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Predictors of Health Outcomes among Children in the RTS,S/AS01E Malaria Vaccine Trial in Siaya, Kenya

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Master of Public Health

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By

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B.A.
Emory University
2015

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An abstract of
A thesis submitted to the Faculty of the Rollins School of Public Health of Emory University in partial fulfillment of the requirements for the degree of Master of Public Health
in the Hubert Department of Global Health
2017

Abstract

Predictors of Health Outcomes among Children in the RTS,S/AS01E Malaria Vaccine Trial in Siaya, Kenya
By Amelia Elizabeth Van Pelt

Malaria causes significant morbidity and mortality around the world, especially in Sub-Saharan Africa. In Kenya, approximately 7.7 million cases of presumed and confirmed malaria occurred in 2015. Researchers are developing a malaria vaccine as a means of prevention. GlaxoSmithKline sponsored a trial that examined the efficacy of the RTS,S/AS01 vaccine candidate among infants (6-12 weeks) and children (5-17 months) in Siaya, Kenya. Using data from this trial, this research assessed the relationship between parasitemia levels (zero, low (1/μL-49,999/μL), medium (50,000/μL-199,999/μL), high (200,000/μL-499,999/μL), and super-high ($\geq$500,000/μL)), vaccination status (RTS,S/AS01 with booster, RTS,S/AS01 without booster, and comparator), and health outcomes (death, severe anemia, severe malaria, stunting, underweight, and wasting). Descriptive statistics and Poisson regression models were used to determine association between the variables. The distribution of parasitemia significantly differed by study arm ($p<0.025$), with median maximal parasite density of 277,919, 95% CI [241,065, 320,409] for RTS,S/AS01 with booster, 314,386, 95% CI [278,354, 355,083] for RTS,S/AS01 without booster, and 377,047, 95% CI [342,283, 415,341] for comparator, respectively; the two RTS,S arms were different from the comparator but not significantly different from each other. Multivariate models with a referent of zero parasitemia indicated that parasitemia significantly predicted severe malaria (super-high parasitemia IRR 7.39, 95% CI [2.35, 23.23]; high parasitemia IRR 4.62, 95% CI [1.45, 14.66]) but not severe anemia, stunting, underweight, or wasting. Unexpectedly, individuals with zero parasitemia had higher rate of death than participants with super-high, high, and medium parasitemia (IRR 0.12, 95% CI [0.05, 0.30]; IRR 0.11, 95% CI [0.04, 0.28]; IRR 0.10, 95% CI [0.01, 0.80], respectively). Study arm did not significantly predict any of the examined outcomes, although parasitemia did. Reassuringly, the highest episodes of parasitemia occurred in the control, and not the vaccine arm, and were not associated with an increased risk of death. This suggests that in this site, the RTS,S/AS01 vaccine candidate was safe. Future large scale pilots will further assess the safety of rare events.

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Acknowledgements

Dr. Gutman, I would like to express my extreme gratitude to you for serving as my thesis advisor. Your patience and guidance enabled me to learn a new statistical analysis without the fear of embarrassment. You exceeded my expectations with your commitment to ensuring that I understood the material. I enjoyed working with you, and I hope to keep in touch.

RTS,S/AS01 Clinical Trial Partnership, thank you for allowing me to explore the data from the trial for my thesis. The ability to examine data post-trial increased my understanding of malaria vaccines from my previous work on malaria vaccine development in the laboratory.

Friends, thank you for support. I would not have succeeded in completing my thesis without your epidemiologic clarifications and our weekly thesis work sessions.

Family, thank you for reminding me to live in the moment rather than looking ahead to the future. Our frequent conversations kept me on track for finishing my thesis on time.

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CHAPTER 1: INTRODUCTION

Context of the Problem

According to the 2016 World Malaria Report, 212 million cases of malaria and 429,000 deaths due to malaria occurred in 2015 (World Health Organization, 2016e), the majority of which occurred in Africa (World Health Organization, 2016b). In Kenya, malaria causes significant mortality and morbidity, with 6.7 million cases of malaria and 4,000 malaria-attributed deaths every year (Centers for Disease Control and Prevention, 2015a). Over 70 percent of the Kenyan population remains at risk of infection (National Malaria Control Programme, 2014).

Malaria can present as an uncomplicated illness with fever, headache, malaise, chills, sweats, body aches, and nausea (Centers for Disease Control and Prevention, 2015b), or as severe malaria, characterized by high levels of parasitemia, severe anemia, cerebral malaria, and death (World Health Organization, 2012). Certain factors such as age and gender can influence an individual's susceptibility to infection and disease severity (World Health Organization, 2007). Artemisinin combination therapies (ACTs) are the standard treatment for uncomplicated malaria (World Health Organization, 2015), while intravenous artesunate is recommended for severe malaria.

Due to the high mortality and morbidity of malaria, WHO recommends controlling malaria through the following key interventions: insecticide-treated bednets (ITNs) and insecticide spraying (World Health Organization, 2016e). The WHO recommends the RTS,S/AS01 vaccine, but countries have not implemented the vaccine into routine practice (World Health Organization, 2016c).

GlaxoSmithKline RTS,S/AS01 Vaccine Trial

GlaxoSmithKline Biologicals, with funding from the PATH Malaria Vaccine Initiative, sponsored a phase three, double-blind, randomized, controlled trial to evaluate the efficacy of the RTS,S/AS01E vaccine candidate (RTS,S Clinical Trials Partnership, 2012) among young children and infants at the time of initial vaccination.

The trial occurred from March 2009-March 2011 at 11 sites in Sub-Saharan Africa. This analysis will focus on data obtained from the Kenya Medical Research Institute (KEMRI)/CDC Research and Public Health Collaboration site in Siaya, Kenya. This site enrolled 820 infants aged 6-12 weeks old and 800 children aged 5-17 months old. Children aged 6-12 weeks and 5-17 months were eligible for enrollment if they had not already received a dose of vaccine against diphtheria, tetanus or pertussis or *Hemophilus influenzae* type B, were > 28 days of age at screening, and the parent/ guardian provided informed consent, and the child had not participated in a previous malaria vaccine trial. Table 1 details criteria for exclusion from enrollment.

Participants were randomized into three groups (RTS,S/AS01 with booster, RTS,S/AS01 without booster, and comparator) and stratified by age-category using a computer generated randomization list (RTS,S Clinical Trials Partnership, 2011). All participants and researchers remained blinded to study allocation throughout the study. Each participant received three vaccinations, which depended on the study group assignment. Both age groups received the RTS,S/AS01E malaria vaccine candidate, but the comparator vaccine differed between groups; the Meningococcal C conjugate vaccine Menjugate™ (Novartis) was used as a comparator for the infants and the rabies vaccine VeroRab™ (Sanofi-Pasteur) was used as a comparator for the children (RTS,S Clinical Trials Partnership, 2011). Vaccines were administered on day zero (visit two), day 30 (visit three), and day 60 (visit four). Multiple endpoints were assessed,

including clinical malaria disease, severe malaria disease, malaria hospitalization, fatal malaria, and severe anemia (Table 2). The primary trial continued for 32 months, while the extension with the inclusion of the booster dose increased the duration of the trial to 56 months (Annez et al., 2008). Additional details of the main trial are reported by the RTS,S Clinical Trials Partnership (RTS,S Clinical Trials Partnership, 2011; RTS,S Clinical Trials Partnership, 2012; RTS,S Clinical Trials Partnership, 2014; RTS,S Clinical Trials Partnership, 2015).

Problem Statement

Preliminary data from the trial indicated high parasite density among the cohorts (RTS,S Clinical Trials Partnership, 2015). Due to the high levels of observed parasitemia, there is a need to assess whether higher levels of parasitemia was associated with vaccine arm or with poorer outcomes among infants and children enrolled in the RTS,S/AS01 malaria vaccine trial in Kenya.

Research Purpose

This research aims to determine the relationship between parasite density (0, 1/μL - 49,999/μL, 50,000/μL -199,999/μL, 200,000/μL-499,999/μL, and $\geq$500,000/μL), vaccination status, and health outcomes among infants and children in the RTS,S/AS01 malaria vaccine trial in Siaya, Kenya. Analyses will compare health outcomes (death, severe anemia, severe malaria, stunting, underweight, and wasting) between infants (6-12 weeks) and children (5-17 months) of low-moderate, high, and super-high parasitemia levels controlling for age, sex, and study arm. A determination of the relationships will enable researchers to identify safety concerns related to the RTS,S/AS01 vaccine.

