Review Article

# Gut Microbiota at the Intersection of Malaria Transmission and Disease: A One Health Perspective for Vector and Host Based Control Strategies

**Rahadi Swastomo[1,2*], Teguh Wahju Sardjono[3], Aulia Rahmi Pawestri[3], Sumarno Reto Prawiro[4]**

*[1]Doctoral Program of Medical Science, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia; [2]Department of Parasitology, Faculty of Veterinary Medicine, Universitas Brawijaya, Malang, Indonesia; [3]Department of Parasitology, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia; [4]Department of Microbiology, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia.*

**Abstract** | Malaria remains a major global health challenge, with persistent transmission driven by ecological heterogeneity, drug and insecticide resistance, and variable host susceptibility. Beyond traditional host–parasite paradigms, accumulating evidence suggests that gut microbiota in both mosquito vectors and vertebrate hosts represents an additional biological layer influencing malaria transmission dynamics, immune regulation, and disease severity. This review critically synthesizes recent literature (2020–2025) examining microbiota–malaria interactions within a One Health framework, integrating evidence from experimental models, human observational studies, and vector ecology. While controlled animal and laboratory studies provide mechanistic insights into microbiota-mediated modulation of parasite development and inflammatory responses, most human and field-based data remain correlational, limiting causal inference; therefore, this review evaluates methodological limitations, statistical rigor, and translational constraints, including ecological variability, population-level heterogeneity, and regulatory challenges. Microbiota-targeted strategies such as probiotics, symbiont manipulation, and paratransgenics are therefore discussed as exploratory and complementary approaches rather than established interventions. Overall, this review highlights the need to move from descriptive associations toward causally robust, ecologically grounded, and policy-aware research to define the realistic role of microbiota-informed strategies in integrated malaria control.

**Keywords** | Malaria, Gut microbiota, Vector competence, Immune regulation, Dysbiosis, Probiotics, Paratransgenics, One health, Translational research, Ecological robustness

**Received** | December 26, 2025; **Accepted** | February 12, 2026; **Published** | July 28, 2026
***Correspondence** | Rahadi Swastomo, Doctoral Program of Medical Science, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia; **Email:** rahadiswastomo@ub.ac.id
**Citation** | Swastomo R, Sardjono TW, Pawestri AR, Prawiro SR (2026). Gut Microbiota at the intersection of malaria transmission and disease: A one health perspective for vector and host based control strategies. Adv. Anim. Vet. Sci., 14(8):1668-1683.
**DOI** | https://dx.doi.org/10.17582/journal.aavs/2026/14.8.1668.1683
**ISSN (Online)** | 2307-8316



## INTRODUCTION

Malaria remains a significant global health burden, particularly in tropical and subtropical regions, where transmission persists despite extensive control efforts. Recent estimates indicate that malaria continues to cause substantial morbidity and mortality worldwide, with children and immunologically vulnerable populations being disproportionately affected (Acheng, 2024; World Health Organization, 2025). Although antimalarial drugs, insecticide-based vector control, and vaccination strategies have reduced disease incidence in some regions,

their long-term effectiveness is increasingly challenged by drug resistance, insecticide resistance, and ecological heterogeneity that shapes transmission dynamics across endemic settings (Naidoo and Oliver, 2025).

Field-based studies from malaria-endemic regions further demonstrate that local environmental conditions, sanitation, and ecological variability substantially influence malaria transmission patterns, underscoring the need for complementary biologically informed control strategies beyond conventional interventions (Oluwafemi and Azuaba, 2021; Ouattara *et al*., 2023).

Traditionally, malaria pathogenesis has been examined primarily through a host–parasite framework, with emphasis on *Plasmodium* biology and host immune responses. However, accumulating evidence over the past decade suggests that the gut microbiota represents an additional biological layer capable of modulating malaria transmission and disease severity in both mosquito vectors and vertebrate hosts (Mukherjee *et al*., 2021; Mandal *et al*., 2021). The gastrointestinal tract of these organisms harbors complex microbial ecosystems that interact dynamically with immune, metabolic, and epithelial pathways relevant to parasite development and host pathology.

Metagenomic analyses across diverse geographic settings consistently report associations between alterations in microbial community structure and malaria severity, suggesting that microbiota-related patterns may accompany disease progression rather than representing isolated observations (Frosch *et al*., 2022). However, most human studies remain observational, limiting causal inference.

In mosquito vectors, the midgut microbiota constitutes a critical ecological interface where *Plasmodium* undergoes essential developmental stages. Experimental and field-based studies indicate that certain bacterial taxa can influence parasite development through immune priming, metabolic competition, and production of antiparasitic metabolites; however, these effects are highly context-dependent and shaped by environmental conditions such as temperature, larval habitat, and blood-meal source (Palinauskas *et al*., 2024; Liu *et al*., 2024; Salgado *et al*., 2024).

In vertebrate hosts, gut microbiota composition is increasingly recognized as a modifier of immune regulation and inflammatory balance during malaria infection. Clinical cohort studies associate gut microbial dysbiosis with increased parasitemia and severe disease outcomes, particularly in pediatric malaria (Van den Ham *et al*., 2024; Bednarsky *et al*., 2025). In contrast, experimental animal models provide stronger causal evidence that manipulation of gut microbiota can alter parasitemia dynamics, immune responses, and tissue pathology, highlighting the microbiota as an active biological modulator under controlled conditions (Mukherjee *et al*., 2021).

Microbiota-targeted nutritional interventions, including probiotics and prebiotics, have therefore been proposed as adjunct strategies for modulating host immune responses during malaria infection. While experimental and small-scale human studies report modulation of inflammatory markers such as IL-6 and IL-10, robust clinical evidence demonstrating reduced malaria incidence, severity, or mortality remains limited. Accordingly, probiotic strategies should be regarded as exploratory and complementary rather than established public health interventions (Mandal *et al*., 2021; Lee *et al*., 2023; Bednarsky *et al*., 2025).

Accordingly, this review critically examines recent literature on the role of gut microbiota in malaria within a One Health framework, integrating evidence from mosquito vectors, vertebrate hosts, and environmental contexts. Emphasis is placed on distinguishing correlation from causation, evaluating methodological limitations, and assessing translational feasibility, with the aim of identifying realistic pathways for microbiota-informed malaria research and control strategies.

While several recent reviews have provided important insights into microbiota–malaria interactions, including comprehensive discussions of host immune modulation and microbial associations with disease severity (Mukherjee *et al*., 2021; Schmidt and Jane, 2023), these studies primarily emphasize biological mechanisms and descriptive patterns. The present review differs conceptually by adopting a critical, translationally grounded perspective that explicitly distinguishes evidence derived from controlled experimental systems from observations obtained in human populations and natural transmission settings. Rather than treating microbiota as an emerging intervention target per se, this review evaluates translational readiness, ecological robustness, and operational feasibility within a One Health framework, integrating constraints related to environmental variability, population heterogeneity, logistics, and policy implementation. By positioning microbiota-mediated processes within these broader biological and operational contexts, this review aims to move beyond mechanistic synthesis toward a more realistic appraisal of how microbiota research may (or may not) inform integrated malaria control strategies.

## Mosquito gut microbiota and vector competence

The gut microbiota of malaria vectors comprises diverse bacterial communities whose composition varies across mosquito species, developmental stages, and ecological settings. Culture-dependent and sequencing-based surveys

consistently identify bacterial taxa belonging predominantly to the phyla Proteobacteria, Firmicutes, and Bacteroidetes, although their relative abundance differs markedly among mosquito populations from different geographic regions (Cansado-Utrilla *et al*., 2021; Liu *et al*., 2024). Field studies further demonstrate that larval habitat characteristics, water quality, seasonal variation, ambient temperature, and blood-meal source exert strong influences on gut microbial composition, resulting in dynamic and often reversible microbial assemblages (Ouattara *et al*., 2023; Salgado *et al*., 2024). These observations collectively indicate that mosquito-associated microbiota are environmentally shaped rather than genetically fixed.

