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Association between malaria and nutrition in Rwanda

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Dedication

This work is dedicated to the memory of my beloved mother, whose life was tragically cut short by malaria when I was just six years old. That loss—so personal, so preventable—planted the seed of my lifelong commitment to fighting this devastating disease. Though I never contracted malaria myself, growing up in a malaria-endemic region exposed me to its relentless toll and instilled in me a lasting determination to make a difference.

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In all things, **in God we trust**.

Summary

Undernutrition and malaria remain major global public health concerns, particularly in low and middle-income countries. Both concerns have consistently remained one of the leading causes of morbidity and mortality, particularly in sub-Saharan Africa, for decades. Malaria infection and outcomes are partly influenced by host immune responses, which are themselves shaped by various factors. In recent years, the status in certain nutrients, specifically micronutrients and macronutrients, has been increasingly recognized for its role in modulating immune function.

Malnutrition, specifically, has been demonstrated to compromise immunological responses and affect susceptibility to malaria. Although nutrient composition is influenced by geographic location, and dietary practices, a significant gap persists in understanding how nutrient intake, assessed through food consumption rather than biochemical markers, influences malaria in Rwanda. Comprehensive Nutrient Gap Assessment and evaluations of biofortified food consumption have provided valuable insights into dietary nutrient gaps in Rwanda, however any study directly investigated the association between food-based nutrient intake and malaria outcomes. Therefore, our project comprised two studies aimed at examining malaria and nutrient intake relationships while accounting for multiple confounding factors in the Rwandan context.

The first study explored the relationship between nutritional status and asymptomatic malaria among children under five years of age in Rwanda, a topic that has received limited attention in local research. Using Demographic and Health Survey (DHS) data and applying both unadjusted and adjusted odds ratios, the analysis identified stunting, underweight, and low household wealth index as significant risk factors for asymptomatic malaria. These results highlight the urgent need for integrated strategies targeting both nutrition and malaria prevention in young children living in endemic areas. Furthermore, the study emphasizes the importance of exploring children's actual dietary intake to better understand how nutrient consumption influences malaria risk and outcomes.

The second study, a case-control study, investigated the relationship between malaria incidence and nutritional status, with a particular focus on stunting and dietary intake across nine malaria-endemic districts in Rwanda. Data were collected in two phases from November 2021 to September 2022 and from May to December 2023 across all four provinces and Kigali City. Using regression models and odds ratios, the study assessed associations between malaria, macronutrient and micronutrient intake, and stunting. Results revealed that dietary patterns were heavily dominated by starchy staples (56.7%), which, while rich in macronutrients, lacked essential micronutrients. Consequently, there was a high prevalence of micronutrient deficiencies in the studied population, including vitamin A (80.4%), vitamin B2 (76.3%), vitamin B12 (76.7%), calcium (82.5%), zinc (71.7%), and selenium (54.4%). Unadjusted analysis showed significant associations between selenium and vitamin E intake and malaria infection. After adjusting for covariates, deficiencies in vitamin E and iron were positively associated with higher odds of malaria, while increasing age, female sex, and selenium deficiency were linked to reduced odds ratio for malaria infection.

The overall study supported that deficiencies in vitamin E, iron, and selenium are linked to either higher or lower risk of malaria infection, highlighting the critical role of nutrition in malaria outcomes. To reduce this risk, there is a need for targeted actions that promote diverse diets, routine assessment of nutritional status, and the development of a national food composition table to guide effective nutrition policies and interventions.

Key words: Malaria Nutrition nutritional status asymptomatic malaria RDHS micronutrients malnutrition in children under five cross sectional study case control Rwanda

List of abbreviations

ACTs	Artemisinin Combined Treatment
AM	Acute malnutrition
ANC	Antenatal Care
AO	Odds Ratio
AOR	Adjusted Odds Ratio
BMI	Body Mass Index
BMR	Basal Metabolic Rate
CFSVA	Comprehensive Food Security Vulnerability Analysis
CHW	Community Health Workers
CI	Confidence Interval
DHS	Demographic and Health Surveys
EE	Energy Expenditure
EI	Energy Intake
EPI	Expanded Programme on Immunization
FCT	Food Composition Table
FFQ	Food Frequency Questionnaire
G6PD	Glucose 6 Phosphate Dehydrogenase
GVIF	Generalized Variance Inflation Factor
HAZ	Height or length-for-Age Z-score
IL	Interleukin
IPTp	Intermittent Preventive Treatment in pregnant women
IPTi	Intermittent Preventive Treatement in infant
IRS	Indoor Residual Spraying
ITNs	Insecticide Treated Nets
LLIN	Long Lasting Insecticide Net
LLINs	Long-Lasting Insecticidal Nets
NCDA	National Child Development Agency

PAL	Physical Activity Level
RBC	Red Blood Cell
RDHS	Rwanda Demographic Health Survey
RDT	Rapid Diagnostic Test
SDGc	Sustainable Development Goals
SMC	Seasonal Malaria Chemoprophylaxis
SP	Sulfadoxine Pyrimethamine
SA	South Africa
SSA	Sub-Saharan Africa
TNF	Tumor Necrosis Factor
TTT	Test, Treat, and Track
UNICEF	United Nations International Children's Emergency Fund
VIF	Variance Inflation Factor
WHO	World Health Organization
WAZ	Weight-for-Age Z-score
WHZ	Weight-for-Height or length Z-score

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INTRODUCTION

Malaria

Malaria is a vector-borne, non-zoonotic disease caused by the *Plasmodium* species, which is transmitted by an infected female anopheles [1,2]. The malaria burden remains a significant and escalating public health concern, with approximately 50% of the world's population exposed to infection risk, particularly in endemic regions. The latest 2024 WHO world report states 263 million malaria cases and 597,000 deaths, with a mortality rate of 13.7 per 100,000 [3]. Sub-Saharan Africa accounted for up to 94% of malaria cases and 95% of deaths, with 75% of these deaths happening in children under five among all malaria fatalities, and this disparity remained similar from the 2023 prior year report [3,4].

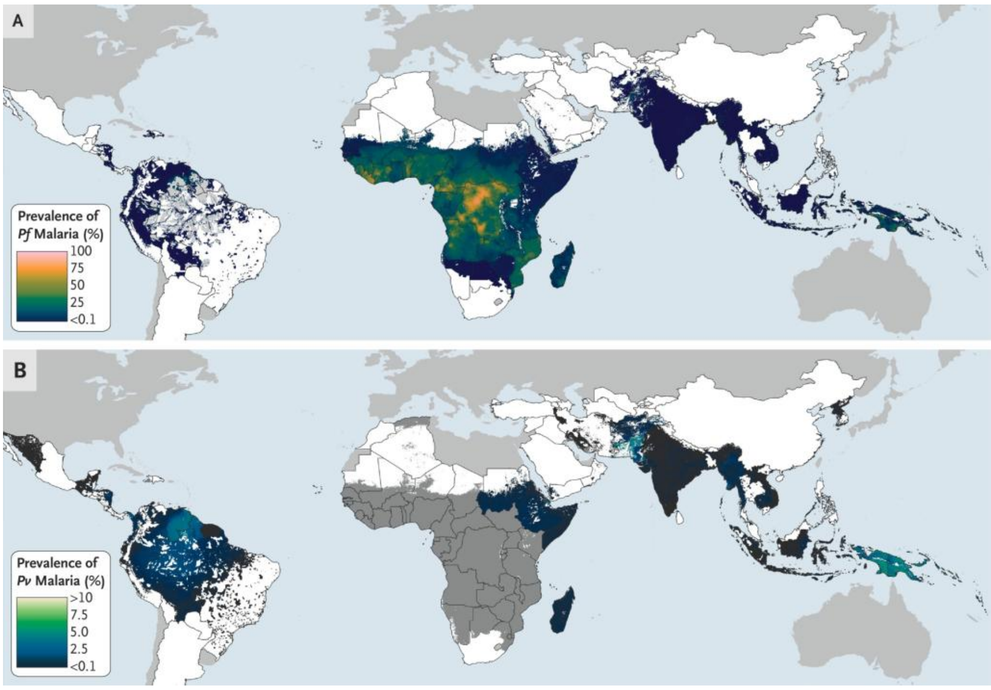


Figure 1. Geographic Distribution of Malaria *Plasmodium* species in 2022, panel A=*Plasmodium falciparum* B=*Plasmodium vivax* [1].

The five *Plasmodium* species (*P.falciparum, P.malariae, P.ovale, P. vivax, and P.knowlesi*) are known to cause malaria infection. Moreover, malaria distribution and severity are also proportionally correlated with the distribution of *Plasmodium* species; the most predominant is *Plasmodium falciparum* (Fig. 1), which causes severe cases and deaths. The latest is mostly observed in Sub-Saharan Africa, which justifies the high morbidity and mortality. The P. *vivax* and P. *ovale* exhibit a dormant liver stage, which poses significant challenges to disease control and eradication due to its reactivation weeks, months, and years following its primary episode [5].

Parasites pathogenesis

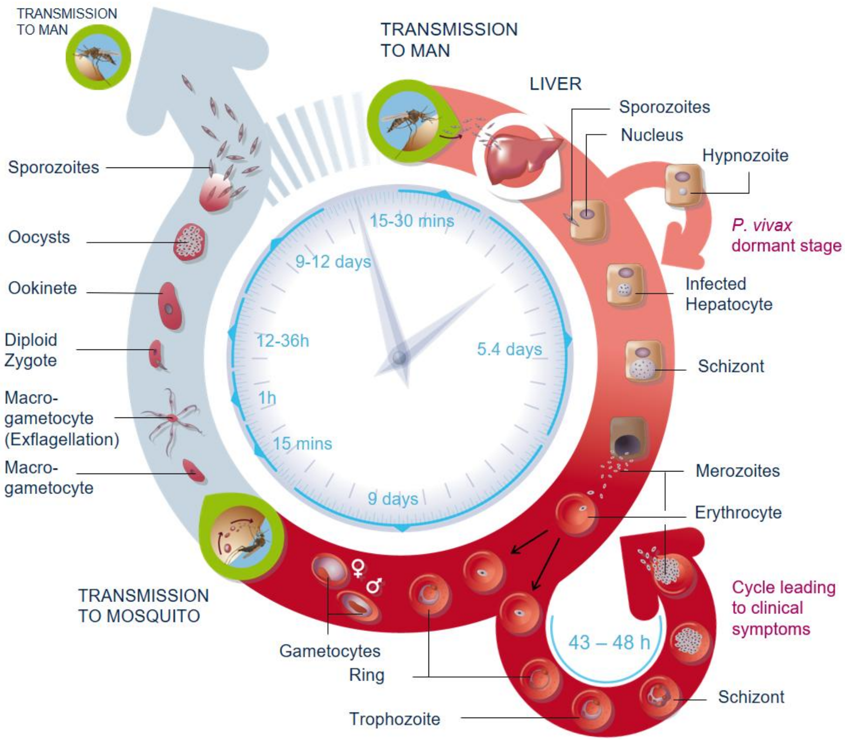


Figure 2. Lifecycle of the malaria parasite [6].

Plasmodium species infecting humans share a similar lifecycle with the same entry point through the blood, followed by liver and further proliferation in the red blood cell [2]. Anopheles females during a blood meal to mature their eggs, ingest sporozoites into the human host, then sporozoites multiply asexually within the liver, and this latent phase varies between 7-14 days [5].Duringthis phase, there are no clinical symptoms. Thereafter, the maturation of schizonts ruptures to merozoites, the latter's invade the red blood cells, the ring stage is characterized by clinical symptoms, specifically fever, following toxin release and pro-inflammatory responses such as TNF-α and IL-1 increase [7]. In some cases, the merozoites replicate in a sexual form (gametocytes), which are subsequently ingested via mosquito bites. Thereafter, the ingested gametocytes progress through further phases in the mosquito gut until the development of sporozoites in the salivary gland of the mosquito [8]. Understanding parasite physiopathology is crucial for efficiently targeting all species at once, using drugs with active action on gametocytes to cut short the transmission cycle [9].

Antimalarial Drug use, Artemether-based combination therapies have been widely used for several decades as a frontline treatment for *Plasmodium falciparum* malaria. However, emerging resistance to artemisinin and its derivatives has become a growing concern. The resistance is characterized by delayed parasite clearance, particularly at the ring stage of the parasite's lifecycle, despite treatment with artemisinin compounds.[1]

Public health strategy for malaria

Recent World Health Organization (WHO) malaria guidelines and standards for malaria control implement a strongly evidence-based framework, with robust recommendations targeting the parasite, the vector, and the human host (Fig. 3). As of 2024, the WHO continues to pursue its long-term vision of drastically reducing the global malaria burden by 2030. The organization has reaffirmed its strategic targets initially set in the *Global Technical Strategy for Malaria 2016–2030* [10], which include:

Achieving a 90% reduction in malaria case incidence compared to 2015 levels,

- Decreasing malaria-related mortality by at least 90% relative to 2015,
- Eliminating malaria in a minimum of 35 countries where the disease was endemic in 2015,
- Preventing the reintroduction of malaria in countries that have already achieved elimination.

To reach these milestones, WHO launched an updated operational strategy in 2024 focused on four main pillars [11]:

- Establishing evidence-based norms and standards and guidance to support national malaria programs;
- Advancing innovation and introducing new tools, such as improved insecticides, diagnostics, and vaccines;
- Enhancing surveillance systems through strategic information for impact to monitor trends better and inform targeted interventions; and
- Providing leadership and fostering partnerships to coordinate global and regional responses.

It has become evident that the recommended evidence-based malaria prevention strategies include vaccines (RTS, S and R21), Seasonal Malaria Chemoprevention (SMC), and Intermittent Preventive Treatments for infants (IPTi) and pregnant women (IPTp) for use in moderate to high-transmission settings across sub-Saharan Africa. However, the applicability of these interventions depends on local transmission intensity, drug resistance patterns, and epidemiological trends. In regions with low malaria endemicity, particularly among children under 3 years old, implementing malaria vaccines such as RTS, S/AS01, and R21/Matrix-M warrants careful consideration[12]. While these vaccines have demonstrated moderate efficacy in areas with high transmission, their effectiveness in low-endemic settings may be reduced due to less frequent exposure to the malaria parasite. This reduction in exposure limits the immunological pressure needed to generate strong protective immunity, potentially rendering the vaccines less effective [4,13].

In contrast, Long Lasting Insecticide Treated Nets (LLINs) remain a highly cost-effective and widely applicable intervention in low-endemic areas, as they continuously provide protection from malaria vector bites, regardless of transmission intensity. LLINs are a proven method for reducing malaria incidence, with a well-established public health benefit, and their impact is not dependent on the frequency of malaria exposure [12].

Furthermore, Rwanda’s experience highlights the importance of adapting malaria prevention strategies to local epidemiological patterns. The country has focused on case management and vector control through LLIN distribution with new generation nets and artemisinin-based combination therapies (ACTs), while refraining from the large-scale implementation of vaccines like RTS, S, and R21. This strategic approach aligns with the fact that the malaria burden in children under 3 years remains low; thus, the cost-effectiveness of introducing vaccines may not be justified. Additionally, Rwanda discontinued the use of IPTp (sulfadoxine-pyrimetamine) in 2008 due to the emergence of SP resistance, further reinforcing the need for targeted interventions rather than blanket vaccination policies [12]. Since 2019, substantial and blanket targeted IRS in 12 highly endemic districts plus focal spraying in 5 districts bordering wih high endemic [14], with universal LLINs and a 2-year replacement, Antenatal Care (ANC), and Expanded Programme on Immunization (EPI) for new family members, have been implemented [15]. These integrated preventive measures, along with focal larviciding in Kigali, have shown a tremendous reduction in malaria cases despite insecticide resistance and antimalarial partial resistance to artemisinin derivatives. Partial resistance has been confirmed by delayed clearance of the parasite [16]. Therefore, Rwanda should adopt multiple first-line ACTs to confuse the parasite and delay resistance, possibly to maintain the susceptibility of partner drugs [17]. Besides, this presents an opportunity to target the parasites and administer a single dose of primaquine to eliminate all parasitic stages and prevent relapse caused by dormant liver forms in *Plasmodium ovale* and *Plasmodium vivax* infections [15]. The World Health Organisation (WHO) recommends a dosage of 1 mg/kg/day for 7 days in patients with at least 70% glucose-6-dehydrogenase (G6PD) deficiency enzyme activity confirmed by diagnostic testing [1].

Malaria Epidemiology: a framework for eradication

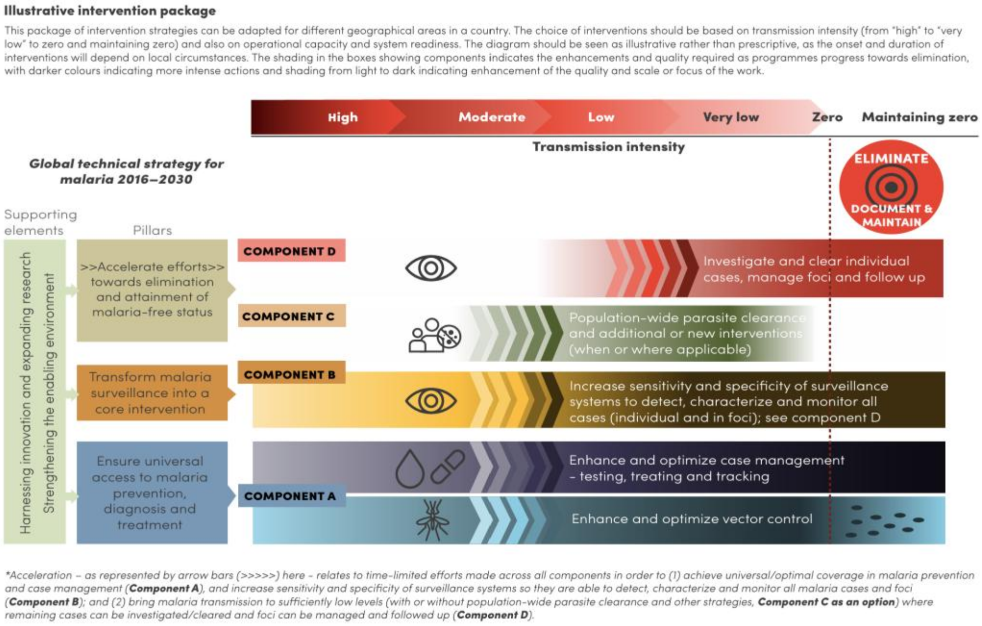


Figure 3. Framework for malaria elimination, illustrative intervention package [13].

To guide countries in their elimination efforts. This framework emphasizes tailored approaches based on local transmission dynamics and local health system settings and capacities. Core strategies include providing quality universal access to diagnosis and treatment, and sustaining vector control using dual insecticides targeting exophilic and exophagic anopheles mosquitoes with larvicides [18]. Notably, it highlights the importance of targeting all malaria species concurrently to enhance the efficiency of elimination efforts and reduce the risk of perennial transmission. Additionally, implementing a comprehensive tracking system as a primary intervention to monitor progress is critical [18,19].

Nutrition

Globally, the 2023 State of Food Security and Nutrition Report emphasizes the rising issue of food insecurity and nutrition. It affects around 30% of the worldwide population. However, almost 60% of the food-insecure population is disproportionately distributed in developing countries, particularly in sub-Saharan Africa and Asia [19,20]. Rwanda has made progress in food security improvement, but challenges in rural areas remain high. Inadequate food security remains a global concern and has direct consequences on nutrition and health outcomes, including stunting [19,21].

Anthropometric indicators are physical measurements that reflect body composition, growth, and nutrition status, including BMI, stunting , eutrophy and wasting.

Wordlwide prevalence of malnutrition

The triple burden of malnutrition includes undernutrition, overweight plus obesity, and micronutrient deficiencies, often referred to as hidden hunger [22]. Undernutrition continues to be a major global health issue, particularly among children under five in low and middle-income countries. Recent global data from 2023 reveals that approximately 148.1 million children under 5 are affected by stunting (22.3% prevalence), 45 million children suffer from wasting (6.8% prevalence), and 37 million children are overweight (5.6% prevalence) [23].Moreover, the maps below show how sub-Saharan Africa and South East Asia are highly affected regions by stunting , with the top 2 prevalence ranges: 30-39.9% and 40% or higher (figure 4).

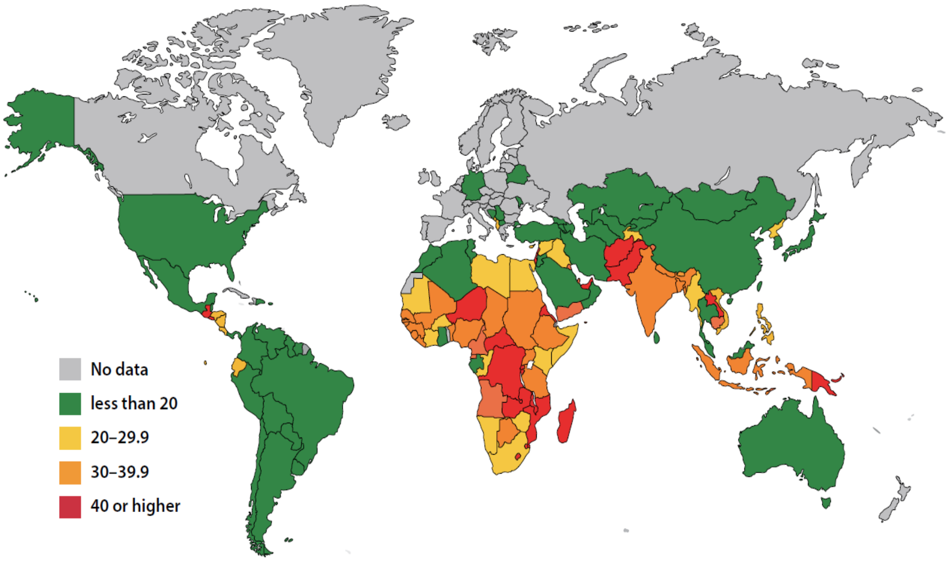


Figure 4. Age-standardized prevalence (%) of stunting in children under 5 years of age, comparable estimates, latest prevalence available [24]

Stunting impairs physical growth, neurocognitive development, and long-term socioeconomic development in adulthood [25]. A longitudinal study in Indonesia further indicates that childhood stunting is associated with reduced to lower educational attainment and cognitive abilities in adulthood, highlighting its long-term effects on human capital growth and development [17].

