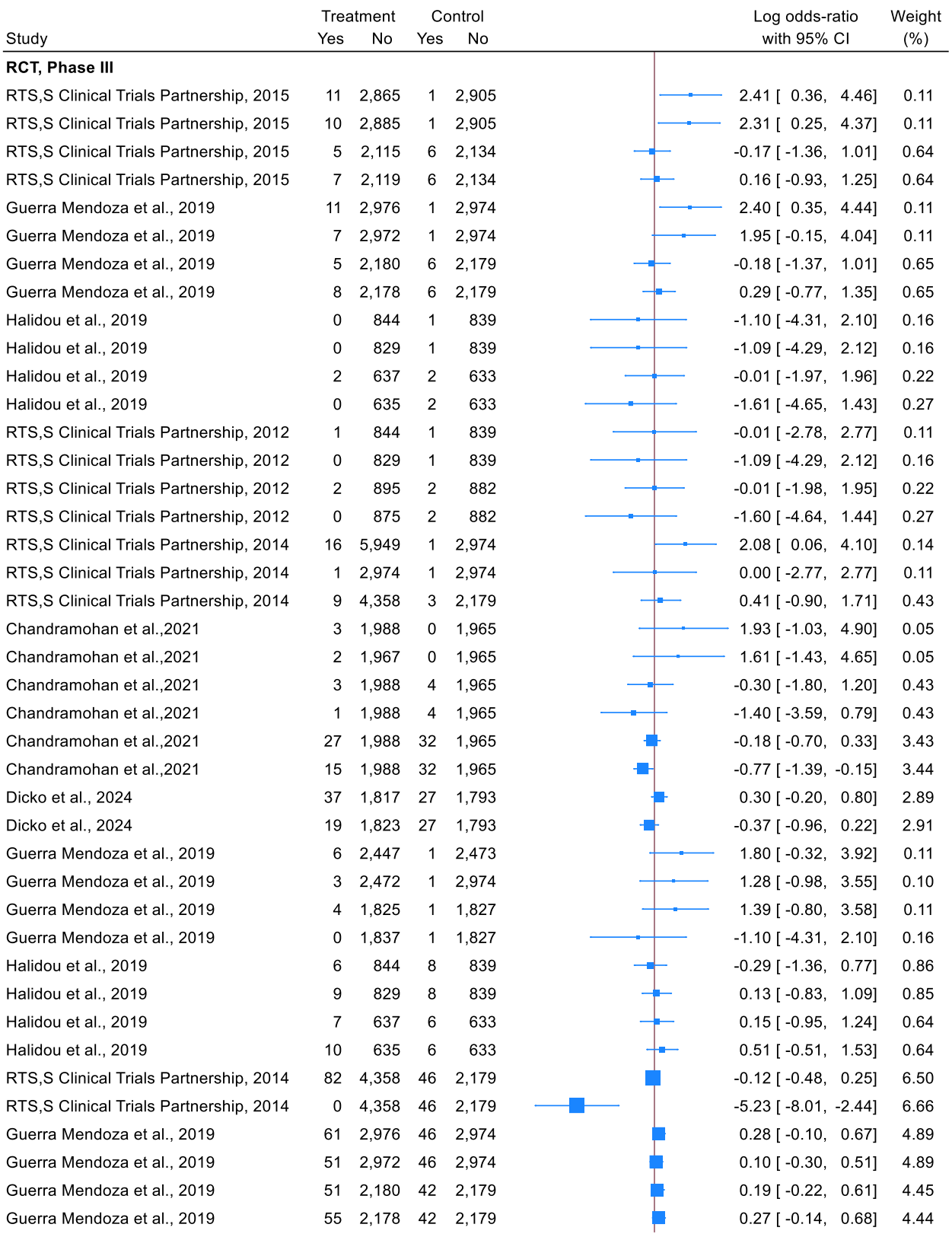


Figure 4.9: Forest plot of subgroup analysis of log OR for adverse events among RTS,S/AS01 vaccine recipients compared with control groups, stratified by study design/trial phase, using a fixed- effects Mantel–Haenszel model

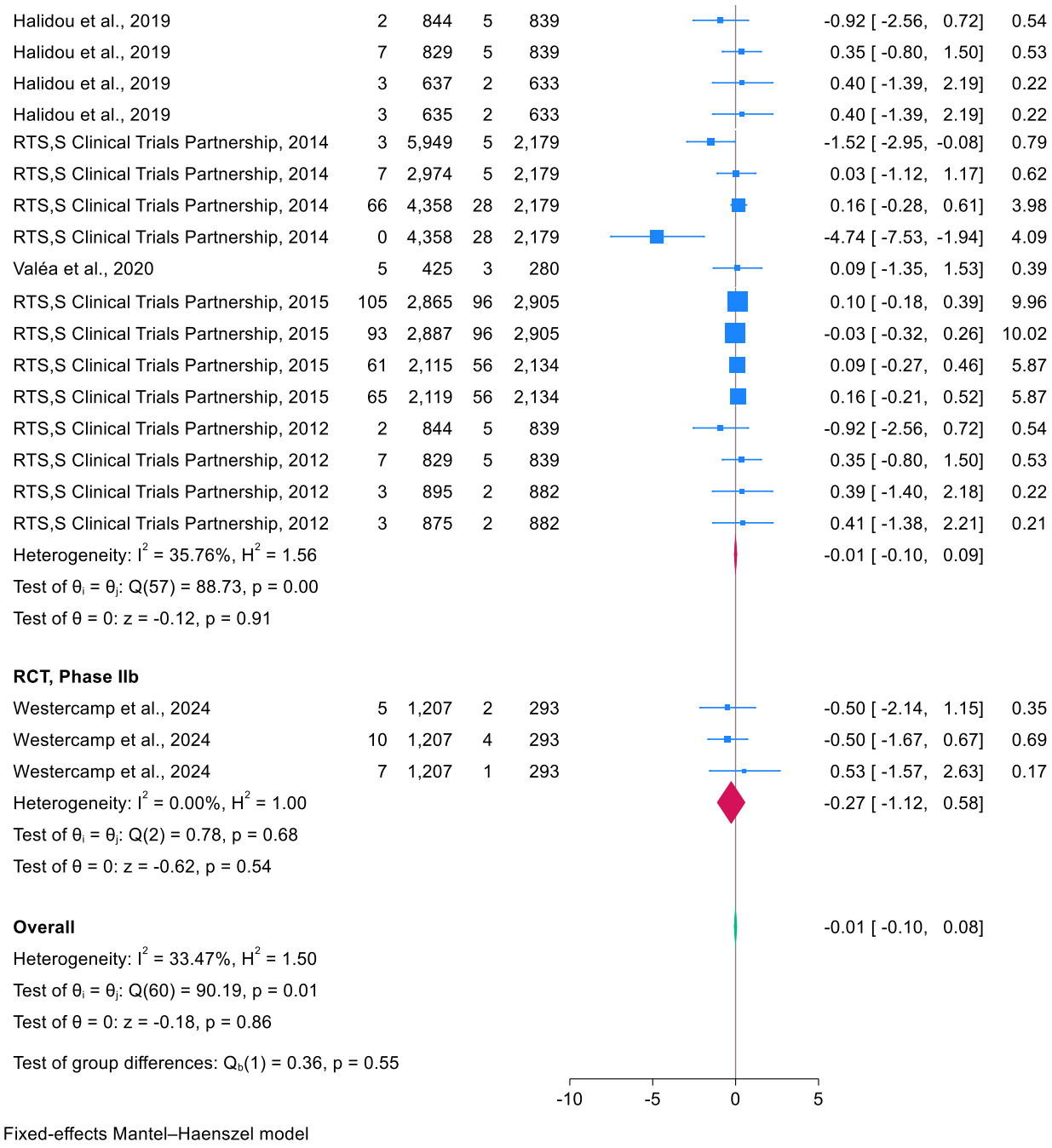


Figure 4.9 continued: Forest plot of subgroup analysis of log OR for adverse events among RTS,S/AS01 vaccine recipients compared with control groups, stratified by study design/trial phase, using a fixed-effects Mantel–Haenszel model

4.4.2.7 Assessment of Publication Bias Using Funnel Plots, Contour-Enhanced Funnel Plots and the Harbord Regression in the Meta-Analysis of RTS,S/AS01 Safety

4.4.2.7.1 Funnel Plot for Assessing Publication Bias in the Meta-Analysis of RTS,S/AS01 Safety

The funnel plot assessing publication bias in the meta-analysis of RTS,S/AS01 safety (Figure 4.10) appears asymmetrical, suggesting a potential risk of publication bias. Studies with smaller standard errors are more dispersed, while those with higher precision cluster around the pooled estimate. The presence of asymmetry may indicate small-study effects.

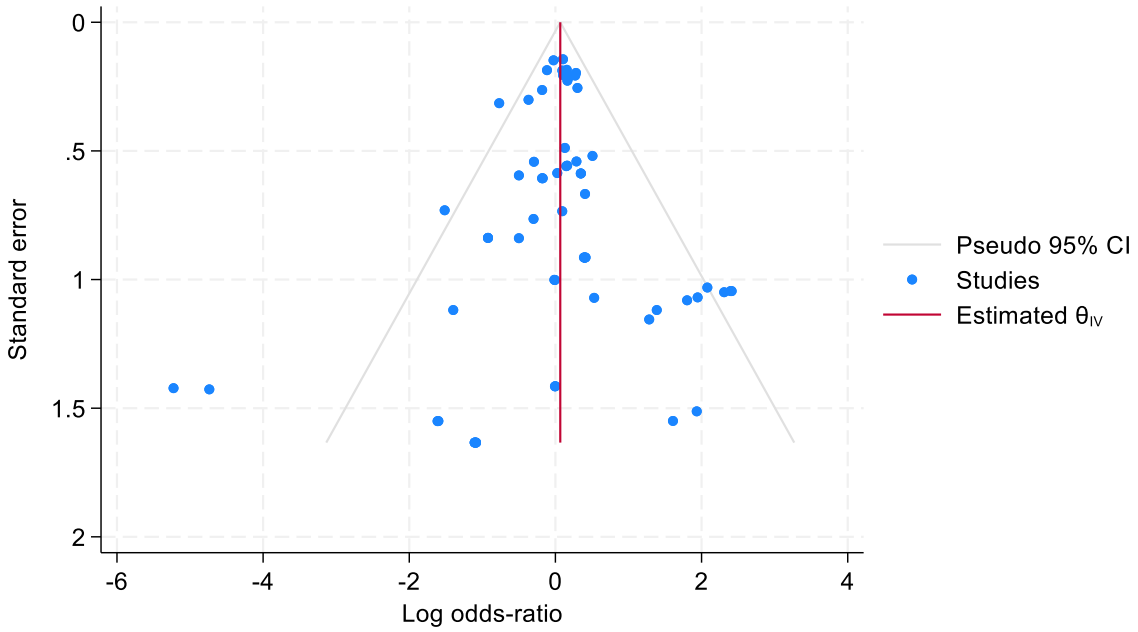


Figure 4.10: The Funnel plot for assessing publication bias in the meta-analysis of RTS,S/AS01 safety

4.4.2.7.2 Contour-Enhanced Funnel for Assessing Publication Bias in the Meta-Analysis of RTS,S/AS01 Safety

The contour-enhanced funnel plot assessing publication bias in the meta-analysis of RTS,S/AS01 safety (Figure 4.11) shows an asymmetrical distribution of studies, suggesting the possibility of publication bias. Studies are more concentrated in areas of higher statistical significance (lighter- shaded regions), which may indicate small-study effects. However, the presence of studies in non- significant areas (darker regions) suggests that asymmetry may not be entirely due to publication bias but could also be influenced by heterogeneity among studies.

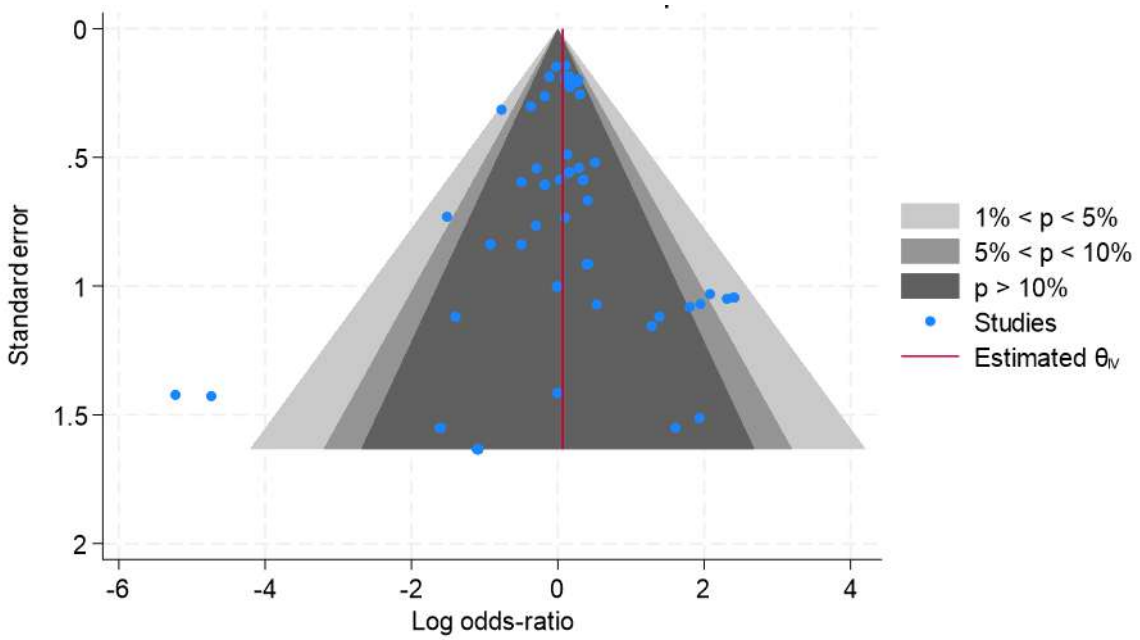


Figure 4.11: The contour-enhanced funnel plot evaluating the potential publication bias in the meta-analysis of RTS,S/AS01 safety studies.

4.4.2.7.3 Harbord Test for Small-Study Effects in the RTS,S Malaria Vaccine Safety Meta- Analysis Safety

The Harbord test for small-study effects using a fixed-effects inverse-variance model showed no evidence of small-study effects in the meta-analysis of RTS,S/AS01 safety (Figure 4.12). The estimated β_1 was -0.07 (SE = 0.211), with a z-score of -0.33 and a p-value of 0.7416, indicating that the null hypothesis of no small-study effects could not be rejected.

```
Regression-based Harbord test for small-study effects
Fixed-effects model
Method: Inverse-variance
H0: beta1 = 0; no small-study effects
      beta1 =   -0.07
   SE of beta1 =   0.211
         z =   -0.33
   Prob > |z| =   0.7416
end of do-file
```

Figure 4.12: Harbord Test for Small-Study Effects in the RTS,S Malaria Vaccine Safety Meta- Analysis

4.4.2.8 Sensitivity Analyses to identify outliers and examine the influence of individual studies on the pooled estimates using Galbraith Plot Analysis of Study Variability in RTS,S/AS01 Safety Trials

4.4.2.8.1 Galbraith plot analysis of study variability in RTS,S/AS01 safety trials

The Galbraith plot assessing study variability in the meta-analysis of RTS,S/AS01 safety trials (Figure 4.13) shows that most studies fall within the 95% confidence interval (shaded region), indicating low to moderate heterogeneity. A few studies deviated from the regression line, suggesting potential outliers that may contribute to variability in effect estimates. The overall distribution suggests that study precision is generally consistent, with no extreme heterogeneity detected.

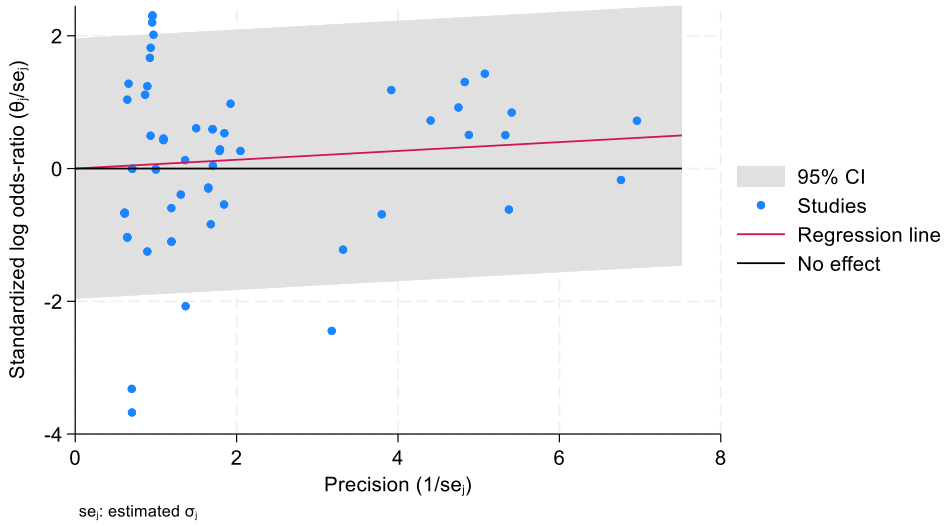


Figure 4.13: Assessment of heterogeneity in the meta-analysis of RTS,S malaria vaccine safety studies using Galbraith plot.