CHAPTER 2: LITERATURE REVIEW

Global Burden of Malaria

According to the World Health Organization (2016e), 91 countries reported malaria transmission in 2015. Regions of the world most affected by malaria include: sub-Saharan Africa, South-East Asia, Latin America, and the Middle East; the greatest burden is in Sub-Saharan Africa, where 90% of the 212 million cases of malaria occurred (World Health Organization, 2016b).

Malaria ranks as the seventh leading cause of death in low-income countries (World Health Organization, 2017). In 2015, malaria caused an estimated 429,000 deaths (World Health Organization, 2016e). Ninety-two percent of the malaria deaths occurred in sub-Saharan Africa (World Health Organization, 2016b). Thirteen countries within sub-Saharan Africa accounted for 76% of cases and 75% of malaria deaths (World Health Organization, 2016b).

Immune response influences the severity of malaria infection. In areas with high transmission of *Plasmodium falciparum*, individuals acquire immunity to the infection (Doolan, Dobaño, & Baird, 2009). Upon infection, the preexisting immunity reduces the severity of disease, so individuals with preexisting immunity often do not present with symptoms from low levels of parasitemia (United Nations Children's Fund, 2000). Thus, parasite density varies depending on the level of transmission in the area. Some populations have an increased risk for malaria infection because of reduced immune response. For example, young children and pregnant women have a greater risk of becoming infected than other populations (Centers for Disease Control and Prevention, 2012). In 2015, 70% of the total number of deaths from malaria occurred in children under five years (World Health Organization, 2016e). The World Health Organization (2016e) articulates that one child under the age of five years dies from malaria

every two minutes. It is estimated that approximately 10,000 pregnant women die from a malaria infection in Africa every year (Guyatt & Snow, 2001).

Malaria in Kenya

Kenya has a substantial burden of disease due to malaria. In 2015, 7,676,980 cases of presumed and confirmed malaria occurred in Kenya, all due to *Plasmodium falciparum.* This equals approximately 3% of the total global cases (World Health Organization, 2016e). Approximately 15,061 cases of malaria resulted in death (World Health Organization, 2016a).

Most regions in Kenya (70%) experience high transmission, defined as greater than one case per 1000 population, while 30% of the country exhibits low transmission, defined as zero to one case per 1000 population (World Health Organization, 2016a). However, all individuals have a risk of becoming infected (World Health Organization, 2016e). The areas around Lake Victoria in western Kenya and the coastal regions have the highest burden of malaria (National Malaria Control Programme, 2014).

Siaya County

Located in the former Nyanza region in the west of Kenya, rural Siaya County contains only 2.2% (984,069 people with 465,748 male and 518,321 female) of the population of Kenya (*Siaya County: Health at a glance*, 2015). Due to the resource constraints, the district has some of the worst health outcomes in the country. Siaya ranks as one of the poorest districts in the country with more than half of the population living below the poverty line (The University of New Mexico School of Medicine Center for Global Health, n.d.). High rates of poverty and unemployment contribute to high rates of severe malnutrition, as 7.8% and 24.7% of the

population are underweight and stunted, respectively (*Siaya County: Health at a glance*, 2015). Further, Siaya has the highest rates of mortality and morbidity from infectious diseases in the country. The most common diseases include HIV/AIDS, respiratory infections, and malaria (The University of New Mexico School of Medicine Center for Global Health, n.d.).

In 2015, Siaya had a higher incidence of malaria compared to the incidence in the entire country (69,761 cases/100,000 people and 20,252 cases/100,000 people, respectively) (*Siaya County: Health at a glance*, 2015. According to the Kenya Health Information System, Siaya has a malaria test positivity rate of 49% (*Siaya County: Health at a glance*, 2015), eight percentage points higher than in Kenya as a whole. At any point in time, approximately 83% of children under 36 months have detectable parasitemia (The University of New Mexico School of Medicine Center for Global Health, n.d.). Following the rainy season, the Siaya District Hospital admits up to 30 children per day with an episode of malaria (The University of New Mexico School of Medicine Center for Global Health, n.d.). In 2015, there were 19,158 hospital admissions for malaria at Siaya District Hospital; this accounted for approximately 11% of the total admissions in the country (*Siaya County: Health at a glance*, 2015).

Malaria Biology

Parasite

There are five species of *Plasmodium* that commonly infect humans: *P. falciparum, P. vivax, P. ovale, P. malariae,* and *P. knowlesi* (Centers for Disease Control and Prevention, 2016b), but *P. falciparum* and *P. vivax* pose the greatest threat of infection (World Health Organization, 2016b).

Humans become infected with malaria following the bite of an infected female *Anopheles*

mosquito (Figure 1). When the mosquito punctures an individual's skin to consume a blood meal, it injects *Plasmodium* sporozoites. The sporozoites invade the liver cells and mature into schizonts, commencing the pre- or ex-erythrocyctic cycle. The schizonts rupture, releasing merozoites into the bloodstream, beginning the erythrocytic cycle. The parasites infect erythrocytes and replicate asexually. Clinical symptoms of malaria manifest at this stage of infection. However, some of the intra-erythrocytic parasites do not replicate but instead develop into gametocytes that circulate throughout the bloodstream. When an *Anopheles* mosquito bites an infected individual, it ingests the gametocytes. In the mosquito's stomach, the male and female gametocytes fuse, generating zygotes, which develop into motile ookinetes. These invade the wall of the mosquito midgut where they develop into oocysts. Once the oocysts are mature, they rupture, releasing sporozoites, which pass through to the mosquito salivary glands and are injected into a human upon the mosquito taking a blood meal to complete the life cycle (Centers for Disease Control and Prevention, 2016a; PATH Malaria Vaccine Initiative, 2017).

Symptoms

Physicians categorize the clinical manifestations of malaria into two categories: uncomplicated and severe (World Health Organization, 2015). The incubation period for malaria differs by *Plasmodium* species (Centers for Disease Control and Prevention, 2015b). For *Plasmodium falciparum*, the onset of symptoms generally occurs eight to fourteen days after an infective bite from the *Anopheles* mosquito (World Health Organization, 2016d). The severity of the disease influences the symptoms.

Uncomplicated *Plasmodium falciparum* malaria manifests with fever, general malaise, body aches, chills, sweats, nausea, vomiting, and headaches (Centers for Disease Control and

Prevention, 2015b). Mild jaundice, enlarged spleen, enlarged liver, and increased respiratory rate can result from malaria infection as well (Centers for Disease Control and Prevention, 2015b). Treatment of uncomplicated malaria results in a positive prognosis (World Health Organization, 2015).

Severe malaria refers to infection complicated by vital organ dysfunction, and is characterized by hyperparasitemia (World Health Organization, 2012). Any of the following symptoms, either alone or in combination, can occur with severe malaria: impaired consciousness, prostration, multiple convulsions (more than two events in 24 hours), acute kidney failure, deep breathing, respiratory distress, acute pulmonary oedema, acute respiratory distress syndrome (ARDS), abnormal bleeding caused by abnormalities in blood coagulation, jaundice, circulatory collapse, shock (systolic blood pressure less than 80mm HG in adults and less than 50mm Hg in children), metabolic acidosis, and hemoglobinuria (Centers for Disease Control and Prevention, 2015b; World Health Organization, 2012). Untreated severe malaria frequently results in death (World Health Organization, 2015). A parasite density greater than 200,000/μl or infection in at least five percent of the erythrocytes is associated with an increased risk of developing severe malaria (Goldman & Schafer, 2015). Typical parasite density varies by the level of transmission of malaria in the region. In areas of high transmission, (World Health Organization, 2012). Table 2 details the levels of parasitemia used for the case definition of severe malaria in this research.

Malaria Health Outcomes

Anemia

Anemia, defined by a hemoglobin of approximately 10-11 g/dL for children under 59

months (World Health Organization, 2011), is a multifactorial condition leading to inadequate oxygen delivery to tissues; severe anemia is defined by a hemoglobin of <5 g/dL (Annez, 2008). Both uncomplicated malaria and severe malaria are associated with anemia, due to destruction of erythrocytes (Centers for Disease Control and Prevention, 2015b) (World Health Organization, 2012). The 2015 Kenya National Malaria Indicator Survey reported that approximately 48% of children six to eight months in Kenya are anemic (National Malaria Control Programme, 2016).