Vector competence, defined as the intrinsic ability of a mosquito to acquire, maintain, and transmit *Plasmodium*, is increasingly recognized as a context-dependent phenotype influenced by microbial and environmental interactions. Comparative field surveys have reported associations between gut microbiota composition and *Plasmodium* infection prevalence in natural mosquito populations, suggesting that microbial communities may contribute to variability in transmission intensity (Gebreslassie *et al*., 2023; Palinauskas *et al*., 2024). However, these field-based observations remain largely correlational, as differences in microbiota composition frequently co-occur with ecological, climatic, and nutritional variables that independently affect parasite development.

**Figure 1:** Conceptual overview of mosquito gut microbiota interactions influencing vector competence and Plasmodium development.

Panel A schematically depicts the mosquito midgut as an ecological niche where gut microbiota may interact with Plasmodium during sporogonic development under the influence of environmental factors such as larval habitat, temperature, and blood-meal source, highlighting context-dependent modulation rather than fixed causal effects. Panel B places these interactions within a broader One Health perspective, emphasizing ecological variability, vector host environment interfaces, and translational constraints. All graphical elements are conceptual and do not represent quantitative effect sizes or translational readiness. This Figure 1 was created by the authors based on synthesis of published literature (Cansado-Utrilla *et al*., 2021; Dong *et al*., 2021; Mancini *et al*., 2022; Tseng *et al*., 2023; Palinauskas *et al*., 2024) and designed using BioRender.com and Adobe Illustrator.

Figure 1 provides a conceptual synthesis of current understanding regarding the role of mosquito gut microbiota in shaping vector competence and Plasmodium development within an ecologically variable transmission system. Panel A depicts the mosquito midgut as a dynamic biological interface in which resident microbial communities coexist with Plasmodium during sporogonic development. Rather than representing a fixed or deterministic pathway, the Figure 1 emphasizes that microbiota–parasite interactions are modulated by environmental context, including larval habitat characteristics, ambient temperature, blood-meal source, and seasonal variation. These factors collectively influence microbial community composition and activity, thereby shaping the local biological conditions encountered by the parasite.

Within this midgut environment, the Figure 1 summarizes several biologically plausible mechanisms through which gut microbiota may influence parasite development. These include immune priming of the mosquito innate immune system, metabolic competition for essential nutrients, and the production of antimicrobial or redox-active molecules by certain bacterial taxa. Importantly, these mechanisms are depicted as interconnected and non-exclusive processes rather than dominant causal pathways. This reflects evidence primarily derived from experimental studies conducted under controlled laboratory conditions, where manipulation of specific microbial taxa or communities has demonstrated effects on parasite survival and development.

The Figure 1 also highlights the context-dependent nature of these interactions, particularly when contrasting laboratory findings with observations from natural mosquito populations. While experimental models provide stronger causal inference, field-based studies predominantly report associations between microbial composition and parasite prevalence that are shaped by ecological variability. As such, Panel A deliberately avoids visual cues that might imply quantitative effect sizes, mechanistic dominance, or universal applicability across mosquito species and transmission settings.

Panel B extends this biological framework into a broader One Health perspective by situating mosquito microbiota within interconnected human, animal, and environmental systems. This panel underscores that vector-associated microbial communities are indirectly influenced by ecosystem-level processes, including land use, sanitation, climate, and interactions among humans, animal reservoirs,

and vectors. These interconnected factors contribute to the complexity of malaria transmission dynamics and constrain the direct translation of microbiota-based findings into operational control strategies.

Collectively, Figure 1 is intended as an integrative conceptual framework rather than a predictive or mechanistic model. It synthesizes diverse lines of evidence to illustrate how mosquito gut microbiota may contribute to variability in vector competence, while explicitly acknowledging current limitations related to causality, ecological robustness, and translational readiness. By doing so, the Figure 1 supports the central argument of this review: that microbiota-mediated processes represent a promising but context-sensitive component of malaria ecology that must be evaluated within integrated, ecosystem-level approaches.

Stronger causal evidence for microbiota-mediated modulation of vector competence is derived from controlled laboratory experiments. Manipulation of mosquito gut microbiota through antibiotic treatment, bacterial supplementation, or gnotobiotic rearing has demonstrated that specific bacterial strains can inhibit or facilitate *Plasmodium* development at different sporogonic stages (Dong *et al.*, 2021; Mancini *et al.*, 2022). These effects have been attributed to mechanisms including immune priming of the mosquito midgut, modulation of oxidative stress, and competition for essential nutrients required for parasite survival. Importantly, such experimental systems allow isolation of microbial effects but do not fully recapitulate the ecological complexity of natural transmission settings.

Microbial effects on *Plasmodium* development are highly heterogeneous and strain-specific. While certain bacterial taxa, such as *Enterobacter* and *Delftia* species, exhibit antiparasitic activity through the production of reactive oxygen species or bioactive metabolites, other members of the gut microbiota may create permissive environments that support parasite survival (Verma *et al.*, 2021; Tseng *et al.*, 2023). This functional diversity cautions against oversimplified interpretations that assign uniform protective or permissive roles to broad microbial taxa and underscores the importance of community-level interactions in shaping vector competence.

From a conceptual perspective, the observed heterogeneity and strain-specificity of microbiota effects on Plasmodium development suggest that simple taxonomic associations are insufficient for predicting functional outcomes. Instead, several working criteria may be considered when evaluating whether a bacterial strain is more likely to exert inhibitory or permissive effects. These include the stability of midgut colonization across environmental conditions, the capacity to prime or modulate mosquito innate immune responses, and metabolic functions relevant to redox balance and nutrient competition within the midgut microenvironment. Importantly, these criteria are not proposed as deterministic rules, but rather as hypothesis-generating considerations that integrate microbial traits with ecological context, including larval habitat characteristics and blood-meal sources, which are known to shape microbiota composition and activity.

A persistent challenge in interpreting field-based associations between mosquito gut microbiota and Plasmodium infection is the difficulty of distinguishing cause from consequence. To address this "chicken-and-egg" problem, future studies would benefit from longitudinal field designs incorporating repeated sampling of individual mosquitoes or well-defined cohorts across transmission seasons. At a minimum, such designs should include baseline microbiota characterization prior to detectable infection, stratification by subsequent infection status, and parallel measurement of key ecological variables such as larval habitat, temperature, and blood-meal source. Comparing microbiota trajectories before and after infection within the same ecological context would provide a more robust framework for assessing whether specific microbial configurations precede, accompany, or follow Plasmodium establishment in natural mosquito populations.

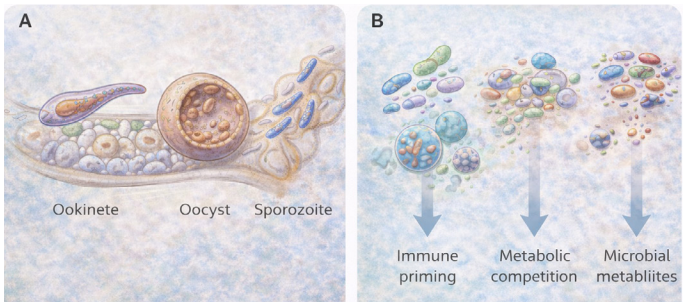


**Figure 2:** Conceptual pathways through which mosquito gut microbiota may influence Plasmodium development.

Panel A outlines key developmental stages of Plasmodium within the mosquito midgut, including the ookinete, oocyst, and sporozoite stages. Panel B summarizes biologically plausible pathways through which mosquito gut microbiota may interact with the parasite, such as immune priming, metabolic competition, and the production of microbiota-derived metabolites. The Figure 2 emphasizes that these interactions are highly context-dependent and are primarily supported by experimental evidence obtained under controlled laboratory conditions rather than definitive field-based causality. All graphical elements are schematic and do not represent quantitative effect sizes, mechanistic dominance, or translational readiness. This Figure 2 was created by the authors based on synthesis of published literature, including studies by Cansado-Utrilla *et al.* (2021), Dong *et al.* (2021), Mancini

*et al*. (2022), Tseng *et al*. (2023), and Palinauskas *et al*. (2024), and was designed as a conceptual illustration using BioRender.com and Adobe Illustrator.