4.4.3 Efficacy Outcomes of RTS,S/AS01

The efficacy of the RTS,S/AS01 malaria vaccine was assessed by comparing the incidence of malaria cases between the vaccine and control groups across various variables. These variables were vaccine regimen, age group, study design and country. The efficacy analysis was conducted using odds ratios presented in a forest plot, which measured the relative reduction in malaria risk among the vaccine group compared to those in the control group.

4.4.3.1 Efficacy Outcome 1: Pooled odds ratios for RTS,S/AS01 efficacy based on case incidence in vaccinated and control groups using a random-effects REML model

The pooled log of OR for the efficacy of RTS,S/AS01 compared with the control group across all included studies was -0.34 (95% CI: -0.47 to -0.22), indicating a significant reduction in case incidence among vaccinated individuals (Figure 4.14). Heterogeneity was high (I^2 = 97.28%), suggesting variability across studies. The test for overall effect was statistically significant ($p < 0.001$), confirming that RTS,S/AS01 was associated with a lower risk of cases compared with the control group.

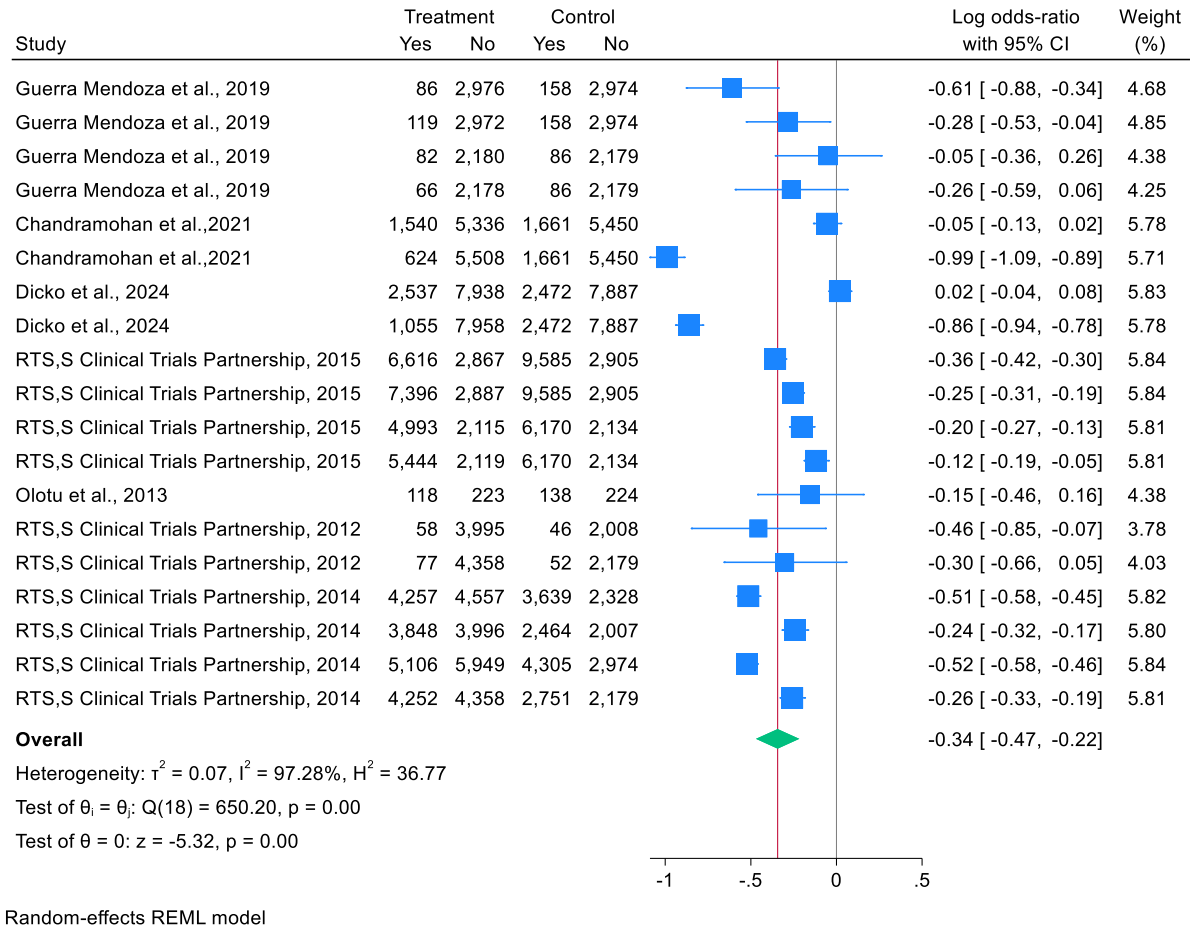


Figure 4.14. Forest plot of pooled log OR for RTS,S/AS01 efficacy based on case incidence in vaccinated and control groups using a random-effects REML model

4.4.3.2 Efficacy Outcome 2: Subgroup analysis of log OR for RTS,S/AS01 efficacy comparing case incidence in vaccinated and control groups, stratified by age group, using a random-effects REML model

The subgroup analysis of RTS,S/AS01 efficacy by age group (Figure 4.15) showed a greater reduction in case incidence among children aged 5–17 months (pooled log of OR: -0.42, 95% CI: -0.61 to -0.23) compared with infants aged 6–12 weeks (pooled log of OR: -0.21, 95% CI: -0.27 to -0.15). The overall pooled odds ratio across all included studies was -0.34 (95% CI: -0.47 to -0.22), indicating a significant reduction in case incidence among vaccinated individuals. Heterogeneity was high (I^2 = 97.28%), reflecting variability among studies. The test for subgroup differences was statistically significant (p = 0.04), suggesting that efficacy may vary by age group.

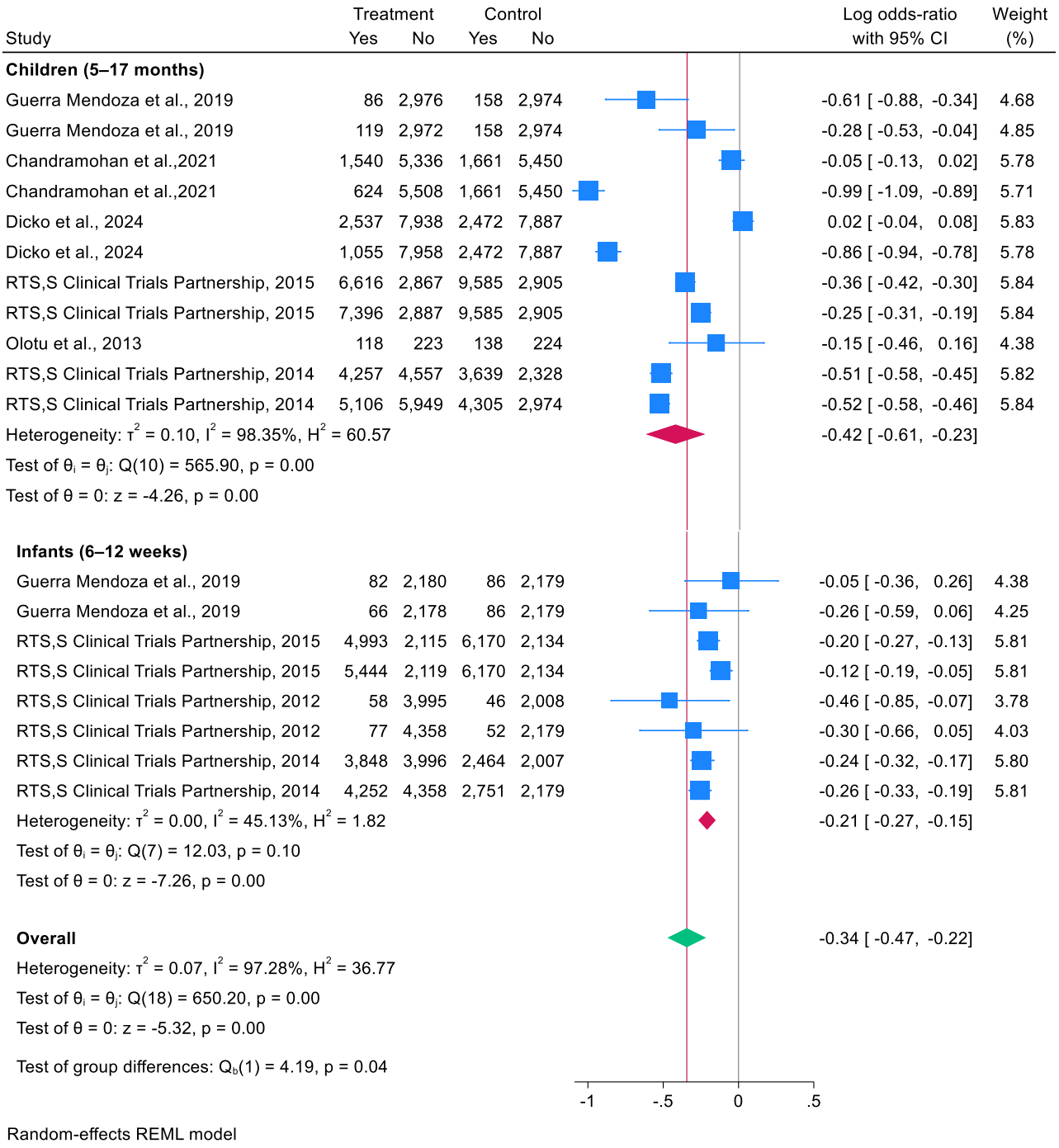


Figure 4.15. Forest plot of subgroup analysis of log OR for RTS,S/AS01 efficacy comparing case incidence in vaccinated and control groups, stratified by age group, using a random-effects REML model.

4.4.3.3 *Efficacy Outcome 3: Subgroup analysis of log OR for RTS,S/AS01 efficacy comparing case incidence in vaccinated and control groups, stratified by vaccine regimen/strategy, using a random-effects REML model*.

The subgroup analysis by vaccine regimen (Figure 4.16) showed that RTS,S/AS01 combined with chemoprevention had the greatest reduction in case incidence (pooled OR: -0.92, 95% CI: -1.05 to - 0.79), followed by RTS,S/AS01 with a booster (pooled OR: -0.34, 95% CI: -0.47 to -0.21) and RTS,S/AS01 alone (pooled OR: -0.21, 95% CI: -0.32 to -0.10). The overall pooled log of OR was -

0.34 (95% CI: -0.47 to -0.22), confirming a significant reduction in case incidence among vaccinated individuals. Heterogeneity remained high (I^2 = 97.28%) and the test for subgroup differences was statistically significant ($p < 0.001$), indicating variation in efficacy across vaccine regimens.

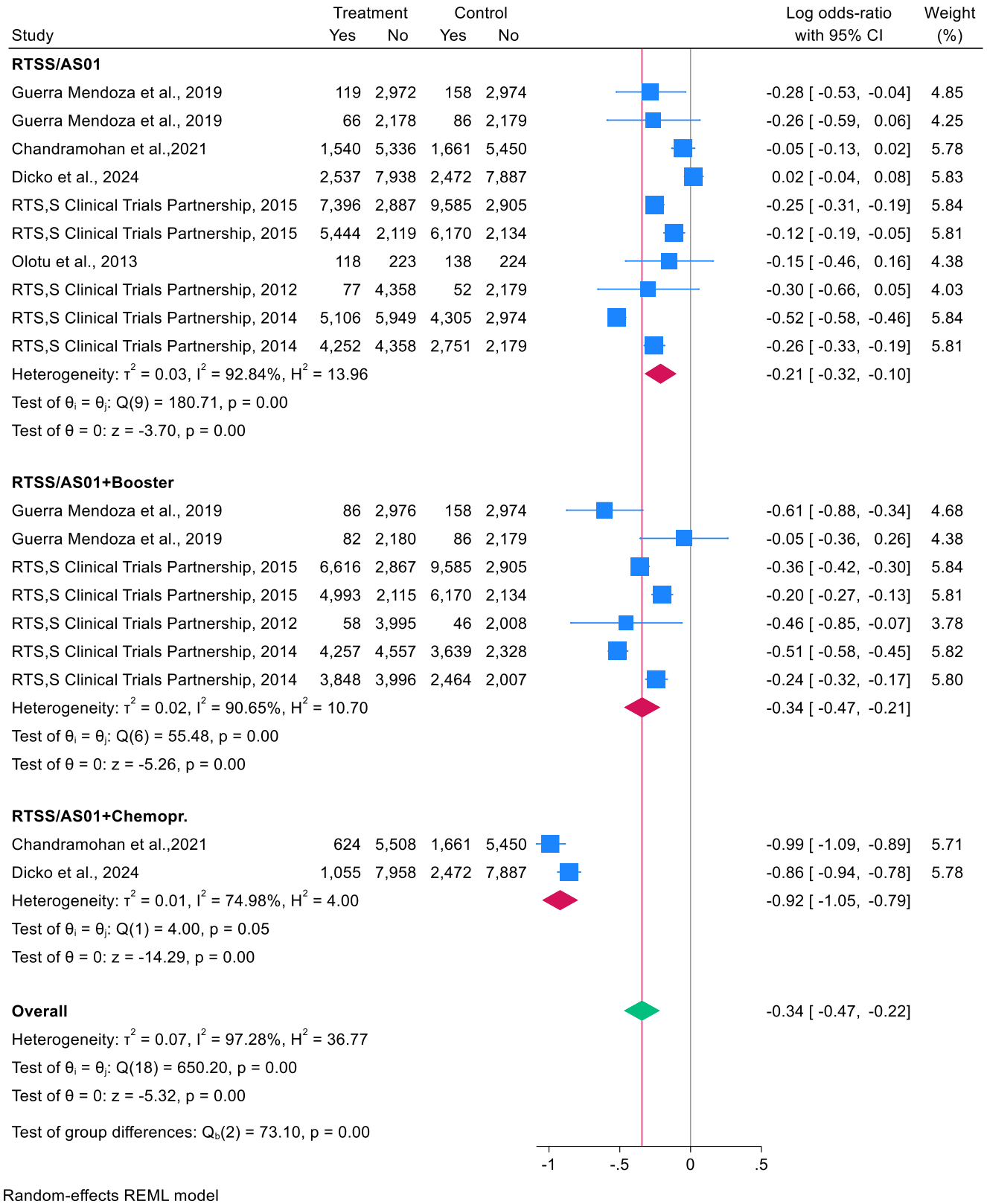


Figure 4.16: The forest plot of subgroup analysis of log OR for RTS,S/AS01 efficacy comparing case incidence in vaccinated and control groups, stratified by vaccine regimen/strategy, using a random-effects REML model.

4.4.3.4 Efficacy Outcome 4: Subgroup analysis of log OR for RTS,S/AS01 efficacy comparing case incidence in vaccinated and control groups, stratified by study location, using a random-effects REML mode*l.*

The subgroup analysis by study location (Figure 4.17) showed variations in RTS,S/AS01 efficacy across different geographical regions. The pooled log of OR indicated the greatest reduction in case incidence in Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, and Tanzania (log of OR: -0.38, 95% CI: -0.51 to -0.26), followed by Burkina Faso, Ghana, Gabon, Kenya, Tanzania, Malawi, Mozambique (log of OR: -0.31, 95% CI: -0.54 to -0.08). The efficacy in Burkina Faso and Mali was not statistically significant (log of OR: -0.47, 95% CI: -0.99 to 0.05, p = 0.07). The overall pooled log of OR was -0.34 (95% CI: -0.47 to -0.22), confirming a significant reduction in case incidence among vaccinated individuals. Heterogeneity remained high (I^2 = 97.28%), and the test for subgroup differences was not statistically significant (p = 0.32), suggesting no strong evidence of variation in efficacy across study locations.