Stunting, characterized by low height-for-age, results from chronic undernutrition during early childhood and has major consequences. It increases the risk of infections due to weakened immunity and can lead to long-term noncommunicable health issues like cardiovascular diseases and metabolic disorders (diabetes) in adulthood [27] [28].

Economically, it reduces adult productivity, hindering national economic growth and perpetuating the cycle of poverty [29]. Stunted children face higher mortality risks, particularly from preventable causes such as malaria [30]. Additionally, stunting exacerbates social inequalities, disproportionately affecting disadvantaged populations. Early interventions, especially during pregnancy and the first two years of life, are crucial to prevent stunting and ensure healthier, more productive future generations [31].

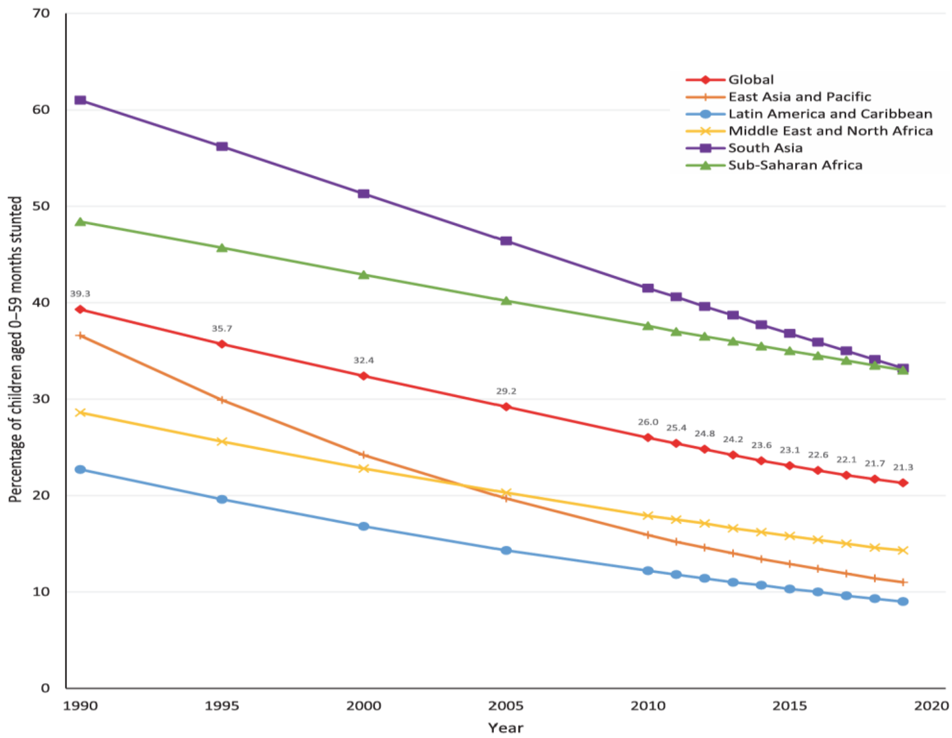


Figure 5. Stunting in childhood: an overview of global burden, trends, determinants, and drivers of decline [32].

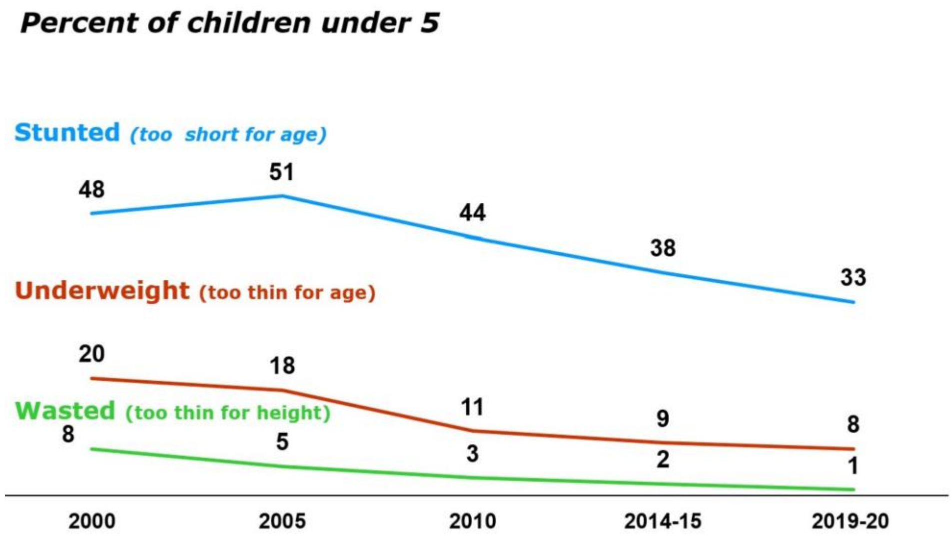
Over the past three decades, global stunting rates have steadily declined, with notable progress across several regions. From 1990 to 2020, global stunting prevalence has declined significantly, with an 18.1% decrease globally. However, regional disparities persist:

- South Asia: Stunting prevalence has declined, but the region continues to experience high stunting rates due to persistent challenges like food insecurity and poor healthcare.
- Sub-Saharan Africa: This region has also seen a decline in stunting prevalence, though progress has been slower than in other regions.

Despite the global reduction in stunting, regional inequalities remain, demonstrating the necessity for sustained, targeted interventions to address the underlying causes of malnutrition, particularly in vulnerable populations[27,32].

The same trends of stunting in Rwanda have been observed despite the country's efforts in implementing a multisectoral two-year plan for malnutrition elimination, focusing on six principal pillars targeting poverty reduction and food security [21,33,34]. Current nutritional data shows that stunting exhibits a slow decline over the decade, from 43% in 2012 to 22.4% in 2021 [35]**.** The same report emphasizes an increase in acute malnutrition(AM), comparing DHS 2020 to CFSVA 2022 reports, AM doubled from 1.1% to 2.4% in one year, with serious disparities in rural areas and districts. The western and southern provinces were mainly affected by both food insecurity and malnutrition [35,36].

Local nutrition context in Rwanda



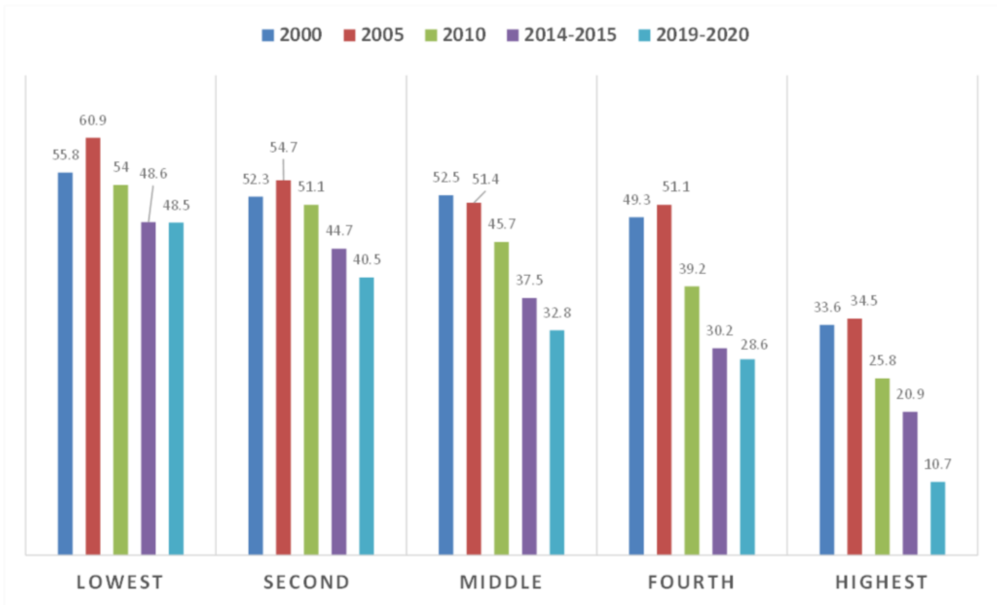


Figure 6. Nutrition trends over time per DHS 2000-2020 (A) and Stunting inequalities accros wealth index (B)

In Rwanda between 2000 and 2019–2020, the relative reduction in stunting prevalence varied markedly across wealth quintiles, with a 13% reduction observed in the lowest quintile compared to a 68% reduction in the highest. This highlights that lower socioeconomic groups have not benefited equally from the national progress in reducing stunting. Similarly, a persistent disparity was observed between urban and rural children, with an absolute difference of 18% in 2000 and 16% in 2019–2020. Despite these differences, growth faltering occurred consistently across all wealth quintiles within the first 36 months of life[36].

1.1.1. *FOOD COMPOSITION*

In Rwanda, the most consumed foods are composed of starchy foods, legumes, and oil, while animal protein sources and fiber are the least consumed [35]**.**

In a balanced diet, carbohydrates, proteins, and fats should contribute 40–70%, 15–20%, and 25–30% of total daily energy intake, respectively [37]. Moreover, daily fiber intake should be at least 25 g a day. A balanced diet should include beneficial foods such as a minimum intake of 400g of fruits and vegetables a day, poultry, , and fiber-rich foods [38,39]. Whole grains should be preferred for its health benefits, dairy products, especially low-fat wich provides micronutrientssuch as calcium and vitamin D for bone health. Quality of oils should be consist of unsaturated fats mostly found in vegetable oils, nuts, and fatty fish, while saturated and trans fats should be limited. While alcohol consumption, shoul be reduce to prevent health risks. Overall, dietary diversity across family food is very important for a balanced diets with nutritional needs to support health conditions [39]

In a healthy women with moderate physical activity, the recommended daily calorie intake is 1800–2000 kcal and should not drop below 1500 kcal to avoid an imbalanced diet and nutrient deficiencies, which may lead to long-term undernutrition. For a healthy man with moderate physical activity, the recommended energy intake is approximately 2,400 to 2,600 kcal per day [40]. It is also important to consider foods with a low glycemic index (GI) to help maintain healthy blood sugar levels [41,42]. Additionally, diets with a higher protein to carbohydrate ratio has been associated with a reduction of abdominal fat, which improve the level of blood sugar, and had a beneficial effects on component ofmetabolic syndrome [43].

Global strategy to mitigate nutrition challenges encompasses: Nutrition before, during, and after pregnancy, focusing on the 1000 Days programme . The first 1000 days of life are considered a critical step in human development. From conception to the second birthday of a child, this period represents a major opportunity for improving nutrition and ensuring optimal growth and development. Malnutrition during this period, particularly undernutrition, can have irreversible consequences, leading to stunting, increasing vulnerability to infections and cognitive impairement as up to 80% of brain development occurs [44]. Proper nutrition before, during, and after pregnancy plays a vital role in preventing these issues and promoting better maternal and child health.

Before pregnancy, nutrition lays the foundation for a healthy conception. Indeed adequate intake of folic acid, iron, vitamin D, and iodine is essential to support both maternal health and fetal development (Rasmussen, 2023). Women of reproductive age should focus on achieving a healthy weight, receiving appropriate micronutrient supplementation, and following a balanced diet to prepare for pregnancy. These interventions can prevent deficiencies that may affect pregnancy outcomes, such as low birth weight or developmental defects [30]**.**

During pregnancy, nutrition is vital for the development of the fetus. A balanced diet with sufficient energy intake per day, including adequate iron, calcium, folic acid, protein, and iodine to support fetal growth and reduces the risk of pregnancy complications such as preterm birth and anemia, mainly micronutrient boost immune system [45]**,** World Health Organization, 2023. Supplementation with iron and folic acid is particularly critical to prevent anemia and reduce the risk of neural tube defects. Calcium supplementation can also help prevent preeclampsia, a pregnancy-related hypertensive disorder (Rasmussen, 2023).

Postpartum nutrition is also crucial for maternal recovery and breastfeeding. Mothers should focus on iron, calcium, and protein intake to rebuild strength and ensure an adequate milk supply for breastfeeding (World Health Organization, 2023). Exclusive breastfeeding is recommended for the first 6 months, followed by continued breastfeeding alongside appropriate complementary (UNICEF, 2022). Proper nutrition supports both health mother and the linear growth of infant, reducing the risks of malnutrition in early childhood.

Malaria and Nutrition

Malaria and stunting are pressing global health issues that impact child mortality, growth, and future economic productivity. These challenges are directly associated with three first Sustainable Development Goals (SDGs), including poverty reduction (SDG 1), hunger eradication (SDG 2), and fostering good health and well-being (SDG 3)[46]. They also affect SDGs aimed at decreasing inequalities (SDG 10), creating sustainable communities (SDG 11), and advancing decent work and economic growth (SDG 8)[47]. The updated WHO Global Technical Strategy for Malaria 2016-2030 sets targets to lower malaria incidence, while WHO and UNICEF have set a goal to decrease stunting by 40% (with 2012 as the reference year) and keep wasting below 5% by 2025. Yet, the latest joint child malnutrition estimates 2023 edition report that 75% of world children are in countries which are off track to achieve the 2030 stunting SDG goal [24,48]. Thus, the level of connectivity between maternal and child nutrition and their long-term health outcomes should be maintained in order to achieve set targets. These global strategies highlight the urgent call for coordinated efforts to accelerate action for the mitigation of the underlying causes of health inequities and promote sustainable development, particularly for vulnerable populations [49,50].

Malaria and nutrition have been studied since 1898. Hence, the Indian Famine Commission stated that malaria deaths were mostly recorded in undernourished children. From 1950, a few quantitative studies were initiated to complement the previous ones based on qualitative data. However, some studies have reported no association between malaria and nutritional conditions. In some cases, a protective effect was noted in severe malaria and death in malnourished children**.** The editors emphasize that quality and diversified food were considered to support immunity and control disease in the 19^{th} century, exhibit that rich caloric food was beneficial with the recognition of vitamin deficiency diseases such as Rickets, Beriberi, and night sight blindness induced by vitamin D, vitamin B2 and vitamin A deficiencies, respectively [51].

Malaria and nutrition tend to have a bidirectional relationship; undernutrition weakens immunity and increases susceptibility to infection, basically due to insufficient intake of calories, proteins, and relatively essential micronutrients such as vitamins and minerals [52]. On the other hand, infection activates immune cells to fight against diseases, and host metabolic functions are regulated by nutrition and gut microbiota [53]. Both macro and micro-nutrients participate in the production of energy through adenosine triphosphate by releasing or using the energy in human cells. Malaria parasites generate reactive oxygen species or reactive nitrogen species, known to increase the oxidative stress in the host, responsible for the inflammatory effect. This leads to malaria being an inflammatory and oxidative disease. Various antimalarial drugs target oxidative stress to limit parasite growth. Moreover, good nutrition with enough micronutrients with antioxidant action, such as minerals and vitamins, respectively, zinc, selenium, and vitamins A, C, and E.

The beneficial action of antioxidants is their ability to protect against chronic inflammation, which may be induced either by the parasite or the host response to the aggression caused by the parasite itself. For example: vitamin A coupled with the host energy are being used by the parasite for its metabolism and growth inside the liver, to invade the RBC the parasite uses retinoic acid the main active metabolite of vitamin A to destabilize the RBC cells [54]. Again vitamin A, iron, vitamins B9 and B12 are essentials nutrients for the erythropoiesis process which happen in the bone marrow for production and maturation of RBC.

Iron deficiency is primary anemia consequences in subsharan African countries caused by soil transmitted helminths, infectious diseases including malaria counting over 50% of anemia cases. To combat anemia deficiency , iron supplementation and biofortification foods are very critical intervention. However, Iron supplement has shown to increase malaria severity and worsen outcomes in children under five [55]. Besides it induces anemia in non iron deficiencies population. Moreover, evidence indicate that people with iron deficiency seems to be protective against malaria infections [56,57]. Over the past decade, Iron biofortified beans in general population and Iron supplementation for pregnant women have been implemented in low middle countries and malaria endemic areas including Rwanda. This policy support the reduction of iron deficiency through the production and consumption of more than 15 varieties of iron rich foods [58] as DHS report high levels of anemiaat 13% in 2020, in women of the reproductive age 15 to 49 years old [36]. The same report revealed a high number of anemia in children under five at 37% at national level. Strategies to control anemia should be taken with caution in both category through improved nutrition

Research methodology for food dietary intake

Accurate measurement of the dietary intakes allows researchers to understand the impact of individual diet effects on human health. Over decades, a variety of methods and technologies have been used, though their for validity and reliability have been challenged. In this thesis, two chapters highlight the use of routine nutritional data and the applicatipon of FFQ, emphasing the importance of converting consumed foods into corresponding nutrient intake data based on food composition table.

This thesis, primarly, explored the existing data and used secondary data analysis from three consecutive demographic health surveys up to the RDHS 2020. The study using unadjusted and adjusted odds ratios explored the relationship between nutritional status and asymptomatic malaria in children under five in Rwanda.

The second phase of this thesis investigated the relationship between malaria prevalence and nutritional status, focusing on stunting and dietary intake in the nine malaria-endemic districts in Rwanda (Fig.7). The malaria incidence decrease noted over years following sustained preventive interventions. The eastern and southern province are located in low altitude settings which is favourable for malaria transmission. The latest were most affected during the project design period, temporal variation was noted following substantial IRS and distribution of Duo insecticide nets coupled with universal home based management of fever for all at villages level by community health worker.

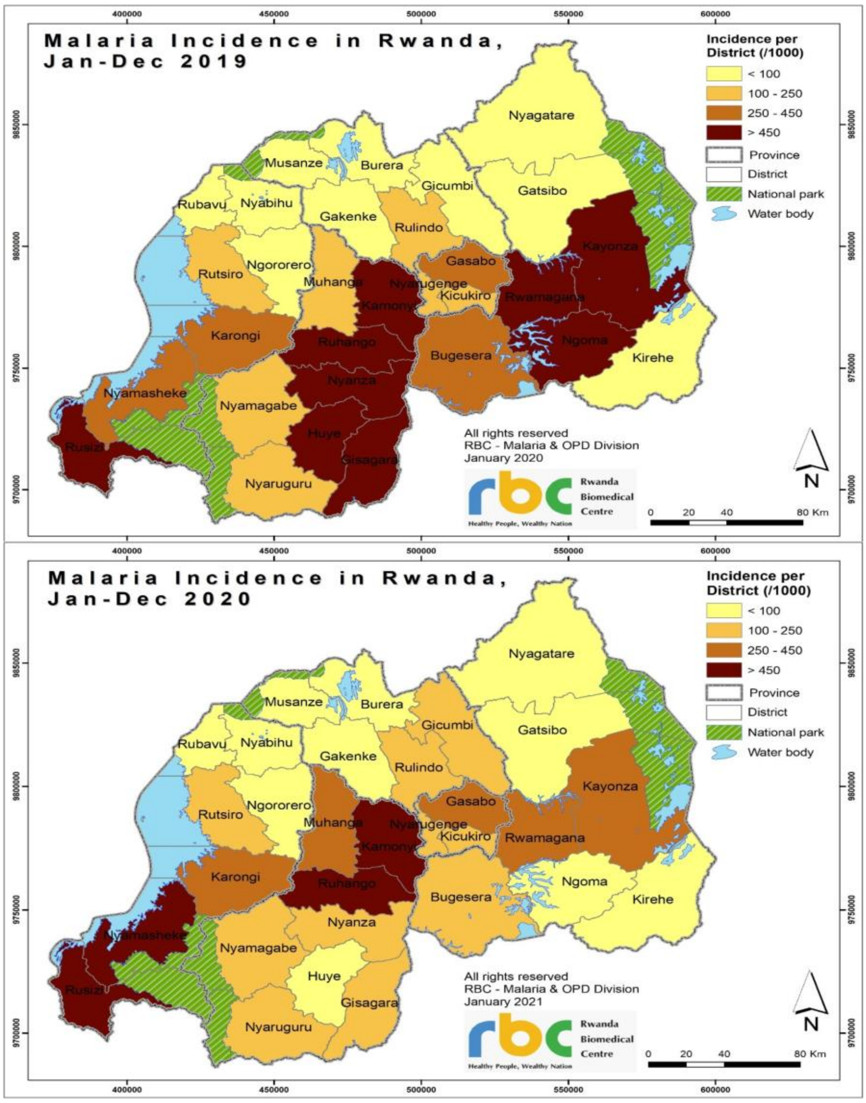


Figure 7: Rwanda Malaria Strategic plan 2020-2024 [59]

1.1.2. DESCRIPTION OF THE STUDY AREA AND SELECTION

Rwanda is a landlocked country located in the eastern region of Africa. The thesis outlined findings from the nine districts with different levels of endemicity. Districts were chosen based on malaria data in the initial phase, and based on malaria interventions, malaria case reduction was observed in many districts at different times. Based on the Rwanda Malaria annual report 2021-2022 [60], the first six districts were selected: Bugesera, Kamonyi, Ruhango, Nyamagabe, Nyamasheke, and Rusizi. The selection was based on the malaria morbidity and high incidence taking into account temporal changes in transmission trends and use of preventive measures indoor residual spraying, long lasting insecticide nets, extended community integrated vector control interventions and as well as the ease accessibility to selected location and proximity of Eastern, Southern, and Western regions, respectively. In the next phase additional 3 districts were added following the endemicity and their location covering the northern province(Gicumbi and Rulindo) and the Kigali City (Gasabo), which at that moment had the top 3 malaria endemic sectors [61].