Figure 2 presents a conceptual framework summarizing how mosquito gut microbiota may interact with Plasmodium across different stages of parasite development within the vector. Panel A depicts key sporogonic stages occurring in the mosquito midgut, highlighting that parasite development is a multi-step process sensitive to local biological conditions. Rather than implying uniform susceptibility, the Figure 2 underscores that developmental success may vary depending on the microbial and ecological context in which these stages occur.

Panel B illustrates several biologically plausible pathways through which gut microbiota may modulate parasite development. These include immune priming of the mosquito innate immune system, competition for essential nutrients, and the production of microbiota-derived metabolites with antiparasitic properties. Importantly, these pathways are not depicted as independent or hierarchically dominant mechanisms but as interconnected processes whose relevance may differ across mosquito species, microbial strains, and environmental settings.

The Figure 2 also emphasizes the distinction between experimental and field-based evidence. Controlled laboratory studies provide stronger support for causal interactions between specific microbial taxa and parasite outcomes, whereas observations from natural mosquito populations predominantly report associations influenced by ecological variability. As such, the schematic deliberately avoids visual cues that could imply quantitative strength or universal applicability of any single pathway.

Overall, Figure 2 serves as an integrative but non-mechanistic representation designed to support discussion of microbiota–parasite interactions without overstating current evidence. By focusing on conceptual pathways rather than predictive models, the Figure 2 aligns with the review's broader emphasis on context-dependence, methodological limitations, and the need for cautious interpretation when extrapolating experimental findings to field-based malaria transmission dynamics.

High-throughput sequencing approaches have substantially expanded descriptive knowledge of mosquito gut microbiota; however, many studies rely primarily on *16S rRNA* gene sequencing, which provides taxonomic resolution but limited functional insight (Chen *et al*., 2022; Schmidt and Jane, 2023). Consequently, associations between microbial taxa and parasite outcomes inferred from 16S data alone should be interpreted cautiously, particularly when mechanistic claims are advanced without complementary functional validation.

Recent advances in shotgun metagenomics, metatranscriptomics, and metabolomics offer improved resolution of microbial gene expression and metabolic activity relevant to parasite development. These approaches have identified microbial pathways involved in redox balance, immune signaling modulation, and metabolite production that may influence parasite permissiveness in mosquitoes (Ankrah *et al*., 2021; Wilmanski *et al*., 2019). Nevertheless, such high-resolution studies remain limited in scale and are underrepresented in longitudinal field investigations from malaria-endemic regions.

Collectively, available evidence supports the concept that mosquito gut microbiota can modulate vector competence; however, most findings remain at the proof-of-concept stage. Ecological variability, microbial instability, and the predominance of laboratory-based causal evidence continue to constrain translation into operational vector control strategies. Addressing these limitations will require integrated field studies capable of linking microbial function, ecological context, and transmission outcomes over time.

## Host gut microbiota, immune regulation, and malaria severity

Substantial evidence from cohort and experimental studies indicates that gut microbiota composition in vertebrate hosts is associated with variability in susceptibility to malaria infection and disease severity. Human cohort studies conducted in malaria-endemic regions consistently report associations between baseline microbial diversity, relative abundance of specific bacterial taxa, and subsequent malaria risk (Mukherjee *et al*., 2021; Van den Ham *et al*., 2024). However, these studies are predominantly observational and therefore cannot establish whether microbiota alterations precede infection or arise as a consequence of malaria-associated inflammation and metabolic stress.

In contrast to human observational studies, experimental animal models provide stronger causal evidence for microbiota-mediated modulation of malaria outcomes. Manipulation of gut microbiota through antibiotic depletion, dietary intervention, or controlled bacterial supplementation in rodent malaria models has been shown to alter parasitemia kinetics, survival rates, and immune response profiles (Mahajan *et al*., 2021; Mukherjee *et al*., 2021). These findings demonstrate that gut microbiota can actively shape host–parasite interactions under controlled conditions, while also highlighting important interspecies differences that limit direct extrapolation to human malaria.

Severe malaria is frequently accompanied by intestinal dysbiosis, characterized by reduced microbial diversity, depletion of beneficial commensals, and enrichment of pro-inflammatory taxa. Clinical studies in pediatric and adult populations report that such dysbiotic profiles are associated with increased parasitemia, systemic inflammation, and adverse clinical outcomes (Frosch *et al*., 2022; Bednarsky *et al*., 2025). Importantly, experimental and clinical evidence supports a bidirectional relationship in which malaria-induced inflammation and intestinal barrier disruption can further exacerbate microbial imbalance, complicating causal inference in human studies.

Disruption of gut microbial homeostasis during malaria infection has been linked to impaired intestinal barrier function and increased microbial translocation. Animal studies demonstrate that malaria-associated dysbiosis may elevate the risk of bacteremia and amplify systemic inflammatory responses through translocation of microbial products into the circulation (He and Qi, 2025). Such processes provide a mechanistic framework linking gut microbiota alterations to severe malaria pathology, although direct validation in large human cohorts remains limited.

Cytokine balance, particularly the balance between pro-inflammatory (e.g., IL-6) and anti-inflammatory (e.g., IL-10) cytokines, plays a central role in malaria immunopathology. Experimental studies suggest that gut microbiota and microbiota-derived metabolites can modulate IL-6 and IL-10 signaling pathways, thereby influencing inflammatory balance and tissue damage during infection (Yawen *et al*., 2022; Hwang *et al*., 2022). However, evidence linking specific microbial taxa or metabolites to consistent modulation of IL-6/IL-10 dynamics in human malaria remains sparse and largely indirect.

Based on experimental findings, microbiota-targeted nutritional interventions, including probiotics and prebiotics, have been proposed as adjunct strategies for modulating host immune responses during malaria infection. Rodent studies demonstrate that supplementation with specific bacterial strains can influence parasitemia levels and cytokine profiles under controlled conditions (Mahajan *et al*., 2021; Guo *et al*., 2022). In humans, available evidence is limited to small-scale and heterogeneous studies reporting modulation of inflammatory markers rather than definitive reductions in malaria incidence, severity, or mortality (Lee *et al*., 2023).

From a conceptual standpoint, the notion of a "universal probiotic" applicable across malaria-endemic populations is inherently problematic. Baseline gut microbiota composition varies substantially across geographic regions, age groups, nutritional states, and patterns of prior exposure, making restoration to a single "healthy" reference state neither biologically realistic nor operationally feasible. A more plausible translational objective is therefore function-targeted modulation, whereby specific microbial functions such as regulation of inflammatory balance or support of epithelial barrier integrity are selectively enhanced within defined ecological and clinical contexts. Framing probiotic strategies in this manner helps to avoid overgeneralization and aligns microbiota-based interventions with the broader emphasis on context-dependence and translational caution articulated throughout this review.