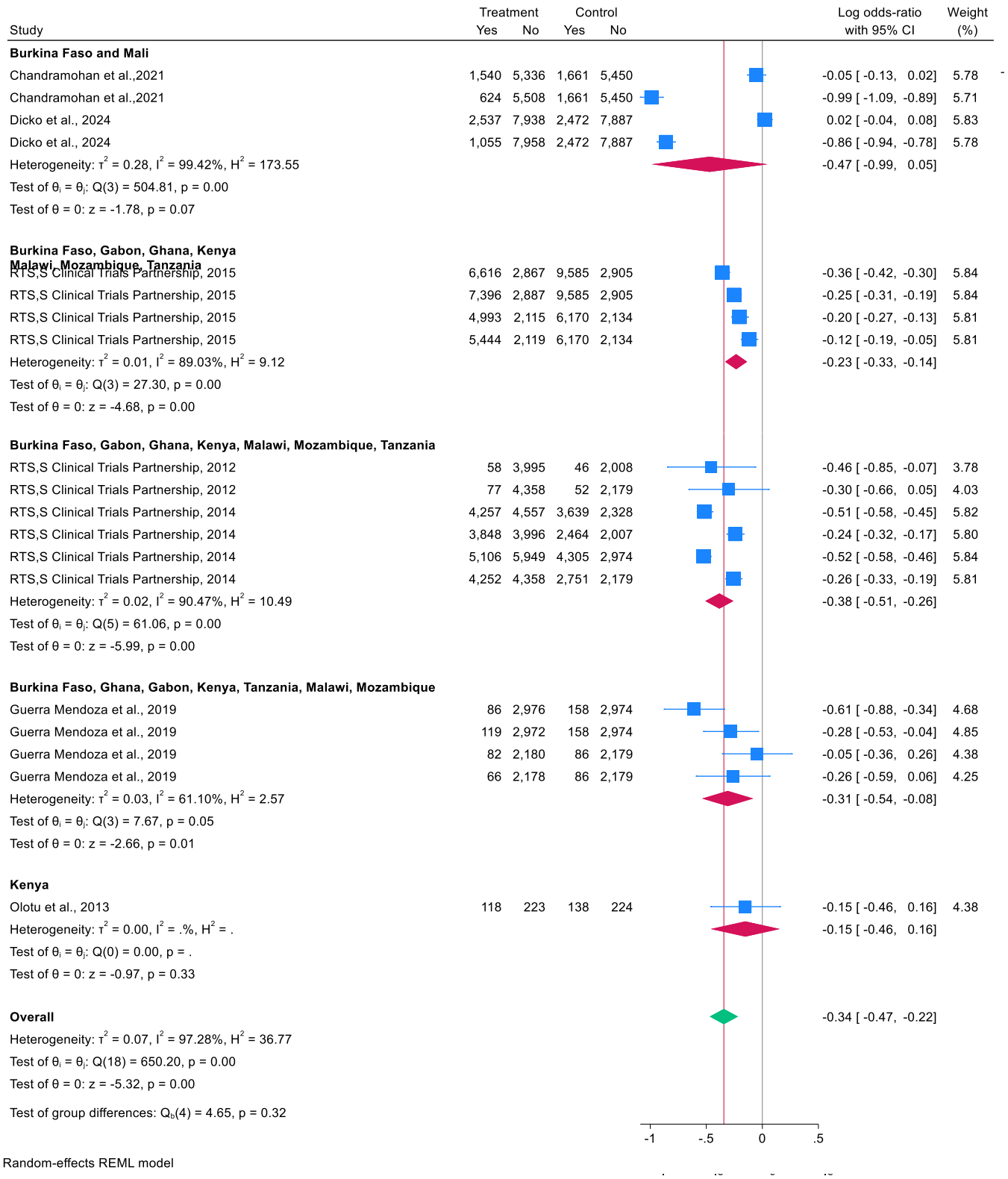


Figure 4.17: The forest plot of subgroup analysis of log OR for RTS,S/AS01 efficacy comparing case incidence in vaccinated and control groups, stratified by study location, using a random-effects REML model.

4.4.3.5 Efficacy Outcome 5: Subgroup analysis of log OR for RTS,S/AS01 efficacy comparing case incidence in vaccinated and control groups, stratified by trial phase, using a random-effects REML model

The subgroup analysis by trial phase (Figure 4.18) showed that RTS,S/AS01 efficacy was higher in Phase III trials (pooled log OR –0.35, 95% CI –0.48 to –0.22) compared with Phase IIb trials, where the estimate was not statistically significant (pooled log OR –0.15, 95% CI –0.46 to 0.16; p = 0.33). The overall (pooled log OR –0.34, 95% CI –0.47 to –0.22), suggesting a significant reduction in case incidence among vaccinated individuals. Heterogeneity remained high (I^2 = 97.28%), and the test for subgroup differences was not statistically significant (p = 0.24), indicating no strong evidence of variation in efficacy between Phase IIb and Phase III trials.

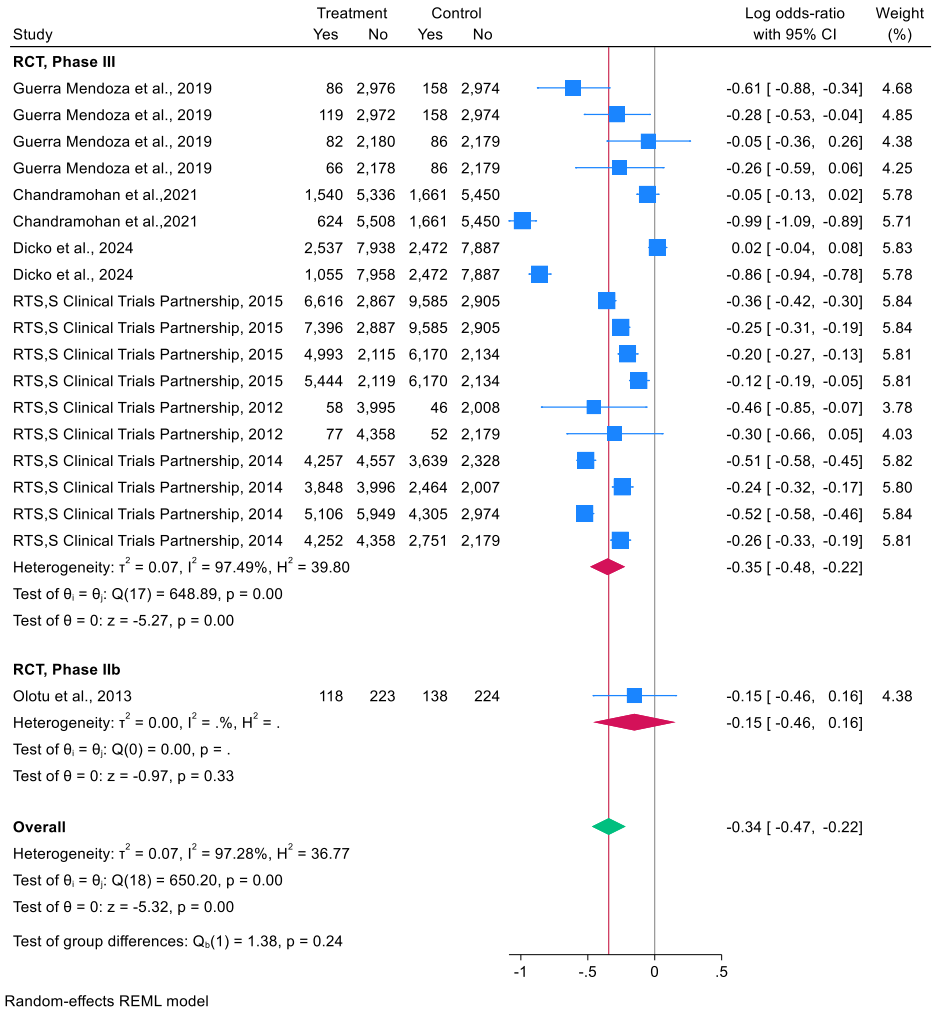


Figure 4.18: Forest plot of subgroup analysis of log OR for RTS,S/AS01 efficacy comparing case incidence in vaccinated and control groups, stratified by trial phase, using a random-effects REML model.

4.4.3.6 Assessment of Publication Bias Using Funnel Plots, Contour-Enhanced Funnel Plots and the Harbord Regression in the Meta-Analysis of RTS,S/AS01 Efficacy

4.4.3.6.1 Funnel Plot for Assessing Publication Bias in the Meta-Analysis of RTS,S/AS01 Efficacy

The funnel plot (Figure 4.19) was used to assess publication bias in the meta-analysis of RTS,S/AS01 efficacy. The distribution of studies appeared asymmetrical, with some deviation from the expected inverted funnel shape, suggesting a potential risk of small-study effects or publication bias. Studies with higher standard errors (smaller sample sizes) showed greater variability in effect estimates.

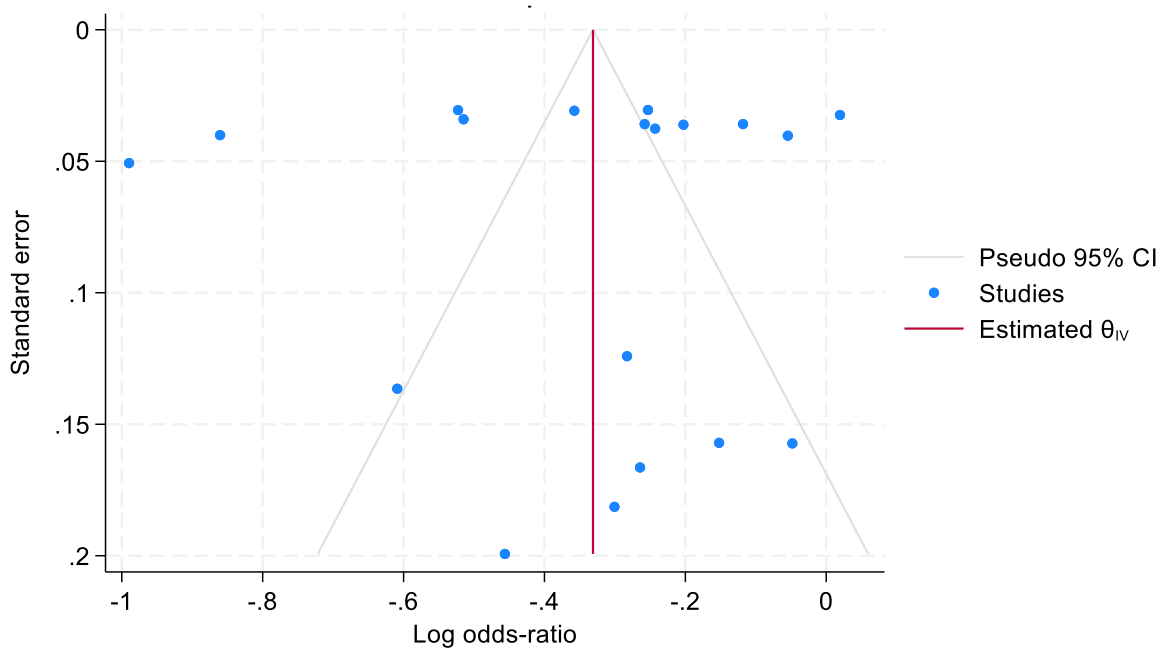
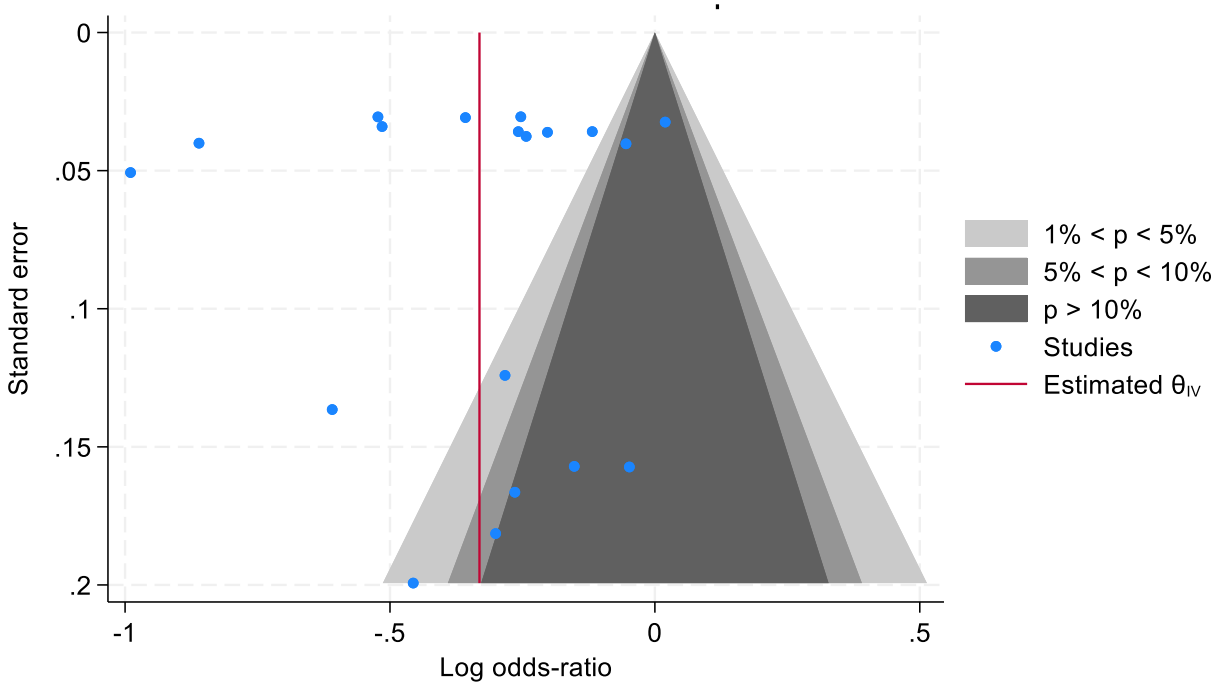


Figure 4.19: The Funnel plot for assessing publication bias in the meta-analysis of RTS,S/AS01 efficacy

4.4.3.6.2 Contour-Enhanced Funnel for Assessing Publication Bias in the Meta-Analysis of RTS,S/AS01 Efficacy

The contour-enhanced funnel plot (Figure 4.20) was used to assess publication bias in the meta- analysis of RTS,S/AS01 efficacy. The plot displays the distribution of study effect sizes against standard errors, with contour shading indicating different significance levels. The



asymmetry observed suggests a potential risk of small-study effects or publication bias. The presence of studies in areas with non-significant p-values (darker regions) indicates that missing studies may be due to factors other than publication bias.

Figure 4.20: The contour-enhanced funnel plot evaluating the potential publication bias in the meta- analysis of RTS,S/AS01 efficacy