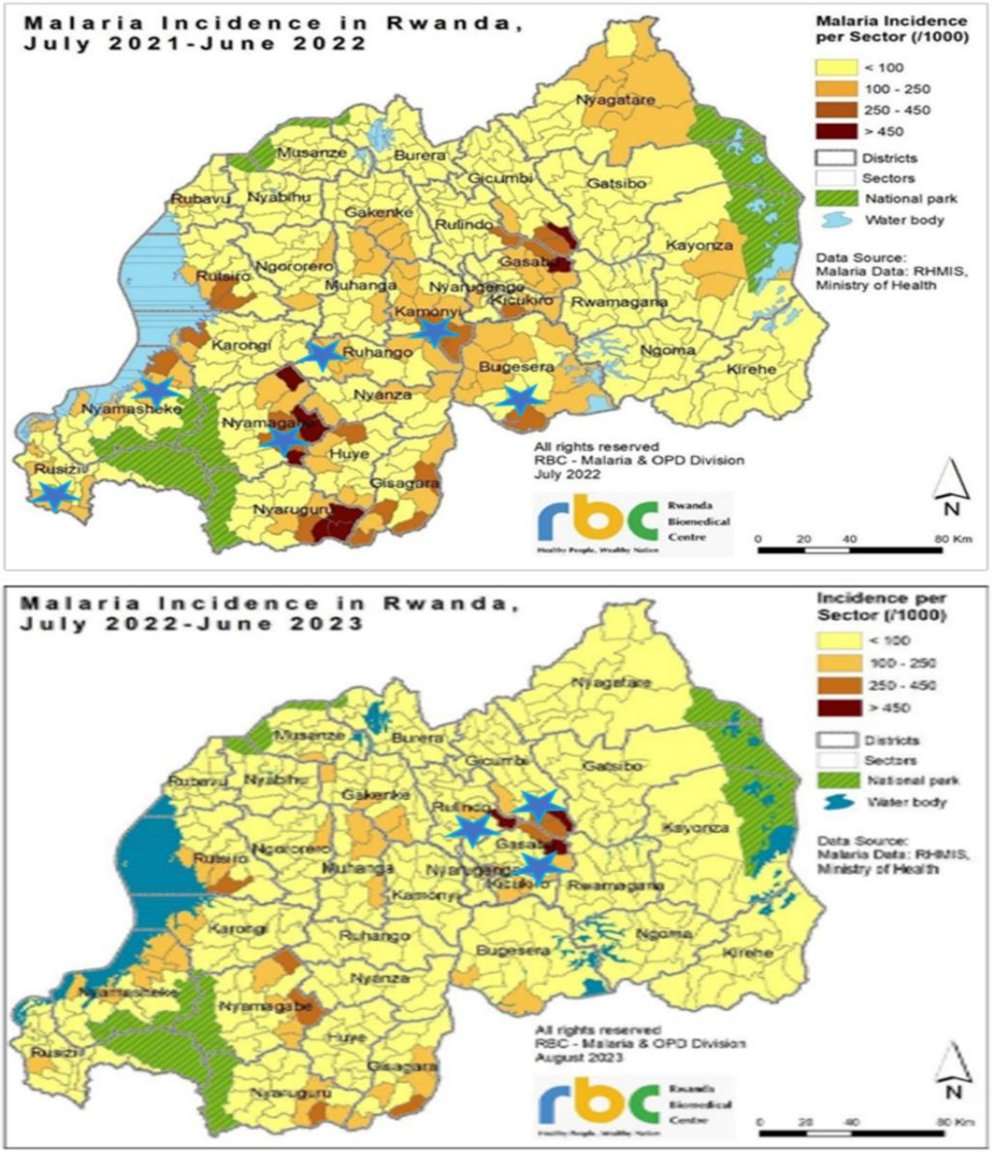


Figure 8: Malaria incidence per sector July2021-June 2022 and Malaria incidence per sector July 2022-June2023 [60,61], sites location are in highlithed blue stars in the two maps.

Malaria is dynamic and seasonal in Rwanda and is also linked with interventions (Fig.7). Malaria is endemic in 19 districts, predominantly in

Eastern and South-eastern [14]. Fig.8, among the 9 districts selected, 3 Blanket IRS districts where districts are fully sprayed : Bugesera, Kamonyi, and Ruhango. Followed by 4 with focal IRS partially sprayed targeting high endemic sector within district: Rusizi, Nyamagabe, Gasabo, and Gicumbi. Whereas, 2 Nyamasheke and Rulindo are in no IRS district [62].

1.1.3. Dietary assessment

Data collection tools for food intake and composition

To establish a relationship between food intake and health conditions in an epidemiological study at the individual level, the food frequency questionnaire (FFQ) is the most commonly used dietary intake tool. The quantitative FFQ is preferred to the 24h recall, because it provides the capacity to assess the eating habits considering frequency, portion size, and can also identify variability in food that a participant can not record within a 24-hour dietary recall. The FFQ has an opportunity to record a large number of food items, which can be more than 100 items in a certain period. The FFQ should be customised for a specific group to maximize the chance of not missing some important food or new food in the specific population; an option to add a new item was allowed [63].

The initial step in achieving the thesis objectives was to create the FFQ and evaluate it in a pilot phase to ensure that quality data could be collected with minimum error. Also, the 24 h recall was conducted to cross-check the accuracy of memory. Then, for every food item report to be taken during the week before being surveyed, with the surveyor's support, the Photographic Food Atlas for Kenyan Adolescents 9-14 years was used to recall food portion and quantities consumed [64].

The study FFQ encompasses 15 food categories, counting over 103 food items. Among the food categories include starches, vegetables rich in vitamin A, with green leaves, and other types. Besides, we have fruits, dairy, legumes, nuts, seeds, fats, sugars, drinks , fish, eggs, meat.

Food composition tables (FCT) were first used in the 1800s and were limited to carbohydrates, fat, protein, moisture, and ash. However, the current FCT displays a high level of heterogeneity due to multiple data sources, terminologies, food types, chemical analysis techniques, and processing methods [54]. The FCT helps track the nutrient quantities in foods consumed during dietary intake in a specific setting. However, Rwanda does not have an FCT. Therefore, we compiled our own FCT for the purpose of this study, using existing data in the African region, which consumes approximately the same food items, provides a comprehensive analysis of nutrients, including principal macronutrients (energy, carbohydrates, proteins, fats), micronutrients, and the most recent FCT. Based on these criteria, the 2019 Western FCT was the first choice, and was used as basis to construct our Rwanda FCT. For missing information, it was supplemented using the 2018 Kenyan FCT, notably containing selenium content. Moreover, we used the 7^{th} edition of the Belgian FCT from 2022 for the composition of foods that were not found in the first two tables to complement the list of the generated Rwandan Food Composition. Thereafter, the generated FCT was used to perform the transcription from food consumed to nutrient value intake for the purpose of our study.

1.1.4. Nutrient Data processing and analysis

After concluding with data collection, the first step was to convert weekly intake of food items (collected through FFQ) into a daily frequency (for calculation of daily intake). In parallel, the edible portion was calculated using the second Belgian edition standardized manual for weight and measurement quantification table [65]. Afterwards, the nutrient value for each food item was calculated using newly created Rwanda FCT. Macronutrient and micronutrient intake were calculated for each participant targeting carbohydrates, proteins, fats, fibers as macronutrients, and minerals and vitamins as follows: calcium, copper, iron, phosphorus, selenium, and zinc. The energy intake of each participant was calculated as well.

1.1.5. Index de Goldberg cut-off and normal BMI

Dietary assessment tools use recall of information, which leads to misreporting of daily intake. To investigate this common issue and in order to minimize potential confounding associations, the Goldberg indice was used, which follows multiple steps taking into account the individual physical activity and basal metabolic rate for total energy requirement, and the estimated energy intake using dietary intake from FFQ [66].

In our study, some participants had inadequate energy intake (below their estimated requirements) despite not exhibiting low body weight defined as a BMI less than 18.5 in adults or a heightI-for-age Z-score below -2 standard deviations according to WHO growth standards for children and adolescents [67].

The basis of the Goldberg cut off rely on the ratio of estimated energy intake based on FFQ data and total energy requirement calculated from BMR times PAL should be equal 1 to that of an individual with a stable weight. In other way, we investigate the limit of the agreement between EI:BMR and PAL [68]. Golberg indice cutoffs follow step for calculation, determination of the constant S, is a factor that takes into account the variation of the energy intale, BMR and PAL.

$$S = \sqrt{\frac{CV^2{}_{wEI}}{d} + CV^2{}_{wB} + CV^2{}_{tP}}$$

Where

- **CVwEI** : the within-subject variation in energy intake
- **d** : the number of days of diet assessment (in our study it is 7)

- **CVwB** : the within-subject variation in repeated BMR measurements reflects both measurement error and temporal variability. This variation affects the precision of estimated BMR when compared with directly measured values [69].

- **CVtP :** he total (between-subject) variation in PAL, but this figure includes also within-subject variation and methodological errors

The revised factors by Black (2000a) should be applied:

- CVwEI = 23%;

- CVwB = 8.5%;

- CVtP=15%.

- d=7

$$s = \sqrt{\frac{23^2}{7} + 8.5^2 + 15^2} = 19.31$$

Upper and lower limit in the equations use:

- SDmin is -2 for the 95% lower confidence limit,

- SDmax is +2 for the 95% upper confidence limit,

n is the number of subject and here is 1

The individual EIrep:BMRest of each subject assigned to the physical activity level is compared with the calculated lower and upper cut-offs for the corresponding activity category.

Example of adult above 65 years, we calculate cut-offs for low physical activity for this age (>65 years) for n=1. We apply PAL= 1.4 which is adopted for low physical activity of adults.

$$Lower\ cut-off\ for\ EIrep{:}BMRest\ > PAL \times \exp\left[SDmin\ \times \frac{S/100}{\sqrt{n}}\right] = \mathbf{0.95}$$

$$Upper\ cut-off\ for\ EIrep{:}BMRest\ < PAL \times \exp\left[SDmax\ \times \frac{S/100}{\sqrt{n}}\right] = \mathbf{2.06}$$

Subjects with calculated values of the ratio EIrep:BMR in the interval 0.95 – 2.06 are classified as plausible reporters.

Subjects with individual EIrep:BMRest < 0.95 are categorized as under-reporters, means the person reports have consumed less than 95% of their energy needs at rest. While, subjects with individual EIrep:BMRest > 2.06 are categorized as over- reporters, means the person report consuming more than two times the energy needs at rest [70].

1.1.6. Physical Activity Level

In this context, the physical activity level (PAL) was not recorded during the survey or self-reported. The PAL is a complex variable that can vary between individuals and days, even for the same person [68]. In practice, the PAL can determine the association between conditions and health outcomes in epidemiology studies [71]. A proxy was determined based on the literature and the daily activity of the population studied. For children without any disabilities attending school, we assume that their lifestyle is similar to that of Belgian children and adolescents up to 17 years old, with a PAL of 1.4, 1.6, and 1.8 for children and adolescents aged 1-3 years, 4-9 years, and 10-17 years, respectively [68]. Additionally, for ages between 18-64 years and above 65 years, the PAL was established based on being moderately active and inactive, at 1.8 and 1.4, respectively, for the two categories [72]

Definition used

For clarity and effective communication of our findings, the key variables used in this research are defined in the following section

Malaria is defined as an occurrence of malaria infection in a person in whom the presence of malaria parasites in the blood has been confirmed by a diagnostic test [73]

Malaria index case is a case of which the epidemiological characteristics trigger additional active case or infection detection. The term "index case" is also used to designate the case identified as the origin of infection of one or a number of introduced cases [73].

Key most vulnerable groups for malaria are pregnant women, children under five, displaced people, old people, prisoner. In addition to this, people living with some disability such as cleft lip and palate children are most concerned for nutrition purpose [59].

Nutrition indicator used for malnutrition, which relates to inadequacies, imbalances or excesses foods intake in term of energy or nutrients. Malnutrition enclosed three major types of conditions undernutrition, micronutrients, and obese. Undernutrition comprises stunting, wasting, underweight and micronutrients deficiencies. These conditions render children in particular at greater risk to disease and mortality.

1. **Anthropometric:**

Stunting (Height-for-Age), indicator of chronic malnutrition
Wasting (Weight-for-Height), indicator of acute malnutrition
Underweight (Weight-for-Age)
BMI (Body Mass Index): underweight, eutrophy, overweight and obese

2. **Dietary:**

Minimum adequate diet (MAD), expresses quality and frequency of consumed foods for children between 6 to 23 months.
Minimum dietary diversity (MDD), reflects foods quanlity consumed by children aged between 6 and 23 months or women in reproductive age.
For both indicators, children aged between 6-23 months and women of reproductive age met the MDD, when they have consumed at minimum 5 out of 8 food groups or 5 out of 10 food groups per day respectively.

AIMS AND OBJECTIVES

2.1. Problem Statement

Malaria is a parasitic inflammatory and oxidative disease and remain a leading cause of morbidity and mortality in the developing countries. It has become evident that the global technical strategy for malaria 2016–2030 has updated its core goal to increase global attention in the elimination of malaria through the evidence based core strategies.

Co-ocurrence of malaria and nutritional conditions are common and their co-existence is frequent in people living in under development countries. Immunologically, it has been shown that, the co-existence, micronutrients modulate protective or induce effects against malaria, potentially affecting its clinical presentation as asymptomatic or mild or else severe disease. Yet, limited leading mechanism responsible to that outcome is not well understood.

The develop and maintain a healthy human body, we have to build a socio economic cultural behavior of consumption of diversified quality and balanced nutritious food, specifically with food with sufficient adequate daily intake of macronutrients and micronutrients. Although the role of high-quality nutrition food in modulating the immune system and supporting healthy metabolism is well recognised, the mechanisms by which nutrition influences malaria susceptibility, and vice versa, remain unclear.

Recent studies also point toward an implication of multiple factors related to perennial malaria infection in developing countries [74–76]. In that optic, we focus on determinants factors of malaria such as nutrients support basal metabolism and immune system, undernutrition, age, sex and spatial locality in Rwanda.

2.2. Research Objectives

The main goal of this PhD project was to analyze the association between malaria and the nutrition profiles in Rwanda.

Specific objectives were to:

I. Analyze nutritional profiles composition among the general population living in malaria-endemic regions, considering host's nutritional habits, location and age;
II. Quantification of daily energy intake, macronutrients, micronutrients from food consumed;
III. Evaluate relationships between the nutrients, nutritional status and malaria episodes considering confounders, in Rwanda;

In other words, we attempted to answer the following questions:

I. Does the nutritional status and/or the nutritional composition of foods affect the prevalence of Malaria ?
II. What are the effects of other factors such as geographic location, nutritional habits or age?
III. Are those links affected by confounders such as sex or age?

2.3. Research Hypothesis

A diverse and high-quality diet that ensures adequate intake of essential nutrients, particularly micronutrients, can modulate the immune system, enhance the efficacy of malaria treatment, and reduce the incidence of malaria.

RESULTS

Chapter 1. Exploring the prevalence and association between nutritional status and asymptomatic malaria in Rwanda among under-5 children: a cross-sectional analysis

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RESEARCH **Open Access**

Exploring the prevalence and association between nutritional status and asymptomatic malaria in Rwanda among under-5 children: a cross-sectional analysis

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Abstract

Background

Undernutrition and severe malaria continue to be major public health concerns worldwide, particularly in African countries. While the association between malaria and malnutrition has been widely studied in various settings, limited research has focused on asymptomatic malaria and its link to nutritional status in Rwanda, leaving a gap in understanding this relationship in the local context. This study aimed to investigate the possible relationship between children's nutritional health and asymptomatic malaria infections. Specifically, the study assessed the prevalence of undernutrition and asymptomatic malaria infection in relation to implemented policies and the link between stunting, wasting, underweight, and asymptomatic malaria infections.

Methods

Data from three Demographic and Health Surveys (DHS) conducted in Rwanda in 2010, 2014-15, and 2019-20 were used in the study, including children aged 6 to 59 months and confirmed malaria diagnoses via blood smear. The odds ratio of stunting, underweight, and wasting on malaria outcomes were calculated using logistic regression, with and without adjusting for factors such as age, gender, mother's education, wealth index, type of residence, and region within each survey. The present study examined data from three DHSs conducted in Rwanda, which included 10,411 children aged less than five years who were tested for malaria and 11,424 children who had anthropometric measurements. Despite this variation, the available sample size (n=10,409) remains robust for drawing meaningful conclusions, and potential biases due to missing data in the analysis were taken into account. This study used unadjusted (OR) and adjusted odds ratios (AOR) to evaluate the relationships between stunting, underweight, age, wealth index, and malaria outcomes. All independent variables with a p-value below 0.05 in the unadjusted regression were included and considered significant in the adjusted regression analysis. A p-value < 0.05 was used to determine statistical significance.

Results

Asymptomatic malaria was found to be present in 1.3% (95% CI: 1.14%–1.59%) of the population (140/10,411). The study also discovered that 38.3% (95% CI: 37.42%–39.21%) of the children were stunted (Z-score <-2.0). The results indicate that malaria was more frequent in children with stunting (OR=1.85, 95% confidence interval (CI)= [1.32;2.59]), p<0.001). Underweight children were also found to have an increased prevalence of malaria (AOR=1.59, 95%CI= [1.14-2.95], p=0.01). Age was also an important variable correlated with malaria infection since the prevalence of malaria was found to be higher in children over 24 months of age (AOR=2.72, 95%CI= [1.78-4.16], p<0.001). Children from the richest families were found to be protected from malaria AOR=0.38[0.24-0.58] p<0.001 in all 3 DHS.

Conclusion

This study revealed that undernutrition indexes such as stunting and underweight as well as poor wealth index are significant risk factors for asymptomatic malaria in children under the age of five years. Malaria itself can worsen nutrition status, creating a vicious cycle. Monitoring and enhancing this dual relationship of nutritional status and malaria highlights the essential needs of children in this age group in malaria-endemic settings.

Keywords Asymptomatic malaria, Nutritional status, Malnutrition in children under 5, Rwanda, RDHS, Cross-sectional study.

Background

Malaria and malnutrition are major contributors to morbidity and mortality, particularly among infants and children under five in sub-Saharan Africa (SSA) [71,72]. Therefore, malaria and stunting are of high global health concerns. These health issues are directly related to achieving several Global Sustainable Development Goals (SDGs); mainly no poverty, zero hunger, good health and well-being, and possibly sustainable communities, reducing inequalities and increasing descent work and economic growth. According to the Global Technical Strategy for Malaria, to improve health, the malaria burden must be reduced by 75% from the baseline level in 2015 by 2030 [77,78]. Additionally, the WHO and UNICEF alongside their stakeholders, aim to reduce the number of stunted children by 40% and wasting to <5% by 2025 [10].

Malaria parasitaemia persists in immune populations, resulting in asymptomatic malaria cases, which contributes to perennial malaria transmission [74] and can also mediate malnutrition and anaemia [72,79]. Stunting, or low height for age determined by the Z scores, is defined as a sign of chronic undernutrition, reflecting insufficient intake of both the quantity and the quality of the recommended diet. Furthermore, while global consumption of red meat and sugary foods has increased, the Comprehensive Food Security and Vulnerability and Nutrition Analysis Survey (CFSVA2022) report revealed that only 2 % of children under five in Rwanda consumed meat and organ meat [80].

This inadequate nutrition compromises daily energy intake, micronutrients, and vitamin intakes, resulting in delayed growth and development, as well as reduced immune system functionality in children under the age of five, which exacerbates infectious diseases such as malaria [72,81].

According to the 2024 World Malaria Report of the World Health Organization (WHO), there were an estimated 263 million cases and 597,000 deaths from malaria in 85 endemic countries and territories worldwide. Unfortunately, over 95% of the malaria burden, including morbidity and mortality, is reported in SSA [3]. Meanwhile, 149.2 million under five children are too short for their age (stunted) and 45.4 million children have low weight for height (wasted) worldwide [82–84]. Similarly, the highest burden of malnutrition is recorded in SSA [85,86]. The coexistence and double burden of malaria and undernutrition are major health issues of concerns, and the co-burden among same individuals is very common, particularly in SSA region [83–86]. Children affected by both conditions have been shown to suffer from long-term complications such as deficits in physical and cognitive development and poor school performance [87,88].