A study that assessed the influence of under-nutrition on malaria among children under five years in southwestern Ethiopia calculated an odds ratio of 1.5 for the relationship between anemia and parasite density (Deribew et al., 2010). Thus, children with malaria had an increased odds of becoming anemic compared to children without malaria, with increasing parasite density associated with greater risk of anemia. Further, an analysis of severe falciparum malaria and health complications among children in India concluded that children under five years had the greatest risk of developing severe anemia out of the ages studied (Satpathy, Mohanty, Nanda, & Samal, 2004).

Death

The severity of the disease determines the health outcome, as not all cases of malaria result in death. However, severe malaria can lead to complications that result in death (Centers for Disease Control and Prevention, 2015b). *Plasmodium falciparum* infection yields the majority of the deaths from severe malaria because of the rapid reproduction of the parasite, and the fact that the parasite is able to infect erythrocytes of all stages, leading to higher levels of parasitemia than with other species (World Health Organization, 2012). Hyperparasitemia can lead to hypoglycemia and severe anemia, impairing tissue oxygenation and metabolic

functioning. As a result, major organ failure and death can occur (World Health Organization, 2015b). In areas of high malaria transmission, individuals with lower immunity, mainly children under five years, pregnant women, and HIV-positive individuals, or those who lack prompt access to treatment carry significant burden (World Health Organization, 2016b).

According to the World Health Organization (2017), malaria ranks as the seventh leading cause of death in low-income countries. In 2015, 429,000 malaria-attributed deaths occurred globally (World Health Organization, 2016e), the majority in sub-Saharan Africa (World Health Organization, 2016b). A systematic review of global child mortality articulates that *P. falciparum* infection accounts for approximately 24% of total child death in sub-Saharan Africa (Murray et al., 2012). In addition, a study that assessed the association between transmission intensity and case fatality of severe *P. falciparum* malaria in Tanzania concluded that areas of low malaria transmission had a significantly higher case-fatality rate than areas of high or moderate transmission (Reyburn et al., 2005).

Stunting

Moderate-severe stunting is defined as being below two standard deviations from median height for age of the reference population (United Nations Children's Fund, n.d.). The Kenya Demographic Health Survey (2015) reports stunting in 26% of children under five years. Children 18-23 months have the highest prevalence of stunting at 36%. Males have a higher prevalence of stunting than females at 30% and 22%, respectively (Demographic Health Survey, 2015). In Siaya, 24.7% of children under five are stunted (Demographic Health Survey, 2015).

Literature reports an association between stunting and malaria. For instance, infants in the Ashanti region of Ghana had an increased risk of stunting for every episode of malaria (0.32 and

95% CI [0.09,1.0], p-value=0.004) (Kang et al., 2013). In addition, among children in the Amazonian region of Brazil, malaria infection was associated with stunting (adjusted ß -0.1 and 95% CI [-0.3,0.0], p-value = 0.035) (Araújo et al., 2015).

Moreover, a bidirectional relationship between malaria and stunting has been described. Stunted children are at a greater risk of developing malaria. In the coastal region of Kenya, stunted children had an increased rate of malaria compared to non-stunted children (incidence rate ratio of 1.91, 95% CI [1.01,3.58], p-value=0.04) (Nyakeriga, et al., 2004). In rural Senegal, children two to fifty-nine months with stunting demonstrated lower antibody levels to *P. falciparum* compared to children without stunting (Fillol et al., 2009).

Underweight

Moderate to severe underweight is defined as being below two standard deviations from the median weight for age of a reference population (weight-for-age Z-score below two standard deviations) (United Nations Children's Fund, n.d.). The Kenya Demographic Health Survey (2015) reports that 11% of all children under five years and 3.7% of children under six months in Kenya are underweight. Males have a higher prevalence of underweight than females at 12.1% and 9.8%, respectively. In Siaya, 7.8% of children under five years are underweight (Demographic Health Survey, 2015).

Literature reports an association between underweight and malaria. For example, HIV-exposed children with malaria under 18 months in Malawi had increased odds of underweight (odds ratio of 1.4 and 95% CI [1,1.9)], p-value=0.04) (Scaracella et al., 2016). In addition, children under 10 years with a *Plasmodium vivax* infection in Vanuatu had increased risk of underweight (IRR, 2.6 and 95% CI [1.5,4.4], p-value= 0.0001) (Williams et al., 1997).

Moreover, a bidirectional relationship between malaria and underweight has been described. Underweight children are at a greater risk of developing malaria. In Ethiopia, underweight children six to fifty-nine months had increased odds of developing malaria compared to children of normal weight (adjusted odds ratio of 1.69 and 95% CI [1.11,2.9]) (Hassen & Ali, 2015). In western Kenya, underweight children had increased odds for malaria compared to normal weight controls (adjusted odds ratio of 2.18 and 95% CI [1.12,4.27], p-value=0.022) (Brooker et al., 2004).

Wasting

Moderate to severe wasting is defined as being below two standard deviations from median weight for height of reference population (weight-for-height Z-score below two standard deviations) (United Nations Children's Fund, n.d.). The Kenya Demographic Health Survey (2015) reports wasting in 4% of children under five years, and 3.7% of children under six months. Males have a higher prevalence of wasting than females at 4.4% and 3.7%, respectively. In Siaya, only 0.2% of children under five were wasted (Demographic Health Survey, 2015).

Literature reports an association between malaria and wasting. Among HIV-exposed children in Malawi, malaria increased the odds of becoming wasted (odds ratio of 2.1, 95% CI [1.2,3.5], p-value=0.005) using a weight-for-length z-score less than negative two, and an odds ratio of 5.7, 95% CI [1.6,20.4], p-value=0.007, using a weight-for-length z-score less than negative three) (Scaracella et al., 2016). However, this association is inconsistent. A study assessing risk factors for malaria among children under five years in rural Gambia concluded that wasting did not increase susceptibility to malaria infection (Deen, Walraven, & von Seidlein, 2002).

Moreover, a bidirectional relationship between malaria and wasting has been described. Wasting is associated with an increased odds of malaria. In Ethiopia, wasted children had an increased risk of malaria compared to children without wasting (adjusted odds ratio = 2.4 and 95% CI [1.146,5.197]) (Hassen & Ali, 2015).

Risk Factors for Malaria

Age

Risk of infection varies by age. During the first few months of life, maternal antibodies transferred through the placenta stimulate the immune system and serve as an infant's protection (Centers for Disease Control and Prevention, 2012). Thus, in a region with high malaria transmission, a mother's repeated malaria infections protects an infant from malaria. However, as the maternal antibodies decrease, a young child's susceptibility to and severity of infection increases. Children under five years have an increased risk of becoming infected with malaria, especially severe malaria (World Health Organization, 2016b). The 2015 World Malaria Report articulates that children accounted for 70% (303,000) of the malaria deaths in 2015 (World Health Organization, 2016e).

A study that assessed clinical manifestations of malaria at various levels of malaria transmission in Tanzania demonstrated that the severity of disease differed by age and level of transmission. The median age of patients admitted for severe malaria in a high-transmission area was one year, in moderate transmission was three years, and in low transmission was five years. In addition, infants less than one year had the highest odds of developing severe malarial anemia (Reyburn et al., 2005).

Sex

The influence of biological sex on the risk of becoming infected with malaria typically manifests at an older age through gender roles and sociocultural norms. For example, men who pursue occupations that require them to work outside or at dusk in endemic countries increase their exposure to mosquitoes (Homan et al., 2016). As a result, in these settings, men have a greater risk of developing malaria than women (Malaria Control Unit, 2015). Among young children, there is not a significant association between biological sex and risk of malaria infection (Crookston et al., 2010).

However, Were et al. (2011) examined health outcomes among children less than 36 months in Siaya District Hospital infected with *Plasmodium falciparum* and concluded that females had lower parasite density than males. In addition, a review of the community health documents in Western and Nyanza province in Kenya revealed a discrepancy between provision of ACT to children under five years by gender. Among those who received ACT, boys accounted for 22%, and girls totaled 78% (Malaria Control Unit, 2015). This suggests that a child's biological sex may influence caregivers' treatment-seeking behaviors and the resulting health outcomes, and thus it is an important factor to examine.