4.4.3.6.3. Harbord Test for Small-Study Effects in the RTS,S Malaria Vaccine Safety Meta- Analysis Efficacy

The Harbord test for small-study effects, using a random-effects REML model, did not detect significant evidence of small-study effects ($\beta = 0.28$, SE = 1.072, $z = 0.26$, $p = 0.7965$). The high p-value suggests no statistically significant small-study effects, which indicates that publication bias or systematic differences between small and large studies are unlikely to be present.

```
Regression-based Harbord test for small-study effects
Random-effects model
Method: REML
H0: beta1 = 0; no small-study effects
        beta1 =    0.28
   SE of beta1 =    1.072
            z =    0.26
    Prob > |z| =    0.7965


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Figure 4.21. Harbord Test for small-study effects in the RTS,S malaria vaccine safety meta-analysis efficacy

4.4.3.7 Sensitivity Analyses to identify outliers and examine the influence of individual studies on the pooled estimates using Galbraith plots

4.4.3.7.1 Galbraith Plot Analysis of Study Variability in RTS,S/AS01 Efficacy Trials

The Galbraith plot confirms the presence of heterogeneity, supporting the need for a random-effects model in the meta-analysis (Figure 4.22). A few studies fall far outside the confidence band, indicating potential outliers or sources of heterogeneity in the dataset. A negative slope of the regression line suggests a protective effect of the RTS,S vaccine, with higher precision studies clustering closer to the regression line, while lower-precision studies (towards the left) show more variation in effect size. The presence of scattered points outside the confidence band suggests that study results vary considerably, possibly due to differences in study design, population, or methodological factors (Figure 4.22).

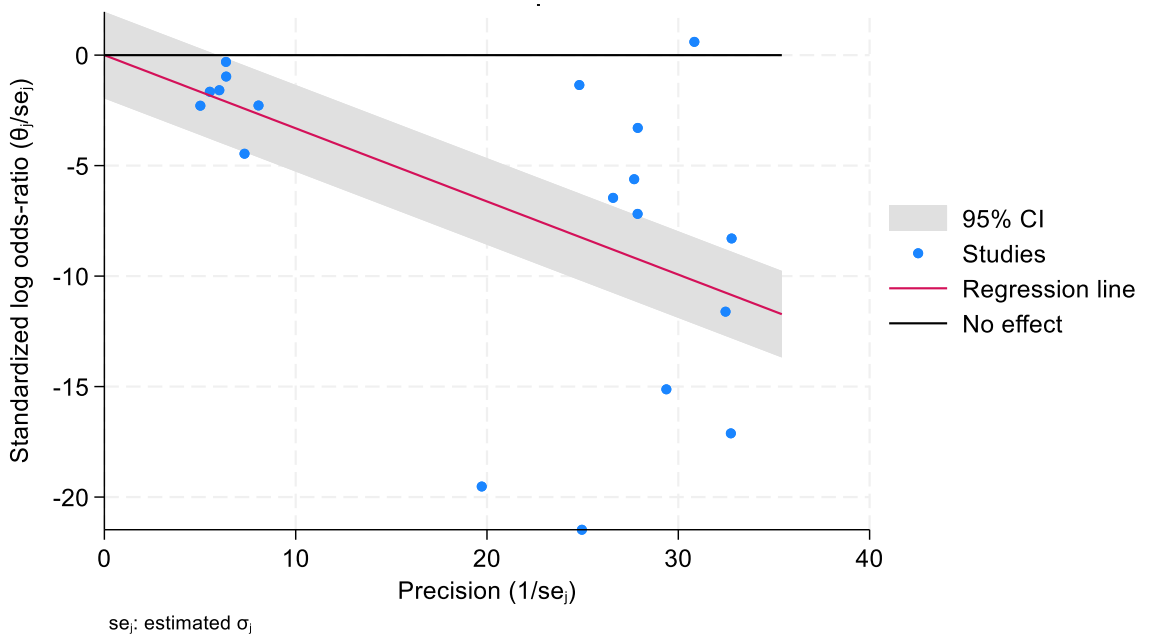

Figure 4.22: Assessment of heterogeneity in the meta-analysis of RTS,S malaria vaccine efficacy using Galbraith Plot.

CHAPTER FIVE – DISCUSSION AND CONCLUSION

5.0 Discussion

The emergence of antimalarial drug resistance and insecticide resistance continues to pose a major challenge to global malaria control efforts, reinforcing the urgent need for safe and effective vaccines to complement existing interventions (Ndiath, 2019; WHO, 2023). Among the various malaria vaccine candidates, RTS,S/AS01 remains the most advanced pre-erythrocytic malaria vaccine and the first to receive WHO approval for large-scale use (Nalova Akua, 2024; WHO, 2024d). However, the success of any vaccine relies not only on its efficacy but also on its safety and immunogenicity,

In this systematic review and meta-analysis, a comprehensive evaluation of the immunogenicity, efficacy and safety of the RTS,S/AS01 malaria vaccine was done across diverse populations. The findings reinforced the moderate protective efficacy of the RTS,S/AS01 while also emphasizing safety concerns and the variability in immune responses.

5.1 Immunogenicity of RTS,S/AS01

The results of the immunogenicity as measured by the GMTs of anti-CSP antibodies align with previous RTS,S trials that reported higher immunogenicity in children aged 5–17 months compared to infants aged 6–12 weeks (RTS,S Clinical Trials Partnership, 2015; White *et al.,* 2014). The highest GMT recorded in this study was 621 EU/mL (95% CI: 592–652), which was in a Phase II trial among children aged 5–17 months (White *et al.,* 2014). This was higher than the 209 EU/mL (95% CI: 197– 222) recorded in infants aged 6–12 weeks (White *et al.,* 2014). These findings reinforced the hypothesis that maternal antibody interference and immune system immaturity in younger infants reduce vaccine responsiveness, consistent with other malaria vaccine trials reported by Edwards (2015), Macià *et al.*, 2022, 2025; Varo *et al*. (2020).

A significant finding in this study was the variation in GMT values across Phase II and Phase III trials, with Phase II trials consistently showing higher GMT values (up to 621 EU/mL) compared to Phase III trials (34.2–383.5 EU/mL). This pattern had been previously documented by RTS,S Clinical Trials Partnership (2015), Sagara *et al*. (2022), White *et al.*

(2015) and Witte *et al*. (2018), where Phase II trials tend to report higher immunogenicity due to controlled conditions, more homogeneous study populations and optimized vaccine administration protocols.

The booster dose strategy significantly enhanced immune responses, though temporarily. One month after the first booster dose, GMTs peaked at 318.2 EU/mL (95% CI: 295.1–343.0) in children aged 5–17 months, but 12 months later, this dropped to 52.4 EU/mL (95% CI: 47.8–57.6). Similarly, among infants, GMT values peaked at 169.9 EU/mL (95% CI: 153.8–187.7) post-booster but declined to 15.9 EU/mL (95% CI: 13.8–18.3) at 12 months. This indicates significant immune waning over time. These findings are comparable to Olotu *et al.* (2014), RTS,S Clinical Trials Partnership (2015), White *et al.* (2014), where antibody titres declined sharply within a year post-vaccination, highlighting the need for additional booster doses to sustain protection.

A significant contribution of the current study is the finding that combining RTS,S with SMC resulted in superior immune responses compared to RTS,S alone. The study showed a GMT of 368.9 EU/mL (95% CI: 317.7–428.4) after three doses of RTS,S + SMC, which surpassed many RTS,S-only regimens. This supports findings from Cairns *et al.* (2022a), Chandramohan *et al.* (2021), Sagara *et al.* (2022), which suggested that RTS,S + SMC enhances both humoral and cellular immunity and may provide extended protection against a malaria infection.

Notable geographic differences were observed in vaccine-induced immunity, an aspect that has been less explored in previous RTS,S trials. GMT values were highest in multi-country Phase II trial regions, particularly in Gambia, Kenya, Mozambique, and Ghana, reaching 621 EU/mL. However, in Nigeria, pre-dose GMTs were extremely low (0.3 EU/mL), though they increased significantly post-vaccination (up to 285.5 EU/mL) (Umeh *et al.,* 2014). These variations may be influenced by host genetic factors, malaria transmission intensity, baseline immunity levels and regional differences in malaria epidemiology, as documented by Bell *et al.* (2022), Dobaño *et al.* (2019a), Juraska *et al.* (2024).

5.2 Safety of RTS,S/AS01

The safety evaluation of the RTS,S/AS01 malaria vaccine is crucial for understanding its risk profile and informing public health decision-making. This meta-analysis assessed adverse events across various study subgroups, including age groups, types of adverse events, vaccine

regimens, geographic locations, and study phases. The overall pooled estimate (log OR –0.01, 95% CI –0.10 to 0.08) indicated no statistically significant difference in adverse events between the vaccinated and control groups, which suggests a favourable safety profile. Moderate heterogeneity ($I^2 = 33.47\%$) was observed, reflecting variability across studies.

The findings in this study aligned with previous large-scale trials, including the Phase III trial conducted by the Guerra Mendoza *et al.*, RTS,S Clinical Trials Partnership (Guerra Mendoza *et al.*, 2019; RTS,S Clinical Trials Partnership, 2015), which reported a comparable safety profile with minimal serious adverse events. However, stratified analyses revealed key differences among subgroups that required further discussion.

The age-stratified analysis suggests that while infants (6–12 weeks) and children (2–26 months) demonstrated no significant increase in adverse events, children aged 5–17 months showed a trend toward a slightly higher rate of adverse events (log OR 0.09, 95% CI –0.03 to 0.21; $p = 0.16$). The difference in immune response among age groups may contribute to these findings, necessitating further investigation into age-dependent vaccine reactogenicity.

The subgroup analyses for adverse events and vaccine regimens revealed significant differences ($p = 0.00$), highlighting variability in outcomes across these groups. The incidence of convulsions was lower in the vaccine group, cases of meningitis were significantly higher; however, no specific microbial pathogen was consistently identified. These findings corroborate earlier studies that noted an increased frequency of meningitis among vaccinated individuals, although no direct causal relationship has been established RTS,S Clinical Trials Partnership, (RTS,S Clinical Trials Partnership, 2015). Additional studies are needed to assess the biological plausibility of this observation.

The vaccine regimen analysis demonstrated that the standard three-dose regimen did not significantly alter adverse event rates, whereas the booster regimen was associated with a slight increase in adverse events (The RTS,S Clinical Trials Partnership, 2012; RTS,S Clinical Trials Partnership, 2015; Guerra Mendoza *et al*., 2019). This suggests that while an additional booster may enhance immunogenicity, it could also lead to increased reactogenicity, as observed in studies evaluating booster effects in malaria vaccines (Syed, 2022). Conversely, the combination of RTS,S/AS01 with seasonal malaria chemoprevention resulted in lower adverse event rates when compared with the control (log OR –0.54, 95% CI –0.95 to –0.13), and this highlights the benefits of integrating vaccine and chemoprevention strategies into routine immunization campaigns.

Geographic variations in adverse events (p = 0.01) were evident, with regions such as Burkina Faso, Ghana, Gabon, Kenya, Tanzania, Malawi, and Mozambique reporting a statistically significant increase in adverse events. These regional disparities may be attributed to genetic, epidemiological or environmental differences. The role of co-endemic infections and differences in healthcare infrastructure should be explored in future research.

The study phase-stratified analysis confirmed consistency in safety outcomes between Phase IIb and Phase III trials (p = 0.55), supporting the robustness of findings across different clinical trial stages. These results reinforce the safety profile of RTS,S/AS01 in large-scale implementation, aligning with the WHO's recommendation for its use in malaria-endemic regions (WHO, 2024a).

The funnel plot and contour-enhanced funnel plot indicated potential publication bias, as evidenced by the asymmetrical distribution of studies. However, the Harbord test (p = 0.7416) suggested no statistically significant evidence of small-study effects, indicating that the observed asymmetry was possibly due to heterogeneity rather than systematic bias. The Galbraith plot analysis further confirmed that most studies fell within the 95% confidence region, suggesting low-to-moderate heterogeneity (Figure 4.22). A few studies with high standard errors show greater variability, but the nearly horizontal regression line indicates that the heterogeneity was primarily due to random variations rather than systematic bias.

5.3 Efficacy of RTS,S/AS01

The efficacy analysis of the RTS,S/AS01 malaria vaccine demonstrated a statistically significant reduction in malaria cases among vaccinated individuals compared to the control group across different subgroups. The forest plots illustrated the overall efficacy of the vaccine, with a pooled log OR of –0.34 (95% CI –0.47 to –0.22), confirming the vaccine's protective benefit. These findings aligned with previous large-scale studies, such as the RTS,S Clinical Trials Partnership (RTS,S Clinical Trials Partnership, 2015), which reported similar levels of efficacy.

The age-stratified meta-analysis revealed that vaccine efficacy is higher in children aged 5–17 months (log OR –0.42, 95% CI –0.61 to –0.23) compared to infants aged 6–12 weeks (log OR –0.21, 95% CI –0.27 to –0.15). This finding is consistent with earlier reports by Guerra Mendoza *et al.* (2019), Moncunill *et al.* (2019a), RTS,S Clinical Trials Partnership (2015) and

White *et al.* (2014). This suggested that immune responses may be stronger in older children, as this age group has a more developed immune system. The high heterogeneity ($I^2 = 98.35\%$) observed in the 5–17 months subgroup may be due to variations in study design, population demographics and malaria transmission intensity across different study sites.

Stratification by vaccine regimen highlighted the impact of different vaccination strategies on efficacy. The RTS,S/AS01+Booster regimen yielded a pooled estimate of (log OR –0.34, 95% CI –0.47 to –0.21), which is higher than the primary RTS,S/AS01 regimen (log OR –0.21, 95% CI –0.32 to –0.10). This finding is in agreement with studies reported by Guerra Mendoza *et al.* (2019), The RTS,S Clinical Trials Partnership (2014, 2015), which suggested that a booster dose enhances long-term protection. Notably, the RTS,S/AS01+Chemoprevention combination demonstrated the highest efficacy (log OR –0.92, 95% CI –1.05 to –0.79), which reinforced the evidence that combining vaccination with seasonal malaria chemoprevention significantly improves protection (Cairns *et al*., 2022a; Chandramohan *et al*., 2021; Dicko *et al*., 2024).

The country-stratified analysis revealed some regional differences in vaccine efficacy. While Burkina Faso and Mali exhibited borderline statistical significance (log OR –0.47, 95% CI –0.99 to 0.05), studies from Ghana, Kenya, Malawi, Mozambique and Tanzania reported a moderate reduction in malaria cases (log OR –0.23, 95% CI –0.33 to –0.14). The heterogeneity in vaccine efficacy across regions may be attributed to differences in malaria transmission intensity, genetic variation in parasite populations, and healthcare infrastructure. Notably, the study by Olutu *et al.* (2013) in Kenya reported a pooled log odds-ratio of (–0.15, 95% CI –0.46 to 0.16), which indicated no significant effect. This result may have been due to high seasonal variability or differences in participant characteristics.

The comparison of efficacy between different study designs indicates that Phase III trials consistently showed significant vaccine protection (log OR –0.35, 95% CI –0.48 to –0.22), whereas Phase IIb studies exhibited a weaker effect (log OR –0.15, 95% CI –0.46 to 0.16). These results align with phase-specific efficacy assessments (Cairns *et al.,* 2022b; RTS,S Clinical Trials Partnership, 2015; The RTS,S Clinical Trials Partnership, 2012), and this highlights the importance of large-scale trials to provide strong efficacy estimates. The high heterogeneity ($I^2 = 97.28\%$) across all studies revealed the need for further standardization in trial methodologies to improve comparability.

The funnel plot analysis suggested moderate asymmetry, raising concerns about potential publication bias. However, the Harbord regression test ($p = 0.7965$) did not indicate strong

statistical evidence of publication bias. This likely could have been due to random variation rather than systematic bias. The contour-enhanced funnel plot further supported this interpretation, with a clustering of studies in statistically significant regions.