Rwanda has recorded a decline in disease burden over the last two decades, as indicated by the substantial progress in controlling malaria and improving nutrition status. This progress has led to a reduction in stunting from 38% in 2015 to 33.1% in 2020 [89]. Similar progress was achieved in malaria control, with prevalence decreasing from 40.5% in 2016 to 5% in 2022. This success is largely attributable to the country's improved healthcare infrastructure, increased deployment of proven malaria interventions like indoor residual spraying of households with insecticide (IRS) and universal coverage with insecticide-treated nets (LLINs), expanded community and stakeholder engagement, and improved at-home case management of childhood illness (pneumonia, diarrhoea, and malaria) [90,91]. Despite these gains, asymptomatic malaria remains a critical public health concern. Unlike symptomatic malaria, which prompts treatment and intervention, asymptomatic infections often go undetected, serving as silent reservoirs that sustain transmission. Given that children with chronic malnutrition may have prolonged parasite carriage, the study focused on asymptomatic malaria to better understand the interplay between nutritional deficiencies and subclinical malaria infections. The investigation of asymptomatic malaria yields valuable insights into hidden reservoirs of infection and contributes to the development of targeted strategies for malaria elimination.

Moreover, evidence shows that the current malaria and nutritional improvements are highly vulnerable, particularly when the health system is confronted with a health emergency like the COVID-19 pandemic crisis. The COVID-19 pandemic has reversed a significant proportion of the progress in reducing malaria and nutrition-related morbidity and mortality[92–94]. Therefore, there is an urgent need to identify novel strategies to support the existing ones.

Despite the high achievements in reducing malaria and undernutrition in Rwanda, little is known about the relationship between nutritional status and malaria, and the currently available data/information are not conclusive [75]. A prospective study conducted by Kateera *et al.* [76] concluded that undernutrition is prevalent in non-malaria endemic district in Rwanda. Unfortunately, the study design did not allow to conclude on a relationship as no nutritional parameters were studied.

Therefore, the primary objective in this study was to explore the association between undernutrition parameters and the prevalence of asymptomatic malaria in children under five in Rwanda. It is worth noting that while malaria and malnutrition often co-exist, this study does not establish a causal relationship between these conditions. Instead, this research examines an association, recognizing that multiple factors — including socio-economic determinants, immune function, and nutritional deficiencies — may contribute to the observed patterns. Further research is needed to explore the bio-physiological and immunological pathways linking these conditions.

While the primary focus of this study is the association between undernutrition and asymptomatic malaria, the authors, also conducted a broader risk factor analysis to identify key determinants influencing asymptomatic malaria prevalence. This approach ensures a more comprehensive understanding of malaria risk beyond nutritional factors, accounting for socio-economic and environmental influences. Consequently, this work focused on policies implementation timing, malaria incidence, and nutrition status with respect to malaria outcomes.

Methods

Data source

All malaria and nutrition data were extracted from three consecutive Demographical Health Surveys (DHSs), that were conducted in Rwanda in 2010-2011, 2014-15, and 2019-20; data is publicly available at http://www.measuredhs.com/. Approval to download and use the data was obtained by ICF Macro.

Setting and population

Data on asymptomatic malaria and nutrition status were extracted from the Rwanda DHS in 2010, 2015, and 2020. Children who were tested for biomarkers and malaria infection and those with an anthropometric and demographic record were selected.

Study design

To enable national representation of important indicators, including rural and urban zones in all five regions, the survey design followed a two-stage selection level. Home-level enumeration, which entailed methodically identifying households in various regions, was the initial step. Proportional representation was ensured in the second step of respondent selection. This indicates that the probability of selecting a household was modified according to its size, where "size" was defined as the total number of people living in each household. The selection was done with a probability proportional to the size of each household. This approach ensures that larger households, which may be more common in certain regions or zones, have a higher chance of being included in the survey, contributing to a sample allowing for unweighted analyses. Specifically, women aged between 15 and 49 who were present in the household on the night of the poll were chosen as respondents. Additionally, all surveys concerning children included optional components and/or biomarkers for a more comprehensive understanding of relevant indicators [89].

For this research, data on malaria and nutrition were combined from three datasets collected between September 2010 and March 2011 (DHS 2010), November 2014 and April 2015 (DHS 2015), and November 2019 and July 2020 (DHS 2020).

Mapping of nutrition and malaria policy, guidelines: implementation period and trends over time

Initially, reviews of national strategic documents, including policies, strategies, guidelines, and annual reports were conducted to consolidate knowledge on essential malaria and nutrition-related issues and their control in Rwanda from 2000 to 2022. This evaluation included published and unpublished reports, such as health-related documents, ministerial instructions, yearly reports, and programme review reports. Given that secondary analyses, such as the DHS, do not account for policy impact, the goal was to explore the influence of key interventions in reducing malaria and nutritional indicators.

Malaria and nutrition variables

This study determined variables of interest, including the definitions, indicators, and other factors related to asymptomatic malaria infection and child nutrition status, according to the WHO guidelines. Then, the relevant data from the DHS surveys were extracted. These selected variables are described in Table 1 [89,95–97].

Definition of malaria variables

Asymptomatic malaria infection was confirmed by a blood smear. Children who were positively diagnosed with malaria without showing clinical symptoms were classified as asymptomatic cases.

Malaria incidence: proportion of malaria cases confirmed by blood smear in a specific population at each time [95].

Nutrition status variables

The undernutrition is defined as a lack of energy-protein intake and nutrients to meet a human/person needs to preserve worthy health [98]. Its indicators were identified by anthropometric variables, mainly height and weight, in combination with age and sex. The age was measured by the difference between the date of the day of the month of the survey and the date of birth [99], the height and weight were measured in centimetres and grams, respectively, using calibrated instruments. Since measurements of children in the field are prone to a small margin of errors that can mislead outcome interpretations, 10% of the measured children were randomly selected for a second measurement. To ensure the accuracy and quality of the collected data, the data collectors were blinded to the previous measurements. Then, using the WHO sex and age distribution as a reference population for standardization, the three primary indices of child development were determined, including ratios of the child's height to age (stunting), body weight to height (wasting), and weight to age (underweight) [87,100].

Stunting, wasting, and underweight were examined as distinct independent variables, each with standalone classifications. Stunting was characterized by children having a height or length-for-age Z-score (HAZ) falling below -2.0, while severe stunting was identified when the HAZ was below -3.0 and normal range values between -2.0 and +2.0. This was classified into binary levels; children with severe or moderate stunting were jointly classified as stunted. Wasting was determined by a weight-for-height or length Z-score (WHZ) below -2.0; likewise, severe wasting was defined by a WHZ below -3.0 and normal range values between -2.0 and +2.0. This was classified into binary levels; a child with severe and moderate wasting was classified as wasted. Underweight was determined in children with a weight-for-age Z-score (WAZ) below -2.0, while severe underweight was indicated when the WAZ fell below -3.0 and normal range values between -2.0 and +2.0. This was classified into binary levels in the same way; children with severe or moderate underweight were classified as underweight [89]. The WHO Global Database on Child Growth standards use the z-score concept applied to the normal curve and assumes the cutoff points of -2.0 or +2.0 to define deficit or excess for a given anthropometric index. The percentage of individuals beyond these cutoff points in the anthropometric pattern is about 2.3%, respectively. A prevalence of 2.3% below or above two for any anthropometric index is considered as "acceptable" since this is the proportion found in the reference population [101].

Anaemia is defined as presenting a haemoglobin ranging below the standard range 11.0g/dl, and it is classified into three categories. It is classified as severe anaemia when the haemoglobin is below 7.0g/dl, moderate when haemoglobin ranges between 7.0 g/dl and 9.9g/dl, and mild anaemia when it is between 10.0 to 10.9g/dl.[102].

Social demographic factors of the child and the mother

The sociodemographic factors include age, gender, residency, region, mother's education, and wealth index. The *age of the child* was classified into three categories based on < 24 months or ≥ 24 months. The *gender* of the child was also categorized as binary according to its cisgender. The *child residency* was classified into urban and rural.

Region: Rwanda is subdivided into five areas according to four provinces (East, North, South, Western) and the City of Kigali. [103,104]

Mother education: A child's mother's educational background includes no education, primary, secondary, and high school; it was categorized into two levels. Non-educated mothers are designated as having no scholarship, and those with primary, secondary, or high school education are categorized as educated [105].

Wealth index: The distribution of the wealth index, encompassing economic strata from the poorest to the richest (poorest, poorer, middle, richer, and richest), was organized into quintiles, providing a graded representation of household socio-economic status. This categorization was grouped into two levels: the first level included the poorest and poorer categories, classified as poor, while the second level included the middle, richer, and richest categories, classified as rich. This classification follows standard DHS methodology for wealth index calculation[89].

Statistical analyses

Multivariable logistic regression analysis and decomposition techniques were used to assess which predictors were statistically significant in the association between asymptomatic malaria prevalence and nutritional status. This study's dependent variable was dichotomous: malaria was either positive or negative in children aged 6 to 59 months and children also could have or not have clinical malaria symptoms.

To determine the malaria risk factors, a full logistic regression model with all variables and calculated the odds ratio along with a 95% confidence interval and p-value were computed. All independent variables with a p-value below 0.05 in the unadjusted regression were included and considered significant in the adjusted regression analysis. The model was adjusted for age, gender, and the wealth index, and tested for multicollinearity among variables. A diagnostic was performed, and tolerance was deemed acceptable with $GVIF^{(1/(2df))} < 2$ for categorical variables and $VIF < 10$ for continuous variables. Then backward stepwise selection was used for retaining only variables that remained significant at an alpha level of 0.05 in the final model [106].

Furthermore, the three distinct datasets were combined to create a dataset for the final regression model for assessing the relationship between malaria and nutrition factors. To account for potential temporal variations in socio-demographic and economic conditions, survey year was included as an adjustment variable in all regression models. Stata/SE version 15.1 was used for statistical analysis.

Ethics

This study used secondary data collected through the national DHS without personal identifiers. The DHS surveys received ethical approval from the Rwanda National Ethics Committee [89] and the ICF Institutional Review Board and all data are publicly available on the website: www.statistics.gov.rw. Therefore, no additional ethical approval was required for this secondary analysis.

Results

Mapping of nutrition and malaria policy, guidelines: implementation period and trends over time

Figure 1 provides two key pieces of information: (1) Policies and guidelines to support nutrition interventions in Rwanda and (2) Trends in stunting prevalence among children under five years from 1992 to 2020. The data shows that Rwanda has made progress in reducing stunting over the past few decades, from 56.8% in 1992 to 33.1% in 2020 reflecting a decline of 23.7%. Various health, nutrition, and development policies and interventions introduced during this period likely contributed to this decline.This figure shows the positive impact of policy initiatives on nutritional outcomes in Rwanda.

Figure 2 shows malaria interventions (LLINs and IRS) in Rwanda (2008–2022), highlighting implementation districts, data from DHS, and epidemiological impacts. Various interventions, such as Long-Lasting Insecticidal Nets (LLINs) and Indoor

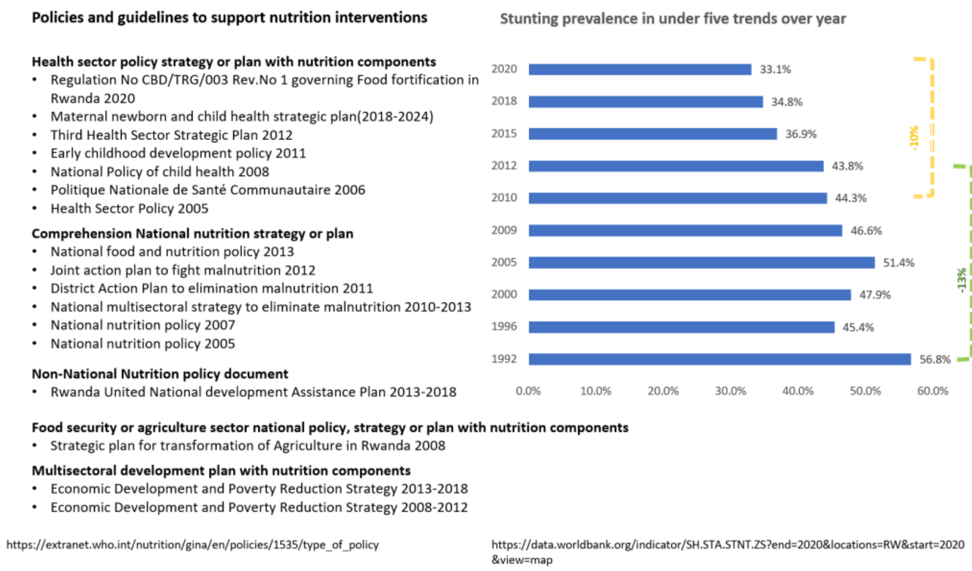

Fig. 1 The development of nutritional policies, strategies and national guidelines over time and the corresponding progress in reducing stunting among children under five in Rwanda between 1992 and 2020

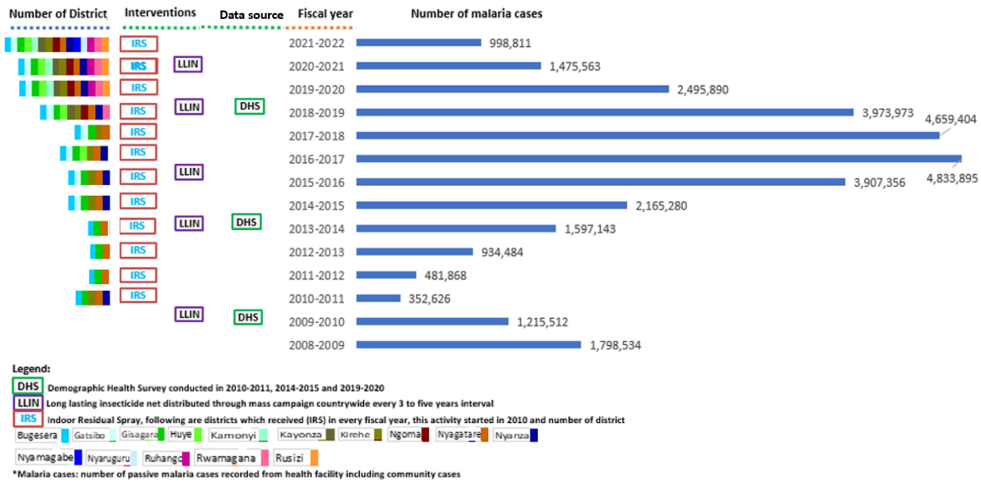

Fig. 2 Malaria interventions (LLINs and IRS) in Rwanda (2008–2022), highlighting implementation districts, data from DHS, and epidemiological impacts

Residual Spraying (IRS), were deployed in different districts during this time. Following 2018, malaria cases gradually decreased, dropping to 998,811 in 2021-2022, likely due to the continuous implementation of these control measures.

Demographics

This study's analysis included 11,571 children under five with a 1:1 male-to-female sex ratio. Among the participants, approximatively one-third (34.8%) were aged between 0-23 months, while two-thirds (65.2%) were between 24-59 months. Regarding economic status, 44.5% of the family wealth index is categorized as poorer. In every district except for the capital city, 81.6% of children under five reside in rural areas (Table 2). Despite the high urbanisation rate in the capital city, the study found that there are still variations in regions, with 89.1%, 75.8%, and 59.1% of children under five living in the urban area in the Kicukiro, Nyarugenge, and Gasabo districts of Kigali city, respectively (Table 3).

Table 2 Demographic and nutritional characteristics of children under five in Rwanda, DHS 2010, 2014–15, and 2019–20 (N = 10,411)

Variables	2010 n(%)	2015 n(%)	2020 n(%)	2010-2020 n (%)
Asymptomatic Malaria (N)	3,739	3,237	3,435	10,411
Negative	3,698 (98.9)	3,169 (97.9)	3,404 (99.1)	10,271 (98.7)
Positive	41 (1.1)	68.0 (2.1)	31 (0.9)	140 (1.3)
Stunting (N)	4,077	3,538	3,809	11,424
Normal	2,297 (56.3)	2,214 (62.6)	2,537 (66.6)	7,048 (61.7)
Stunted	1,780 (43.7)	1,324 (37.4)	1,272 (33.4)	4,376 (38.3)
Child's age (N)	3,713	3,222	3,376	10,311
0-23 months	1,214 (32.7)	1,171 (36.3)	1,203 (35.6)	3,588 (34.8)
24-59 months	2,499 (67.3)	2,051 (63.7)	2,173 (64.4)	6,723 (65.2)
Sex of child (N)	4,133	3,615	3,821	11,569
Male	2,082 (50.4)	1,848 (51.1)	1,927 (50.4)	5,857 (50.6)
Female	2,051 (49.6)	1,767 (48.9)	1,894 (49.6)	5,712 (49.4)
Residence (N)	4,135	3,615	3,821	11,571
Urban	555 (13.4)	783 (21.7)	796 (20.8)	2,134 (18.4)
Rural	3,580 (86.6)	2,832 (78.3)	3,025 (79.2)	9,437 (81.6)
Regions (N)	4,133	3,615	3,821	11,569
Kigali city	441 (10.7)	418 (11.6)	432 (11.3)	1,291 (11.2)
South	1,032 (25)	926 (25.6)	878 (23)	2,836 (24.5)
West	1,007 (24.4)	887 (24.5)	977 (25.6)	2,871 (24.8)
North	657 (15.9)	496 (13.7)	611 (16)	1,764 (15.2)
East	996 (24.1)	888 (24.6)	923 (24.2)	2,807 (24.3)
Wealth Index (N)	4,135	3,615	3,821	11,571
Poor	1,805 (43.7)	1,643 (45.4)	1,699 (44.5)	5,147 (44.5)
Rich	2,330 (56.3)	1,972 (54.6)	2,122 (55.5)	6,424 (55.5)
Mother Education (N)	4,135	3,615	3,821	11,571
No Education	776 (18.8)	504 (13.9)	453 (11.9)	1,733 (15.0)
Primary	2,972 (71.9)	2,605 (72.1)	2,457 (64.3)	8,034 (69.4)
Secondary + High	387 (9.4)	506.0 (14.0)	911 (23.8)	1,804 (15.6)
Underweight (N)	4,077	3,538	3,811	11,426
Normal	3,617 (88.7)	3,221 (91.0)	3,524 (92.5)	10,362 (90.7)
Underweight	460 (11.3)	317 (9.0)	287 (7.5)	1,064 (9.3)
Wasting (N)	4,077	3,538	3,809	11,424
Normal	3,960 (97.1)	3,460 (97.8)	3,764 (98.8)	11,184 (97.9)
Wasted	117 (2.9)	78 (2.2)	45 (1.2)	240 (2.1)
Anemia	3,733	3,230	3,436	10,399
Anaemic	1,428 (38.3)	1,158 (35.9)	1,257 (36.6)	3,843 (37)
Not Anaemic	2,305 (61.7)	2,072 (64.1)	2,179 (63.4)	6,556 (63)

Table 3 Distribution of children under five by urban and rural areas across districts, Rwanda DHS 2010, 2014–15, and 2019–20 (N = 10,411)

District	Urban		Rural	
	Freq	%	Freq	%
Nyarugenge	319	75.8	102	24.2
Gasabo	275	59.1	190	40.9
Kicukiro	361	89.1	44	10.9
Nyanza	45	12.4	318	87.6
Gisagara	35	8.9	359	91.1
Nyaruguru	27	6.9	364	93.1
Huye	44	12.5	309	87.5
Nyamagabe	23	6.6	328	93.4
Ruhango	45	13.8	282	86.2
Muhanga	64	19.9	258	80.1
Kamonyi	51	15.2	284	84.8
Karongi	55	15.9	290	84.1
Rutsiro	37	8.3	409	91.7
Rubavu	111	24.6	341	75.4
Nyabihu	46	11.7	348	88.3
Ngororero	25	6.7	349	93.3
Rusizi	69	16.4	352	83.6
Nyamasheke	32	7.3	407	92.7
Rulindo	31	9.2	306	90.8
Gakenke	25	7.4	313	92.6
Musanze	73	19.6	299	80.4
Burera	26	7.2	337	92.8
Gicumbi	39	11	315	89
Rwamagana	42	11	339	89
Nyagatare	60	13.9	373	86.1
Gatsibo	33	8.1	373	91.9
Kayonza	35	8.8	361	91.2
Kirehe	25	6.6	353	93.4
Ngoma	40	10.5	340	89.5
Bugesera	40	9.2	393	90.8
Total	2,133	18.4	9,436	81.6

Regarding education, 69.4% of mothers attended primary school, followed by 15.6% who had secondary and a high school diploma, and 15% had no education (Table 2).

Malaria data

According to the DHS from 2010, 2015, and 2020, 1.1%, 2.1%, and 0.9% of under-five children had asymptomatic malaria, respectively (Table 2).

Nutritional data

During the three periods of the demographic and health survey (DHS), the prevalence of stunting (chronic malnutrition) in children under five years old decreased from 43.7% and 37.4% to 33.4% in 2010, 2015, and 2020, respectively. Additionally, the prevalence of wasting (acute malnutrition) declined steadily from 2.9% in 2010 to 2.2% in 2015 to 1.2% in 2020. There was also a slight reduction in the prevalence rate of underweight children (too thin for their age), reduced from 11.3% and 9% to 7.5% in 2010, 2015, and 2020, respectively (Table 2).