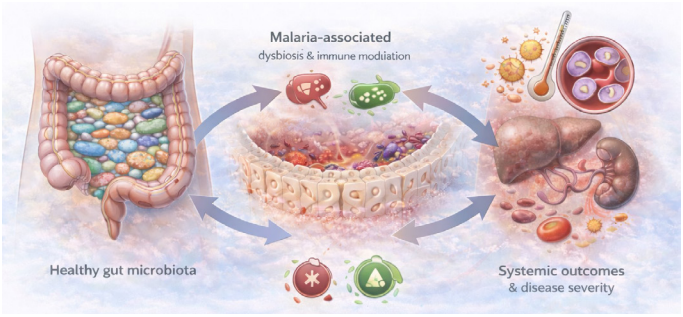


**Figure 3:** Host gut microbiota dysbiosis as a contextual determinant of malaria severity and immune regulation.

Figure 3 presents a conceptual synthesis of current evidence linking host gut microbiota composition to immune regulation and malaria severity. It illustrates the transition from a balanced gut microbial ecosystem to malaria-associated dysbiosis, highlighting bidirectional interactions between intestinal barrier integrity, immune modulation, and systemic inflammatory outcomes. The relationships depicted are context-dependent and non-deterministic, integrating observational human studies with mechanistic insights from experimental animal models, without implying direct causality or quantitative effect sizes. This schematic focuses on upstream immune regulatory processes and host–microbiota interactions, rather than downstream pathological or clinical outcomes, which are addressed separately. Figure 3 was created by the authors based on synthesis of published literature (Mukherjee *et al*., 2021; Mahajan *et al*., 2021; Frosch *et al*., 2022; Yawen *et al*., 2022; Guo *et al*., 2022; Van den Ham *et al*., 2024; Bednarsky *et al*., 2025; He and Qi, 2025) using BioRender and refined with Adobe Illustrator

Accumulating evidence from human cohort studies conducted in malaria-endemic regions indicates that baseline gut microbiota composition is associated with inter-individual variability in malaria susceptibility and disease severity. Reduced microbial diversity and altered relative abundance of specific bacterial taxa have been correlated with increased malaria risk and adverse clinical outcomes. Importantly, disentangling the independent contribution of gut microbiota from major host-level

confounders particularly malnutrition, co-infections, prior malaria exposure, and antimicrobial use requires more than cross-sectional association analyses. Future studies should prioritize longitudinal cohort designs with baseline microbiota characterization and apply multivariable statistical models that explicitly adjust for these confounding factors. Where feasible, stratified analyses and within-individual comparisons over time would further strengthen causal inference by reducing bias introduced by inter-individual heterogeneity.

Experimental animal models provide stronger support for a functional role of gut microbiota in shaping host–parasite interactions. Controlled manipulation of microbial communities through antibiotic depletion, dietary modification, or defined bacterial supplementation has been shown to alter parasitemia kinetics, immune activation profiles, and survival outcomes in rodent malaria models. These findings demonstrate that gut microbiota can actively modulate host immune responses during infection, while also underscoring important interspecies differences that constrain direct extrapolation to human malaria.

Malaria infection itself is increasingly recognized as a driver of intestinal dysbiosis. Severe malaria is frequently accompanied by disruption of gut microbial homeostasis, characterized by loss of beneficial commensals, expansion of pro-inflammatory taxa, and impaired intestinal barrier integrity. Such dysbiosis has been linked to increased microbial translocation and systemic exposure to microbial products, thereby amplifying inflammatory responses and contributing to malaria-associated immunopathology. This bidirectional relationship complicates causal inference in clinical settings, as microbiota alterations may function both as determinants and consequences of disease severity.

Cytokine balance represents a key mechanistic link between gut microbiota and malaria outcomes. Experimental evidence suggests that microbiota-derived signals and metabolites can influence the regulation of pro-inflammatory cytokines, such as IL-6, and anti-inflammatory mediators, such as IL-10, thereby shaping the magnitude and duration of inflammatory responses during infection. While these mechanisms are well supported in experimental systems, evidence directly linking specific microbial taxa or metabolites to consistent cytokine modulation in human malaria remains limited and largely indirect.

Finally, microbiota-targeted nutritional strategies, including probiotics and prebiotics, have been proposed as adjunct approaches for modulating host immune responses during malaria infection. Although rodent studies demonstrate immunomodulatory and parasitological effects under controlled conditions, available human data are sparse, heterogeneous, and insufficient to support clinical efficacy claims. Accordingly, this Figure 3 emphasizes the role of gut microbiota as a contextual and dynamic modulator of malaria severity rather than a validated therapeutic target, aligning with current reviewer expectations for cautious interpretation and conceptual framing.

Collectively, current evidence supports a role for gut microbiota in shaping malaria severity through immune modulation and metabolic interactions; however, translational application remains constrained by substantial heterogeneity in microbiota composition across individuals and populations. Variability in diet, co-infections, antimicrobial exposure, and environmental context limits the feasibility of universal microbial signatures or one-size-fits-all probiotic interventions. Consequently, microbiota-targeted strategies should be regarded as exploratory and complementary components within broader malaria control frameworks rather than as standalone clinical solutions.

## Integrated host vector microbiota interactions within a One Health framework

Malaria transmission emerges from a complex ecological network involving parasites, mosquito vectors, vertebrate hosts, and their associated microbial communities. While host and vector microbiota are often studied independently, growing evidence suggests that these microbial systems are shaped by shared environmental exposures, including climate, land use, water sources, and human–animal interactions (Cansado-Utrilla *et al*., 2021; Palinauskas *et al*., 2024). Such shared ecological drivers raise the possibility that host and vector microbiota may be indirectly coupled through environmental reservoirs rather than through direct microbial exchange.

Operationalizing a One Health approach in malaria–microbiota research requires intentional integration across disciplines from the outset, rather than parallel data collection interpreted post hoc. In practice, this would involve coordinated study designs in which clinical teams define host phenotypes and collect longitudinal human samples, veterinary scientists monitor animal reservoirs and shared environmental exposures, entomologists conduct synchronized vector sampling and infection assessments, and environmental microbiologists characterize microbial reservoirs in larval habitats, water sources, and surrounding ecosystems. Crucially, these data streams should be linked through shared metadata standards and joint analytical frameworks, allowing hypotheses regarding microbiota-mediated interactions to be tested across host, vector, and environmental compartments simultaneously. Framing One Health research in this operational manner moves beyond conceptual alignment and enables more realistic

evaluation of microbiota-informed strategies within complex malaria transmission systems.

Direct empirical evidence demonstrating functional microbial or metabolite exchange between vertebrate hosts and mosquito vectors influencing malaria outcomes in vivo remains limited. Most current support for cross-system microbiota interactions derives from theoretical frameworks and indirect observations, including shared bacterial taxa detected in environmental reservoirs and convergent microbial responses to ecological pressures (Ankrah *et al*., 2021; Salgado *et al*., 2024). Consequently, proposed host–vector microbiota linkages should be interpreted cautiously and distinguished from experimentally validated mechanisms.

Despite fundamental biological differences between insects and vertebrates, certain regulatory themes recur across host and vector systems. These include immune priming, metabolic competition, and microbiota-derived modulation of inflammatory signaling pathways that influence parasite development or clearance (Dong *et al*., 2021; Hwang *et al*., 2022). However, the molecular architectures underlying these processes differ substantially between mosquitoes and vertebrates, underscoring that apparent mechanistic parallels do not necessarily imply functional equivalence.