Malaria Prevention

The Sustainable Development Goals (SGD) articulate the importance of focusing on malaria prevention. Goal three states, "Ensure healthy lives and promote well-being for all at all ages," (United Nations, n.d.). Further, Target 3.3 states, "By 2030, end the epidemics of AIDS, tuberculosis, malaria and neglected tropical diseases and combat hepatitis, water-borne diseases and other communicable diseases," (United Nations, n.d.). Thus, all countries should consider

malaria reduction as a priority. To this end, WHO recommends the use of insecticide-treated mosquito nets (ITNs), indoor residual spraying (IRS), and antimalarial medication as means of prevention (World Health Organization, 2016b). In addition, researchers are currently conducting pre-clinical and clinical trials for malaria vaccine candidates.

Vaccination

Vaccination serves as an effective method of prevention of disease, however, a highly effective malaria vaccine has been elusive. Most vaccine candidates remain in the early stages of vaccine development (World Health Organization, 2016c). The RTS,S/AS01 vaccine candidate, a recombinant protein vaccine, is the farthest along in development (World Health Organization, 2016c). The European Medicines Agency has adopted a positive scientific opinion, under the Article 58 process, for RTS,S in children aged 6 weeks to 17 months, and the WHO has recommended large-scale pilot implementations of RTS,S in children 5 months to 9 months of age moderate-to-high transmission settings in Africa (PATH Malaria Vaccine Initiative, 2016). RTS,S/AS01 targets the pre-erythrocytic stage of the *Plasmodium falciparum* circumsporozoite protein in the malaria cycle (World Health Organization, 2016d). Phase three randomized controlled trials in multiple sites in Africa demonstrated vaccine efficacy of 27% and 46% against clinical malaria among infants and young children in Africa, respectively (RTS,S Clinical Trials Partnership, 2014). The inclusion of a booster dose prevented a substantial number of cases of malaria, but some adverse health outcomes were observed (RTS,S Clinical Trials Partnership, 2015).

CHAPTER 3: METHODOLOGY

A secondary data analysis on data from the GlaxoSmithKline RTS,S/AS01E vaccine trial was conducted. Participants included 820 infants (6-12 weeks) and 799 children (5-17 months) in the Siaya, Kenya site who met the inclusion criteria for the main trial. Refer to the Introduction for an explanation of the randomization process, included vaccines, and vaccination schedule.

This analysis aimed to assess the relationship between maximum parasitemia level and outcomes of death, severe malaria, severe anemia, stunting, underweight, and wasting among the participants enrolled in the study controlling for age at enrollment, biological sex, and study arm.

Dataset Compilation

The datasets were acquired from the GlaxoSmithKline RTS,S/AS01E vaccine trial's database. Six spreadsheets (demographics, efficacy, follow-up, hospitalization admissions, mortality, and severe malaria) that contained the variables of interest for the research question were merged together to create a master dataset for this analysis.

New variables were created to measure the predictors and health outcomes of interest. Rather than assessing all episodes of parasitemia, the single highest parasite density value was used. Originally, parasite density was categorized into three levels (low-moderate, high, super-high) based on the levels outlined in the GlaxoSmithKline RTS,S/AS01 protocol (Greenwood & Armstrong, 1991). After reviewing the distribution across the levels, the categories were modified to none (0), low (1/μL -49,999/μL), medium 50,000/μL -199,999/μL), high 200,000/μL-499,999/μL), and super-high ($\geq$500,000/μL). Variables for stunting, underweight, and wasting were created from measurements taken at both baseline and endline, at 32 months after the initial vaccination, according to standard definitions (Annez et al., 2008; Onis &

Blössner, 1997). Hemoglobin was assessed for all participants at baseline, at 20 months post-vaccination, as well as any time a patient was hospitalized; the outcome variable for severe anemia incorporated measurements at 20 months as well as at any hospitalization. A variable for person-time was calculated by subtracting the start date for follow-up from the end date of follow-up to compute the number of days in the study; the log of the person time was used in the analysis.

Statistical Analysis

Descriptive Statistics

Descriptive analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC). Two-by-two tables were generated to determine the association between each health outcome and the following participant characteristics: parasitemia level (zero, low, medium, high, and super-high), age (infants and children), sex (female and male), and study arm (RTS,S/AS01 without booster, RTS,S/AS01 with booster, comparator). P-values were calculated using Pearson's Chi-Square statistic to determine the significance of the association between the variables.

Poisson Regression

Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC). Poisson regression models were used. The parameterization method for the classification variables was reference with the first reference designated. To account for the variance in the number of days each participant was enrolled in the trial, analyses were offset by person-time.

Univariate models were performed for each independent variable (age, parasitemia, sex,

and study arm) and each of the health outcomes (death, severe anemia, severe malaria, stunting, underweight, and wasting). For the growth measures (stunting, underweight, and wasting) and severe anemia, the respective baseline Z-score or baseline hemoglobin level was included in the multivariate model to account for pre-existing status. Multivariate models were performed for each health outcome. Individuals without parasitemia were excluded from the analysis for severe malaria, so for this analysis, the low parasitemia level was used as the referent. Due to the significant statistical association determined by univariate analysis and biological association reported by literature, age, parasitemia, sex, and study arm were all included in the multivariate models.

Ethical Considerations

The CDC and the KEMRI granted Institutional Review Board (IRB) approval for the RTS,S/AS01E vaccine trial (Annez et al., 2008). As this current analysis made use of a fully de-identified dataset, with no additional participant contacts, an IRB exemption was granted from Emory University.

Limitations

Limitations in the analyses resulted from missing observations. Table 3 shows the frequency of observations for each variable. The majority of the variables had an observation for every individual. However, approximately 27% of individuals did not have a recorded Z-score for stunting, underweight, or wasting, but a sufficient number of observations existed for analysis. In addition, 41 individuals did not have a recorded parasite density from the efficacy dataset. Due to the distribution of parasitemia in the sample from the original categorization, the

parasite density cutoffs were modified to ensure sufficient participants in each parasitemia level. However, the levels still closely reflected the clinical cutoffs.

CHAPTER 4: RESULTS

This research aimed to determine the relationship between parasite density, vaccination status, and health outcomes among children in the GlaxoSmithKline RTS,S/AS01 malaria vaccine trial in Siaya, Kenya. A secondary data analysis on the 1,619 participants and 11 variables were used for descriptive analyses and Poisson regression modelling.

Study Population

This analysis included 820 infants (6-12 weeks) and 799 children (5-17 months), with approximately equal numbers of females and males. There were 539 participants in both the RTS,S/AS01 groups (with and without booster) and 541 participants in the comparator group. A total of XX% of participants never had parasitemia. The medium parasitemia level and super-high parasitemia level were comprised of more females than males, while the other parasitemia levels consisted of more males than females; however, the geometric mean parasite levels did not differ significantly by sex. The geometric mean parasite density was significantly different when comparing either RTS,S group to the comparator, but not when comparing between the RTS,S groups with or without booster (geometric mean 277,919, 95% CI [241,065,320,409] for RTS,S/AS01 with booster; 314,386, 95% CI [278,354,355,083] for RTS,S/AS01 without booster; 377,047, 95% CI [342,283,415,341] for comparator; p-value=0.0251). Figure 2 illustrates the distribution of parasite density by study arm.

Unadjusted, Univariate, and Multivariate Modelling Results

Death

In unadjusted analysis, surprisingly, a greater proportion of participants in the zero

parasitemia group (6.8%) died compared to the low (4.6%), medium (1.2%), high (1.5%), and super-high (1.7%) parasitemia groups (overall p value=0.002). Age, sex, and study arm were not significantly associated with death (Table 5).

Compared to individuals who never had parasitemia, super-high, high, and medium parasite density were significantly associated with a decreased rate of death in both univariate (IRR 0.12, 95% CI [0.05,0.29], p-value= <0.0001; IRR 0.11, 95% CI [0.04,0.28], p-value=<0.0001; IRR 0.10, 95% CI [0.01,0.79], p-value=0.03, respectively) and multivariate analyses (IRR 0.12, 95% CI [0.05,0.30], p-value=<0.0001; IRR 0.11, 95% CI [0.04,0.28], p-value=<0.0001; IRR 0.10, 95% CI [0.01,0.80], p-value=0.03, respectively) (Table 6).

Severe Anemia

In unadjusted analysis, age, sex, study arm, and parasitemia were not significantly associated with severe anemia (Table 5).

In univariate models, none of the examined predictors were significantly associated with severe anemia. In multivariate analyses, being an infant at the time of enrollment was significantly associated with an increased rate of severe anemia compared to being a child (IRR 2.52, 95% CI [1.20,5.32], p-value=0.02) (Table 7).