Risk factors for asymptomatic malaria

For the years 2010, 2015, and 2020, tables 4, 5, and 6 provide summaries of the unadjusted and modified odds that were used to compare asymptomatic malaria episodes to nutritional conditions. In the 3 DHS surveys, age correlated with increased asymptomatic malaria cases in children over 24 months compared to children under 23 months (Tables 4,5,6)

In 2010, children aged 24 months and above had a higher prevalence of asymptomatic malaria than those who were under 23 months old (AOR=2.37, 95% CI 1.13-4.99). Regarding anaemia, children identified as anaemic had a higher prevalence than non-anaemic children (AOR=7.56, 95% CI 3.43-16.68). Furthermore, regarding the household wealth index, individuals classified as rich had a lower malaria prevalence than those categorized as poor (AOR=0.39, 95% CI 0.19-0.79). Specifically, regarding nutrition indicators, being underweight was associated with a higher malaria prevalence (AOR=1.39, 95% CI 0.55-3.54), but it was not statistically significant. More details are provided in Table 4..

In 2015, children aged 24 months or more had a higher prevalence of malaria than the youngest (AOR=3.34, 95% CI 1.76-6.31). The rich wealth index had a lower malaria prevalence than the poor (AOR = 0.33, 95% CI 0.17-0.62). Anaemia was highly correlated with malaria; the prevalence of malaria was higher in anaemic children (AOR = 8.17, 95% CI (4.36-15.31). Interestingly, salt with iodine negatively correlated with asymptomatic malaria cases, according to the 2015 survey, with an OR of 0.40 and 95% CI (0.21-0.78). Concerning nutritional status, underweight children had more asymptomatic malaria than children with normal percentiles for their age and sex (AOR=2.64, 95% CI 1.33-5.26), Table 5.

DHS 2020 was the only survey where stunting showed a relationship with asymptomatic malaria (AOR=2.51; 95% CI 1.11-5.67). Similarly, to 2010 and 2015, malaria prevalence was higher in children aged 24 months or more (AOR=2.51; 95% CI 1.06-5.96) and in children identified as anaemic (AOR=6.28, 95% CI 2.66-14.85) and in underweight children (OR=2.31, 95% CI 0.88-6.05). Besides the adjusted odds ratio, the association with underweight became non-significant after adjusting for confounding variables (Table 6).

Table 1. Association between asymptomatic malaria infection and nutritional status in 2010: Unadjusted and adjusted analyses (N=3,739).

Risk factors 2010	Asymptomatic malaria cases n/N	% 	univariate OR (95%CI)	P value	Adjusted OR (95%CI)	P value
Total	41/3737	1.1				
Age (months)				**0.44**		**0.023**
6-23 months	11/1195	0.92	1		1	
24-59 months	30/2489	1.21	1.31(0.66-2.63)		2.37(1.13-4.99)	
Residence				**0.53**		**0.88**
Urban	4/489	0.82	1		1	
Rural	37/3248	1.14	1.39(0.49-3.94)		0.93(0.31-2.81)	
Maternal Education				**0.23**		**0.57**
No education	11/709	1.55	1		1	
Primary	27/2689	1	0.64(0.32-1.30)		0.82(0.39-1.68)	
Sec + High	3/339	0.88	0.57(0.16-2.04)		0.73(0.14-2.67)	
Anemia				**<0.001**		**<0.001**
Not anemic	147406	0.35	1		1	
Anemic	33/1426	2.31	6.79(3.13-14.76)		7.56(3.43-16.68)	
Wealth index				**0.005**		**0.009**
Poor	27/1630	1.66	1		1	
Rich	14/2107	0.66	0.39(0.21-0.76)		0.39(0.19-0.79)	
Stunting				**0.65**		**0.57**
Normal	20/1978	1.01	1		1	
Stunted	20/1718	1.16	1.15(0.62-2.15)		0.82(0.41-1.64)	
Underweight				**0.27**		**0.49**
Normal	33/3260	1.01	1		1	
Underweight	7/436	1.61	1.59(0.70-3.63)		1.39(0.55-3.54)	
Wasting				**0.95**		**0.66**
Normal	39/3598	1.08	1		1	
Wasted	Jan-98	1.02	0.9(41.13-6.92)		0.62(0.08- 5.11)	

Nutritional risk factors for asymptomatic malaria

As shown in Table 7, this study combined the three cross-sectional analyses in 2010, 2015, and 2020 to examine the association between nutritional factors and asymptomatic malaria in this study. Overall, results indicated that underweight was associated with an increased prevalence of asymptomatic malaria (AOR=1.59 95% CI 1.14-2.95). Stunting was also associated with the prevalence of asymptomatic malaria, with an OR of 1.85 (95% CI 1.32-2.59) but this association was lost with the AOR. For individual DHS survey analysis, increasing age was associated with an increased prevalence of asymptomatic malaria (AOR=2.72, 95% CI 1.78-4.16). Female gender and primary or advanced maternal education were not associated with an increased malaria prevalence. There was also a linear trend with the wealth index, as malaria prevalence decreased with increasing wealth index from the poorest to the wealthiest children.

Discussion

Rwanda’s efforts to eliminate malaria and improve nutrition faced significant setbacks in the early 1990s due to disruptions in healthcare services, increased disease burden, and widespread malnutrition [107]. However, over the past three decades, the country has rebuilt a resilient healthcare system through strong leadership, evidence-based strategies, and well-defined priorities. A desk review revealed that in 2000, Rwanda adopted a national decentralization policy to strengthen local healthcare systems as part of broader post-genocide reconstruction efforts. This policy aimed to enhance community engagement, customize services to local needs, and improve health outcomes.

Table 5. Association between asymptomatic malaria infection and nutritional status in 2014–15: Unadjusted and adjusted analyses (N = 3237)

Risk factors	Asymptomatic malaria cases		Unadjusted	P value	Adjusted	P value
2014-2015	n/N	%	OR(95%CI)		OR(95%CI)	
Total	68/3237	2.1				
Age (months)				0.006		<0.001
6-23 months	13/1154	1.13	1		1	
24-59 months	53/2043	2.59	2.34(1.27-4.31)		3.34(1.76-6.31)	
Residence				0.004		0.2
Urban	4/682	0.59	1		1	
Rural	64/2555	2.5	4.35(1.58-12.00)		1.95(0.68-5.64)	
Maternal Education				0.01		0.5
No education	15/466	3.22	1		1	
Primary	50/2351	2.13	0.65(0.36-1.17)		0.91(0.49-1.70)	
Sec + High	3/420	0.71	0.22(0.06-0.75)		0.59(0.16-2.20)	
Anemia				<0.001		<0.001
Not anemic	13/2068	0.63	1			
Anemic	55/1158	4.75	7.88 (4.29-14.49)		8.17 (4.36-15.31)	
Wealth index				<0.001		0.001
Poor	53/1489	3.56	1		1	
Rich	15/1748	0.86	0.23(0.13-0.41)		0.33(0.17-0.62)	
Underweight				<0.001		0.006
Normal	50/2886	1.73	1			
Underweight	17/303	5.61	3.37(1.9-5.9)		2.64(1.33-5.26)	
Stunting				0.025		0.35
Normal	31/1905	1.63	1		1	
Stunted	36/1284	2.8	1.74(1.07-2.83)		0.75 (0.42-1.36)	
Wasting				0.79		0.31
Normal	66/3127	2.11	1		1	
Wasted	Jan-62	1.61	0.76(0.10-5.57)		0.34(0.04-2.72)	
Iodine in salt						
No iodine	11/239	4.6	1	0.007		0.071
Iodine present	57/2991	1.91	0.40(0.21-0.78)		0.52(0.26-1.05)	

By empowering local authorities and communities to plan and implement interventions, Rwanda created a more responsive healthcare system, particularly in tackling malaria and malnutrition. Community Health Workers (CHWs) play a critical role in expanding healthcare access, delivering preventive and curative services at the household level, and reducing barriers related to gender, socioeconomic status, and geographic location. To further decentralize healthcare, the Ministry of Health redistributed trained personnel from the central level to districts, where district health officers oversee malaria control, nutrition programmes, and other health indicators. The fight against malnutrition in Rwanda is guided by evidence-based interventions addressing undernutrition, overnutrition, and micronutrient deficiencies. In 2020, the government established the National Child Development Agency (NCDA) with a mandate to eradicate malnutrition and stunted growth among children. One of its key initiatives is the 1000 Days Campaign, launched in 2013 in collaboration with stakeholders, focusing on reducing infant, child, and maternal mortality through improved antenatal and postnatal care, iron and folic acid supplementation, exclusive breastfeeding, complementary feeding, growth monitoring, and community-based nutrition education.

Table 6 Association between asymptomatic malaria infection and nutritional status in 2019–20: Unadjusted and adjusted Analyses (N = 3,435)

Risk factors 2019-2020	Asymptomatic malaria cases n/N	%	Unadjusted OR(95%CI)	P value	Adjusted OR(95%CI)	P value
Total	31/3435	0.90				
Age (months)				0.14		0.04
6-23 months	7/1199	0.58	1.00		1.00	
24-59 months	24/2168	1.11	1.91(0.82-4.44)		2.51(1.06-5.96)	
Residence				0.13		0.76
Urban	3/720	0.42	1.00		1.00	
Rural	28/2715	1.03	2.49(0.76-8.22)		1.29(0.37-4.56)	
Maternal Education				0.01		0.26
No education	9/410	2.20	1.00		1.00	
Primary	18/2223	0.81	0.36(0.16-0.82)		0.55(0.24-1.25)	
Sec + High	4/802	0.50	0.22(0.07-0.73)		0.57(0.16-2.05)	
Anemia				<0.001		<0.001
Not anemic	102086.00	0.32	1.00			
Anemic	24/1256	1.91	6.04 (2.59-14.06)		6.28 (2.66-14.85)	
Wealth index				0.09		0.08
Poor	23/1538	1.50	1.00		1.00	
Rich	8/1897	0.42	0.28(0.12-0.6)		0.45(0.19-1.08)	
Underweight				0.00		0.69
Normal	26/3167	0.82	1.00			
Underweight	5/267	1.87	2.31(0.88-6.05)		1.22(0.44-3.39)	
Stunting				<0.001		0.03
Normal	118979.00	0.45	1.00		1.00	
Stunted	21/1207	1.74	3.92(1.84-8.36)		2.51 (1.11-5.67)	

Additionally, the government introduced sustainable nutrition programmes, such as Akarima Kigikoni (kitchen gardens) in 2014 to promote household vegetable production and Girinka (One Cow per Poor Family) in 2006 to improve food security and combat malnutrition. The Health Sector Strategic Plan (2018–2024) emphasizes high-quality, accessible healthcare and prioritizes preventive measures, including IRS, LLINs, and public health campaigns to improve healthcare-seeking behaviour. Through evidence-based policies and decision-making, Rwanda has increased domestic investment in health programmes and strengthened partnerships with local and international stakeholders to ensure affordable, sustainable healthcare services. Furthermore, the government continues to enhance community engagement by empowering CHWs to lead malaria control efforts, particularly in IRS implementation and early Test, Treat, and Track (TTT) strategies at the household level. This analysis explored the relationship between asymptomatic malaria and nutritional indices adjusted by socio-demographic and economic factors and household parameters using the demographic and health surveys conducted in Rwanda in 2010, 2015, and 2020. It was observed that children under five who had an undernutrition index (stunted and underweight) had a statistically significant increased likelihood of developing asymptomatic malaria.

The findings from this study reveal a significant association between nutritional status and asymptomatic malaria in children under five. Stunted and underweight children had a greater probability of having asymptomatic malaria, reinforcing the importance of considering nutrition in malaria control efforts. Undernutrition weakens immune defenses, thereby increasing susceptibility to infections, including malaria. Similarly, malaria infections can exacerbate undernutrition by impairing appetite, inducing metabolic alterations, and increasing nutrient losses. A study conducted in Ethiopia also found that malaria was associated with stunting and wasting [108], further supporting the observed trends in Rwanda. While previous research has primarily focused on symptomatic malaria, this study highlights the importance of asymptomatic malaria, which contributes to sustained transmission and may hinder child growth and development. While unadjusted analyses suggested a strong association between stunting and asymptomatic malaria, this association lost statistical significance in adjusted models. This suggests that socioeconomic factors such as maternal education, wealth index, and access to malaria prevention measures play a key role in shaping malaria risk among stunted children. Similar findings were reported by Taye *et al.* (2018) [72] where malaria was identified as a risk factor for stunting and wasting, emphasizing the need to consider broader social determinants when analysing the interplay between malnutrition and malaria.

Table 7 Pooled analysis of the association between asymptomatic malaria infection and nutritional status across three DHS Surveys (2010,2014-15,2019-20)(N=10,411)

Risk factors	Asymptomatic malaria cases n/N	%	Unadjusted OR (95%CI)	P value	Adjusted OR (95%CI)	P value
Total	140/10409	1.34				
Age (months)				**0.00**		**<0.001**
6-23 months	31/3548	0.87	1.00		1.00	
24-59 months	107/6700	1.60	1.84(1.23-2.75)		2.72(1.78-4.16)	
Year				**<0.001**		**0.90**
2 010	41/3737	1.10	1.00		1.00	
2015	68/3237	2.10	1.93(1.31-2.85)		197(1.31-2.96)	
2020	31/3435	0.90	0.82(0.51-1.31)		1.01(0.62-1.64)	
Region				**0.08**		**0.25**
Kigali	3/1145	0.26	1.00		1.00	
South	57/2563	2.22	8.66(2.71-27.7)		4.01(1.17-13,75)	
West	22/2581	0.85	3.27(0.98-10.96)		1.55(0.43-5.52)	
North	1/1600	0.06	0.24(0.02-2.29)		0.13(0.01-1.34)	
East	57/2520	2.26	8.81(2.75-28.19)		4.68(1.37-16.02)	
Residence				**0.00**		**0.51**
Urban	11/1891	0.58	1.00		1.00	
Rural	129/8518	1.51	2.63(1.42-4.87)		1.04(0.53-2.04)	
Mother Education				**<0.001**		**0.18**
No education	35/1585	2.21	1.00		1.00	
Primary	95/7263	1.31	0.59(0.39-0.87)		0.76(0.49-1.14)	
Sec + High	10/1561	0.64	0.29(0.14-0.58)		0.65(0.29-1.46)	
Sex				**0.63**		**0.98**
Male	74/5291	1.40	1.00		1.00	
Female	66/5118	1.29	0.92(0.66-1.29)		0.99(0.70-1.42)	
Anaemia				**<0.001**		
Not Anaemic	28/6550	0.43	1.00		1.00	<0.001
Anaemic	112/3840	2.92	6.99(4.62-10.61)		7.34(4.79- 11.27)	
Wealth index				**<0.001**		**<0.001**
Poor	103/4657	2.21	1.00		1.00	
Rich	37/5752	0.64	0.29(0.19-0.42)		0.38(0.24-0.58)	
	Stunting			**<0.001**		**0.84**
Normal	61/6108	1.00	1.00		1.00	
Stunted	77/4209	1.83	1.85(1.32-2.59)		1.15(0.77-1.71)	
Underweight				**<0.001**		**0.01**
Normal	109/9313	1.17	1.00		1.00	
Underweight	29/1006	2.88	2.51(1.66-3.79)		1.59(1.14-2.95)	
Wasting				**0.69**		**0.28**
Normal	136/10120	1.34	1.00		1.00	
Wasted	2/198	1.01	0.75(0.18-3.05)		0.49(0.11-2.09)	
Salt Iodine						
No iodine	13/525	2.48	1.00	0.08	1.00	0.29
Iodine present	96/6442	1.49	0.59(0.33-1.07)		0.66(0.35-1.24)	

Globally, in 2015, malaria increased and was most prevalent in sub-Saharan Africa [105,106]. The same situation was observed in Rwanda, where malaria morbidity started progressively increasing from 2011 until it reached ten times the previous levels in 2016 [107]. Furthermore, this findings mirror the same trends in asymptomatic malaria cases, which increased by nearly 90.9% in 2015 compared to 2010. Moreover, this study confirmed the decline of asymptomatic malaria prevalence to 57.1% from 2015 to 2020. Mother's education increased over time, from 81% to 88% between 2010 and 2020, and this may be an explanation of the corresponding decrease in asymptomatic malaria, along with the improvements in malaria prevention and nutritional status [109,110]. The prevalence of stunting has declined over the years, dropping from 43.7% in 2010 to 37.4% in 2015 and further decreasing to 33.4% in 2020. However, this is still higher than the prevalence of stunting in Eastern Africa, which is also significantly higher than the global average of 22.0% [111].

This study demonstrated that the incidence of asymptomatic malaria increased with age, particularly among older children aged 24 months and above, where there were 107 asymptomatic malaria cases out of 6700 in contrast to younger children below 24 months, who had 31 cases out of 3548. This is consistent with the gradually observed increased risk of exposure to mosquito bites compared to younger children who are more likely to sleep soundly under ITNs because they still share a bed with their mothers[99]. Moreover, children exposed to *Plasmodium falciparum* develop acquired immunity by producing antibodies against *P. falciparum*, which confer resistance to clinical malaria[100], thus increasing the prevalence of asymptomatic malaria episodes [76,82].

According to the study's findings, asymptomatic malaria was more prevalent among children residing in the eastern and southern regions, which have a higher endemicity with 57 cases each out of around 2500, than in the City of Kigali. This might be due to perennial parasite exposure; children in areas with a high prevalence of malaria developed a strong immunity and typically appear with asymptomatic malaria [75,76].

In 2010, 2015, and 2020, the odds ratios of asymptomatic malaria were lower in non-anaemic children, and this difference was statistically significant, suggesting a potential association between non-anaemic children and lower rates of asymptomatic malaria. However, this does not imply a protective effect. Prior research indicates that malaria itself is a major cause of anaemia in children, and this association may be reflective of the impact of recurrent malaria infections on haemoglobin levels rather than a direct protective mechanism. Nonetheless, considering that prior studies have discovered a high association between anaemia and symptomatic malaria, this finding is not surprising. This indicates that malaria infection is the primary cause of anaemia in children. This study revealed a potential association between iodine in salt and asymptomatic malaria. Considering that iodine is essential for growth, cognitive development, and overall good health [112,113], this is comparable to these findings where children with the presence of iodine in salt consumed in their household tend to have a lower prevalence of malaria at 1.91% compared to 4.60% for children with no iodine; the odds ratio for children with iodine was 0.40 in the year 2015. Moreover, it is important to mention that research examining the effects of acute and chronic malaria episodes on the release of thyroid hormones found that both acute and chronic infections showed inhibition of iodine hormone with no rebound effect, particularly in chronic malaria (i.e. with parasitaemia persistence) [114]. Therefore, further studies investigating the causal link between iodine in salt and malaria protection are warranted to produce additional evidence.

This study had two limitations. First, the study used secondary data collected by routine surveillance rather than prospectively carrying out a longitudinal cohort study that more powerfully investigates exposure and outcome. Additionally, this study focused exclusively on asymptomatic malaria, as these infections often go undiagnosed and contribute to sustained malaria transmission. However, authors acknowledge that including symptomatic malaria cases would provide a more complete picture of how nutritional status influences malaria risk. Future research should examine both symptomatic and asymptomatic malaria cases using longitudinal cohort designs to establish stronger evidence on causal mechanisms.

Conclusion

In conclusion, the association between nutrition and malaria in Rwanda has been overlooked until now. This study highlights an association between nutritional status and asymptomatic malaria. Nevertheless, additional prospective longitudinal studies are required to conduct an in-depth analysis of the impact of nutrition on malaria outcomes. For instance, additional research should investigate the relationship between malaria protection and iodine in salt. Given the complex relationship between nutrition and asymptomatic malaria in Rwandan children, future DHSs should thoroughly assess dietary determinants, particularly micronutrient parameters, to better understand their effects on infectious diseases such as malaria. Moreover, since other confounding factors seem to impact malaria infection, education, comprehensive monitoring, and evaluation of interventions such as maternal education programs and malaria preventive measures are required. Finally, targeted interventions should be directed by variations observed within regions in asymptomatic and symptomatic malaria cases.

List of abbreviations

AOR	Adjusted Odds Ratio
CHW	Community Health Workers
CI	Confidence Interval
DHS	Demographic and Health Surveys
HAZ	Height or length-for-Age Z-score
IRS	Indoor Residual Spraying
LLINs	Long-Lasting Insecticidal Nets
NCDA	National Child Development Agency
OR	Odds Ratio
SDGc	Global Sustainable Development Goals
SSA	Sub-Saharan Africa
VIF	Variance Inflation Factor
UNICEF	United Nations International Children's Emergency Fund
WHO	World Health Organization
WAZ	Weight-for-Age Z-score
WHZ	Weight-for-Height or length Z-score

Declarations

Ethic

Data was extracted from Rwanda's Demographical Health Survey (DHS), which is publicly available at www.measuredhs.com, after receiving written approval to use it from Macro.

Data availability

All data supporting study findings are available for download from the DHS MEASURE website, conditional on DHS approval.

Author Contributions

All authors contributed to the writing of the manuscript and approved the final draft of the manuscript.