The Galbraith plot analysis confirmed substantial heterogeneity, with some studies falling outside the confidence bands, suggesting potential outliers. This result highlighted the variability in study conditions, including differences in follow-up durations, participant demographics and malaria transmission levels. The need for a random-effects model in the meta-analysis was justified, given this high heterogeneity

5.4 Strengths of this study

One of the study's foremost strengths was its comprehensive assessment of immunogenicity across diverse populations, age groups, and study phases, which provides a well-rounded understanding of the performance of RTSS/AS01. Unlike previous reviews that focused primarily on RCTs, this study integrates findings from both RCTs and observational studies, which ensures a more complete picture of real-world effectiveness.

This study confirmed that RTS,S/AS01 demonstrated significant protective efficacy against malaria, particularly in children aged 5–17 months, who exhibited GMTs of anti-CSP antibodies compared to infants (6–12 weeks). The inclusion of data from multiple clinical trial phases (Phase II, III, and IV) ensured consistency in findings across different levels of vaccine evaluation.

5.5 Limitations

Some studies included in this review assessed immune responses over varying time frames, and a considerable number of participants were lost to follow-up in extended analyses. This attrition could have influenced GMT decay estimates, leading to an incomplete understanding of antibody persistence over time. A more standardized approach to long-term participant retention was essential for accurately evaluating the duration of vaccine-induced immunity.

Another major limitation was the focus of most studies on short to mid-term efficacy, leading to gaps in knowledge regarding the long-term durability of vaccine protection. While many trials reported robust immune responses within the first few months post-vaccination, fewer

studies extended beyond one year, making it difficult to determine the persistence of immunity over longer durations. Given the potential for waning immunity, longer-term follow-up studies are necessary to assess the true duration of protection conferred by vaccination.

Furthermore, the significant heterogeneity among studies (I^2 = 97.28%), especially in the efficacy analysis suggested considerable variation in effect sizes, which was likely influenced by differences in population characteristics, transmission settings and study designs. This high degree of heterogeneity may have affected the generalizability of the findings, as vaccine efficacy could vary across different demographic and epidemiological contexts. Additionally, inconsistencies in case definitions, diagnostic methodologies, and follow-up protocols across studies may have further contributed to variability in reported efficacy estimates.

5.6 Policy implications

The findings of this study provide critical insights for policymakers in malaria-endemic regions, particularly regarding the strategic implementation of RTS,S/AS01 in national immunization programs. The study highlighted the moderate but time-limited immunity conferred by the vaccine, which reinforces the importance of booster doses and combination strategies to sustain long-term protection.

Given the rapid waning of vaccine-induced immunity, optimizing booster dose schedules should be a priority in order to enhance durability and effectiveness. Policymakers should also consider integrating RTS,S/AS01 with SMC, as the study demonstrates that this combined approach enhances both humoral and cellular immune responses. Countries with high seasonal malaria transmission should incorporate RTS,S+SMC strategies into their malaria control programs to maximize the vaccine's impact.

Furthermore, the evidence from this study suggested higher vaccine efficacy in children aged 5–17 months compared to infants, indicating that vaccination programs should prioritize this age group to achieve maximum benefit. This targeted approach could optimize resource allocation and improve overall malaria control efforts.

The favourable safety profile of RTS,S/AS01, particularly when combined with chemoprevention, supported its inclusion in routine immunization programs. However, continued post-implementation safety monitoring through pharmacovigilance was very important to assess potential rare or long-term adverse events.

Therefore, the successful introduction of RTS,S/AS01will depend on both health system readiness and supply chain strength. Sustained coverage requires investments in cold chain infrastructure, a trained workforce, and integration of the vaccine into existing expanded programme on immunization schedules. These programme capacities must be matched with reliable forecasting, procurement, and last-mile delivery, which are often most challenging in rural and high-burden areas. The added complexity of coordinating RTS,S/AS01 with seasonal malaria chemoprevention further highlights the need for efficient planning and strong system support, to ensure that the efficacy observed in trials is realized in routine practice.

5.7 Future Research

Despite the significant contributions of this study to understanding the RTS,S/AS01 malaria vaccine, several areas warrant further research to optimize vaccine efficacy, durability and safety. Future studies should focus on the long-term durability of vaccine-induced immunity. Current evidence suggested a significant decline in GMTs of anti-CSP antibodies within the first-year post-vaccination. Therefore, optimized booster dose schedules must be explored to sustain immunity over extended periods.

Furthermore, additional research is needed to understand the immunological mechanisms underlying the higher vaccine efficacy observed in children aged 5–17 months compared to infants. Investigating factors such as T-cell responses and memory B-cell dynamics could provide deeper insights into age-related differences in vaccine performance.

Combination strategies involving RTS,S/AS01 and SMC have shown enhanced protective efficacy. However, further research should explore alternative vaccine adjuvants and their potential to enhance cellular immunity, given that the current formulation primarily elicits humoral responses.

Future studies should also examine age- and region-specific variations in adverse events. Although RTS,S/AS01 has demonstrated a favourable safety profile, the increased incidence of meningitis and slightly higher adverse event rates in older children warrant continued surveillance and further investigation into potential risk factors.

5.8 Conclusions

RTS,S/AS01 induced moderate but time-limited protection, with peak immunogenicity following the primary series and subsequent antibody waning, emphasizing the need for booster doses to sustain long-term immunity. The efficacy in preventing malaria is highest among children aged 5–17 months compared to infants (6–12 weeks). RTS,S/AS01 exhibits an acceptable safety profile, however, there is a potential association with increased cases of febrile seizures and suspected meningitis. This highlights the need for continued pharmacovigilance, further research to clarify causality and risk-benefit assessments to inform vaccination policies and ensure patient safety.

REFERENCES

Adepoju, P. (2024). Routine malaria vaccinations begin. *The Lancet*, *403*(10425), 423. https://doi.org/10.1016/S0140-6736(24)00194-6

Afolabi, M. O., Ale, B. M., Dabira, E. D., Agbla, S. C., Bustinduy, A. L., Ndiaye, J. L. A., & Greenwood, B. (2021). Malaria and helminth co-infections in children living in endemic countries: A systematic review with meta-analysis. *PLOS Neglected Tropical Diseases*, 15(2), e0009138. https://doi.org/10.1371/journal.pntd.0009138

Ajua, A., Lell, B., Agnandji, S. T., Asante, K. P., Owusu-Agyei, S., Mwangoka, G., Mpina, M., Salim, N., Tanner, M., Abdulla, S., Vekemans, J., Jongert, E., Lievens, M., Cambron, P., Ockenhouse, C. F., Kremsner, P. G., & Mordmüller, B. (2015). The effect of immunization schedule with the malaria vaccine candidate RTS,S/AS01E on protective efficacy and anti-circumsporozoite protein antibody avidity in African infants. *Malaria Journal*, *14*(1), 72. https://doi.org/10.1186/s12936-015-0605-7

Al-Awadhi, M., Ahmad, S., & Iqbal, J. (2021). Current Status and the Epidemiology of Malaria in the Middle East Region and Beyond. Microorganisms, 9(2), 338. https://doi.org/10.3.390/microorganisms9020338.

Amani, A., Mboussou, F., Impouma, B., Cabore, J., & Moeti, M. R. (2024). Introduction and rollout of malaria vaccines in Cameroon and Burkina Faso: Early lessons learned. *The Lancet Global Health*, 12(5), e740–e741. https://doi.org/10.1016/S2214-109X(24)00101-3

Andrade, M. V., Noronha, K., Diniz, B. P. C., Guedes, G., Carvalho, L. R., Silva, V. A., Calazans, J. A., Santos, A. S., Silva, D. N., & Castro, M. C. (2022). The economic burden of malaria: A systematic review. *Malaria Journal*, 21(1), 283. https://doi.org/10.1186/s12936-022-04303-6

Asante, K. P., Ansong, D., Kaali, S., Adjei, S., Lievens, M., Nana Badu, L., Agyapong Darko, P., Boakye Yiadom Buabeng, P., Boahen, O., Maria Rettig, T., Agutu, C., Benard Ekow Harrison, S., Ntiamoah, Y., Adomako Anim, J., Adeniji, E., Agordo Dornudo, A., Gvozdenovic, E., Dosoo, D., Sambian, D., … Ofori-Anyinam, O. (2020). Immunogenicity and safety of the RTS,S/AS01 malaria vaccine co-administered with measles, rubella and yellow fever vaccines in Ghanaian children: A phase IIIb, multi-center, non-inferiority, randomized, open, controlled trial. *Vaccine*, *38*(18), 3411–3421. https://doi.org/10.1016/j.vaccine.2020.03.014

Asante, K. P., Mathanga, D. P., Milligan, P., Akech, S., Oduro, A., Mwapasa, V., Moore, K. A., Kwambai, T. K., Hamel, M. J., Gyan, T., Westercamp, N., Kapito-Tembo, A., Njuguna, P., Ansong, D., Kariuki, S., Mvalo, T., Snell, P., Schellenberg, D., Welega, P., … Sillah, J. S. (2024). Feasibility, safety, and impact of the RTS,S/AS01E malaria vaccine when implemented through national immunisation programmes: Evaluation of cluster-randomised introduction of the vaccine in Ghana, Kenya, and Malawi. *The Lancet*, *403*(10437), 1660–1670. https://doi.org/10.1016/S0140-6736(24)00004-7

Balaji, S., Deshmukh, R., & Trivedi, V. (2020). Severe malaria: Biology, clinical manifestation, pathogenesis and consequences. *Journal of Vector Borne Diseases*, 57(1), 1. https://doi.org/10.4103/0972-9062.308793

Bell, G. J., Goel, V., Essone, P., Dosoo, D., Adu, B., Mensah, B. A., Gyaase, S., Wiru, K., Mougeni, F., Osei, M., Minsoko, P., Sinai, C., Niaré, K., Juliano, J. J., Hudgens, M., Ghansah, A., Kamthunzi, P., Mvalo, T., Agnandji, S. T., … Emch, M. (2022). Malaria Transmission Intensity Likely Modifies RTS, S/AS01 Efficacy Due to a Rebound Effect in Ghana, Malawi, and Gabon. *The Journal of Infectious Diseases*, *226*(9), 1646–1656. https://doi.org/10.1093/infdis/jiac322

Bell, G. J., Gyaase, S., Goel, V., Adu, B., Mensah, B., Essone, P., Dosoo, D., Osei, M., Niare, K., Wiru, K., Brandt, K., Emch, M., Ghansah, A., Asante, K. P., Mvalo, T., Agnandji, S. T., Juliano, J. J., & Bailey, J. A. (2023). Background malaria incidence and parasitemia during the three-dose RTS,S/AS01 vaccination series do not reduce magnitude of antibody response nor efficacy against the first case of malaria. *BMC Infectious Diseases*, *23*(1), 716. https://doi.org/10.1186/s12879-023-08699-7

Bell, G. J., Loop, M. S., Mvalo, T., Juliano, J. J., Mofolo, I., Kamthunzi, P., Tegha, G., Lievens, M., Bailey, J., Emch, M., & Hoffman, I. (2020). Environmental modifiers of RTS,S/AS01 malaria vaccine efficacy in Lilongwe, Malawi. *BMC Public Health*, *20*(1), 910. https://doi.org/10.1186/s12889-020-09039-z

Björkman, A., Benn, C. S., Aaby, P., & Schapira, A. (2023). RTS,S/AS01 malaria vaccine—Proven safe and effective? *The Lancet Infectious Diseases*, *23*(8), e318–e322. https://doi.org/10.1016/S1473-3099(23)00126-3

Cairns, M., Barry, A., Zongo, I., Sagara, I., Yerbanga, S. R., Diarra, M., Zoungrana, C., Issiaka, D., Sienou, A. A., Tapily, A., Sanogo, K., Kaya, M., Traore, S., Diarra, K., Yalcouye, H., Sidibe, Y., Haro, A., Thera, I., Snell, P., … Ouedraogo, J. B. (2022a). The

duration of protection against clinical malaria provided by the combination of seasonal RTS,S/AS01E vaccination and seasonal malaria chemoprevention versus either intervention given alone. *BMC Medicine*, *20*(1), 352. https://doi.org/10.1186/s12916-022-02536-5

Cairns, M., Barry, A., Zongo, I., Sagara, I., Yerbanga, S. R., Diarra, M., Zoungrana, C., Issiaka, D., Sienou, A. A., Tapily, A., Sanogo, K., Kaya, M., Traore, S., Diarra, K., Yalcouye, H., Sidibe, Y., Haro, A., Thera, I., Snell, P., … Ouedraogo, J. B. (2022b). The duration of protection against clinical malaria provided by the combination of seasonal RTS,S/AS01E vaccination and seasonal malaria chemoprevention versus either intervention given alone. *BMC Medicine*, *20*(1), 352. https://doi.org/10.1186/s12916-022-02536-5

Casares, S., Brumeanu, T.-D., & Richie, T. L. (2010). The RTS,S malaria vaccine. *Vaccine*, *28*(31), 4880–4894. https://doi.org/10.1016/j.vaccine.2010.05.033

Centre for Disease Control and Pevention. (2020, April 9). *Malaria*. https://www.cdc.gov/malaria/about/distribution.html

Chandramohan, D., Zongo, I., Sagara, I., Cairns, M., Yerbanga, R.-S., Diarra, M., Nikièma, F., Tapily, A., Sompougdou, F., Issiaka, D., Zoungrana, C., Sanogo, K., Haro, A., Kaya, M., Sienou, A.-A., Traore, S., Mahamar, A., Thera, I., Diarra, K., … Greenwood, B. (2021). Seasonal Malaria Vaccination with or without Seasonal Malaria Chemoprevention. *New England Journal of Medicine*, 385(11), 1005–1017. https://doi.org/10.1056/NEJMoa2026330

Chaudhury, S., Regules, J. A., Darko, C. A., Dutta, S., Wallqvist, A., Waters, N. C., Jongert, E., Lemiale, F., & Bergmann-Leitner, E. S. (2017). Delayed fractional dose regimen of the RTS,S/AS01 malaria vaccine candidate enhances an IgG4 response that inhibits serum opsonophagocytosis. *Scientific Reports*, 7(1), 7998. https://doi.org/10.1038/s41598-017-08526-5

Cohen, J., Nussenzweig, V., Vekemans, J., & Leach, A. (2010). From the circumsporozoite protein to the RTS,S/AS candidate vaccine. *Human Vaccines*, 6(1), 90–96. https://doi.org/10.4161/hv.6.1.9677

Dicko, A., Ouedraogo, J.-B., Zongo, I., Sagara, I., Cairns, M., Yerbanga, R. S., Issiaka, D., Zoungrana, C., Sidibe, Y., Tapily, A., Nikièma, F., Sompougdou, F., Sanogo, K., Kaya,

M., Yalcouye, H., Dicko, O. M., Diarra, M., Diarra, K., Thera, I., … Greenwood, B. (2024). Seasonal vaccination with RTS,S/AS01E vaccine with or without seasonal malaria chemoprevention in children up to the age of 5 years in Burkina Faso and Mali: A double-blind, randomised, controlled, phase 3 trial. *The Lancet Infectious Diseases*, *24*(1), 75–86. https://doi.org/10.1016/S1473- 3099(23)00368-7