Severe Malaria

In unadjusted analysis, a greater percentage of participants with super-high parasitemia had severe malaria (19.9%) compared to individuals with low (2.0%), medium (6.2%), and high (12.0%) parasitemia (overall p-value= <0.0001). Age, sex, and study arm were not significantly associated with severe malaria (Table 5).

Super-high and high parasite density were significantly associated with an increased rate for severe malaria compared to individuals who had low parasitemia in both univariate analyses (IRR 7.39, 95% CI [2.35,23.23], p-value=0.001 and IRR 4.62, 95% CI [1.45,14.66], p-value=0.01, respectively) and multivariate analyses (IRR 7.45, 95% CI [2.37,23.45], p-value=0.001 and IRR 4.62, 95% CI [1.45,14.70], p-value=0.01, respectively) (Table 8).

Stunting

In unadjusted analysis, stunting was more common among infants than children (39.4% and 24.3%, respectively, p-value= <0.0001) and among males than females (35.8% and 27.7%, respectively, p-value= 0.003). Study arm and parasitemia were not significantly associated with stunting (Table 5).

In univariate models, but not multivariate models, being female was significantly associated with a decreased rate of stunting compared to being male (IRR 0.78, 95% CI [0.63,0.95], p-value=0.01). In addition, being an infant at the time of enrollment was significantly associated with an increased rate for stunting at 32 months post-vaccination compared to being a child at the time of enrollment in both univariate analyses (IRR 1.67, 95% CI [1.32,1.99], p-value=<0.0001) and multivariate analyses (IRR 2.08, 95% CI [1.68,2.58], p-value=<0.0001). Parasite density was not significantly associated with stunting (Table 9).

Underweight

In unadjusted analysis, age, sex, study arm, and parasitemia were not significantly associated with underweight (Table 5).

Being an infant at enrollment was significantly associated with being underweight at 32

months post-vaccination compared to being a child at enrollment in multivariate analyses (IRR 1.64, 95% CI [1.18,2.28], p-value=0.003). Parasite density was not significantly associated with the rate for underweight (Table 10).

Wasting

In unadjusted analysis, age, sex, study arm, and parasitemia were not significantly associated with wasting (Table 5).

None of the examined predictors were significantly associated with wasting in univariate or multivariate analyses (Table 11).

CHAPTER 5: DISCUSSION

Conclusions

As expected, increased parasite density was associated with an increased rate of severe malaria, but surprisingly, death was more common among participants with no or low parasitemia compared to those with higher levels of parasitemia. Parasite density was not associated with severe anemia, stunting, wasting, or underweight.

The rate of death among individuals with medium to super high parasitemia was approximately 0.10-0.12 times the rate of death among participants with zero parasitemia. Multiple hypotheses may explain these findings. First, very few (2.3%) participants died. Thus, a small sample size could have resulted in an inability to detect the true difference between groups. Second, participants could have died from other causes. The leading causes of death in Siaya include HIV/AIDS, respiratory infections, and malnutrition (The University of New Mexico School of Medicine Center for Global Health, n.d.); any of these diseases may have lead to the death of children in the trial. The overall death rate for participants in the trial was lower than that of the community overall (infant mortality rate is 80-200 deaths per 1,000 births, The University of New Mexico School of Medicine Center for Global Health, n.d.), suggesting that overall care was improved by being in the trial. This might have influenced the causes of deaths and reduced malaria related deaths. A study among children in Uganda found that children with negative malaria blood smears had a higher case fatality rate than children with positive malaria blood smears (OR 1.59 and 95% CI [1.29,1.96], p-value=<0.001) (Opoka, Xia, Bangirana, & John, 2008). Prompt treatment by individuals with malaria infection may have improved not only malaria infection but other health outcomes as well, which could have decreased the rate of death from other causes. Third, the selection of the single, highest parasite density value for each participant, without consideration for the number of episodes, may have influenced the results.

Individuals with higher parasitemia may have had repeated infections, which could have induced immunity. Fourth, participants with higher parasitemia could have received treatment that decreased their risk of death.

As expected, parasitemia significantly predicted severe malaria. Participants with high and super-high parasitemia had approximately 4.6 and 7.4 times the rate of severe malaria as individuals with low parasitemia. A study that examined *Plasmodium falciparum* density among children in Tanzania concluded that on average, parasite density was higher during cases of severe malaria than mild cases (Gonçalves et al., 2014). In addition, research among children under five years in Kisumu, Kenya found that individuals with severe malaria had a higher parasite load than those with mild malaria (Kituyi, Nyakoe, Ngeranwa, Runo, & Waitumbi, 2014).

Also surprisingly, parasitemia was not associated with an increased risk for severe anemia. The lack of statistical significance might have been a result of the small sample size used to analyze severe anemia, as only 43 participants experienced severe anemia at any point. Further, the cases of anemia among the participants may have resulted from malnutrition, as many individuals in Siaya are malnourished (The University of New Mexico School of Medicine Center for Global Health, n.d.). Additional analyses looking at alternate cut-offs for mild and moderate anemia should be explored to further assess for relationships between parasitemia and anemia.

This research has multiple strengths. To begin with, the large sample size results in strong statistical power, which ensures greater validity in the statistical significance of the analyses. In addition, the multivariate analyses included both biologically significant according to literature

and statistically significant according to univariate analysis variables in the models, which increases the reliability of the statistical analyses.

Refer to the Methodology for an explanation of the limitations in the conduct of the statistical analyses. An additional limitation surrounds the generalizability of the results and conclusions presented in this research. This research only analyzed data on 1,619 individuals in Siaya, Kenya. The conclusions drawn may differ among different populations because of varying characteristics. Thus, further research is needed to increase the confidence in the generalizability of the results.

Public Health Implications

The Sustainable Development Goal 3.3 proposes to reduce the incidence of malaria. To contribute to the reduction, malaria vaccine candidates, such as RTS,S/AS01, are being tested. This analysis adds to conclusions about the safety of the vaccine: the lack of significant association between study arm and any of the health outcomes indicates that the vaccine did not significantly increase the rate of such outcomes. Thus, researchers can conclude that the vaccine does not pose safety concerns with regard to the outcomes studied, but further research is necessary to generalize these findings.

Future Research

Additional analyses would contribute to this research and the greater public health implications. This analysis examined whether the single maximal density of parasitemia experienced by a participant was associated with the health outcomes. However, this does not allow the maximal use of the available longitudinal data. Additional analyses could explore

whether the number of episodes of malaria affected outcomes and the effect of each episode of parasitemia experienced by the participant. For outcomes such as severe malaria and severe anemia which could have occurred multiple times throughout the course of follow-up, these analyses could assess incidence, rather than occurrence of outcomes. Such longitudinal analysis would provide more detailed information on the association between parasitemia and outcomes than is presented here.

This research examined stunting, underweight, and wasting as health outcomes. However, literature shows that these conditions can influence the manifestation and severity of malaria as well (Fillol et al., 2009; Brooker et al., 2004; Hassen & Ali, 2015). Thus, although this research controlled for baseline the measures, future research could assess the influence of stunting, underweight, and wasting as predictors for death, severe anemia, and severe malaria.

In addition, the trial collected measures on other health outcomes, such as neurological outcomes, pneumonia, sepsis, and gastroenteritis, that could be assessed for association with parasitemia or the RTS,S/AS01 vaccine.

Finally, a replication of the analyses of the outcomes and predictors included in this research on the data from all 11 sites in the RTS,S/AS01E trial would increase both the power and the generalizability of the results.