Declaration of interest

A.E. and P.D.C. are inventors on patent applications dealing with the use of A. muciniphila and its components in the treatment of metabolic disorders. A. E. and P.D.C. are inventors on patent applications dealing with gut microbes in food reward dysregulations. P.D.C. was cofounder of The Akkermansia company SA and Enterosys. All other authors declare they have no competing interests

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Chapter 2. Associations between undernutrition and malaria infection: A case-control study from Rwanda

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Abstract

Undernutrition and malaria remain major global public health challenges. The relationship between nutritional status and malaria infection is complex. Better understanding of their association is needed to improve prevention and control of these health conditions. Undernutrition can be assessed by evaluating nutrient intake (macronutrient and micronutrient intake) measured through Food Frequency Questionnaire or by using indicators for chronic undernutrition including the stunting parameter, defined by WHO as child being too short for their age. This study aims to investigate associations between inadequate nutrient intake or indicators for undernutrition and malaria infection diagnosed by a positive blood smear. The analysis compares malaria cases against controls composed of other malaria free people living in the same households with the cases (same household grouping). Data collection was conducted between November 2021 and December 2023 across 9 endemic districts located in all four provinces and Kigali City. Regression models were developed to investigate the association between undernutrition (i.e. inadequate nutrient intake or stunting) and malaria infection.

Despite numerous nutrition interventions aimed at reducing the burden of undernutrition, the dietary patterns observed in this study remain predominantly imbalanced. The food composition was predominantly made up of starchy staples, accounting for 56.7% of total energy intake. Such foods are typically rich in macronutrients but low in essential micronutrients. The findings revealed a high prevalence of micronutrient deficiency risk, with a reported risk of vitamin A, B2, B12, calcium, zinc, and selenium deficiency between 50% and 80% in the studied population. Regarding stunting, even if the percentage of severe chronic stunting was higher in malaria cases (17%) compared to controls (10,6%), the association between stunting and malaria infection was not statistically significant.

In unadjusted analysis, intake of selenium and vitamin E were respectively negatively and positively associated with malaria infection ($p<0.05$). After adjusting for covariates, risk of vitamin E deficiency and risk of iron deficiency were positively associated with malaria (aOR=7.46; 95% CI: 4.43–12.58; $p<0.001$ and aOR=1.80; 95% CI: 1.11–2.93; $p=0.017$, respectively). Conversely, age, sex, and risk of selenium deficiency intake were inversely associated with malaria. Increasing age (aOR=0.58;

95% CI: 0.36-0.95; $p<0.04$), female sex (aOR=0.67; 95% CI: 0.46–0.97; $p<0.001$), and selenium deficiency (aOR=0.62; 95% CI: 0.43–0.91; $p=0.013$) were all linked to lower odds of malaria infection.

These findings highlight the critical role of nutrient imbalances in influencing malaria infections, therefore tackling these preventable deficiencies is required through targeted strategies as guided by UNICEF conceptual framework 2020-2030. For instance, improving dietary diversity, regularly monitoring nutritional status, and establishing a comprehensive national food composition database could support such strategies. These approaches will support effective nutrition policies and interventions.

Background

Undernutrition and malaria remain major global public health concerns, particularly in low- and middle-income countries [115]. Although global undernutrition has declined by 4% over the past decade, it still contributes to half of all deaths in children under five [37,116,117]. Sub Saharan Africa and South Asia bear over 90% of the burden, with East Africa showing particularly high rates of undernutrition [37,116], ranking above the WHO cut-off of >30% [118,119]. Undernutrition weakens immunity and increases vulnerability to diseases like malaria [120–122].

Indicators for chronic undernutrition include the stunting parameter, defined by WHO as child being too short for their age. In Rwanda, childhood stunting has decreased from 42% in 2000 to 33% in 2020 [104,123], yet malnutrition is a critical public health issue [122]. Contributing factors to undernutrition include maternal education, household wealth, child age, low birth weight, limited access to health services [122,124–126], and food insecurity [127]. Despite agriculture being the main livelihood, food insecurity affects up to 35% of households in some areas, especially in the Western Province [128], due to recurrent flooding and limited dietary diversity [127]. Globally, food security access to safe nutritious food, remains a challenge, with over 820 million people affected in 2018 [129,130]. Climate change, conflict, and economic instability are primary drivers [37,131,132]. Food insecurity affects human and planetary health and must be tackle through better food system governance to meet sustainable developments goals [129,133]. While interventions, such as increasing education, women's empowerment, and home gardening, have shown some improvements, rural diets remain heavily reliant on nutrient poor staples [134].

In addition to food access, dietary diversity plays a vital role in nutrient adequacy. Micronutrient deficiencies remain widespread, highlighting the need for stronger food policies and diversified agriculture to improve nutrition outcomes [135]. A study found that 81.8% of women in agriculture had low dietary diversity, with 22.1% affected by aneamia [135]. Interestingly, recent analysis of Rwandan staple foods like red kidney beans, amaranth leaves, sweet potatoes, and carrots shows they are rich in nutrients. However, the absence of comprehensive food composition table limits the development of diverse and nutritious diets [136–138].

Micronutrient deficiencies, particularly iron, vitamin A, and zinc, are common probably due to plant-based diets and are linked with poor immunity [135,139,140]. Although Rwanda has implemented interventions like vitamin A supplementation and micronutrient powder distribution covering 87% of children aged 6-23 months, gaps are remaining in some districts [141,142].

Socioeconomic disparities and limited data on micronutrient intakes hinder progress toward the 2025 nutrition targets set by the World Health Assembly and Sustainable Development Goals [125,126,143–145]. Studies in East Africa, including Rwanda, reveal poor dietary habits among children between 6 and 23 months. Only 22% of children meet the minimum acceptable diet (MAD), and just 8.4% consume eggs regularly [144–147], despite eggs being rich in protein, energy and essential nutrient like vitamin B12, choline, selenium, and zinc [148]. Anaemia affects 36.6% of children, due to essential micronutrient deficiencies [145]. Minimum dietary diversity (MDD) is also low at 10.47% across East Africa, though Rwanda shows relatively better rates [126]. These findings highlight the urgent need for targeted nutritional interventions based on studies investigating population's micronutrient deficiencies [144,145].

Malaria continues to be a major health threat, with 263 million cases and 597,000 deaths globally in 2023, 94% occurring in the Africa region [3,132]. Children under five and pregnant women are most vulnerable due to weak immunity [149,150].

Malnutrition and malaria are closely linked, with undernutrition, especially deficiencies in iron, vitamin A, and zinc, heightening the severe malaria and complications [139].

Food insecurity contributes to this vulnerability, as shown in studies from Haiti and Uganda, where it correlated with increased malaria incidence. Malnutrition weakens immune function, increasing malaria-related morbidity and mortality [151–153], while malaria can worsen nutritional deficiencies, creating a vicious cycle [154–159]. This bidirectional relationship is particularly concerning in children, where chronic undernutrition leads to severe malaria outcomes like aneamia and high parasitaemia [160]. Addressing food insecurity and nutritional deficiencies may reduce malaria risk and improve child health in resource limited settings [141,160]. Integrating culturally appropriate nutrition programs into malaria interventions is recommended [156]. While Rwanda has studied dietary habits to inform policy, no research has explored the relationship between dietary nutrient intake, nutritional status, and malaria. This study addresses that gap by examining how macronutrient intake, micronutrient intake and undernutrition indicators like stunting are related to malaria outcomes.

Methods

Study design

This case-control study was conducted in the Republic of Rwanda. The study was implemented by identifying malaria cases from the immediate notification by the case management at healthcare facilities. Then, cases were invited to voluntary participate in the study, and following their consent, the research team visit their household to collect demographic and nutrition status data of the case and their household companions.

The cohort was analyzed to estimate whether it is representative of the Rwandan population. Rwanda has a predominantly young demographic population, with 70.3% of its population under the age of 30, according to the Fifth Rwanda Population and Housing Census of 2022 [128,161]. In comparison, the 2012 census reported that 78.7% of the population was under the age of 30 [162]. In the present cohort, 67.02 % were under the age of 30. In this study, the age distribution is slightly lower to that observed in the general population in census 2022, 67.3% versus 70.3% for the population aged below 30 years old. This difference may be caused by the period of data collection, the recruitment method, as in the census, is the general population, while in the study, the index case is positive malaria cases. Moreover, out of the 1025 participants, only 15 were children below the age of 3 (1.5%), suggesting that this category of age was underrepresented in the present cohort and was excluded to avoid misinterpretation of the data (Fig. 1).

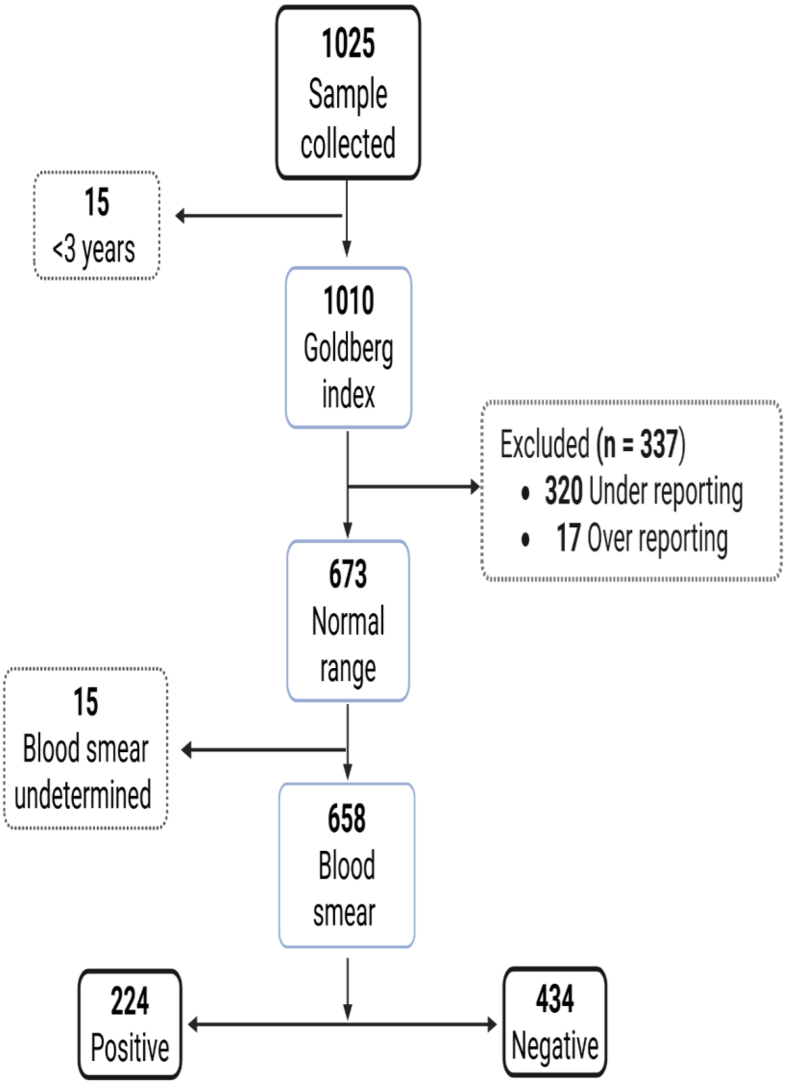


Figure 1. Participant data collection flowchart based on energy intake and malaria diagnosis validation

Study Sites

Administratively, Rwanda is divided into four provinces and the City of Kigali, comprising a total of 30 districts. The majority of the population (72.1%) resides in rural areas, while nearly half of the urban population is concentrated in Kigali, the capital city. The country topography and climate change increase environmental suitability and favourable condition for the breeding and population growth of the disease vectors (mosquito's). In Rwanda, malaria is high endemic in 11 out of 30 districts and the remaining 19 districts are prone to epidemics [163]. These areas, located in altitudes generally below 1,500 meters above sea level, are marked by warmer climate and are characterised by vast marshy plains [14]. Generally varying between 980 meters up to 2800 meters in the highland of Nyamagabe and Gicumbi. This study was conducted in nine districts of Rwanda namely Rusizi, Nyamasheke, Nyamagabe, Ruhango, Kamonyi, Bugesera, Gicumbi, Rulindo, and Gasabo spanning both urban and rural environments. The area experiences two high malaria transmission peaks associated with the dry seasons, typically observed after the rainy seasons, from November to December and May to July [164].

Participants, and samples

This study involved Western, Southern, Northern, Eastern provinces and Kigali of Rwanda, which are classified as malaria-endemic with stable transmission. A total of 1025 participants were voluntary enrolled in our study. Data collection was performed between 1st of November 2021 until December 2023, also the period encompasses three malaria peak season. Study participant recruitment took place at either a health facility (cases) or within their household (controls). This convenience sampling strategy was adopted to ensure enrolment of sufficient malaria positive participants despite the reduction in malaria cases in response to the commendable depletion of malaria cases, thanks to robust interventions (e.g., indoor residual spraying) deployed by the government of Rwanda in targeted areas at the time of our study. Cases of malaria-infected participants were identified at district hospitals or health centres in their respective catchment areas. Control participants were selected from the same households as the cases, and were visited at their houses on the same day as cases. Case were defined as individuals who tested positive for malaria and controls were those tested negative for malaria. There were no age or gender restrictions for each group. However, to exclude participants with antimalarial, antibiotic or deworming treatments, both case and control were asked to respond either they have been given or not antimalarials, antibiotics, and/or deworming in the past two weeks before sample collection.

No other restrictions were considered during the period of recruitment. Upon receiving their signed consent forms, a demographic questionnaire was filled out for each participant. On the same day, for each participant, a confirmatory (for cases met in health facilities) or screening (for controls met in households) rapid diagnostic test (RDT) was performed, and blood smears were prepared for final malaria diagnosis. 1025 subjects were recruited, and the flow of our study participants is displayed in Fig. 1. Children under 3 years old were excluded due to underrepresentation. After analysis of reported dietary habits to detect under reporters and over reporters using the Goldberg index, 337 participants were excluded. Then participants with adequacy between energy expenditure (EE) and energy intake (EI) were retained. Finally underdetermined blood smear analysis were excluded from the cohort and 658 participants were remaining with 224 positive as cases and 434 negative as controls (Fig. 1). All malaria positive cases were confirmed by RDT at health facilities or households and were treated in accordance with national malaria treatment guidelines by a trained surveyor All samples were collected prior antimalarial drugs administration.

Nutritional questionnaires and Anthropometric measurements

Food intake was evaluated using a 7-day Food Frequency Questionnaire (FFQ) and a 24-hour recall questionnaire. FFQ and 24h recall questionnaires were combined to minimize error in recording data related to food items consumed and to enhance complete and accurate food recall [165]. Study participants (both cases and controls) were challenged to report their dietary habits (FFQ) as well as to recall and list all the foods and drinks they had consumed the day before (24-hour recall questionnaire). Participants used visual aids provided to support recall of the portion of food consumed from “Photographic Food Atlas for Kenyan adolescents (9-14 years)” to evaluate the serving sizes of various foods, both for the FFQ and 24h recall. For the FFQ, four categories of consumption frequency were checked for each food item: never or rarely, 1 – 3 times per week, 4 – 7 times per week. Information related to the frequency of the food consumed per day was noted either once or twice per day, and 3 times per day. The total quantities of items consumed by study participants were recorded per 7 days. To obtain daily quantities, recorded amounts were divided by 7 before multiplying the result by the number of times the food item was consumed during the week. FFQ was used to estimate energy intake as well as macronutrients and micronutrients consumption. To convert the quantities of each food consumed in nutrients (macronutrients and micronutrients), a Rwanda Food Composition Table (FCT) was established based on the West Africa FCT 2019 and supplemented with the Kenya FCT 2018. Finally, the 7th edition of the Belgian FCT 2022 was used to complete the Rwanda FCT with a few items that were not present in the two previously mentioned tables. The West Africa FCT 2019 and Kenya FCT 2018 were chosen based on the closest proximate in food items and preparation for any given food composition. Thereafter, the Rwanda FCT generated was used to assess the macronutrients content of each food (protein (g), lipids (g), carbohydrates (g) and fibers (g)) that allowed to calculate energy intake (kcal). Micronutrients minerals intake (calcium, copper, iron, phosphorus, selenium, zinc) and vitamins intake (vitamin A, vitamin B2, vitamin B6, vitamin B9, vitamin B12, vitamin C, vitamin D, vitamin E) were also evaluated from the FFQ data using the Rwanda FCT.

To estimate the energy requirements of the participants, Basal Metabolic Rate (BMR) defined as the energy expended at rest based on weight, height, age, and sex, was calculated using Henry formulas [166]. This equation encompasses energy expenditure due to body maintenance for essential physiological functions at rest [167]. In addition, Physical Activity Level (PAL) was attributed to each participant based on age, and activity categories using estimation based on high activity level related to farming, walking long distance since over 70 % of the population lives in rural areas. Therefore these PAL values were used: 1,4 from 1 to 3 years old; 1,6 from 4 to 9 years; 1,8 from 10 to 17 years old, 1.8 for adult above 18 years old up to 64 years; and 1.4 above 65 years as the retirement age is at 65 years old where activity level is considered as reduced. All those PALs were attributed to the different age categories based on criteria mentioned above. Then, the total energy requirement was estimated considering both BMR and physical activity level (PAL) by multiplying the participant's basal metabolic rate by their level of physical activity.

Then, Goldberg index cut-offs were used to identify under-reporters and over-reporters of energy intake by considering BMR and PAL. This validation using the

Goldberg index is based on the fundamental principle that, energy intake matches energy expenditure when body weight is maintained stable [168,169].

The BMI was calculated by dividing the participant's weight (in kg) by the square of their height (in meters) and was expressed in kg/m^2 for adults aged above 18 years old. From the two key anthropometric measurements weight and height recorded for each person, the WHO instruments were used to assess the nutritional status, classifying them as underweight, eutrophic, overweight, and obese [119]. Following WHO recommendations, sex- and age-specific BMI-for-age Z-scores were used for participants aged 2-19, where the observed BMI were compared with the WHO growth reference for the same age and sex [170,171]. Stunting was defined as a length- or height-for-age Z-score below -2.0, with moderate stunting between -2.0 and -3.0, and severe stunting below -3.0 [34]. Our study population was categorized into four age groups (0-2, 3-10, 11-18, and 18+ years old), and those categories were attributed based on nutritional needs corresponding to different growth stages [172].

Ethical Considerations

Approval to conduct the study was sought and obtained (reference number 031/CMHS IRB/2021 issued on the 2nd of February 2021, reference number 217/CMHS IRB/2022 issued on the 2nd of February 2022, and reference 257/CMHS IRB/2023 issued on the 2nd of February 2023) from the Institutional Review Board of the University of Rwanda college of Medicine and Health Sciences. Written informed consent was obtained from participants aged 18 years and above, or from parents, relatives, or guardians of younger participants.

Statistical analysis

The nutrition data were analyzed for descriptive statistics to perform characterisation of the study population. Frequencies and means were calculated with standards deviations for categorical and numerical variables, respectively. Pearson chi square test was also performed to assess associations between categorical variables by comparing an association between observed and expected frequencies with p.value less than 0.05 if considered significant. Regression logistic analyses were performed where odds ratio with 95% confidence intervals were done to test the association between nutrition parameters in malaria cases versus their control. Adjusted OR were also generated from multivariate logistic regression, including variables with p.values <0.1 from univariate logistic regression in the model. The significance was set at 5% using StataSE 15 (64-bit).

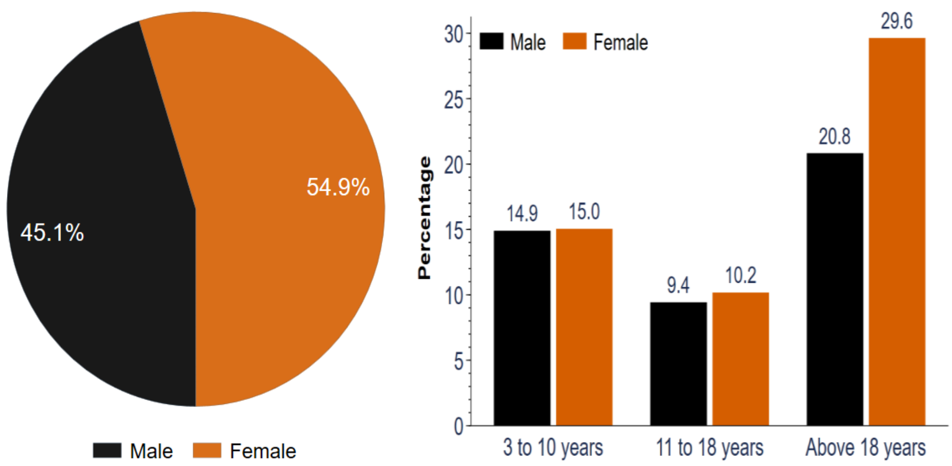
Results

Characteristics of study participants

A total of 658 participants were included in the analysis. The participants were categorized into three age groups based on different nutritional needs over the life growth: 197 participants were aged 3 to 10 years, 129 participants were aged 11 to 18 years, and 332 participants were over 18 years old.

The characteristics of study participants showed the following gender distribution; for every 1 male, there are approximately 1.22 females (45%/55%) with variations across age groups (Fig. 2A). Nearly equal proportion of males and females were represented in the group of 3 to 10 years old and 11 to 18 years old group respectively. A notable difference was observed in the adult group (above 18 years), where 20.8% of males and 29.6% of females were represented, indicating a higher proportion of females in this age category (Fig. 2B).