Dobaño, C., Santano, R., Vidal, M., Jiménez, A., Jairoce, C., Ubillos, I., Dosoo, D., Aguilar, R., Williams, N. A., Díez-Padrisa, N., Ayestaran, A., Valim, C., Asante, K. P., Owusu-Agyei, S., Lanar, D., Chauhan, V., Chitnis, C., Dutta, S., Angov, E., … Moncunill, G. (2019a). Differential Patterns of IgG Subclass Responses to *Plasmodium falciparum* Antigens in Relation to Malaria Protection and RTS,S Vaccination. *Frontiers in Immunology*, *10*, 439. https://doi.org/10.3389/fimmu.2019.00439

Dobaño, C., Sanz, H., Sorgho, H., Dosoo, D., Mpina, M., Ubillos, I., Aguilar, R., Ford, T., Díez- Padrisa, N., Williams, N. A., Ayestaran, A., Traore, O., Nhabomba, A. J., Jairoce, C., Waitumbi, J., Agnandji, S. T., Kariuki, S., Abdulla, S., Aponte, J. J., … Daubenberger, C. (2019b). Concentration and avidity of antibodies to different circumsporozoite epitopes correlate with RTS,S/AS01E malaria vaccine efficacy. *Nature Communications*, *10*(1), 2174. https://doi.org/10.1038/s41467-019-10195-z

Dobaño, C., Ubillos, I., Jairoce, C., Gyan, B., Vidal, M., Jiménez, A., Santano, R., Dosoo, D., Nhabomba, A. J., Ayestaran, A., Aguilar, R., Williams, N. A., Díez-Padrisa, N., Lanar, D., Chauhan, V., Chitnis, C., Dutta, S., Gaur, D., Angov, E., … Moncunill, G. (2019c). RTS,S/AS01E immunization increases antibody responses to vaccine-unrelated *Plasmodium falciparum* antigens associated with protection against clinical malaria in African children: A case-control study. *BMC Medicine*, *17*(1), 157. https://doi.org/10.1186/s12916-019-1378-6

Duffy, P. E., & Patrick Gorres, J. (2020). Malaria vaccines since 2000: Progress, priorities, products. *Npj Vaccines*, *5*(1), 48. https://doi.org/10.1038/s41541-020-0196-3

Edwards, K. M. (2015). Maternal antibodies and infant immune responses to vaccines. *Vaccine*, *33*(47), 6469–6472. https://doi.org/10.1016/j.vaccine.2015.07.085

Egbewande, O. M. (2022). The RTS,S malaria vaccine: Journey from conception to recommendation. *Public Health in Practice*, 4, 100283. https://doi.org/10.1016/j.puhip.2022.100283

El-Moamly, A. A., & El-Sweify, M. A. (2023). Malaria vaccines: The 60-year journey of hope and final success—lessons learned and future prospects. *Tropical Medicine and Health*, *51*(1), 29. https://doi.org/10.1186/s41182-023-00516-w

Feng, G., Kurtovic, L., Agius, P. A., Aitken, E. H., Sacarlal, J., Wines, B. D., Hogarth, P. M., Rogerson, S. J., Fowkes, F. J. I., Dobaño, C., & Beeson, J. G. (2022). Induction, decay, and determinants of functional antibodies following vaccination with the RTS,S malaria vaccine in young children. *BMC Medicine*, *20*(1), 289. https://doi.org/10.1186/s12916-022-02466-2

Ghattas, M., Dwivedi, G., Lavertu, M., & Alameh, M.-G. (2021). Vaccine Technologies and Platforms for Infectious Diseases: Current Progress, Challenges, and Opportunities. *Vaccines*, *9*(12), 1490. https://doi.org/10.3390/vaccines9121490

Grant, J., Gyan, T., Agbokey, F., Webster, J., Greenwood, B., & Asante, K. P. (2022). Challenges and lessons learned during the planning and early implementation of the RTS,S/AS01E malaria vaccine in three regions of Ghana: A qualitative study. *Malaria Journal*, *21*(1), 147. https://doi.org/10.1186/s12936-022-04168-9

Graves, P. M., & Gelband, H. (2006). Vaccines for preventing malaria (SPf66). *Cochrane Database of Systematic Reviews*. https://doi.org/10.1002/14651858.CD005966

Greenwood, B. M., Bojang, K., Whitty, C. J., & Targett, G. A. (2005). Malaria. *The Lancet*, *365*(9469), 1487–1498. https://doi.org/10.1016/S0140-6736(05)66420-3

Guerra Mendoza, Y., Garric, E., Leach, A., Lievens, M., Ofori-Anyinam, O., Pirçon, J.-Y., Stegmann, J.-U., Vandoolaeghe, P., Otieno, L., Otieno, W., Owusu-Agyei, S., Sacarlal, J., Masoud, N. S., Sorgho, H., Tanner, M., Tinto, H., Valea, I., Mtoro, A. T., Njuguna, P., … Schuerman, L. (2019). Safety profile of the RTS,S/AS01 malaria vaccine in infants and children: Additional data from a phase III randomized controlled trial in sub-Saharan Africa. Human Vaccines & Immunotherapeutics, 15(10), 2386–2398. https://doi.org/10.1080/21645515.2019.1586040

Guggisberg, A. M., Amthor, R. E., & Odom, A. R. (2014). Isoprenoid Biosynthesis in Plasmodium falciparum. Eukaryotic Cell, 13(11), 1348–1359. https://doi.org/10.1128/EC.00160-14

Hasson, S. S. A. A., Al-Busaidi, J. K. Z., & Sallam, T. A. (2015). The past, current and future trends in DNA vaccine immunisations. *Asian Pacific Journal of Tropical Biomedicine*,

5(5), 344–353. https://doi.org/10.1016/S2221-1691(15)30366-X

Horowitz, A., Hafalla, J. C. R., King, E., Lusingu, J., Dekker, D., Leach, A., Moris, P., Cohen, J., Vekemans, J., Villafana, T., Corran, P. H., Bejon, P., Drakeley, C. J., Von Seidlein, L., & Riley,

E. M. (2012). Antigen-Specific IL-2 Secretion Correlates with NK Cell Responses after Immunization of Tanzanian Children with the RTS,S/AS01 Malaria Vaccine. *The* Journal of Immunology, 188(10), 5054–5062. https://doi.org/10.4049/jimmunol.1102710

Iwasaki, A., & Omer, S. B. (2020). Why and How Vaccines Work. *Cell*, *183*(2), 290–295. https://doi.org/10.1016/j.cell.2020.09.040

Juraska, M., Early, A. M., Li, L., Schaffner, S. F., Lievens, M., Khorgade, A., Simpkins, B., Hejazi, N. S., Benkeser, D. A., Wang, Q., Mercer, L. D., Adjei, S., Agbenyega, T., Anderson, S., Ansong, D., Bii, D. K., Buabeng, P. B. Y., English, S., Fitzgerald, N., … Neafsey, D. E. (2023). *Baseline malaria infection status and RTS,S/AS01E malaria vaccine efficacy*. https://doi.org/10.1101/2023.11.22.23298907

Juraska, M., Early, A. M., Li, L., Schaffner, S. F., Lievens, M., Khorgade, A., Simpkins, B., Hejazi, N. S., Benkeser, D., Wang, Q., Mercer, L. D., Adjei, S., Agbenyega, T., Anderson, S., Ansong, D., Bii, D. K., Buabeng, P. B. Y., English, S., Fitzgerald, N., … Neafsey, D. E. (2024). Genotypic analysis of RTS,S/AS01E malaria vaccine efficacy against parasite infection as a function of dosage regimen and baseline malaria infection status in children aged 5–17 months in Ghana and Kenya: A longitudinal phase 2b randomised controlled trial. The Lancet Infectious Diseases, S1473309924001798. https://doi.org/10.1016/S1473-3099(24)00179-8

Kaslow, D. C., & Biernaux, S. (2015). RTS,S: Toward a first landmark on the Malaria Vaccine Technology Roadmap. Vaccine, 33(52), 7425 - 7432 https://doi.org/10.1016/j.vaccine.2015.09.061

Kayser, V., & Ramzan, I. (2024). Vaccines and vaccination: History and emerging issues. Human Vaccines & Immunotherapeutics 17(12), 5255-5268. https://doi.org/10.1080/21645515.2021.1977057

Keating, C. (2020). The history of the RTS,S/AS01 malaria vaccine trial. *The Lancet*, *395*(10233), 1336–1337. https://doi.org/10.1016/S0140-6736(20)30815-1

Kurtovic, L., Agius, P. A., Feng, G., Drew, D. R., Ubillos, I., Sacarlal, J., Aponte, J. J.,

Fowkes, F. J. I., Dobaño, C., & Beeson, J. G. (2019). Induction and decay of functional complement-fixing antibodies by the RTS,S malaria vaccine in children, and a negative impact of malaria exposure. *BMC Medicine*, *17*(1), 45. https://doi.org/10.1186/s12916-019-1277-x

Kurtovic, L., Atre, T., Feng, G., Wines, B. D., Chan, J.-A., Boyle, M. J., Drew, D. R., Hogarth, P. M., Fowkes, F. J. I., Bergmann-Leitner, E. S., & Beeson, J. G. (2021a). Multifunctional Antibodies Are Induced by the RTS,S Malaria Vaccine and Associated With Protection in a Phase 1/2a Trial. *The Journal of Infectious Diseases*, *224*(7), 1128–1138. https://doi.org/10.1093/infdis/jiaa144

Kurtovic, L., Reiling, L., Opi, D. H., & Beeson, J. G. (2021b). Recent clinical trials inform the future for malaria vaccines. *Communications Medicine*, *1*(1), 26. https://doi.org/10.1038/s43856-021- 00030-2

Laurens, M. B. (2018). The Promise of a Malaria Vaccine—Are We Closer? *Annual Review of Microbiology*, *72*(1), 273–292. https://doi.org/10.1146/annurev-micro-090817-062427

Laurens, M. B. (2020). RTS,S/AS01 vaccine (Mosquirix^{TM}): An overview. *Human Vaccines & Immunotherapeutics*, *16*(3), 480–489. https://doi.org/10.1080/21645515.2019.1669415

Laurens, M. B. (2021). Novel malaria vaccines. *Human Vaccines & Immunotherapeutics*, *17*(11), 4549–4552. https://doi.org/10.1080/21645515.2021.1947762

Leroux-Roels, G., Leroux-Roels, I., Clement, F., Ofori-Anyinam, O., Lievens, M., Jongert, E., Moris, P., Ballou, W. R., & Cohen, J. (2014). Evaluation of the immune response to RTS,S/AS01 and RTS,S/AS02 adjuvanted vaccines: Randomized, double-blind study in malaria-naïve adults. *Human Vaccines & Immunotherapeutics*, *10*(8), 2211–2219. https://doi.org/10.4161/hv.29375

Macià, D., Campo, J. J., Jairoce, C., Mpina, M., Sorgho, H., Dosoo, D., Agnandji, S. T., Kusi, K. A., Molinos-Albert, L. M., Kariuki, S., Daubenberger, C., Mordmüller, B., Moncunill, G., & Dobaño, C. (2025). The effect of *Plasmodium falciparum* exposure and maternal anti-circumsporozoite protein antibodies on responses to RTS,S/AS01E vaccination in infants and children: An ancillary observational immunological study to a phase 3, randomised clinical trial. *The Lancet Infectious Diseases*, *25*(3), 335–345. https://doi.org/10.1016/S1473-3099(24)00527- 9

Macià, D., Campo, J. J., Moncunill, G., Jairoce, C., Nhabomba, A. J., Mpina, M., Sorgho, H., Dosoo, D., Traore, O., Kusi, K. A., Williams, N. A., Oberai, A., Randall, A., Sanz, H., Valim, C., Asante, K. P., Owusu-Agyei, S., Tinto, H., Agnandji, S. T., … Dobaño, C. (2022). Strong off-target antibody reactivity to malarial antigens induced by RTS,S/AS01E vaccination is associated with protection. *JCI Insight*, *7*(10), e158030. https://doi.org/10.1172/jci.insight.158030

Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G., & for the PRISMA Group. (2009). Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *BMJ*, *339*(jul21 1), b2535–b2535. https://doi.org/10.1136/bmj.b2535

Moncunill, G., De Rosa, S. C., Ayestaran, A., Nhabomba, A. J., Mpina, M., Cohen, K. W., Jairoce, C., Rutishauser, T., Campo, J. J., Harezlak, J., Sanz, H., Díez-Padrisa, N., Williams, N. A., Morris, D., Aponte, J. J., Valim, C., Daubenberger, C., Dobaño, C., & McElrath, M. J. (2017a). RTS,S/AS01E Malaria Vaccine Induces Memory and Polyfunctional T Cell Responses in a Pediatric African Phase III Trial. *Frontiers in Immunology*, *8*, 1008. https://doi.org/10.3389/fimmu.2017.01008

Moncunill, G., Mpina, M., Nhabomba, A. J., Aguilar, R., Ayestaran, A., Sanz, H., Campo, J. J., Jairoce, C., Barrios, D., Dong, Y., Díez-Padrisa, N., Fernandes, J. F., Abdulla, S., Sacarlal, J., Williams, N. A., Harezlak, J., Mordmüller, B., Agnandji, S. T., Aponte, J. J., … Dobaño, C. (2017b). Distinct Helper T Cell Type 1 and 2 Responses Associated With Malaria Protection and Risk in RTS,S/AS01E Vaccinees. *Clinical Infectious Diseases*, *65*(5), 746–755. https://doi.org/10.1093/cid/cix429

Moon, J. E., Greenleaf, M. E., Regules, J. A., Debois, M., Duncan, E. H., Sedegah, M., Chuang, I., Lee, C. K., Sikaffy, A. K., Garver, L. S., Ivinson, K., Angov, E., Morelle, D., Lievens, M., Ockenhouse, C. F., Ngauy, V., & Ofori-Anyinam, O. (2021). A phase IIA extension study evaluating the effect of booster vaccination with a fractional dose of RTS,S/AS01E in a controlled human malaria infection challenge. *Vaccine*, *39*(43), 6398–6406. https://doi.org/10.1016/j.vaccine.2021.09.024

Moon, J. E., Ockenhouse, C., Regules, J. A., Vekemans, J., Lee, C., Chuang, I., Traskine, M., Jongert, E., Ivinson, K., Morelle, D., Komisar, J. L., Lievens, M., Sedegah, M., Garver, L. S., Sikaffy, A. K., Waters, N. C., Ballou, W. R., Ofori-Anyinam, O., RTS,S Malaria Vaccine Working Group, Murray, L. (2020). A Phase IIa Controlled Human Malaria Infection and Immunogenicity Study of RTS,S/AS01E and RTS,S/AS01B Delayed

Fractional Dose Regimens in Malaria-Naive Adults. *The Journal of Infectious Diseases*, *222*(10), 1681–1691. https://doi.org/10.1093/infdis/jiaa421

Mugo, R. M., Mwai, K., Mwacharo, J., Shee, F. M., Musyoki, J. N., Wambua, J., Otieno, E., Bejon, P., & Ndungu, F. M. (2021). Seven-year kinetics of RTS, S/AS01-induced anti-CSP antibodies in young Kenyan children. *Malaria Journal*, *20*(1), 452. https://doi.org/10.1186/s12936-021- 03961-2

Mumtaz, H., Nadeem, A., Bilal, W., Ansar, F., Saleem, S., Khan, Q. A., Tango, T., Farkouh, C., Belay, N. F., Verma, R., Farkouh, M., & Saqib, M. (2023). Acceptance, availability, and feasibility of RTS, S/AS01 malaria vaccine: A review. *Immunity, Inflammation and Disease*, *11*(6), e899. https://doi.org/10.1002/iid3.899

Nalova Akua. (2024, January 22). A turning point: Cameroon rolls out world's first malaria vaccine. *Gavi*. https://www.gavi.org/vaccineswork/turning-point-cameroon-rolls-out-worlds-first- malaria-vaccine

Ndiath, M. O. (2019). Insecticides and Insecticide Resistance. In F. Ariey, F. Gay, & R. Ménard (Eds.), *Malaria Control and Elimination* (Vol. 2013, pp. 287–304). Springer New York. https://doi.org/10.1007/978-1-4939-9550-9_18

Ndungu, F. M., Mwacharo, J., Kimani, D., Kai, O., Moris, P., Jongert, E., Vekemans, J., Olotu, A., & Bejon, P. (2012). A Statistical Interaction between Circumsporozoite Protein-Specific T Cell and Antibody Responses and Risk of Clinical Malaria Episodes following Vaccination with RTS,S/AS01E. *PLoS ONE*, *7*(12), e52870. https://doi.org/10.1371/journal.pone.0052870

Ndungu, F. M., Mwacharo, J., Wambua, J., Njuguna, P., Marsh, K., Drakeley, C., & Bejon, P. (2019). A seven-year study on the effect of the pre-erythrocytic malaria vaccine candidate RTS,S/AS01E on blood stage immunity in young Kenyan children. *Wellcome Open Research*, *4*, 42. https://doi.org/10.12688/wellcomeopenres.15002.1

Olotu, A., Clement, F., Jongert, E., Vekemans, J., Njuguna, P., Ndungu, F. M., Marsh, K., Leroux- Roels, G., & Bejon, P. (2014). Avidity of Anti-Circumsporozoite Antibodies following Vaccination with RTS,S/AS01E in Young Children. *PLoS ONE*, *9*(12), e115126. https://doi.org/10.1371/journal.pone.0115126

Olotu, A., Fegan, G., Wambua, J., Nyangweso, G., Awuondo, K. O., Leach, A., Lievens, M., Leboulleux, D., Njuguna, P., Peshu, N., Marsh, K., & Bejon, P. (2013). Four-Year Efficacy of RTS,S/AS01E and Its Interaction with Malaria Exposure. *New England*

Journal of Medicine, *368*(12), 1111–1120. https://doi.org/10.1056/NEJMoa1207564

Olotu, A., Fegan, G., Wambua, J., Nyangweso, G., Leach, A., Lievens, M., Kaslow, D. C., Njuguna, P., Marsh, K., & Bejon, P. (2016). Seven-Year Efficacy of RTS,S/AS01 Malaria Vaccine among Young African Children. *New England Journal of Medicine*, *374*(26), 2519–2529. https://doi.org/10.1056/NEJMoa1515257

Ouattara, A., & Laurens, M. B. (2015). Vaccines Against Malaria. *Clinical Infectious Diseases*, *60*(6), 930–936. https://doi.org/10.1093/cid/ciu954

Pallikkuth, S., Chaudhury, S., Lu, P., Pan, L., Jongert, E., Wille-Reece, U., & Pahwa, S. (2020). A delayed fractionated dose RTS,S AS01 vaccine regimen mediates protection via improved T follicular helper and B cell responses. *eLife*, *9*, e51889. https://doi.org/10.7554/eLife.51889