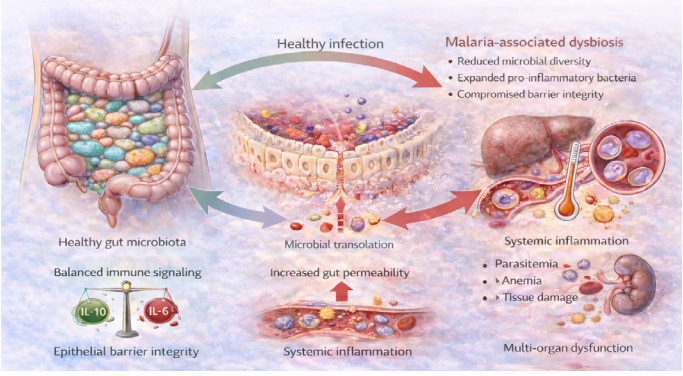


**Figure 4:** Gut microbiota dysbiosis as a mechanistic contributor to malaria pathogenesis and disease severity.

Figure 4 illustrates gut microbiota dysbiosis as a downstream contributor to malaria-associated immunopathology and disease severity. Under healthy conditions, a diverse gut microbiota supports intestinal barrier integrity and balanced immune signaling. During malaria infection, dysbiosis characterized by reduced microbial diversity and expansion of pro-inflammatory taxa compromises epithelial barrier function, promoting microbial translocation and systemic inflammation. The interactions depicted are context-dependent and integrate evidence from clinical observations and experimental models without implying linear causality or quantitative effect sizes. In contrast to Figure 3, this schematic emphasizes downstream pathophysiological processes and clinical disease severity, rather than upstream immune regulatory dynamics. Figure 4 was created by the authors based on synthesis of published literature (Mukherjee *et al*., 2021; Frosch *et al*., 2022; Schmidt and Jane, 2023; Van den Ham *et al*., 2024; Bednarsky *et al*., 2025; He and Qi, 2025) using BioRender and refined with Adobe Illustrator.

Gut microbiota dysbiosis is increasingly recognized as a key feature accompanying malaria infection, particularly in severe disease. Clinical studies consistently report reduced microbial diversity and altered taxonomic composition in patients with high parasitemia and adverse outcomes. However, these findings primarily reflect associative relationships, as human studies cannot reliably determine whether dysbiosis precedes infection or arises secondary to malaria-induced inflammation and metabolic stress.

Experimental models provide mechanistic insight into how dysbiosis may contribute to malaria pathogenesis. Disruption of gut microbial homeostasis impairs epithelial tight junction integrity, increasing intestinal permeability and facilitating translocation of microbial products into the systemic circulation. This process amplifies innate immune activation and promotes excessive inflammatory responses that are central to malaria-associated immunopathology.

Microbial translocation has been linked to heightened production of pro-inflammatory cytokines, including IL-6 and TNF-α, which exacerbate anemia, endothelial dysfunction, and tissue injury. These processes contribute to multi-organ involvement characteristic of severe malaria, reinforcing the concept that gut-derived inflammatory signals may amplify disease severity beyond parasite burden alone.

Importantly, the Figure 4 emphasizes bidirectionality: malaria-induced inflammation can further disrupt gut microbial homeostasis, creating a self-reinforcing cycle of dysbiosis and immune dysregulation. This dynamic relationship complicates causal inference and underscores the need for longitudinal and mechanistic studies to disentangle drivers from consequences in human malaria.

Figure 5 presents a conceptual integration of vertebrate host microbiota, mosquito vector microbiota, and Plasmodium parasites within a One Health framework. It highlights how shared environmental pressures shape microbial communities across host and vector systems, and how bidirectional exchanges during blood-feeding generate cross-kingdom microbial and immunological interactions. The Figure 5 emphasizes malaria transmission as an ecosystem-driven process regulated by interconnected microbial and immunometabolic networks rather than isolated host–parasite interactions. Directional

arrows represent conceptual linkages and shared ecological influences, and do not imply direct microbial transfer, functional exchange, or demonstrated causality between host and vector systems. Figure 5 was created by the authors based on synthesis of recent literature (Schmidt and Jane, 2023; Palinauskas *et al*., 2024; Asare and Duntu, 2024; Van den Ham *et al*., 2024; Kumar *et al*., 2024; He and Qi, 2025; Riithi *et al*., 2025) using BioRender and refined with Adobe Illustrator.

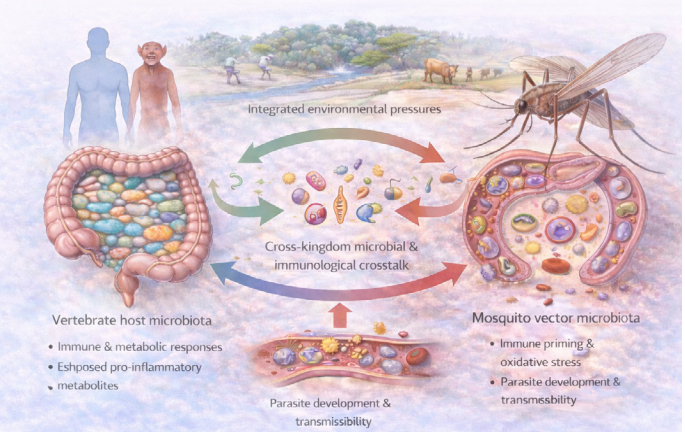


**Figure 5:** Integrated host–vector–microbiota networks regulating malaria transmission within a One Health framework.

Malaria transmission emerges from interactions among parasites, mosquito vectors, vertebrate hosts, and their associated microbial communities. Although host and vector microbiota are often investigated separately, increasing evidence indicates that both systems are shaped by shared environmental exposures, including climate, land use, water sources, and human–animal contact patterns. These ecological drivers may indirectly couple host and vector microbiota through environmental reservoirs rather than direct microbial transfer.

During blood-feeding, mosquitoes acquire not only Plasmodium parasites but also host-derived immune mediators, microbial components, and metabolites. These exchanges create a dynamic interface in which microbial and immunological signals converge, influencing parasite survival and development. However, direct in vivo evidence demonstrating functional microbial exchange between host and vector remains limited, warranting cautious interpretation.

Within the mosquito, symbiotic bacteria modulate immune priming, oxidative stress responses, and midgut environmental conditions that govern parasite permissiveness. Cooperative and competitive interactions among microbial symbionts further shape vector competence, underscoring the importance of microbial ecology in transmission dynamics.

From a One Health perspective, this integrated network highlights malaria as an ecosystem level phenomenon. Understanding transmission therefore requires coordinated investigation across human, animal, vector, and environmental compartments, rather than reductionist focus on single biological systems.

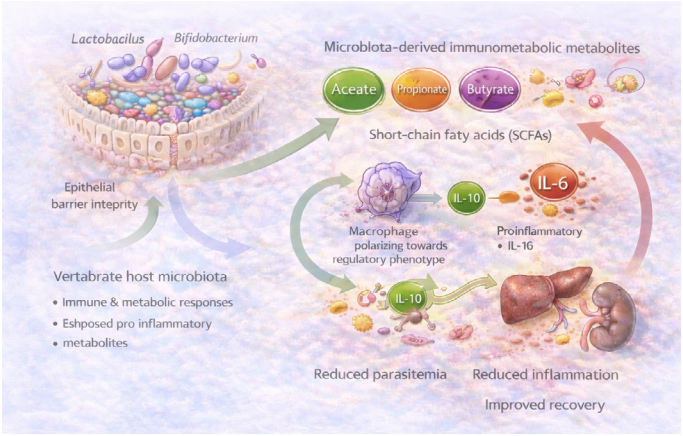


**Figure 6:** Protective roles of gut microbiota and microbiota-derived metabolites in modulating malaria outcomes.

Figure 6 illustrates the protective roles of gut microbiota and microbiota-derived metabolites in supporting host resilience and favorable clinical outcomes during malaria infection. Beneficial microbial communities contribute to epithelial barrier repair, immunometabolic regulation, and balanced cytokine responses that limit excessive inflammation. Figure 6 emphasizes microbiota-mediated protection as context-dependent and complementary rather than as a standalone therapeutic strategy. These protective effects are primarily supported by experimental and observational studies, and their magnitude and clinical relevance in diverse human malaria settings remain to be fully established. Figure 6 was created by the authors based on synthesis of published literature (Hwang *et al*., 2022; Lee *et al*., 2023; Schmidt and Jane, 2023; Kumar *et al*., 2024; Carlos *et al*., 2025; Bednarsky *et al*., 2025) using BioRender and refined with Adobe Illustrator.