A **B**



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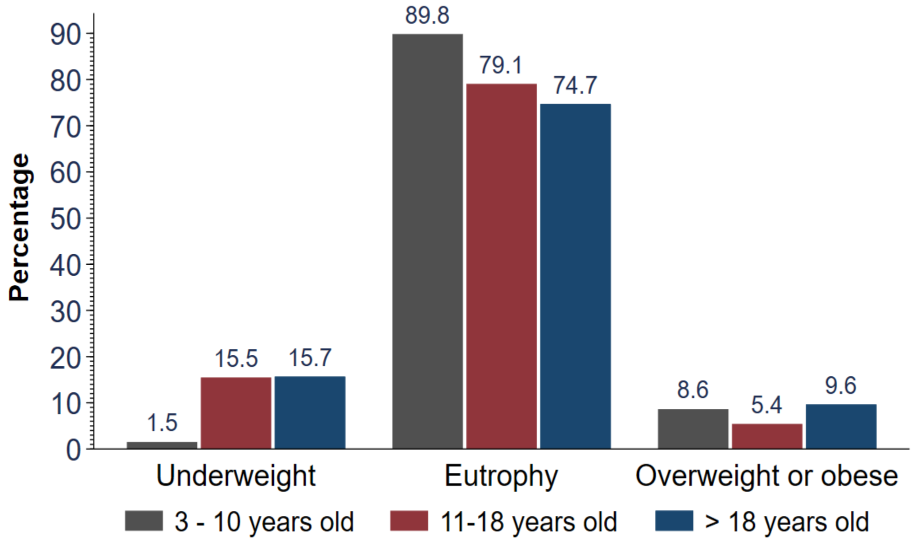

Figure 2 : Characteristic of the cohort population.

Characteristics of participants in negative and positive cases are shown in Table 1. Compared with controls, malaria cases were less likely in Kigali and Northern province while malaria cases were very high in Eastern, Southern, and Western province and the difference is statistically significant (p<0.001) (Table 1).

Table 1. Socio- demographics characteristics of the participants

Demographic & Nutrition N(%)	Malaria Negative n=434	%	Malaria Positive n=224	%	*p*-value*
Province					**<0.001**
Est	62	14.3	47	21.0	
Kigali City	263	60.6	100	44.6	
North	99	22.8	30	13.4	
West	4	0.9	22	9.8	
South	6	1.4	25	11.2	
Age group					**0.024**
3 to 10 years	115	26.5	82	36.6	
11 to 18 years	87	20.1	42	18.8	
Above 18 years	232	53.4	100	44.6	
Sex					0.102
Male	186	42.9	111	49.6	
Female	248	57.1	113	50.4	
Stunting (3-19 years old)					**0.063**
Normal	91	21.0	52	23.2	
Moderate chronic stunting	65	15.0	34	15.2	
Severe chronic stunting	46	10.6	38	17.0	
Not analysed**	232	53.4	100	44.6	
Body Mass (Based on BMI/Z-score)					0.934
Eutrophy	348	80.2	179	79.9	
Obesity	86	19.8	45	20.1	

*Pearson's chi squared

**Not analysed, Stunting calculated up 19 years old as per WHO standards, above 19 years old not analysed.

Dietary patterns influence body composition and associated health risks, contributing to conditions such as underweight, eutrophy, overweight, and obesity. In this dataset, nutritional status varied by age group: the prevalence of underweight increased from 1.5% in children aged 3–10 years to 15.5% in adolescents (11–18 years) and 15.7% in adults (>18 years) (Figure 2C). Conversely, eutrophy declined with age, from 89.8% in the youngest group to 79.1% in adolescents and 74.7% in adults.

In this study, overall malaria morbidity was 34.04 %; these findings are not comparable to malaria cases observed in the general population due to the participant selection for this study based on malaria cases versus control cases.

Dietary patterns of study participants

Participant's dietary patterns were analysed from the FFQ questionnaires after excluding 337 participants presenting under-reported or over-reported of their energy intake (Fig. 1).

The dietary habits of the study population were predominantly composed of starchy foods, which accounted for 56.7% of total intake. Fats contributed a moderate 16%, while vegetables and legumes made up 9.4% and 9.3%, respectively. Animal-source proteins were consumed at notably low levels: dairy products at 2.5%, and meat, poultry, fish, and eggs collectively at just 1.1% (Fig. 3).

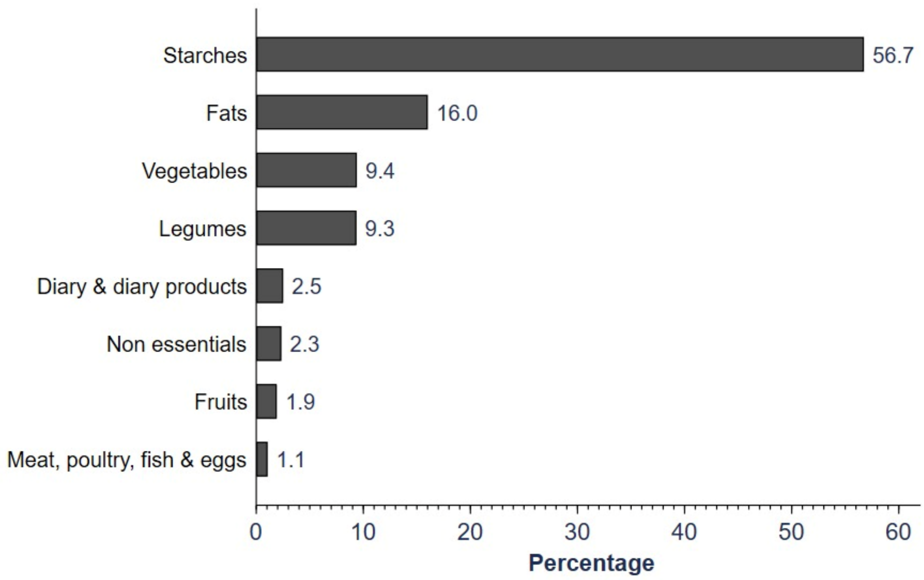

Figure 3: Distribution of food groups in the cohort: an analysis of dietary sources

Legend: Dietary distribution of starches, fats, vegetables, legumes, and animal protein sources, including meat, poultry, fish, dairy, and eggs.

The coverage between participant's dietary patterns and energy requirements was investigated. For the overall cohort, 58.2% of participants reported caloric intake below 90% of the daily recommended intake (Table 2). A total of 61.5% of female participants versus 54.2% of male participants reported energy intake below 90%

of their estimated daily energy requirements (Table 2). The mean daily caloric intake for females was 1706.6 kcal (SD = 408.1), while for males, it was 1771.8 kcal (SD = 526.8) (Table 2). The proportion of participants with reported intake below 90% increased progressively with advancing age, with the highest prevalence observed among adolescents (11-18 years old) at 76.7% (Table 2). Provincial variation was also noted, with the highest proportion of inadequate energy intake in Kigali (63.4%), followed by the Eastern Province (55%), and the lowest in the Southern Province (45%) (Table 2).

Table 2. Coverage of dietary requirements across the study cohort. Mean values, standard deviations, and percentages per sex, age, and province of the 658 participants classified according to the coverage of energy requirement based on their intake reports.

	Average daily caloric intake (kcal/day), mean(sd)	**Reported intake as a percentage of actual energy requirements**		
		< 90	**90-110**	**> 110**
Sex				
Male (n=297)	1771.8 (526.8)	54.2	26.3	19.5
Female (n=361)	1706.6 (408.1)	61.5	29.4	9.1
Age				
3 to 10 years (n=197)	1263.0 (287.3)	36.0	34.5	29.4
11 to 18 years (n=129)	1810.4 (316.6)	76.7	19.4	3.9
Above 18 years (n=332)	1987.8 (380.7)	64.2	27.4	8.4
Province				
East (n=109)	1640.9 (449.4)	55.0	25.7	19.3
Kigali (n=363)	1703.5 (447.8)	63.4	25.6	11.0
North (n=129)	1904.1 (478.2)	51.2	30.2	18.6
West (n=26)	1618.1 (485.5)	50.0	34.6	15.4
South (n=31)	1850.9 (499.0)	45.2	48.4	6.5
Total (n=658)	**1736.0 (466.20)**	**58.2**	**28.0**	**13.8**

Associations of nutrition characteristics with malaria outcomes

Tables 1 and table 3 up to 5 report % of different variable categories across cases compared to % of different variable categories across control subjects whereas table 6 indicates significant associations between nutrition characteristics and malaria. First, age distribution in malaria cases compared to control cases was investigated: 36.6% of the malaria cases were in the younger age group aged between 3 to 10 years old whereas controls in this age group were at 26.55%. In the opposite, the proportion of 11 to 18 years and above 18 years positive for malaria was lower compared to the proportion of controls negative case in this category. This higher representation of positive cases in 3 to 10 years old is in accordance higher odds ratio in the 3 to 10 years old group compared to older groups. (Table 6). Indeed, malaria cases decrease with an increase in age (aOR=0.58; 95% CI: 035-0.98 for 11 to 18 years and aOR=0.58; 95% CI: 0.36-0.95 above 18 years; p<0.04) (Table 6). Moreover, being female was negatively associated malaria (aOR=067; 95% CI: 0.46-0.97, p<0.001) (Table 6).

Stunting analysis was conducted up to 19 years since stunting condition is characterizing an impaired growth and is defined as to low height for age. While comparing to controls, severe chronic stunting is higher in positive cases reaching 17 % versus 10.6% in control (Table 1). However, moderate chronic malnutrition in malaria positive were similar to the proportion observed in negative cases. Besides, in normal stature, a slight increase of 2.2% for positive cases versus negative cases was observed, p=0.063 (Table 1). However, even if an increase odds ratio was observed in severe chronic malnutrition (aOR=1.23; 95% CI: 0.67-2.28), the association between malaria and stunting severity was not statistically significant (Table 6).

An association was highlighted between macronutrient intake and malaria, particularly lipid consumption. The proportion of individuals with low lipids intake was higher in malaria cases compared to controls, p<0.001 (Table 3). Interestingly, the risk of vitamin B12 deficiency among malaria cases was lower compared to controls (67% versus 76.7%, p=0.007) (Table 4). Although, the risk of vitamin C deficiency was low in the study population, it was significantly higher in malaria cases when compared to controls (3.1% versus 0.5%, p=0.005) (Table 4).

Moreover, compared to controls, vitamin E insufficient intake was a higher in malaria positive (31.3%) compared to the proportion of vitamin E deficiency in control cases 6.2 %, with p<0.001 (Table 4). For selenium, the prevalence of risk of deficiency is reduced in malaria cases (42,9%) compared to the risk of deficiency among negative cases (54.4%), p<0.005. (Table 5). In the unadjusted univariate analysis, the insufficient intake of selenium and vitamin E were respectively negatively and positively significantly associated with the presence of malaria ($p<0.05$) (Table 6) and this remained significant after adjusting for other covariates using multivariate logistic regression with an aOR at 7.46 (95% CI 4.43-12.58; $p<0,001$) for the risk of vitamin E deficiency and an aOR at 0.62 (95% CI 0.43-0.91; $p=0.013$) for the risk of selenium deficiency (Table 6). In addition, iron deficiency was positively associated with the presence of malaria (aOR =1.80; 95% CI 1.11-2.93; $p=0.017$) (Table 6).

Table 3. Malaria Proportions according to macronutrient variables

	Malaria				
Macronutrient	**Negative**		**Positive**		***p*-value**
N(%)	n=434	%	n=224	%	
Carbohydrates					0.811
Optimal Intake	423	97.5	219	97.8	
Low Intake	11	2.5	5	2.2	
Fibers					0.249
Optimal Intake	382	88.0	190	84.8	
Low Intake	52	12.0	34	15.2	
Proteins					0.748
Optimal Intake	356	82.0	186	83.0	
Low Intake	78	18.0	38	17.0	
lipids					**<0.001**
Optimal Intake	281	64.7	112	50.0	
Low Intake	153	35.3	112	50.0	

Table 4. Malaria Proportions according to vitamin nutrient variables

Vitamin Variables	Malaria Negative (N=434) Freq	%	Positive (N=224) Freq	%	*p*-value
Vitamin A					0.499
Optimal Intake	85	18.6	39	17.4	
Risk of Deficiency	349	80.4	185	82.6	
Vitamin B2					0.627
Optimal Intake	103	23.7	57	25.5	
Risk of Deficiency	331	76.3	167	74.5	
Vitamin B6					0.480
Optimal Intake	308	71.0	153	68.3	
Risk of Deficiency	126	29.0	71	31.7	
Vitamin B9					0.448
Optimal Intake	258	59.5	140	62.5	
Risk of Deficiency	176	40.5	84	37.5	
Vitamin B12					**0.007**
Optimal Intake	101	23.3	74	33.0	
Risk of Deficiency	333	76.7	150	67.0	
Vitamin C					**0.005**
Optimal Intake	432	99.5	217	96.9	
Risk of Deficiency	2	0.5	7	3.1	
Vitamin D					0.507
Optimal Intake	5	1.1	4	1.8	
Risk of Deficiency	429	98.9	220	98.2	
Vitamin E					**<0.001**
Optimal Intake	407	93.8	154	68.7	
Risk of Deficiency	27	6.2	70	31.3	

Table 5. Malaria Proportions according to minerals nutrient variables

Mineral Variables	Malaria Negative Freq	%	Positive Freq	%	*p*-value
Calcium					
Optimal Intake	76	17.5	46	20.5	0.344
Risk of Deficiency	358	82.5	178	79.5	
Iron					
Optimal Intake	342	78.8	178	79.5	0.843
Risk of Deficiency	92	21.2	46	20.5	
Copper					
Optimal Intake	431	99.3	219	97.8	0.087
Risk of Deficiency	3	0.7	5	2.2	
Zinc					
Optimal Intake	123	28.3	59	26.3	0.586
Risk of Deficiency	311	71.7	165	73.7	
Phosphorus					
Optimal Intake	338	77.9	168	75.0	0.406
Risk of Deficiency	96	22.1	56	25.0	
Selenium					
Optimal Intake	198	45.6	128	57.1	**0.005**
Risk of Deficiency	236	54.4	96	42.9	

Table 6. Regression pooled analysis of the association between malaria infection and nutritional status micronutrient and macronutrient

Malaria (n=224)	Positive Freq	%	Odds ratio	[95% conf. interval]	*p*-value	Adjusted Odds ratio	[95% conf. interval]	*p*-value
Age group					**0.025***			**0.041**
3 to 10 years	82	41.6	1			1		
11 to 18 years	42	32.6	0.68	0.425 - 1.077		0.58	0.347 - 0.979	
Above 18 years	100	30.1	0.60	0.418 - 0.873		0.58	0.355 - 0.954	
Sex					0.102			**0.036**
Male	111	37.4	1			1		
Female	113	31.3	0.76	0.552 - 1.055		0.67	0.455 - 0.973	
Vitamin E					**<0.001**			**<0.001**
Optimal Intake	154	27.5	1			1		
Risk of Deficiency	70	72.2	6.85	4.235 - 11.086		7.46	4.426 - 12.581	
Vitamin B9					0.448			0.083
Optimal Intake	140	35.2	1			1		
Risk of Deficiency	84	32.3	0.88	0.631 - 1.225		1.42	0.956 - 2.103	
Stunting (3 -19 years)*					0.170			0.552
Normal	52	36.4	1			1		
Moderate chronic stunting	34	34.3	0.92	0.535 - 1.566		1.02	0.571 -1.813	
Severe chronic stunting	38	45.2	1.45	0.835 - 2.501		1.23	0.667 - 2.284	
Selenium					**0.005**			**0.013**
Optimal Intake	128	39.3	1			1		
Risk of Deficiency	96	28.9	0.63	0.455 - 0.871		0.62	0.427 - 0.905	
Iron					0.843			**0.017**
Optimal Intake	178	34.2	1			1		
Risk of Deficiency	46	33.3	0.96	0.646 - 1.430		1.80	1.111 - 2.930	

Discussion

This study aims to investigate the association between nutritional intake and malaria infection in rural areas of Rwanda. These findings indicate that undernutrition, characterised by the risk of macronutrient and micronutrient deficiencies, was common in these rural settings. Moreover, the prevalence of chronic undernutrition evaluated through stunting indicator was important (32.2%, Table 1). This is in agreement with the 2020 Rwanda Demographic and Health Survey that reported stunting prevalence of 33% [89]. The study findings only revealed marginally insignificant association between stunting and malaria in the model. However, the frequencies of severe stunting among malaria positive cases tend to be higher compared to control cases with a p=0.063. This is in accordance with the previous study evaluating the relationship between nutritional status and malaria revealing a significant association between malaria and stunting based on DHS data 2010-2020. That said, the relationship between malaria and nutritional status, specifically stunting, remains complex and varies among studies [173–175].

Despite several nutritional interventions to address nutritional burden [176], this study reveals inadequate intake in micronutrients for vitamin A in 81.2%, vitamin B2 in 75.7%, vitamin B12 in 73.4%, calcium in 81.5%, zinc in 72.3%, and selenium in 50.5% of the participants (Table 4 and 5). These findings corroborate with Arsenault et al., 2018 [139], reporting risk of deficiencies in calcium at 75%, vitamin A at 60%, vitamin B12 at 55% and vitamin B2 at 33% in Rwanda. Additionally, inadequate intake of micronutrients in the study population is in accordance with Goto et al., 2024 [177] reporting inadequate intake up to 90% in mainland of Zanzibar in the absence of food fortification, whereas a slight decrease in insufficient vitamin A intake from 88% to 66% was observed after fortification. Finally, Mbunga et al., 2022 [178] study analysed the levels of minerals in serum and reported relatively high selenium deficiency 84.1% [95% CI 81.4-87], indicating low intake of selenium-containing food products and/or low selenium absorption in the region. Possible explanations resulting in these deficiencies include food insecurity and lack of diversified food [141,179]. This evidence suggest the need for upscaling and improving the implementation of currently ongoing strategies to prevent and control undernutrition in the country including biannual micronutrient powder supplementation [112,139], vitamin A distribution for children under five, and deworming (Albendazole, Mebendazole) for children under 15 years through Maternal and Child Health Week [139].

This study also reported that composition of foods consumed are mainly based on starches (56.7% of total food intake) which are rich in macronutrients but low in micronutrients [136]. Evidence from the current study indicates that fruits and animal source product consumption were extremely low accounting for 1.9% and 1.1% of total food intake, respectively. This dietary pattern may contribute to the micronutrient deficiency. Al-Bayyari et al. 2024 [180] observed that anaemia and micronutrient deficiencies in pregnant women were linked to low intake of animal products, starchy diets, and limited seasonal consumption of fruits and vegetables.

Based on multivariate analysis, this study found that the proportion of the population at risk of selenium deficiency was significantly higher in malaria negative individuals compared to malaria positive individuals. This suggests that selenium deficiency is inversely associated with malaria, as indicated by the adjusted odds ratio (aOR = 0.62; 95% CI: 0.43–0.91; P < 0.013) (Table 6). One hypothesis supporting this surprising result could be that selenium deficiency induced oxidative stress, which reduced the parasite *Plasmodium falciparum* multiplication in the host. Supporting this hypothesis the antimalarial drugs also contribute to the oxidative stress as an effective way of parasite clearance within the red blood cell [181]. However, this hypothesis is totally speculative and remains to be investigated.

In Rwanda, the consumption of iron bean biofortified has been shown to improve iron intake [182], which is also seen in this study reporting appropriate iron intake in almost 80% of the study population. However, even if the risk of iron deficiency remains low it was positively associated with malaria in adjusted odds supporting a positive link between malaria and insufficient iron intake. Importantly, these findings suggest that inadequate iron intake may contribute to anaemia in individuals with malaria in addition to the direct effect of parasite on anaemia [34].

Finally, the regression model revealed that the risk vitamin E deficiency was associated with malaria as indicated by the adjusted odds ratio (aOR=7.46; 95% CI 4.43-12.58). Similarly, in the systematic review and meta-analysis conducted by Kotepui et al.,2023 [183], insufficient vitamin E intake were also significantly associated with an increased likelihood of malaria.

Strengths and limitations

Major strengths of this study comprised the highlighting of nutritional determinants including selenium, iron, and vitamin E intake that are associated with the risk of malaria infection. These results will contribute to develop targeted nutrition strategies to improve public health practice which is in alignment with public health programs in Rwanda. Therefore, integration of both dietary intake and infection data is also an important strength of this study. Additionally, including the household companions of the cases as control, reduced the risk of participant selection and reporting bias as well as limited the risk of potential unforeseen lifestyle confounding factors. Furthermore, the use of adjusted odds ratios to control for confounding empowered this study contributing to the limited literature on micronutrient and malaria associations in low-transmission settings in sub Saharan Africa including Rwanda. That said, this study also presents some limitations. A limitation of this study was the small number of malaria cases in children under three years of age, primarily due to significant changes in malaria epidemiology in Rwanda resulting from successful malaria control interventions. This low number of malaria cases led to a small sample size in this subgroup, limiting the statistical power to detect meaningful associations, therefore children under three years old subgroup was excluded from the final analysis. Moreover, it is also important to keep in mind that two studies, one is cross sectional and the second is a case control study, both studies limit the causality of the effect between nutritional intake and malaria infection. Additional studies such as longitudinal and intervention studies are required to address the causality effect. Finally, a recall bias due to the retrospective information related to nutrition intake cannot be excluded and could lead to potential risk of error from participants in reporting their dietary habits.