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TABLES AND FIGURES

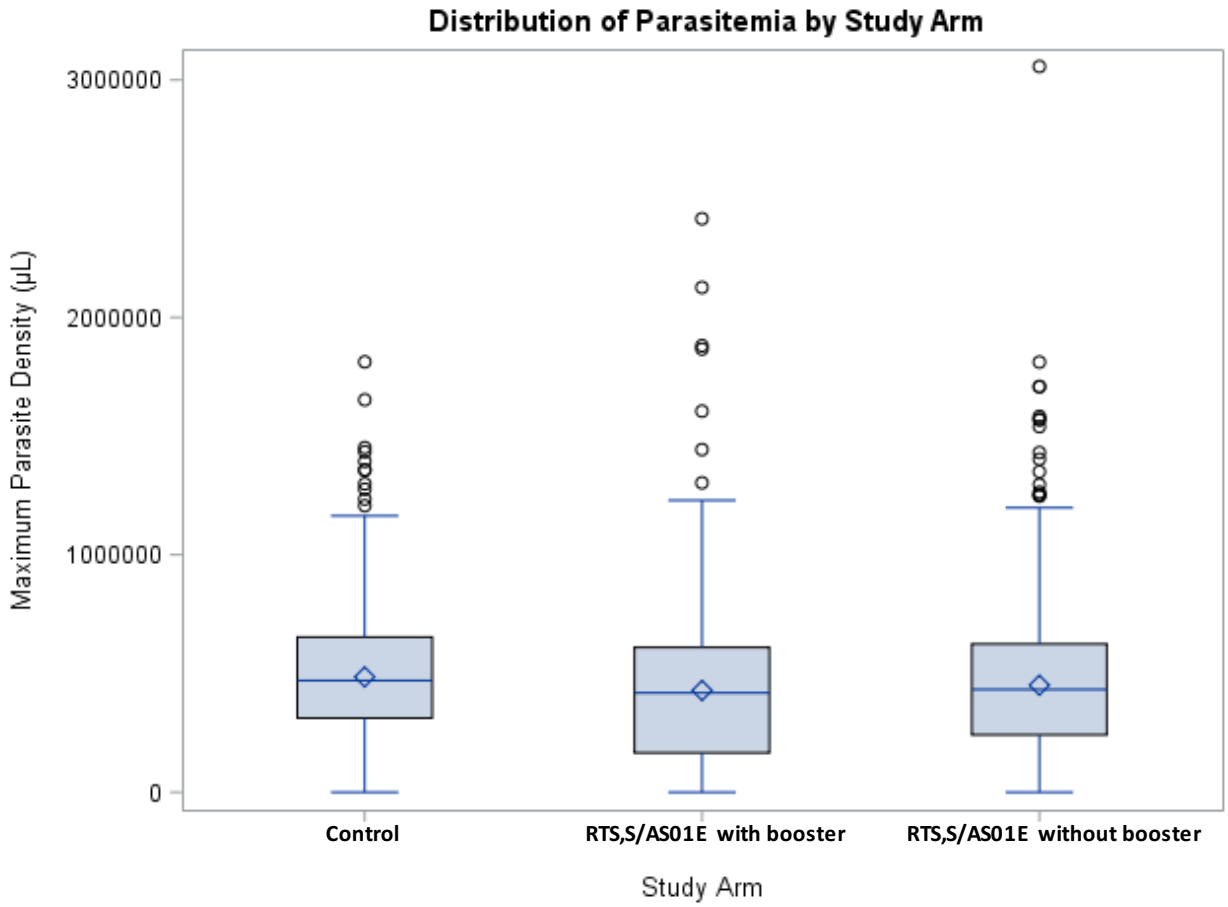

Figure 2. Distribution of parasitemia significantly differs by study arm. Geometric mean of 377,047, 95% CI [342,283, 415,341] for control, 277,919, 95% CI [241,065, 320,409] for RTS,S/AS01 with booster, and 314,386, 95% CI [278,354, 355,083] for RTS,S/AS01 without booster; overall p-value= 0.0251. The difference between the vaccine with booster and without booster was not statistically significant.

TABLE 1
Exclusion criteria for enrollment into RTS,S/AS01 vaccine trial

Acute disease at the time of enrolment (acute disease is defined as the presence of a moderate or severe illness with or without fever). All vaccines can be administered to persons with a minor illness such as diarrhea, mild upper respiratory infection with or without low-grade febrile illness, i.e. axillary temperature < 37.5°C).
Acute or chronic, clinically significant pulmonary, cardiovascular, hepatic or renal functional abnormality, as determined by physical examination or laboratory screening tests.
Anemia defined as: - hemoglobin < 5.0 g/dL. - or hemoglobin < 8 g/dL associated with clinical signs of heart failure or severe respiratory distress.
Major congenital defects.
History of allergic reactions, significant IgE-mediated events or anaphylaxis to previous immunizations.
Children with a past history of a neurological disorder or atypical febrile seizure (a febrile seizure is atypical if it meets one of the following criteria: not associated with fever; lasts > 5 minutes; focal (not generalized); followed by transient or persistent neurological abnormality; occurs in a child < 6 months of age).
Children with malnutrition requiring hospital admission.
Children currently meeting the criteria for HIV disease of Stage III or Stage IV severity as defined by the World Health Organization [4WHO, 2005] (please refer to 4Appendix I for current guidelines). NB: a previous history of having Stage III or Stage IV HIV disease is NOT an exclusion criterion.
History of allergic disease or reactions likely to be exacerbated by any component of the vaccine.
Concurrently participating in another clinical study, at any time during the study period, in which the subject has been or will be exposed to a drug or vaccine that is not licensed for that indication (by one of the following authorities: FDA or EU member state or WHO [with respect to prequalification]) with the exception of studies with the objective of improving the drug treatment or clinical management of severe malaria disease.
Use of a drug or vaccine that is not approved for that indication (by one of the following authorities: FDA or EU member state or WHO [with respect to prequalification]) other than the study vaccines within 30 days preceding the first dose of study vaccine, or planned use during the study period.
Previous participation in any other malaria vaccine trial.
Receipt of a vaccine within the preceding 7 days
Any other findings that the investigator feels would increase the risk of having an adverse outcome from participation in the trial.
Any other findings that the investigator feels would result in data collected being incomplete or of poor quality.

TABLE 2
Case definitions for clinical endpoints in the RTS,S/AS01 vaccine trial

Clinical Endpoint	Definition(s)
Clinical malaria disease	*P. falciparum* asexual parasitemia > 5000 parasites per μL AND presence of fever (axillary temperature ≥ 37.5°C) at the time of presentation AND occurring in a child who is unwell and brought for treatment to a healthcare facility OR A case of malaria meeting the primary case definition of severe malaria disease *P. falciparum* asexual parasitemia > 0 AND presence of fever (axillary temperature ≥ 37.5°C) at the time of presentation or history of fever within 24 hours of presentation AND occurring in a child who is unwell and brought for treatment to a healthcare facility *P. falciparum* asexual parasitemia >500 parasites/μL AND presence of fever (axillary temperature ≥ 37.5°C) at the time of presentation AND occurring in a child who is unwell and brought for treatment to a healthcare facility *P. falciparum* asexual parasitemia > 20000 parasites/μL AND presence of fever (axillary temperature ≥ 37.5°C) at the time of presentation AND occurring in a child who is unwell and brought for treatment to a healthcare facility
Severe malaria disease	*P. falciparum* > 5000 parasites per μL AND with one or more marker of disease severity • Prostration • Respiratory distress • Blantyre score≤ 2 • Seizures 2 or more • Hypoglycemia <2.2 mmol/L • Acidosis BE ≤ -10.0 mmol/L • Lactate ≥ 5.0 mmol/L • Anemia < 5.0 g/dL AND without diagnosis of a co-morbidity • Radiographically proven pneumonia • Meningitis on cerebral spinal fluid examination • Positive blood culture • Gastroenteritis with dehydration
Malaria hospitalization	A medical hospitalization with confirmed *P, falciparum* > 5000 parasites/μL (excludes planned admissions for medical investigation/care or elective surgery and trauma) A hospitalization which, in the judgement of the principal investigator, *P. falciparum* infection was the sole or a major contributing factor to the presentation
Fatal malaria	A case of severe malaria meeting the primary case definition of severe malaria disease (see *primary case definition of severe malaria disease* [above]) with a fatal outcome (i.e. is restricted to hospital mortality and does not include cause ascribed by verbal autopsy)

TABLE 3
Frequency of variable observations

Variable	Number of Observations	Number of Missing Observations
Age	1,619	0
Death	1,619	0
Parasitemia	1,578	41
Severe anemia	1,619	0
Severe malaria	1,619	0
Sex	1,619	0
Study Arm	1,619	0
Stunting	1,189	430
Underweight	1,191	428
Wasting	1,187	432

TABLE 4
Participant characteristics % (n)