Park, S., Nixon, C. E., Miller, O., Choi, N.-K., Kurtis, J. D., Friedman, J. F., & Michelow, I. C. (2020). Impact of Malaria in Pregnancy on Risk of Malaria in Young Children: Systematic Review and Meta-Analyses. *The Journal of Infectious Diseases*, *222*(4), 538–550. https://doi.org/10.1093/infdis/jiaa139

Praet, N., Asante, K. P., Bozonnat, M.-C., Akité, E. J., Ansah, P. O., Baril, L., Boahen, O., Mendoza, Y. G., Haine, V., Kariuki, S., Lamy, M., Maleta, K., Mungwira, R., Ndeketa, L., Oduro, A., Ogutu, B., Olewe, F., Oneko, M., Orsini, M., … Pirçon, J.-Y. (2022). Assessing the safety, impact and effectiveness of RTS,S/AS01E malaria vaccine following its introduction in three sub-Saharan African countries: Methodological approaches and study set-up. *Malaria Journal*, *21*(1), 132. https://doi.org/10.1186/s12936-022-04144-3

Regules, J. A., Cicatelli, S. B., Bennett, J. W., Paolino, K. M., Twomey, P. S., Moon, J. E., Kathcart, A. K., Hauns, K. D., Komisar, J. L., Qabar, A. N., Davidson, S. A., Dutta, S., Griffith, M. E., Magee, C. D., Wojnarski, M., Livezey, J. R., Kress, A. T., Waterman, P. E., Jongert, E., … Vekemans, J. (2016). Fractional Third and Fourth Dose of RTS,S/AS01 Malaria Candidate Vaccine: A Phase 2a Controlled Human Malaria Parasite Infection and Immunogenicity Study. *Journal of Infectious Diseases*, *214*(5), 762–771. https://doi.org/10.1093/infdis/jiw237

RTS,S Clinical Trials Partnership. (2015). Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: Final results of a phase 3, individually randomised, controlled trial. *The Lancet*, *386*(9988), 31–45. https://doi.org/10.1016/S0140- 6736(15)60721-8

Sagara, I., Zongo, I., Cairns, M., Yerbanga, R. S., Mahamar, A., Nikièma, F., Tapily, A., Sompougdou, F., Diarra, M., Zoungrana, C., Issiaka, D., Haro, A., Sanogo, K., Aziz Sienou, A., Kaya, M., Traore, S., Thera, I., Diarra, K., Dolo, A., … Greenwood, B. (2022). The Anti-Circumsporozoite Antibody Response of Children to Seasonal Vaccination With the RTS,S/AS01E Malaria Vaccine. *Clinical Infectious Diseases*, *75*(4), 613–622. https://doi.org/10.1093/cid/ciab1017

Sallam, M., Al-Khatib, A. O., Al-Mahzoum, K., Abdelaziz, D. H., & Sallam, M. (2024). *Current Developments in Malaria Vaccination: A Concise Review on Implementation, Challenges, and Future Directions*. https://doi.org/10.20944/preprints202412.1635.v1

Samuels, A. M., Ansong, D., Kariuki, S. K., Adjei, S., Bollaerts, A., Ockenhouse, C., Westercamp, N., Lee, C. K., Schuerman, L., Bii, D. K., Osei-Tutu, L., Oneko, M., Lievens, M., Attobrah Sarfo, M. A., Atieno, C., Morelle, D., Bakari, A., Sang, T., Jongert, E., … Ivinson, K. (2022). Efficacy of RTS,S/AS01E malaria vaccine administered according to different full, fractional, and delayed third or early fourth dose regimens in children aged 5–17 months in Ghana and Kenya: An open- label, phase 2b, randomised controlled trial. *The Lancet Infectious Diseases*, *22*(9), 1329–1342. https://doi.org/10.1016/S1473-3099(22)00273-0

Sánchez, L., Vidal, M., Jairoce, C., Aguilar, R., Ubillos, I., Cuamba, I., Nhabomba, A. J., Williams, N. A., Díez-Padrisa, N., Cavanagh, D., Angov, E., Coppel, R. L., Gaur, D., Beeson, J. G., Dutta, S., Aide, P., Campo, J. J., Moncunill, G., & Dobaño, C. (2020). Antibody responses to the RTS,S/AS01E vaccine and *Plasmodium falciparum* antigens after a booster dose within the phase 3 trial in Mozambique. *Npj Vaccines*, *5*(1), 46. https://doi.org/10.1038/s41541-020-0192-7

Sinnis, P., & Zavala, F. (2012). The skin: Where malaria infection and the host immune response begin. *Seminars in Immunopathology*, *34*(6), 787–792. https://doi.org/10.1007/s00281-012- 0345-5

Stoute, J. A., Slaoui, M., Heppner, D. G., Momin, P., Kester, K. E., Desmons, P., Wellde, B. T., Garçon, N., Krzych, U., Marchand, M., Ballou, W. R., & Cohen, J. D. (1997). A Preliminary Evaluation of a Recombinant Circumsporozoite Protein Vaccine against *Plasmodium falciparum Malaria*. *New England Journal of Medicine*, *336*(2), 86–91. https://doi.org/10.1056/NEJM199701093360202

Suau, R., Vidal, M., Aguilar, R., Ruiz-Olalla, G., Vázquez-Santiago, M., Jairoce, C.,

Nhabomba, A. J., Gyan, B., Dosoo, D., Asante, K. P., Owusu-Agyei, S., Campo, J. J., Izquierdo, L., Cavanagh, D., Coppel, R. L., Chauhan, V., Angov, E., Dutta, S., Gaur, D., … Dobaño, C. (2021). RTS,S/AS01E malaria vaccine induces IgA responses against CSP and vaccine-unrelated antigens in African children in the phase 3 trial. *Vaccine*, *39*(4), 687–698. https://doi.org/10.1016/j.vaccine.2020.12.038

Suscovich, T. J., Fallon, J. K., Das, J., Demas, A. R., Crain, J., Linde, C. H., Michell, A., Natarajan, H., Arevalo, C., Broge, T., Linnekin, T., Kulkarni, V., Lu, R., Slein, M. D., Luedemann, C., Marquette, M., March, S., Weiner, J., Gregory, S., … Alter, G. (2020). Mapping functional humoral correlates of protection against malaria challenge following RTS,S/AS01 vaccination.

Science Translational Medicine 12(553), eabb4757. https://doi.org/10.1126/scitranslmed.abb4757

Syed, Y. Y. (2022). RTS,S/AS01 malaria vaccine (Mosquirix®): A profile of its use. *Drugs & Therapy Perspectives*, *38*(9), 373–381. https://doi.org/10.1007/s40267-022-00937-3

Talapko, Škrlec, Alebić, Jukić, & Včev. (2019). Malaria: The Past and the Present. *Microorganisms*, *7*(6), 179. https://doi.org/10.3390/microorganisms7060179

The Lancet. (2021). Malaria vaccine approval: A step change for global health. *The Lancet*, *398*(10309), 1381. https://doi.org/10.1016/S0140-6736(21)02235-2

The RTS,S Clinical Trials Partnership. (2011). First Results of Phase 3 Trial of RTS,S/AS01 Malaria Vaccine in African Children. *New England Journal of Medicine*, *365*(20), 1863–1875. https://doi.org/10.1056/NEJMoa1102287

The RTS,S Clinical Trials Partnership. (2012). A Phase 3 Trial of RTS,S/AS01 Malaria Vaccine in African Infants. *New England Journal of Medicine*, *367*(24), 2284–2295. https://doi.org/10.1056/NEJMoa1208394

The RTS,S Clinical Trials Partnership (2014). (2014). Efficacy and Safety of the RTS,S/AS01 Malaria Vaccine during 18 Months after Vaccination: A Phase 3 Randomized, Controlled Trial in Children and Young Infants at 11 African Sites. *PLoS Medicine*, *11*(7), e1001685. https://doi.org/10.1371/journal.pmed.1001685

Tinto Halidou., Otieno, W., Gesase, S., Sorgho, H., Otieno, L., Liheluka, E., Valéa, I., Sing'oei, V., Malabeja, A., Valia, D., Wangwe, A., Gvozdenovic, E., Guerra Mendoza, Y., Jongert, E., Lievens, M., Roman, F., Schuerman, L., & Lusingu, J. (2019). Long-term

incidence of severe malaria following RTS,S/AS01 vaccination in children and infants in Africa: An open-label 3-year extension study of a phase 3 randomised controlled trial. *The Lancet Infectious Diseases*, *19*(8), 821–832. https://doi.org/10.1016/S1473-3099(19)30300-7

Tsoumani, M. E., Voyiatzaki, C., & Efstathiou, A. (2023). Malaria Vaccines: From the Past towards the mRNA Vaccine Era. *Vaccines*, *11*(9), 1452. https://doi.org/10.3390/vaccines11091452

Ubillos, I., Ayestaran, A., Nhabomba, A. J., Dosoo, D., Vidal, M., Jiménez, A., Jairoce, C., Sanz, H., Aguilar, R., Williams, N. A., Díez-Padrisa, N., Mpina, M., Sorgho, H., Agnandji, S. T., Kariuki, S., Mordmüller, B., Daubenberger, C., Asante, K. P., Owusu-Agyei, S., … Dobaño, C. (2018). Baseline exposure, antibody subclass, and hepatitis B response differentially affect malaria protective immunity following RTS,S/AS01E vaccination in African children. *BMC Medicine*, *16*(1), 197. https://doi.org/10.1186/s12916-018-1186-4

Umeh, R., Oguche, S., Oguonu, T., Pitmang, S., Shu, E., Onyia, J.-T., Daniyam, C. A., Shwe, D., Ahmad, A., Jongert, E., Catteau, G., Lievens, M., Ofori-Anyinam, O., & Leach, A. (2014). Immunogenicity and safety of the candidate RTS,S/AS01 vaccine in young Nigerian children: A randomized, double-blind, lot-to-lot consistency trial. *Vaccine*, *32*(48), 6556–6562. https://doi.org/10.1016/j.vaccine.2014.07.067

Umumararungu, T., Nkuranga, J. B., Habarurema, G., Nyandwi, J. B., Mukazayire, M. J., Mukiza, J., Muganga, R., Hahirwa, I., Mpenda, M., Katembezi, A. N., Olawode, E. O., Kayitare, E., & Kayumba, P. C. (2023). Recent developments in antimalarial drug discovery. *Bioorganic & Medicinal Chemistry*, *88–89*, 117339. https://doi.org/10.1016/j.bmc.2023.117339

Valéa, I., Adjei, S., Usuf, E., Traore, O., Ansong, D., Tinto, H., Owusu Boateng, H., Some, A. M., Buabeng, P., Vekemans, J., Kotey, A., Vandoolaeghe, P., Cullinane, M., Traskine, M., Ouedraogo, F., Sambian, D., Lievens, M., Tahita, M. C., Jongert, E., … Agbenyega, T. (2020). Long-term immunogenicity and immune memory response to the hepatitis B antigen in the RTS,S/AS01 E malaria vaccine in African children: A randomized trial. *Human Vaccines & Immunotherapeutics*, *16*(6), 1464–1470. https://doi.org/10.1080/21645515.2019.1695457

Varo, R., Chaccour, C., & Bassat, Q. (2020). Update on malaria. *Medicina Clínica*, *155*(9),

395–402. https://doi.org/10.1016/j.medcli.2020.05.010

Venkatesan, P. (2024). The 2023 WHO World malaria report. *The Lancet Microbe*, S2666524724000168. https://doi.org/10.1016/S2666-5247(24)00016-8

Von Seidlein, L., Hanboonkunupakarn, B., Jittamala, P., Pongsuwan, P., Chotivanich, K., Tarning, J., Hoglund, R. M., Winterberg, M., Mukaka, M., Peerawaranun, P., Sirithiranont, P., Doran, Z., Ockenhouse, C. F., Ivinson, K., Lee, C., Birkett, A. J., Kaslow, D. C., Singhasivanon, P., Day, N. P. J., … Pukrittayakamee, S. (2020). Combining antimalarial drugs and vaccine for malaria elimination campaigns: A randomized safety and immunogenicity trial of RTS,S/AS01 administered with dihydroartemisinin, piperaquine, and primaquine in healthy Thai adult volunteers. *Human Vaccines & Immunotherapeutics*, *16*(1), 33–41. https://doi.org/10.1080/21645515.2019.1643675

Westercamp, N., Osei-Tutu, L., Schuerman, L., Kariuki, S. K., Bollaerts, A., Lee, C. K., Samuels, A. M., Ockenhouse, C., Bii, D. K., Adjei, S., Oneko, M., Lievens, M., Attobrah Sarfo, M. A., Atieno, C., Bakari, A., Sang, T., Kotoh-Mortty, M. F., Otieno, K., Roman, F., … Ofori-Anyinam, O. (2024). Could Less Be More? Accounting for Fractional-Dose Regimens and Different Number of Vaccine Doses When Measuring the Impact of the RTS,S/AS01E Malaria Vaccine. *The Journal of Infectious Diseases*, jiae075. https://doi.org/10.1093/infdis/jiae075

White, M. T., Bejon, P., Olotu, A., Griffin, J. T., Bojang, K., Lusingu, J., Salim, N., Abdulla, S., Otsyula, N., Agnandji, S. T., Lell, B., Asante, K. P., Owusu-Agyei, S., Mahama, E., Agbenyega, T., Ansong, D., Sacarlal, J., Aponte, J. J., & Ghani, A. C. (2014). A combined analysis of immunogenicity, antibody kinetics and vaccine efficacy from phase 2 trials of the RTS,S malaria vaccine. *BMC Medicine*, *12*(1), 117. https://doi.org/10.1186/s12916-014-0117-2

White, M. T., Verity, R., Griffin, J. T., Asante, K. P., Owusu-Agyei, S., Greenwood, B., Drakeley, C., Gesase, S., Lusingu, J., Ansong, D., Adjei, S., Agbenyega, T., Ogutu, B., Otieno, L., Otieno, W., Agnandji, S. T., Lell, B., Kremsner, P., Hoffman, I., … Ghani, A. C. (2015). Immunogenicity of the RTS,S/AS01 malaria vaccine and implications for duration of vaccine efficacy: Secondary analysis of data from a phase 3 randomised controlled trial. *The Lancet Infectious Diseases*, *15*(12), 1450–1458. https://doi.org/10.1016/S1473-3099(15)00239-X

WHO. (2023). *World Malaria Report 2023*. World Health Organizatio, Geneva.

https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2023

WHO. (2024a). Malaria vaccine: WHO position paper-May 2024. https://www.who.int/publications/i/item/who-wer-9919-225-248.

WHO. (2024b). *The Global Health Observatory, Explore a world of health data*. https://www.who.int/data/gho/data/themes/malaria

WHO. (2024c). *World Malaria Report 2024. Addressing inequity in the global malaria response*. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2024

WHO. (2024d, January 17). *Malaria vaccines (RTS,S and R21)*. https://www.who.int/news- room/questions-and-answers/item/q-a-on-rts-s-malaria-vaccine#:~:text=The%20RTS%2CS%20vaccine%20was%20prequalified%20by%20WHO%20in,vaccine%20was%20prequalified%20by%20WHO%20in%20December%20203

Witte, D., Cunliffe, N. A., Turner, A. M., Ngulube, E., Ofori-Anyinam, O., Vekemans, J., Chimpeni, P., Lievens, M., Wilson, T. P., Njiram'madzi, J., Mendoza, Y. G., & Leach, A. (2018). Safety and Immunogenicity of Seven Dosing Regimens of the Candidate RTS,S/AS01E Malaria Vaccine Integrated Within an Expanded Program on Immunization Regimen: A Phase II, Single-Center, Open, Controlled Trial in Infants in Malawi. *Pediatric Infectious Disease Journal*, *37*(5), 483–491. https://doi.org/10.1097/INF.0000000000001937

Yihunie, W., Kebede, B., Tegegne, B. A., Getachew, M., Abebe, D., Aschale, Y., Belew, H., & Bahiru, B. (2023). Systematic Review of Safety of RTS,S with AS01 and AS02 Adjuvant Systems Using Data from Randomized Controlled Trials in Infants, Children, and Adults. *Clinical Pharmacology: Advances and Applications*, *Volume 15*, 21–32. https://doi.org/10.2147/CPAA.S400155

Zavala, F. (2022). RTS,S: The first malaria vaccine. *Journal of Clinical Investigation*, *132*(1), e156588. https://doi.org/10.1172/JCI156588

Yin, J., Zhang, L., Wang, C., Qin, C., & Miao, M. (2024). Immunogenicity and safety of ebolavirus vaccines in healthy adults: A systematic review and meta-analysis of randomized controlled trials. *Expert Review of Vaccines*, 23(1), 148–159. https://doi.org/10.1080/14760584.2023.2296937

APPENDICES

APPENDIX A: Detailed breakdown of the search strategy, number of records retrieved (as of 30 June 2024).

Search	Search terms	Number of Articles Retrieved
#1	RTS, S/AS01 [Mesh]	**PubMed=1788** **Cochrane = 101** **Clinical trials= 27** **Scopus =338**
#2	((RTS, S/AS01 vaccine OR RTS, S vaccination trials OR RTS, S vaccination program OR RTS,S immunization OR RTS,S immunization program OR Malaria vaccination program OR Malaria immunization) OR ("RTS, S/AS01 vaccine" OR "RTS, S vaccination trials" OR "RTS, S vaccination program*" OR "RTS,S immunization" OR "RTS,S immunization program" OR "Malaria vaccination program*" OR "Malaria immunization")) OR ("RTS, S/AS01 vaccine" OR "RTS, S vaccination trials" OR "RTS, S vaccination program*" OR "RTS,S immuni?ation" OR "RTS,S immuni?ation program" OR "Malaria vaccination program*" OR "Malaria immuni?ation")	**PubMed = 9231** **Cochrane = 534** **Scopus = 204**
#3	Safety OR efficacy OR risks and benefits OR advantages and disadvantages,,,"""safety""[MeSH Terms] OR ""safety""[All Fields] OR ""safeties""[All Fields] OR (""efficacies""[All Fields] OR ""efficacious""[All Fields] OR ""efficaciously""[All Fields] OR ""efficaciousness""[All Fields] OR ""efficacy""[All Fields]) OR (""risk assessment""[MeSH Terms] OR (""risk""[All Fields] AND ""assessment""[All Fields]) OR ""risk assessment""[All Fields] OR (""risks""[All Fields] AND ""benefits""[All Fields]) OR ""risks and benefits""[All Fields]) OR ((""advantage""[All Fields] OR ""advantageous""[All Fields] OR ""advantageously""[All Fields] OR ""advantages""[All Fields]) AND (""disadvantage""[All Fields] OR ""disadvantageous""[All Fields] OR ""disadvantageously""[All Fields] OR ""disadvantages""[All Fields] OR ""disadvantaging""[All Fields] OR ""vulnerable populations""[MeSH Terms] OR (""vulnerable""[All Fields] AND ""populations""[All Fields]) OR ""vulnerable populations""[All Fields] OR ""disadvantaged""[All Fields]))	**PubMed = 30** **Cochrane = 57** **Scopus = 26**