Conclusion

These results emphasize the association between nutrient intake particularly vitamin E, selenium, and iron, and the susceptibility to malaria infection. This suggests that addressing micronutrient deficiencies may be a valuable strategy in malaria control efforts. Improving nutrition status, with an emphasis on food composition and a balanced diet, could further strengthen immunity for the control of infectious diseases including malaria. Therefore, integration or close collaboration between national programs for nutrition and infectious diseases control are highly recommended.

In perspective, a comprehensive understanding of *Plasmodium* pathogenesis, and particularly the impact of specific micronutrient levels in the host on this pathogenesis, is essential to improving disease prevention and control. Such insights are critical not only for elucidating the parasite's survival and reproduction strategies but also for guiding effective interventions aimed at its elimination.

Author contributions

Conceptualization: A.E, L.M., J.P.C., P.D.C. and N.R.; methodology, A.U., H.A., A.R., L.M. and A.E.; investigation and interpretation: A.U., H.A., J.A.M., J.P.C., L.M. and A.E.; writing—original draft: A.U., L.M. and A.E.; writing—review & editing: A.U., H.A., J.A.M., A.R., N.R., P.D.C., J.P.C., L.M. and A.E.; supervision: L.M. and A.E.; funding acquisition: N.R., P.D.C., J.P.C., L.M. and A.E.

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Competing interests

A.E. and P.D.C. are inventors on patent applications dealing with the use of A. muciniphila and its components in the treatment of metabolic disorders. A.E. and P.D.C. are inventors on patent applications dealing with gut microbes in food reward dysregulations. A.E. is inventor on patent applications dealing with the use of bacteria metabolites in the prevention or treatment of respiratory viral infections P.D.C. was cofounder of The Akkermansia company SA and Enterosys. All other authors declare they have no competing interests.

DISCUSSION AND PERSPECTIVES

In Rwanda, malaria is a public health concern and remains a high priority. Several evidence-based interventions have been adopted over time and have shown good impact; however, sustainability has been hindered by issues related to emerging parasite resistance to artemisinin derivatives and vector resistance to pyrethroid and the re-emergence of the *Anopheles gambiae* and environmental effects related to air temperature increased, including the socioeconomic aspects [184,185]. Due to limited resources, the country has adopted and is encouraging researchers to foster a new innovative approach to support existing malaria control measures. Accordingly, our thesis aimed to assess the nutritional status and asymptomatic malaria cases. It also focused on assessing nutrient composition and its association with malaria cases in Rwanda endemic areas. The first study revealed the important role of asymptomatic malaria in maintaining the perennial malaria transmission and child growth and development. It further identified the association between nutritional status and asymptomatic malaria.

The second study revealed the cricial role of micronutrients status in malaria infection outcomes, highlighting the public health importance of addressing widespread micronutrient deficiencies in malaria endemic settings. This chapter focuses on major findings and their impact, study limitations, and considerations.

Major findings and their impact

First study

- *Undernutrition and asymptomatic malaria:*
  Undernutrition was significantly associated with a higher risk of asymptomatic malaria in children under five, indicating a potential underestimation of public health concern in malaria control strategies.

- *Potential Protective Role of Iodized Salt:*
  The use of iodized salt showed a reduction of malaria cases in unadjusted models, suggesting that specific micronutrients like iodine may play a role in lowering malaria risk.

- *Anemia and Micronutrient Deficiency:*

The presence of anemia indicates iron, vitamin B9 or B12 deficiencies as a proxy, was found to be associated with asymptomatic malaria, reinforcing the importance of addressing underlying micronutrient gaps.

- *Influence of Socioeconomic Factors*:
  Maternal education, household wealth, and other socioeconomic factors were associated with malaria outcomes, emphasizing the significance of broader social determinants in child health and malaria prevention.

Second study

- *High Levels of Micronutrient Deficiencies:*
  The study revealed that participants had low levels of essential nutrients such as Vitamin A, B2, B12, calcium, zinc, and selenium, highlighting that micronutrient deficiencies remain a major concern in rural Rwanda.

- *Limited Dietary Diversity:*
  Most diets were dominated by starchy foods (56.7%), with very little consumption of fruits (1.9%) and animal-based foods (1.1%), which likely contributes to the elevated rates of nutrient deficiencies.

- *Vitamin E and Malaria Risk:*
  Children deficient in Vitamin E were seven times more prone to contracting malaria, suggesting that this nutrient may play an important role in combating infections.

- *Female Sex as possible protective factor*
  Being female were linked to a reduced risk of malaria when accounting for other nutrients and age, indicating they could have a protective effect.

Strengths and limitations

- Effective and timely vector control interventions in the country result in epidemiological change. Thus, a notable malaria decrease was noted in children under 3 years, causing a delay in data collection.

- The study utilized Physical Activity Levels (PAL) based on data from existing literature, rather than direct measurements, which may not fully reflect the physical activity habits of the participants.
- Energy intake was estimated using a Food Frequency Questionnaire (FFQ), a method that, despite being commonly used, is prone to recall bias and inaccuracies, potentially affecting the reliability of dietary intake data.
- Secondary data analysis of surveillance data used, collected at one specific time, with limited ability to conclude on the causality effect.

4.1. MAJOR FINDINGS AND THEIR IMPACT

The above-mentioned major findings reveal initial evidence for a relationship between micronutrients from dietary intake and malaria in Rwanda. Although one study conducted in 2015 by Kateera et al. measured the association between malaria and malnutrition, the authors used only anthropometric measurements [76]. A systematic review conducted by Das et al. in 2018 [186] also reveals mixed findings on malnutrition outcomes related to malaria risk. While wasting exhibits no evident association with malaria risk, stunting was repeatedly associated with increased prevalence of severe malaria and anemia. Additionally, among the 33 studies analyzed, individual micronutrient analyses were not conducted [186] [187].

The first evaluation of the DHS data revealed that stunting, underweight, and children living in low wealth index households had significant risk factors for asymptomatic malaria. Besides, this interconnectivity may be exacerbated with reduced immunity caused by hidden hunger due to chronic malnutrition.

In endemic areas, children are often exposed to *Plasmodium falciparum* from an early age, which confers partial or acquired immunity or sometimes natural (innate) immunity [188,189]. Asymptomatic malaria remains a neglected concern, despite its significant role in sustaining perennial transmission, which poses a major challenges for malaria elimination [190]. Li et al [191] evaluated factors associated with child stunting, wasting, and underweight in 35 low and middle-income countries using DHS (2007-2018). These authors concluded on the relevance of sociodemographic factors (wealth index, mother education, household dietary habits) for child stunting. Besides, they found that households with a history of no iodized salt and infectious diseases were associated with child stunting. However, country-specific orientation and adoption of policy should be taken with caution. These findings corroborate our findings from our first evaluation [34].

Anemia is a condition characterized by a reduction of RBC due to multiple factors related to parasite infections, including *Plasmodium falciparum*, low absorption of iron from diets, and poor dietary habits. Similarly, our results from the DHS analysis mirrored those from the study conducted in Uganda, where secondary analysis of the Malaria Indicator Survey (MIS) 2018-2019 in children under five highlighted the interlinkage between anemia and malaria [181]. In asymptomatic malaria cases, parasite density is very low. However, parasites are recognized by the strong host-acquired immunity, and thus do not cause symptoms. Mostly, parasites invade at the ring stage and rarely burst to invade the RBC, where cytokines, TNF alpha, and IL6 are released to cause signs and symptoms, which is why asymptomatic carriers do not seek treatment. Yet, few RBCs remain compromised, underscoring the importance of targeting asymptomatic carriers with seasonal chemoprophylaxis and antimalarial drugs that tarovaleget the sexual cycle to complete available integrated interventions [181]. Although anemia is caused by multifaceted factors and actions taken by the WHO and countries, it continues to affect approximately millions worldwide and mostly affects 4 out of 10 children under five globally, disproportionately distributed and mostly affecting SSA and SA countries [182]. Therefore, strategies should target the underlying causes in different settings, both in clinical situations and for public health purposes [183]. Besides, anemia affects economic development, induced by fatigue, which reduces productivity. An economic analysis showed that investing 1 $ in the control of anemia in pregnant women, adolescent girls, and children under would return 12$ from human production [192].

The second study assessed the relationship between nutrition and malaria through an in depth assessment of dietary intake, in order to understand the possible contribution related to inadequate micronutrient intake to malaria risk. Findings showed that limited dietary diversity is highly distributed in our study population, with diets composed predominantly of starches at almost 57%, fruits, and animal source foods at 1.9% and 1.1%, respectively. Common source of micronutrient dense foods, such as fruits, animal source foods, vegetables, and legumes were among the least consumed in our study population, which likely contributes to the high proportion of individuals at risk of micronutrient deficiencies. The risk of inadequate intake observed, where mainly essential in micronutrients: vitamins, and minerals, such as vitamin A, vitamin B2, vitamin B12, zinc, and calcium ranged between 67% - 82.6%. This issue was emphasized in 2024 by the findings from the global estimation of dietary micronutrient inadequacies conducted by Simone et al [193]. Similarly, a modelling analysis from 185 countires using individual level dietary dataset of food intake which revealed that estimated inadequate intakes were high in calcium, vitamin E, iodine ranging between 66% and 68% in more than 5 billion and for more than 40 billion the consumption was also very low ranging between 53% to 65% for vitamin, vitamin B9, vitamin B2 and Iron. This shows how the issue of hidden hunger is crucial and the importance of food fortification programs, especially in low and middle-income countries, and should be applied in emergencies [194]. This issue continues to rise as food insecurity follows the same trends, and dramatically continues to rise disproportionately in Africa and South Asia. Warnings have been made by UN agencies (FAO, IFAD, UNICEF, WHO) related to the progress trends [195].

Nutrition plays a crucial role in the optimal control of infectious diseases and the promiotion of well-being, Both macronutrients and micronutrients are necessary for body metabolism and the immune system support. In our second study, participants with vitamin E inadequate intake, were seven times more prone to get malaria episodes, suggesting that this nutrient may play an important role in combating infections. Besides, the risk of iron deficiency and being a female may protect against malaria episodes. Adjusting for other nutrients, sex, and age, and being female were each linked to a reduced risk of malaria. These findings suggest possible interaction effect when combined and enhance protection against malaria. Given the proven immune role of zinc and selenium, our study population showed that high concern of risk deficiency which may compromise diseases control and severe malaria and concommitent complications in a broader extent [196,197]. Secondary analysis will be conducted to roll out, nutritional status changes are associated with malaria parasite density in order to ascertain possible correlation

4.2. STRENGTHS AND LIMITATIONS

Dietary habits and food intake are oftenly being studied in term of economic growth aspect in Rwanda, but the linkage with infectious diseases and specifically malaria seemed underresearched. Specifically when the linkage target the importance of micronutrient in malaria control. Assessment of food intake using a quantitative FFQ to investigate the linkage between malaria and micronutrients in Rwanda seems to be the first, after a search using mesh term in PubMed, search for micronutrients and infectious diseases in Africa, retrieved 865 papers: ("micronutrients"[Pharmacological Action] OR "micronutrients"[Supplementary Concept] OR "micronutrients"[All Fields] OR "micronutrients"[MeSH Terms] OR "micronutriments"[All Fields] OR "trace elements"[Pharmacological Action] OR "trace elements"[Supplementary Concept] OR "trace elements"[All Fields] OR "micronutrient"[All Fields] OR "trace elements"[MeSH Terms] OR ("trace"[All Fields] AND "elements"[All Fields])) AND ("communicable diseases"[MeSH Terms] OR ("communicable"[All Fields] AND "diseases"[All Fields]) OR "communicable diseases"[All Fields] OR ("infectious"[All Fields] AND "diseases"[All Fields]) OR "infectious diseases"[All Fields]) AND ("africa"[MeSH Terms] OR "africa"[All Fields] OR "africa s"[All Fields] OR "africas"[All Fields]). Following the search, zero papers were retrieved after adding the mesh terms vitamins, minerals, and Rwanda.

Our second study helped to elucidate the nutritional determinants, such as selenium, iron, and vitamin E intake in addition to demographic factors like age and sex, in relation to the risk of malaria episodes in the context of food secutiry.

We experienced data collection delays due to malaria patterns changes following effective and timely vector control interventions (IRS in 12 targeted endemic districts and universal LLINs with pyrethroids and PBOs nets) in the country resulting in epidemiological change; a notable malaria decrease was noted in children under 3 years. Umugwaneza et al. confirmed the epidemiological change and reduction of 84% in children under five from 2018 up to 2023 and 88% reduction in the general population [185]. The concern age group, below 3 years old is the most age affected by malnutrition, this study may limit the accuracy of the overall undernutrition indicators.

Additionally, this study used attributed physical activity level (PAL) values based on existing data and literature, rather than collecting PAL directly using the questionnaires, which are usually used in epidemiological nutrition research. While numerous tools exist to measure PAL, such as self-report questionnaires, direct observation, and devices such as accelerometers, pedometers, heart rate monitors, armbands, their accuracy have been also questioned. All these tools are not accurate, no single tool can comprehensively provide all the aspect of PA, besides devices based methods often require some knowledge and are expensive [198]. Expert in epidemiology nutrition study suggest to use multiple PA measurement tools to complement the missing part in one another where deem appropriate to capture accurate measurement of PAL with available financial resource and adequate time.

Another important component is the energy intake estimated using a FFQ, despite being an easy commonly used tool, it is prone to frequent undereporting biais which my result from recall bias or intentional omission due social desirability, both generate frequent inaccuracies in frequencies and portion of food consumed over weekdays in end compromise the quality of data. This may potentially affect the reliability of dietary habits and intake information data. Besides, the study used a generated FCT due to the unavailability of the Rwanda specific version, this may affect the accuracy of estimation in nutrients, which highlights the need to have a country specific validated food composition table. Integrating both dietary intake and malaria infection information is major advantage of our study. Moreover, considering household companions as controls mitigate selection bias and potential confounding.

Correlation between underweight and stunting interpretation

Stunting in underweight 87.9% (934/1063) compared to stunting in normal 33.2% (3,440/10,357)

Among 11,420 children, 38.3% were stunted and 9.3 % were underweight. Among 4,374 stunted children, 21.4% were underweight were also not underweight, and only 1.83% were underweight. Showing an important association where a child is stunted has a high chance to be underweight,

The difference was significant, chi-squared = , p-value <0.001.

Table 1. The contingency table of association between malaria and stunting for people who are underweight or of normal weight

	Asymptomatic malaria			
	n/N	**%**	**OR (95% CI)**	**P value**
Underweight = Yes				
Stunting = Yes	26/898	2.89	1.03 (0.3, 3.47)	0.957
Stunting = No	3/107	2.80	1	
Underweight = No				
Stunting = Yes	51/3,311	1.54	1.60 (1.10, 2.34)	0.015
Stunting = No	58/6,001	0.97	1	

In underweight children, the prevalence of asymptomatic malaria is high, 2.89% and 2.80% in both stunted and non-stunted children. While in normal children, asymptomatic malaria is higher in stunted (1.54%) than in non-stunted children (0.97%)

We investigated whether underweight is a potential effect modifier in this study. We stratified the crude data into two groups—underweight and normal weight—and calculated the odds ratio (OR) for each category. The OR for underweight individuals was 1.03 (95% CI: 0.30–3.47), while for those with normal weight, it was 1.60 (95% CI: 1.10–2.34). These results suggest that underweight is an effect modifier.

Correlation describes the strength of the relationship between two variables. A correlation coefficient ranges from -1 to +1. +1 indicates a perfect positive linear relationship, and -1 indicates a perfect negative linear relationship. Zero indicates the variables are uncorrelated and there is no linear relationship [199,200]

Table 2. Scatter correlation matrix of all covariates

	Malaria	Stunting	Underweight	Wasting	Age	Year	Region	Residence	Education	Sex	Anaemia	Wealth
Malaria	1											
Stunting	0.0355	1										
Underweight	0.0442	0.3266	1									
Wasting	-0.004	-0.0103	0.2996	1								
Age	0.0299	0.0763	0.0038	-0.0729	1							
Year	-0.006	-0.0881	-0.0538	-0.0488	-0.0261	1						
Region	0.0175	0.0503	0.0145	-0.0187	0.0085	0.0026	1					
Residence	0.0312	0.1447	0.0664	0.0001	0.0065	-0.0806	0.3105	1				
Education	-0.0318	-0.0967	-0.0553	-0.0032	-0.0456	0.0807	-0.0468	-0.1065	1			
Sex	-0.0047	-0.0845	-0.0329	-0.0104	0.0022	-0.0006	-0.0077	0.0161	0.0124	1		
Anaemia	0.1042	0.0284	0.0633	0.0517	-0.2476	-0.0147	0.0138	0.0345	-0.029	-0.0331	1	
Wealth	-0.0677	-0.1823	-0.1059	-0.0151	0.0092	-0.0071	-0.0432	-0.2825	0.1638	0.0034	-0.0565	1

Legend:

-1 0 +1

Based on the scatter plot matrix and the correlation matrix, the Pearson correlation coefficients indicated a weak positive association between malaria and both stunting (r = 0.0355) and underweight (r = 0.0442), stunting and underweight exhibited a positive correlation (r = 0.3266) , supporting the interpretation that, when one is more prevalent , then the other one is more prevalent.

Our studies are cross sectional study and case control study, both collected information at one specific time. There are observational study without assigned interventions, both studies are limited to determine the causal inference and can only identify an association between different groups. Therefore, the recommendation is to perform strong, well-designed randomized controlled trials (RCT) or longitudinal studies that control bias through random sampling but due to the complexity of nutrition assessment in real world, RCT are not preferable. For example, provide different groups with fortified food and control.

4.3.IMPLICATIONS FOR MALARIA INTERVENTIONS

Critical highlights from the present thesis elucidate the linkage between micronutrients and malaria in Rwanda settings. These findings may orient the fortification of the crops program to complement available malaria prevention interventions. For example, the iron-rich foods biofortified programme in rural settings. Also, coupled with the available 14 iron biofortified bean types and varieties, clear monitoring and evaluation of the program should be put in place, as a randomised controlled trial has shown an improvement in micronutrient status by consumption of biofortified foods in a targeted population.

Further studies are needed to accurately determine how much iron biofortified beans should contain in cooked meals, to provide a minimum dietary diversity and a minimum acceptable diet. This will be possible if targeted areas and populations are repeatedly followed either in a randomized controlled trials or in a longitudinal cohort studies, using more precise tools to measure the PAL, and calorimetry tools. To be effective in a sustained manner, policies and guidelines should serve as a foundation to ensure minimum dietary diversity and food security, especially in a lean seasons. A recent cross-sectional study showed that more than half of the pregnant women followed had inadequate minimum dietary diversity in a health facility cross-sectional study in a southern province in Rwanda [201]. This finding revealed a very important concern of the 1000 Days campaign of life. Sustainability of prompt and accurate use of multiple micronutrient supplements in antenatal care should be implemented countrywide with the support of an awareness campaign through community radio.

Perhaps the most promising area of exploration is the mechanism of the role of micronutrients in protection against malaria through alleviation of oxidative stress and the inflammation induced by the parasites or the host. This step will enhance the use of supplements adequately or biofortified crops, this dual approach would support the ultimate protection of the host while minimizing the interference of micronutrients in the immune system.

4.4 General Conclusion

Malaria remains a major public health concern in Rwanda, driven by factors such as parasite resistance and environmental changes. This study examined the link between nutrition and malaria, with a focus on asymptomatic malaria and the nutritional status of vulnerable groups, particularly children under five.

Findings revealed a strong association between undernutrition and increased risk of asymptomatic malaria, underscoring the need to integrate nutritional strategies into malaria control efforts. Anaemia and micronutrient deficiencies—especially Vitamin E—were found to significantly influence malaria outcomes. The widespread issue of hidden hunger, caused by poor dietary diversity, further exacerbates these deficiencies, suggesting that targeted nutritional interventions could bolster malaria prevention.

Socioeconomic determinants, including maternal education and household wealth, also emerged as key risk factors, supporting a multi-sectoral approach that incorporates nutrition and education into health strategies.

Despite offering valuable insights, the study faced limitations, such as reliance on secondary data and potential biases in dietary assessments. Future research should prioritise longitudinal studies to establish causality and explore the biological mechanisms linking micronutrients to malaria susceptibility. Evaluating biofortification programmes may also offer promising avenues for improving nutrition and reducing malaria risk.

The findings advocate for integrated malaria control strategies that combine nutritional interventions with existing measures, alongside community education on the role of nutrition in malaria prevention. Addressing nutritional deficiencies is essential for effective malaria control and improving health outcomes among Rwanda's most vulnerable populations are ranging between 5 to 14 years old, this category is most affected age due to shifts in malaria epidemiology and impede immunity following extensive integrated malaria control.

Take-Home Messages

- **Undernutrition is strongly linked to increased risk of asymptomatic malaria**, especially in children under five and pregnan.
- **Micronutrient deficiencies**, particularly Vitamin E and anaemia, significantly affect malaria outcomes.
- **Hidden hunger**, due to limited dietary diversity, it worsens nutritional deficiencies and hunders malaria vulnerability.
- **Socioeconomic factors**, such as maternal education and household wealth, influence malaria risk.
- **Integrated strategies** combining nutrition, education, and malaria control are essential.
- **Future research** should focus on longitudinal studies and the role of biofortification in improving nutritional status and in malaria control include epigenetic
- **Community education** on balanced and adequate nutrition in malaria prevention is vital for improved health and wealth conditions.

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