	Parasitemia					P-value[Δ]
	None	Low*	Medium[+]	High^	Super-High[†]	
Age						**0.02**
Infants	62.7 (74)	55.3 (84)	45.7 (37)	49.9 (291)	47.2 (304)	
Children	37.3 (44)	44.7 (68)	54.3 (44)	50.1 (292)	52.8 (340)	
Sex						0.23
Female	51.7 (57)	43.4 (66)	51.9 (42)	49.2 (287)	53.3 (343)	
Male	48.3 (61)	56.6 (86)	48.1 (39)	50.8 (296)	46.7 (301)	
Study Arm						**0.01**
RTS,S/AS01 w/ booster	43.2 (51)	40.8 (62)	35.8 (29)	31.4 (183)	30.0 (193)	
RTS,S/AS01 w/o booster	32.2 (38)	33.6 (51)	38.3 (31)	34.1 (199)	32.6 (210)	
Comparator	24.6 (29)	25.6 (39)	25.9 (21)	34.5 (201)	37.4 (241)	

*1/μL -49,999/μL
[+]50,000/μL -199,999/μL
^ 200,000/μL-499,999/μL
[†] ≥500,000/μL
[Δ]P-value calculated by Pearson Chi-Square statistic

TABLE 5
Frequency of health outcomes among participants, % (n)

Outcome	Age			Sex			Study Arm				Parasitemia					
	Infants	Children	P-value	Female	Male	P-value	RTS,S/AS01 w/ booster	RTS,S/AS01 w/o booster	Comparator	P-value	None	Low*	Medium+	High^	Super-high†	P-value^Δ
Total N	820	799		816	803		539	539	541		118	152	81	583	644	
Death																
Yes	2.9 (24)	1.6 (13)	0.08	2.0 (16)	2.6 (21)	0.38	2.0 (11)	2.6 (14)	2.2 (12)	0.82	6.8 (8)	4.6 (7)	1.2 (1)	1.5 (9)	1.7 (11)	**0.002**
No	97.1 (796)	98.4 (786)		98.0 (800)	97.4 (782)		98.0 (528)	97.4 (525)	97.8 (529)		93.2 (110)	95.4 (145)	98.8 (80)	98.5 (574)	98.3 (633)	
Severe anemia																
Yes	3.1 (25)	2.2 (18)	0.32	2.2 (18)	3.1(25)	0.26	2.4 (13)	3.2 (17)	2.4 (13)	0.68	0.9 (1)	1.3 (2)	1.2 (1)	2.2 (13)	4.0 (26)	0.09
No	96.9 (795)	97.8 (781)		97.8 (798)	96.9 (778)		97.6 (526)	96.8 (522)	97.6 (528)		99.1 (117)	98.7 (150)	98.8 (80)	97.8 (570)	96.0 (618)	
Severe malaria	13.8 (110)	11.7 (96)	0.21	11.6 (95)	13.8 (111)	0.19	11.3 (61)	13.0 (70)	13.9 (75)	0.44	0 (0)	2.0 (3)	6.2 (5)	12.0 (70)	19.9 (128)	**<.0001**
Yes	86.2(689)	88.3 (724)		88.4 (721)	86.2 (692)		88.7 (478)	87.0 (469)	86.1 (466)		100 (118)	98.0 (149)	93.8 (76)	88.0 (513)	80.1 (516)	
No																
Stunting																
Yes	39.4 (229)	24.3 (148)	**<.0001**	27.7 (167)	35.8 (210)	**0.003**	31.0 (125)	32.0 (130)	31.1 (122)	0.80	19.4 (6)	32.9 (24)	32.0 (16)	32.7 (153)	31.4 (178)	0.65
No	60.6 (352)	75.7 (460)		72.3 (435)	64.2 (377)		69.0 (278)	67.0 (264)	68.9 (270)		80.6 (25)	67.1 (49)	68.0 (34)	67.3 (315)	68.6 (389)	
Under-weight																
Yes	13.7 (80)	12.0 (73)	0.39	11.6 (70)	14.1 (83)	0.19	11.7 (47)	13.2 (52)	13.7 (54)	0.66	6.5 (2)	12.3 (9)	12.0 (6)	11.5 (54)	14.5 (82)	0.51
No	86.3 (504)	88.0 (534)		88.4 (534)	85.9 (504)		88.3 (356)	86.8 (343)	86.3 (339)		93.5 (29)	87.7 (64)	88.0 (44)	88.5 (416)	85.5 (485)	
Wasting																
Yes	2.8 (16)	4.1 (25)	0.20	3.3 (20)	3.6 (21)	0.82	3.2 (13)	4.3 (17)	2.8 (11)	0.49	3.2 (1)	2.7 (2)	2.0 (1)	4.1 (19)	3.2 (18)	0.90
No	97.2 (565)	95.9 (581)		96.7 (580)	96.4 (566)		96.8 (390)	95.7 (376)	97.2 (380)		96.8 (30)	97.3 (71)	98.0 (49)	95.9 (448)	96.8 (548)	

TABLE 6
Poisson Regression Results for Death

	Univariate		Multivariate˅	
	Mean Estimate (95% CI)	P-Value	Mean Estimate (95% CI)	P-Value
Parasitemia				
Super-High†	0.12 (0.05,0.29)	**<.0001**	0.12 (0.05,0.30)	**<.0001**
High^	0.11 (0.04,0.28)	**<.0001**	0.11 (0.04,0.28)	**<.0001**
Medium+	0.10 (0.01,0.79)	**0.03**	0.10 (0.01,0.80)	**0.03**
Low*	0.43 (0.16,1.18)	0.10	0.42 (0.15,1.15)	0.09
None	referent		referent	
Age				
Infant	1.87 (0.95,3.67)	0.07	1.55 (0.78,3.07)	0.21
Child	referent		referent	
Sex				
Female	0.74 (0.39,1.42)	0.36	0.68 (0.35,1.33)	0.26
Male	referent		referent	
Study Arm				
RTS,S/AS01E with booster	0.94 (0.41,2.12)	0.87	0.67 (0.29,1.56)	0.35
RTS,S/AS01E without booster	1.78 (0.54,2.55)	0.68	1.02 (0.47,2.23)	0.95
Comparator	referent		referent	

*1/μL -49,999/μL
+50,000/μL -199,999/μL
^ 200,000/μL-499,999/μL
† ≥500,000/μL
˅Multivariate model includes parasitemia, age, sex, and study arm

TABLE 7
Poisson Regression Results for Severe Anemia

	Univariate		Multivariate˘	
	Mean Estimate (95% CI)	P-Value	Mean Estimate (95% CI)	P-Value
Parasitemia				
Super-High†	2.21 (0.30,16.26)	0.44	2.27 (0.31,16.83)	0.42
High^	1.26 (0.16,9.63)	0.82	1.24 (0.16,9.53)	0.84
Medium+	0.79 (0.05,12.60)	0.87	0.79 (0.05,12.64)	0.87
Low*	0.98 (0.09,10.80)	0.99	0.95 (0.09,10.44)	0.96
None	referent		referent	
Age				
Infant	1.40 (0.77,2.57)	0.27	2.52 (1.20,5.32)	**0.02**
Child	referent		referent	
Sex				
Female	0.70 (0.38,1.28)	0.25	0.72 (0.39,1.33)	0.30
Male	referent		referent	
Study Arm				
RTS,S/AS01E with booster	1.02 (0.47,2.20)	0.96	1.03 (0.48,2.24)	0.93
RTS,S/AS01E without booster	1.32 (0.64,2.72)	0.45	1.31 (0.63,2.70)	0.47
Comparator	referent		referent	

*1/μL -49,999/μL
+50,000/μL -199,999/μL
^ 200,000/μL-499,999/μL
† ≥500,000/μL
˘Multivariate model includes parasitemia, age, sex, and study arm

TABLE 8
Poisson Regression Results for Severe Malaria

	Univariate		Multivariate˅	
	Mean Estimate (95% CI)	P-Value	Mean Estimate (95% CI)	P-Value
Parasitemia				
Super-High†	7.39 (2.35,23.23)	**0.001**	7.45 (2.37,23.45)	**0.001**
High^	4.62 (1.45,14.66)	**0.01**	4.62 (1.45,14.70)	**0.01**
Medium+	2.68 (0.64,11.22)	0.18	2.72 (0.65,11.40)	0.17
Low*	referent			
Age				
Infant	0.88 (0.67,1.16)	0.37	0.94 (0.71,1.23)	0.65
Child	referent		referent	
Sex				
Female	0.83 (0.63,1.09)	0.18	0.80 (0.61,1.05)	0.11
Male	referent		referent	
Study Arm				
RTS,S/AS01E with booster	0.83 (0.59,1.16)	0.28	0.91 (0.65,1.28)	0.58
RTS,S/AS01E without booster	0.94 (0.68,1.30)	0.72	0.99 (0.71,1.37)	0.95
Comparator	referent		referent	

*1/μL -49,999/μL
+50,000/μL -199,999/μL
^ 200,000/μL-499,999/μL
† ≥500,000/μL
˅Multivariate model includes parasitemia, age, sex, and study arm
Those with a parasite density of zero were excluded from the model, as no one would have severe malaria.