Gut microbiota can exert protective effects during malaria by supporting intestinal barrier integrity and regulating host immune responses. Experimental studies demonstrate that enrichment of beneficial bacterial taxa promotes epithelial repair and limits microbial translocation, thereby reducing systemic inflammatory burden during infection.

Microbiota-derived metabolites, particularly short-chain fatty acids such as acetate, propionate, and butyrate, function as key immunometabolic mediators. These metabolites influence macrophage polarization, regulatory T-cell activity, and cytokine balance, favoring IL-10–mediated immune regulation while restraining excessive pro-inflammatory signaling.

Through coordinated effects on immune modulation and metabolic homeostasis, gut microbiota contribute to reduced parasitemia, attenuation of tissue damage, and improved recovery trajectories in experimental models. However, translation of these findings to human malaria remains limited by heterogeneous study designs and lack of large-scale clinical validation.

Accordingly, microbiota-targeted strategies are best viewed as adjunctive components within integrated malaria control frameworks. Aligning microbiota research with One Health principles prioritizes ecological robustness, ethical feasibility, and long-term sustainability over rapid deployment of unvalidated interventions.

Operationalizing a One Health approach to microbiota-informed malaria research requires coordinated surveillance across human, animal, vector, and environmental compartments. This includes harmonized sampling protocols, shared metadata standards, and longitudinal monitoring capable of capturing temporal dynamics in microbial composition, parasite prevalence, and ecological conditions (Chen *et al*., 2022; Schmidt and Jane, 2023). Such integrated designs are essential for disentangling causal relationships from coincident ecological associations.

Within this integrated framework, microbiota-based interventions should be prioritized as complementary components of malaria control rather than as standalone solutions. Emphasis should be placed on interventions that demonstrate ecological robustness, ethical feasibility, and compatibility with existing control measures, including vector management, chemoprevention, and vaccination. Aligning microbiota research with One Health principles thus reshapes priorities toward ecosystem-level understanding and long-term sustainability rather than rapid technological deployment.

## Emerging microbiota-based strategies and translational readiness

Microbiota-based strategies targeting mosquito vectors have been proposed as innovative complements to conventional vector control approaches. Experimental studies demonstrate that manipulation of mosquito-associated microbial communities, including symbiont enrichment and bacterial supplementation, can alter *Plasmodium* development under laboratory conditions (Mancini *et al*., 2022; Tseng *et al*., 2023). However, most of these approaches remain confined to controlled experimental settings, and their effectiveness under heterogeneous field conditions has yet to be robustly demonstrated.

Paratransgenic strategies, which involve engineering symbiotic bacteria to express antiparasitic molecules within mosquito vectors, represent a promising but early-stage intervention concept. Proof-of-concept studies indicate that genetically modified symbionts can reduce parasite development in mosquitoes (Ben Ami *et al*., 2023; Mancini *et al*., 2022). Nevertheless, empirical data from long-term or large-scale field trials assessing ecological stability, reversibility, and biosafety are currently lacking, necessitating cautious interpretation of translational potential.

The deployment of engineered or manipulated microbial communities in natural ecosystems raises significant ecological and biosafety concerns. Potential risks include unintended ecological effects, disruption of native microbial networks, and horizontal gene transfer to non-target organisms (Lo and Chan, 2022). Addressing these risks will require staged evaluation frameworks, rigorous environmental monitoring, and compliance with national and international biosafety regulations prior to any field implementation.

In vertebrate hosts, microbiota-targeted nutritional interventions, including probiotics and prebiotics, have been explored as adjunct strategies for modulating immune responses during malaria infection. Experimental animal studies suggest that supplementation with specific bacterial strains can influence parasitemia and cytokine profiles, including IL-6 and IL-10, under controlled conditions (Mahajan *et al*., 2021; Guo *et al*., 2022). In humans, however, available evidence is limited to small-scale and heterogeneous studies, precluding definitive conclusions regarding clinical efficacy (Lee *et al*., 2023).

Recent studies have explored the use of artificial intelligence and machine-learning approaches to integrate microbiome, environmental, and clinical data for predicting malaria susceptibility and transmission risk. While such models demonstrate potential in retrospective analyses, reported performance metrics are often derived from internal validation and may not generalize across diverse geographic and ecological contexts (Singh *et al*., 2023; Patel *et al*., 2024). At present, microbiota-informed AI models remain exploratory research tools with no established role in operational malaria control programs.

The convergence of host, vector, and environmental microbiota research reinforces the importance of One Health frameworks in malaria control. Environmental hygiene, land-use practices, and climate variability influence microbial ecosystems that shape both mosquito and host susceptibility (Oluwafemi and Azuaba, 2021; Palinauskas *et al*., 2024). Integrating microbiota considerations into malaria surveillance and control programs may improve

resilience against ecological and epidemiological change.

From a policy perspective, microbiota-based interventions require interdisciplinary collaboration across microbiology, veterinary sciences, clinical medicine, public health, and ecology. Ethical governance, biosafety assessment, and community engagement are essential components for translating microbiota-driven strategies into field applications (Caragata and Shorts, 2022).

Ethical analyses emphasize that deployment of microbial interventions in vector control must balance innovation with ecological preservation and cultural acceptance, highlighting the need for transparent regulatory frameworks and stakeholder engagement (Lo and Chan, 2022).

Collectively, these approaches highlight the microbiota as a modifiable biological interface that bridges environmental management, vector biology, and host health.

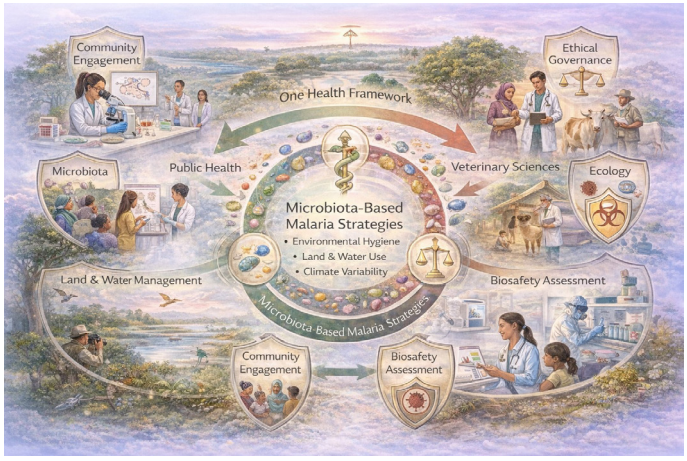


**Figure 7:** Emerging microbiota-based strategies for sustainable malaria control within a One Health framework.

Figure 7 conceptualizes microbiota-based strategies as an integrative component of sustainable malaria control within a One Health framework. It illustrates how environmental management, vector and host microbiota research, public health surveillance, and ethical governance intersect to shape malaria resilience under changing ecological conditions. Figure 7 emphasizes interdisciplinary coordination, ecological robustness, and regulatory oversight rather than direct intervention efficacy, synthesizing evidence from recent experimental, ecological, and policy-oriented studies. Figure 7 was created by the authors based on synthesis of published literature (Caragata and Shorts, 2022; Kumar *et al.*, 2024; Asare and Duntu, 2024; Palinauskas *et al.*, 2024; Bednarsky *et al.*, 2025; Lee *et al.*, 2023; Naidoo and Oliver, 2025) using BioRender and refined with Adobe Illustrator.