	((("RTS,S-AS01 vaccine" [Supplementary Concept] OR RTS,S-AS01 vaccine) AND (Immunogenicity OR immune response OR antibodies involved)) AND (Safety OR efficacy OR risks and benefits OR advantages and disadvantages)) AND (((RTS, S/AS01 vaccine OR RTS, S vaccination trials OR RTS, S	**PubMed = 41** **Cochrane = 44** **Scopus = 6**

#4	vaccination program OR RTS,S immunization OR RTS,S immunization program OR Malaria vaccination program OR Malaria immunization) OR ("RTS, S/AS01 vaccine" OR "RTS, S vaccination trials" OR "RTS, S vaccination program*" OR "RTS,S immunization" OR "RTS,S immunization program" OR "Malaria vaccination program*" OR "Malaria immunization")) OR ("RTS, S/AS01 vaccine" OR "RTS, S vaccination trials" OR "RTS, S vaccination program*" OR "RTS,S immuni?ation" OR "RTS,S immuni?ation program" OR "Malaria vaccination program*" OR "Malaria immuni?ation"))	

APPENDIX B: Excel sheet for Efficacy data extraction

Author	Vaccine Regimen/Strategy	Efficacy Against	Study Design/Phase	Country	Age group	Vaccine Cases	Vaccine Participants	Control Cases	Control Participants
Guerra Mendoza *et al.,* 2019	RTS,S/AS01+Booster	Malaria Cases	RCT, Phase III	Burkina Faso, Ghana, Gabon, Kenya, Tanzania, Malawi, Mozambique	Children (5–17 months)	86	2,976	158	2,974
Guerra Mendoza *et al.,* 2019	RTS,S/AS01	Malaria Cases	RCT, Phase III	Burkina Faso, Ghana, Gabon, Kenya, Tanzania, Malawi, Mozambique	Children (5–17 months)	119	2,972	158	2,974
Guerra Mendoza *et al.,* 2019	RTS,S/AS01+Booster	Malaria Cases	RCT, Phase III	Burkina Faso, Ghana, Gabon, Kenya, Tanzania, Malawi, Mozambique	Infants (6–12 weeks)	82	2,180	86	2,179
Guerra Mendoza *et al.,* 2019	RTS,S/AS01	Malaria Cases	RCT, Phase III	Burkina Faso, Ghana, Gabon, Kenya, Tanzania, Malawi, Mozambique	Infants (6–12 weeks)	66	2,178	86	2,179
Chandramohan *et al.,*2021	RTS,S/AS01	Malaria Cases	RCT, Phase III	Burkina Faso and Mali	Children (5–17 months)	1540	5336	1661	5,450
Chandramohan *et al.,*2021	RTS,S/AS01+Chemopr.	Malaria Cases	RCT, Phase III	Burkina Faso and Mali	Children (5–17 months)	624	5,508	1661	5,450
Dicko *et al.,* 2024	RTS,S/AS01	Malaria Cases	RCT, Phase III	Burkina Faso and Mali	Children (5–17 months)	2537	7938	2472	7887
Dicko *et al.,* 2024	RTS,S/AS01+Chemopr.	Malaria Cases	RCT, Phase III	Burkina Faso and Mali	Children (5–17 months)	1055	7958	2472	7887
RTS,S Clinical Trials Partnership, 2015	RTS,S/AS01+Booster	Malaria Cases	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya Malawi, Mozambique, Tanzania	Children (5–17 months)	6616	2867	9585	2905
RTS,S Clinical Trials Partnership, 2015	RTS,S/AS01	Malaria Cases	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya Malawi, Mozambique, Tanzania	Children (5–17 months)	7396	2887	9585	2905
RTS,S Clinical Trials Partnership, 2015	RTS,S/AS01+Booster	Malaria Cases	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya Malawi, Mozambique, Tanzania	Infants (6–12 weeks)	4993	2,115	6170	2134
RTS,S Clinical Trials Partnership, 2015	RTS,S/AS01	Malaria Cases	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya Malawi, Mozambique, Tanzania	Infants (6–12 weeks)	5444	2,119	6170	2134
Olotu *et al.,* 2013	RTS,S/AS01	Malaria Cases	RCT, Phase IIb	Kenya	Children (5–17 months)	118	223	138	224

RTS,S Clinical Trials Partnership, 2012	RTS,S/AS01+Booster	Malaria Cases	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Infants (6–12 weeks)	58	3995	46	2008
RTS,S Clinical Trials Partnership, 2012	RTS,S/AS01	Malaria Cases	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Infants (6–12 weeks)	77	4358	52	2179
RTS,S Clinical Trials Partnership, 2014	RTS,S/AS01+Booster	Malaria Cases	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Children (5–17 months)	4257	4,557	3639	2,328
RTS,S Clinical Trials Partnership, 2014	RTS,S/AS01+Booster	Malaria Cases	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Infants (6–12 weeks)	3848	3,996	2464	2,007
RTS,S Clinical Trials Partnership, 2014	RTS,S/AS01	Malaria Cases	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Children (5–17 months)	5106	5,949	4305	2,974
RTS,S Clinical Trials Partnership, 2014	RTS,S/AS01	Malaria Cases	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Infants (6–12 weeks)	4252	4,358	2751	2,179

APPENDIX C: Excel sheet for safety data extraction

Adverse Event	Vaccine Regimen/Strategy	Author	Study design/Phase	Country	Age Group	Vaccine Cases	Vaccine Participants	Control Cases	Control Participants
Meningitis	RTS,S/AS01+Booster	RTS,S Clinical Trials Partnership, 2015	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Children(5-17months)	11	2865	1	2905
Meningitis	RTS,S/AS01	RTS,S Clinical Trials Partnership, 2015	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Children(5-17months)	10	2885	1	2905
Meningitis	RTS,S/AS01+Booster	RTS,S Clinical Trials Partnership, 2015	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Infants (6–12 weeks)	5	2115	6	2134
Meningitis	RTS,S/AS01	RTS,S Clinical Trials Partnership, 2015	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Infants (6–12 weeks)	7	2119	6	2134
Meningitis	RTS,S/AS01+Booster	Guerra Mendoza *et al.,* 2019	RCT, Phase III	Burkina Faso, Ghana, Gabon, Kenya, Tanzania, Malawi, Mozambique	Children(5-17months)	11	2,976	1	2974
Meningitis	RTS,S/AS01	Guerra Mendoza *et al.,* 2019	RCT, Phase III	Burkina Faso, Ghana, Gabon, Kenya, Tanzania, Malawi, Mozambique	Children(5-17months)	7	2972	1	2974
Meningitis	RTS,S/AS01+Booster	Guerra Mendoza *et al.,* 2019	RCT, Phase III	Burkina Faso, Ghana, Gabon, Kenya, Tanzania, Malawi, Mozambique	Infants (6–12 weeks)	5	2180	6	2179
Meningitis	RTS,S/AS01	Guerra Mendoza *et al.,* 2019	RCT, Phase III	Burkina Faso, Ghana, Gabon, Kenya, Tanzania, Malawi, Mozambique	Infants (6–12 weeks)	8	2178	6	2179
Meningitis	RTS,S/AS01+Booster	Tinto Halidou et al., 2019	RCT, Phase III	Burkina Faso, Kenya and Tanzania	Children(5-17months)	0	844	1	839
Meningitis	RTS,S/AS01	Tinto Halidou et al., 2019	RCT, Phase III	Burkina Faso, Kenya and Tanzania	Children(5-17months)	0	829	1	839
Meningitis	RTS,S/AS01+Booster	Tinto Halidou et al., 2019	RCT, Phase III	Burkina Faso, Kenya and Tanzania	Infants (6–12 weeks)	2	637	2	633

Meningitis	RTS,S/AS01	Tinto Halidou et al., 2019	RCT, Phase III	Burkina Faso, Kenya and Tanzania	Infants (6–12 weeks)	0	635	2	633
Meningitis	RTS,S/AS01+Booster	RTS,S Clinical Trials Partnership, 2012	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Children(5-17months)	1	844	1	839
Meningitis	RTS,S/AS01	RTS,S Clinical Trials Partnership, 2012	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Children(5-17months)	0	829	1	839
Meningitis	RTS,S/AS01+Booster	RTS,S Clinical Trials Partnership, 2012	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Infants (6–12 weeks)	2	895	2	882
Meningitis	RTS,S/AS01	RTS,S Clinical Trials Partnership, 2012	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Infants (6–12 weeks)	0	875	2	882
Meningitis	RTS,S/AS01+Booster	RTS,S Clinical Trials Partnership, 2014	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Children(5-17months)	16	5,949	1	2,974
Meningitis	RTS,S/AS01	RTS,S Clinical Trials Partnership, 2014	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Children(5-17months)	1	2,974	1	2,974
Meningitis	RTS,S/AS01+Booster	RTS,S Clinical Trials Partnership, 2014	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Infants (6–12 weeks)	9	4,358	3	2,179
Meningitis	RTS,S/AS01	Westercamp *et al.,* 2024	RCT, Phase IIb	Ghana and Kenya	Children(5-17months)	5	1207	2	293
Convulsion	RTS,S/AS01	Westercamp *et al.,* 2024	RCT, Phase IIb	Ghana and Kenya	Children(5-17months)	10	1207	4	293
Death	RTS,S/AS01	Westercamp *et al.,* 2024	RCT, Phase IIb	Ghana and Kenya	Children(5-17months)	7	1207	1	293
Convulsion	RTS,S/AS01	Chandramohan *et al.,*2021	RCT, Phase III	Burkina Faso and Mali	Children(5-17months)	3	1988	0	1965
Convulsion	RTS,S/AS01+Chemopr.	Chandramohan *et al.,*2021	RCT, Phase III	Burkina Faso and Mali	Children(5-17months)	2	1967	0	1965
Meningitis	RTS,S/AS01	Chandramohan *et al.,*2021	RCT, Phase III	Burkina Faso and Mali	Children(5-17months)	3	1988	4	1965

Meningitis	RTS,S/AS01+Chemopr.	Chandramohan *et al.,*2021	RCT, Phase III	Burkina Faso and Mali	Children(5-17months)	1	1988	4	1965
Death	RTS,S/AS01	Chandramohan *et al.,*2021	RCT, Phase III	Burkina Faso and Mali	Children(5-17months)	27	1988	32	1965
Death	RTS,S/AS01+Chemopr.	Chandramohan *et al.,*2021	RCT, Phase III	Burkina Faso and Mali	Children(5-17months)	15	1988	32	1965
Death	RTS,S/AS01	Dicko *et al.,* 2024	RCT, Phase III	Burkina Faso and Mali	Children(5-17months)	37	1817	27	1793
Death	RTS,S/AS01+Chemopr.	Dicko *et al.,* 2024	RCT, Phase III	Burkina Faso and Mali	Children(5-17months)	19	1823	27	1793
Convulsion	RTS,S/AS01+Booster	Guerra Mendoza *et al.,* 2019	RCT, Phase III	Burkina Faso, Ghana, Gabon, Kenya, Tanzania, Malawi, Mozambique	Children(5-17months)	6	2,447	1	2473
Convulsion	RTS,S/AS01	Guerra Mendoza *et al.,* 2019	RCT, Phase III	Burkina Faso, Ghana, Gabon, Kenya, Tanzania, Malawi, Mozambique	Children(5-17months)	3	2472	1	2974
Convulsion	RTS,S/AS01+Booster	Guerra Mendoza *et al.,* 2019	RCT, Phase III	Burkina Faso, Ghana, Gabon, Kenya, Tanzania, Malawi, Mozambique	Infants (6–12 weeks)	4	1825	1	1827
Convulsion	RTS,S/AS01	Guerra Mendoza *et al.,* 2019	RCT, Phase III	Burkina Faso, Ghana, Gabon, Kenya, Tanzania, Malawi, Mozambique	Infants (6–12 weeks)	0	1837	1	1827
Convulsion	RTS,S/AS01+Booster	Tinto Halidou et al., 2019	RCT, Phase III	Burkina Faso, Kenya and Tanzania	Children(5-17months)	6	844	8	839
Convulsion	RTS,S/AS01	Tinto Halidou et al., 2019	RCT, Phase III	Burkina Faso, Kenya and Tanzania	Children(5-17months)	9	829	8	839
Convulsion	RTS,S/AS01+Booster	Tinto Halidou et al., 2019	RCT, Phase III	Burkina Faso, Kenya and Tanzania	Infants (6–12 weeks)	7	637	6	633
Convulsion	RTS,S/AS01	Tinto Halidou et al., 2019	RCT, Phase III	Burkina Faso, Kenya and Tanzania	Infants (6–12 weeks)	10	635	6	633
Convulsion	RTS,S/AS01+Booster	RTS,S Clinical Trials Partnership, 2014	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Infants (6–12 weeks)	82	4,358	46	2,179
Convulsion	RTS,S/AS01	RTS,S Clinical Trials Partnership, 2014	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Infants (6–12 weeks)	0	4,358	46	2,179

Death	RTS,S/AS01+Booster	Guerra Mendoza *et al.,* 2019	RCT, Phase III	Burkina Faso, Ghana, Gabon, Kenya, Tanzania, Malawi, Mozambique	Children(5-17months)	61	2,976	46	2974
Death	RTS,S/AS01	Guerra Mendoza *et al.,* 2019	RCT, Phase III	Burkina Faso, Ghana, Gabon, Kenya, Tanzania, Malawi, Mozambique	Children(5-17months)	51	2972	46	2974
Death	RTS,S/AS01+Booster	Guerra Mendoza *et al.,* 2019	RCT, Phase III	Burkina Faso, Ghana, Gabon, Kenya, Tanzania, Malawi, Mozambique	Infants (6–12 weeks)	51	2180	42	2179
Death	RTS,S/AS01	Guerra Mendoza *et al.,* 2019	RCT, Phase III	Burkina Faso, Ghana, Gabon, Kenya, Tanzania, Malawi, Mozambique	Infants (6–12 weeks)	55	2178	42	2179
Death	RTS,S/AS01+Booster	Tinto Halidou *et al.,* 2019	RCT, Phase III	Burkina Faso, Kenya and Tanzania	Children(5-17months)	2	844	5	839
Death	RTS,S/AS01	Tinto Halidou *et al.,* 2019	RCT, Phase III	Burkina Faso, Kenya and Tanzania	Children(5-17months)	7	829	5	839
Death	RTS,S/AS01+Booster	Tinto Halidou *et al.,* 2019	RCT, Phase III	Burkina Faso, Kenya and Tanzania	Infants (6–12 weeks)	3	637	2	633
Death	RTS,S/AS01	Tinto Halidou *et al.,* 2019	RCT, Phase III	Burkina Faso, Kenya and Tanzania	Infants (6–12 weeks)	3	635	2	633
Death	RTS,S/AS01+Booster	RTS,S Clinical Trials Partnership, 2014	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Children(5-17months)	3	5,949	5	2,179
Death	RTS,S/AS01	RTS,S Clinical Trials Partnership, 2014	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Children(5-17months)	7	2,974	5	2,179
Death	RTS,S/AS01+Booster	RTS,S Clinical Trials Partnership, 2014	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Infants (6–12 weeks)	66	4,358	28	2,179
Death	RTS,S/AS01	RTS,S Clinical Trials Partnership, 2014	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Infants (6–12 weeks)	0	4,358	28	2,179
Death	RTS,S/AS01	Valéa *et al.,* 2020	RCT, Phase III	Burkina Faso and Ghana	Infants (2–26months)	5	425	3	280
Death	RTS,S/AS01+Booster	RTS,S Clinical Trials	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique,	Children(5-17months)	105	2865	96	2905

		Partnership, 2015		Tanzania					
Death	RTS,S/AS01	RTS,S Clinical Trials Partnership, 2015	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Children(5-17months)	93	2887	96	2905
Death	RTS,S/AS01+Booster	RTS,S Clinical Trials Partnership, 2015	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Infants (6–12 weeks)	61	2115	56	2134
Death	RTS,S/AS01	RTS,S Clinical Trials Partnership, 2015	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Infants (6–12 weeks)	65	2119	56	2134
Death	RTS,S/AS01+Booster	RTS,S Clinical Trials Partnership, 2012	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Children(5-17months)	2	844	5	839
Death	RTS,S/AS01	RTS,S Clinical Trials Partnership, 2012	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Children(5-17months)	7	829	5	839
Death	RTS,S/AS01+Booster	RTS,S Clinical Trials Partnership, 2012	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Infants (6–12 weeks)	3	895	2	882
Death	RTS,S/AS01	RTS,S Clinical Trials Partnership, 2012	RCT, Phase III	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	Infants (6–12 weeks)	3	875	2	882

APPENDIX D: Ethical Clearance Certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (NON-MEDICAL)
Registration number: REC-101114-044

23 February 2024

Re: Mr. Darlington Ugwu (2745465)

Waiver letter number: HRECNMW24/03/06

To whom it may concern,

Mr. Ugwu is a registered Masters' student in the School of Pathology at the University of the Witwatersrand, Johannesburg. This letter is to confirm that, at the time of writing, Mr. Ugwu does not need ethical clearance for his Masters' study entitled *'A Systematic Review of the immunogenicity, safety and efficacy of the RTS, S/AS01 Malaria Vaccine'*. This decision has been reached based upon a description of the project supplied by Mr. Ugwu to the University Human Research Ethics Committee (Non-Medical), which has been evaluated by the Chairs and Deputy Chairs. If, however, Mr. Ugwu changes the methods of data collection and analysis for this study, this decision may no longer be valid. If such changes take place, this should be communicated to the University Human Research Ethics Committee (Non-Medical) as soon as possible. This waiver letter is valid until 22 February 2027.

Please feel free to contact me should you require any further information.

Thank you.

Yours sincerely,
S Schoeman

Shaun Schoeman (Administrative Officer)
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