TABLE 9
Poisson Regression Results for Stunting

	Univariate		Multivariate˅	
	Mean Estimate (95% CI)	P-Value	Mean Estimate (95% CI)	P-Value
Parasitemia				
Super-High†	1.64 (0.73,3.70)	0.23	1.38 (0.61,3.13)	0.44
High^	1.71 (0.76,3.87)	0.20	1.32 (0.58,3.01)	0.50
Medium+	1.67 (0.65,4.26)	0.29	1.30 (0.51,3.34)	0.58
Low*	1.71 (0.70,4.18)	0.24	1.43 (0.58,3.51)	0.43
None	referent			
Age				
Infant	1.67 (1.32,1.99)	**<.0001**	2.08 (1.68,2.58)	**<.0001**
Child	referent			
Sex				
Female	0.78 (0.63,0.95)	**0.01**	0.88 (0.72,1.09)	0.24
Male	referent			
Study Arm				
RTS,S/AS01E with booster	1.00 (0.78,1.28)	0.98	0.96 (0.75,1.24)	0.78
RTS,S/AS01E without booster	1.06 (0.83,1.36)	0.63	1.06 (0.83,1.36)	0.63
Comparator	referent			

*1/μL -49,999/μL
+50,000/μL -199,999/μL
^ 200,000/μL-499,999/μL
† ≥500,000/μL
˅Multivariate model includes parasitemia, age, sex, and study arm

TABLE 10
Poisson Regression Results for Underweight

	Univariate		Multivariate˘	
	Mean Estimate (95% CI)	P-Value	Mean Estimate (95% CI)	P-Value
Parasitemia				
Super-High†	2.27 (0.56,9.22)	0.25	1.62 (0.40,6.60)	0.50
High^	1.80 (0.44,7.39)	0.41	1.23 (0.30,5.06)	0.78
Medium+	1.88 (0.38,9.30)	0.44	1.53 (0.31,7.59)	0.60
Low*	1.92 (0.42,8.91)	0.40	1.40 (0.30,6.49)	0.67
None	referent			
Age				
Infant	1.14 (0.83,1.56)	0.43	1.64 (1.18,2.28)	**0.003**
Child	referent			
Sex				
Female	0.82 (0.60,1.13)	0.22	0.99 (0.72,1.37)	0.97
Male	referent			
Study Arm				
RTS,S/AS01E with booster	0.85 (0.57,1.26)	0.41	0.89 (0.60,1.32)	0.55
RTS,S/AS01E without booster	0.96 (0.66,1.40)	0.83	0.88 (0.60,1.29)	0.51
Comparator	referent			

*1/μL -49,999/μL
+50,000/μL -199,999/μL
^ 200,000/μL-499,999/μL
† ≥500,000/μL
˘Multivariate model includes parasitemia, age, sex, and study arm

TABLE 11
Poisson Regression Results for Wasting

	Univariate		Multivariateˇ	
	Mean Estimate (95% CI)	P-Value	Mean Estimate (95% CI)	P-Value
Parasitemia				
Super-High†	1.00 (0.13,7.47)	1.00	0.88 (0.12,6.64)	0.90
High^	1.28 (0.17,9.54)	0.81	1.00 (0.13,7.59)	1.00
Medium+	0.63 (0.04,10.00)	0.74	0.62 (0.04,9.95)	0.74
Low*	0.86 (0.08,9.43)	0.90	0.73 (0.07,8.04)	0.79
None	referent			
Age				
Infant	0.67 (0.36,1.25)	0.21	0.91 (0.48,1.71)	0.77
Child	referent			
Sex				
Female	0.93 (0.51,1.72)	0.83	1.16 (0.61,2.18)	0.65
Male	referent			
Study Arm				
RTS,S/AS01E with booster	1.15 (0.51,2.56)	0.74	1.33 (0.59,2.98)	0.49
RTS,S/AS01E without booster	1.54 (0.72,3.29)	0.26	1.30 (0.60,2.83)	0.50
Comparator	referent			

*1/μL -49,999/μL
+50,000/μL -199,999/μL
^ 200,000/μL-499,999/μL
† ≥500,000/μL
ˇMultivariate model includes parasitemia, age, sex, and study arm

APPENDICES

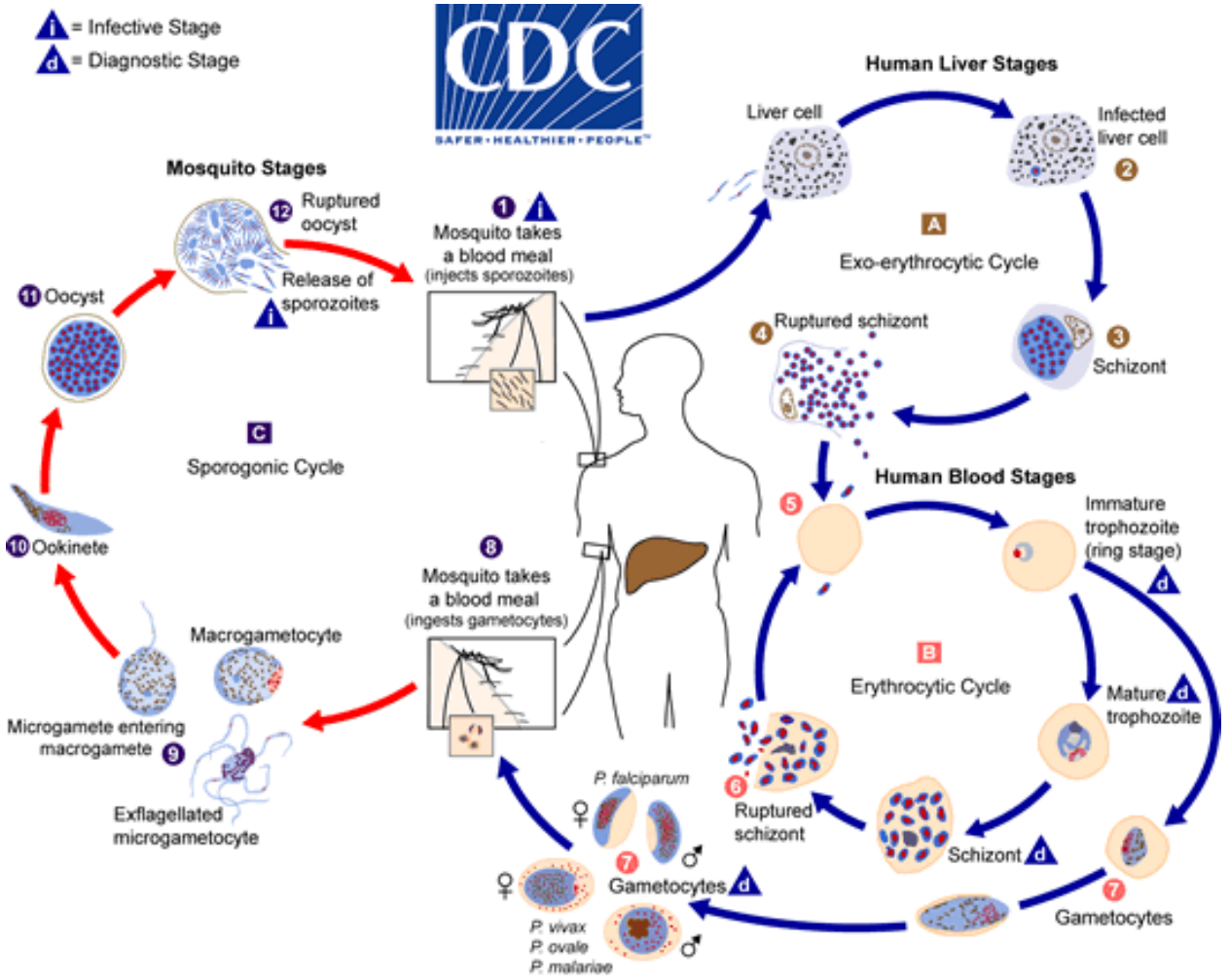

Figure 1. Life cycle of malaria in both the mosquito and human stages. (Centers for Disease Control and Prevention, 2016a)