The Figure 7 positions microbiota as a modifiable biological interface linking environmental conditions, vector biology, and host health within a unified One Health perspective. Environmental hygiene, land-use practices, and climate variability shape microbial ecosystems in soil, water, animals, humans, and mosquito breeding habitats. These shared ecological pressures influence both host susceptibility and vector competence indirectly, reinforcing the concept that malaria transmission dynamics are embedded within broader environmental and microbial networks rather than isolated host–parasite interactions.

At the interface of host and vector systems, microbiota-based research highlights convergent regulatory themes, including immune priming, metabolic modulation, and inflammatory balance. While vertebrate and mosquito immune architectures differ fundamentally, microbiota-mediated processes in both systems can influence parasite development and transmission potential. Importantly, the Figure 7 reflects current consensus that evidence for direct microbial exchange or transferable microbiota-mediated protection across host and vector systems remains limited, and that observed linkages are largely shaped by shared environmental reservoirs and ecological drivers.

From a public health and veterinary perspective, the Figure 7 underscores the necessity of integrated surveillance approaches that incorporate microbiota monitoring alongside traditional parasitological and entomological indicators. Coordinated sampling across human, animal, vector, and environmental compartments can enhance detection of ecological shifts that may precede changes in malaria risk, thereby improving system-level resilience to epidemiological and climatic perturbations.

The Figure 7 further highlights ethical governance and biosafety assessment as essential pillars for translating microbiota-informed concepts into field-relevant strategies. Deployment of microbiota-based approaches particularly those involving vector manipulation or environmental modification must be guided by transparent regulatory frameworks, rigorous ecological risk assessment, and sustained community engagement. Ethical analyses emphasize balancing innovation with ecosystem preservation, cultural acceptance, and long-term sustainability.

Collectively, this Figure 7 frames microbiota-based strategies not as standalone solutions, but as complementary components within ecosystem-informed malaria control programs. By aligning microbiota research with One Health principles, future malaria control efforts can prioritize interdisciplinary collaboration, ecological integrity, and adaptive policy design over narrow, technology-driven interventions.

Across both vector- and host-targeted approaches, a substantial translational gap persists between experimental findings and field-ready interventions. Challenges include ecological variability, population-level heterogeneity in microbiota composition, logistical constraints in resource-limited settings, and regulatory complexity. Consequently, microbiota-based strategies should be positioned as complementary components within integrated malaria control portfolios rather than as standalone or replacement solutions.

## Methodological advances, statistical rigor, and research standards in malaria–microbiota studies

Early malaria–microbiota studies relied heavily on descriptive profiling of microbial community composition, providing valuable initial insights into taxonomic diversity across hosts and vectors. However, taxonomic shifts alone do not necessarily reflect functional consequences relevant to parasite development or disease severity. Consequently, strong mechanistic claims based solely on compositional data should be interpreted cautiously.

A substantial proportion of published studies continue to rely on *16S rRNA* gene sequencing, which offers limited resolution for inferring microbial function. While 16S-based approaches are useful for characterizing broad community structure, they cannot reliably capture strain-level variation, gene expression, or metabolic activity. As a result, associations between specific taxa and malaria outcomes inferred from 16S data should not be equated with causal functional mechanisms in the absence of complementary validation (Chen *et al*., 2022; Schmidt and Jane, 2023).

Shotgun metagenomics, metatranscriptomics, metaproteomics, and metabolomics provide improved resolution of microbial functional potential and activity, enabling identification of metabolic pathways and immune-modulatory processes relevant to malaria infection. These approaches have revealed microbial genes and metabolites associated with redox balance, nutrient competition, and immune signaling (Ankrah *et al*., 2021; Wilmanski *et al*., 2019). Nevertheless, their application remains limited by high cost, computational demands, and challenges in standardization across laboratories and field sites.

Microbiome-wide association studies face inherent statistical challenges due to high dimensionality, sparse data structures, and extensive multiple testing. Many studies are underpowered to detect modest but biologically meaningful effect sizes, increasing the risk of false-positive findings. Rigorous statistical frameworks including appropriate correction for multiple comparisons, transparent reporting of effect sizes and confidence intervals, and pre-registration of analytical pipelines are therefore essential for improving reproducibility and interpretability.

Technical artifacts, including batch effects and laboratory contamination, pose significant challenges in malaria–microbiota research, particularly when analyzing low-biomass samples such as mosquito midguts or blood-associated microbial DNA. Without stringent controls, background contaminants may be misinterpreted as biologically meaningful signals. Implementation of negative controls, standardized DNA extraction protocols, and contamination-aware analytical pipelines is critical for ensuring data reliability.

Interpretation of microbiota–malaria associations is further complicated by numerous confounding variables, including diet, age, prior exposure, co-infections, antimicrobial use, and environmental conditions. Failure to adequately measure and adjust for these factors can obscure true biological relationships. Comprehensive metadata collection and transparent reporting of confounder adjustment strategies should therefore be considered minimum requirements for future studies.

Machine-learning approaches offer potential for integrating complex, multi-dimensional datasets in malaria–microbiota research. However, predictive models are highly sensitive to study design, feature selection, and validation strategy. Many reported models rely on internal cross-validation without independent external cohorts, limiting generalizability across geographic regions with distinct microbiota profiles (Singh *et al*., 2023). Accordingly, machine-learning outputs should be viewed as hypothesis-generating tools rather than definitive predictors of malaria risk.

To advance the field beyond descriptive associations, standardized reporting frameworks are urgently needed. Recommended minimum standards include transparent reporting of alpha and beta diversity metrics, sequencing depth, effect sizes, statistical thresholds, confounder adjustment, and metadata availability. Adoption of harmonized protocols and open data practices would facilitate cross-study comparison, meta-analysis, and cumulative knowledge building in malaria–microbiota research.

Future malaria–microbiota studies should prioritize longitudinal designs, multi-site field investigations, and integration of functional validation experiments capable of resolving causality. Aligning methodological rigor with ecological realism will be essential for translating

microbiota research into biologically meaningful and policy-relevant insights.

## Translational, ecological, regulatory, and policy implications

Despite substantial progress in elucidating microbiota–malaria interactions under experimental conditions, significant translational gaps persist between laboratory findings and field-ready interventions. Most mechanistic insights are derived from controlled animal models or simplified vector systems that do not fully capture the ecological complexity, host heterogeneity, and environmental variability characteristic of malaria-endemic settings. Consequently, extrapolation of experimental results to population-level impact should be approached with caution.

The long-term effectiveness of microbiota-based strategies depends critically on ecological robustness. Seasonal variation, climate-driven environmental change, land-use patterns, and sanitation conditions can rapidly reshape microbial communities in both mosquito vectors and vertebrate hosts. Without sustained ecological stability, microbiota-targeted interventions may exhibit transient or inconsistent effects, limiting their durability as malaria control tools.

Operational deployment of microbiota-informed interventions presents additional challenges in resource-limited settings where malaria burden is highest. Requirements for cold-chain storage, strain standardization, repeated dosing, or complex diagnostic infrastructure may constrain feasibility relative to established interventions such as insecticide-treated bed nets, rapid diagnostic tests, and antimalarial chemotherapy. Cost-effectiveness and logistical simplicity must therefore be central considerations in evaluating translational potential.

Microbiota-based interventions, particularly those involving engineered symbionts or paratransgenic approaches, require rigorous regulatory oversight prior to field deployment. Approval pathways may involve national biosafety authorities, environmental protection agencies, and international advisory mechanisms coordinated by organizations such as the World Health Organization. Comprehensive risk assessment, post-release monitoring, and contingency planning are essential to ensure biosafety and environmental protection.

Ethical governance and community engagement are critical components of microbiota-informed malaria control strategies. Interventions that modify microbial ecosystems whether in vectors, humans, or animals raise questions regarding consent, environmental stewardship, and long-term societal impact. Transparent communication, inclusion of local stakeholders, and culturally sensitive engagement strategies are necessary to build trust and ensure social acceptability.

Within a One Health framework, microbiota research should be integrated into broader malaria control policies that encompass human health, veterinary surveillance, vector ecology, and environmental management. Rather than replacing existing interventions, microbiota-based approaches are best positioned as complementary components that may enhance resilience and sustainability when aligned with established control measures.

Future priorities should focus on longitudinal, multi-site field studies capable of resolving causal relationships between microbiota composition, functional activity, and malaria outcomes. Integration of ecological data, standardized methodologies, and robust statistical frameworks will be essential for identifying interventions that are both biologically effective and operationally feasible. Such efforts will ultimately determine whether microbiota-informed strategies can transition from experimental concepts to meaningful contributors within integrated malaria control programs.

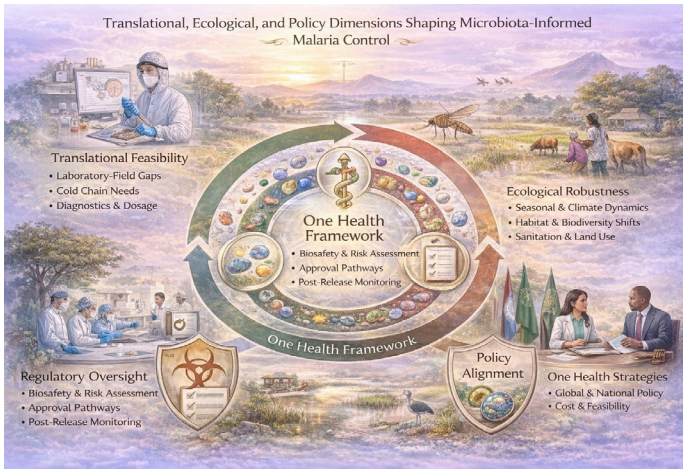


**Figure 8:** Translational, ecological, and policy dimensions shaping microbiota-informed malaria control.

Figure 8 conceptualizes microbiota-informed malaria control as a multidimensional process integrating translational feasibility, ecological robustness, regulatory oversight, and policy alignment within a One Health framework. It highlights key constraints that shape the transition from experimental microbiota–malaria research to field-relevant applications, emphasizing responsible innovation, environmental context, and governance rather than direct claims of intervention efficacy. The Figure 8 was created by the authors based on synthesis of recent peer-reviewed literature (Caragata and Shorts, 2022; Liu *et al*., 2024; Palinauskas *et al*., 2024; Asare and Duntu,

2024; Van den Ham *et al*., 2024; Bednarsky *et al*., 2025; Naidoo and Oliver, 2025) using BioRender and refined with Adobe Illustrator.

Figure 8 frames microbiota-informed malaria control as a continuum extending from laboratory discovery to policy-level implementation, rather than as a linear pathway toward immediate intervention. At the translational interface, it emphasizes the persistent gap between mechanistic insights derived from controlled animal or vector models and the ecological complexity of malaria-endemic settings. Host heterogeneity, environmental variability, and fluctuating transmission intensity impose substantial uncertainty on the population-level relevance of experimental findings, necessitating cautious interpretation and staged validation.

Ecological robustness represents a central determinant of long-term effectiveness for microbiota-based approaches. Seasonal dynamics, climate variability, land-use change, and sanitation conditions continuously reshape microbial communities in hosts, vectors, and environmental reservoirs. The Figure 8 illustrates that without sustained ecological stability, microbiota-targeted strategies may yield transient or inconsistent effects, limiting their durability compared with established malaria control measures. This perspective aligns with reviewer expectations that microbiota should be viewed as environmentally embedded rather than technically fixed targets.

Operational and logistical constraints are highlighted as critical translational filters. In many high-burden settings, requirements for cold-chain storage, strain standardization, repeated administration, or advanced diagnostics may restrict feasibility relative to simpler and well-established interventions such as insecticide-treated nets, rapid diagnostic tests, and antimalarial drugs. Accordingly, the Figure 8 situates microbiota-based strategies as complementary components whose value depends on cost-effectiveness, logistical simplicity, and compatibility with existing control infrastructures.

Regulatory oversight and ethical governance form another essential dimension of the framework. Microbiota-based interventions particularly those involving engineered symbionts or paratransgenic vectors require rigorous biosafety assessment, transparent approval pathways, and post-deployment monitoring. The Figure 8 underscores the role of national regulatory authorities, environmental agencies, and international bodies in balancing innovation with ecological protection and public trust. Ethical considerations, including informed consent, environmental stewardship, and long-term societal impact, are depicted as integral rather than peripheral elements.

Finally, the Figure 8 situates microbiota research within a One Health policy context that integrates human health, veterinary surveillance, vector ecology, and environmental management. Rather than displacing existing interventions, microbiota-informed strategies are framed as potential enhancers of system resilience when aligned with established malaria control programs. The Figure 8 thus reinforces the conclusion that future progress depends on longitudinal, multi-site field studies, standardized methodologies, and policy-aware research design capable of translating microbiota insights into sustainable, socially acceptable contributions to malaria control.

## CONCLUSIONS

This review highlights gut microbiota associated with mosquito vectors and vertebrate hosts as an important, yet context-dependent, biological layer influencing malaria transmission and disease severity. While experimental studies provide valuable mechanistic insights into microbiota-mediated modulation of parasite development, immune regulation, and inflammatory balance, much of the available human and field-based evidence remains correlational. A key priority for future research is therefore the transition from descriptive associations toward robust causal inference through longitudinal designs, functional validation, and multi-site field studies. Although microbiota-based strategies including symbiont manipulation, paratransgenics, and probiotic supplementation offer promising conceptual avenues, their current translational readiness is limited, with substantial challenges related to ecological robustness, biosafety, regulatory oversight, and operational feasibility. Within a One Health framework, integrating microbiota research across human, animal, vector, and environmental domains may enhance ecosystem-level understanding of malaria ecology, positioning microbiota-informed approaches as complementary rather than standalone components of integrated malaria control strategies.

## ACKNOWLEDGMENTS

The authors would like to express their sincere gratitude to the Indonesia Endowment Fund for Education (Lembaga Pengelola Dana Pendidikan, LPDP) for providing financial support for the doctoral study of Rahadi Swastomo. This article was prepared as part of the academic requirements for the completion of the Doctoral (PhD) program. The authors also acknowledge the valuable academic guidance and institutional support provided by the coaching team of the Faculty of Veterinary Medicine, Brawijaya University, under the supervision of Dr. Ajeng Erika and Dr. Fidi Nur Aini, during the preparation of this manuscript.

## NOVELTY STATEMENT

This review provides a comprehensive One Health perspective on malaria–gut microbiota interactions by integrating evidence from mosquito vectors, vertebrate hosts, and environmental ecosystems within a single conceptual framework. Unlike previous reviews that primarily focused on biological mechanisms, this review critically evaluates the strength of current evidence, distinguishes causal findings from associative observations, and emphasizes translational readiness, ecological robustness, methodological rigor, biosafety considerations, and policy implications. This integrated perspective offers a practical framework for guiding future microbiota-informed malaria control strategies.

## AUTHORS' CONTRIBUTION

RS conceptualized the review, developed the overall framework and scope of the manuscript, performed the systematic literature search and synthesis, and prepared the original draft. TWS contributed to conceptual refinement, critical scientific evaluation of the content, and substantive revision of the manuscript for intellectual coherence and accuracy. ARP contributed to thematic organization, critical interpretation of the reviewed literature, and refinement of the manuscript structure and scientific narrative. SRP provided overall academic supervision, strategic guidance, critical appraisal of the manuscript, and final approval of the version to be published. All authors contributed to manuscript revision, read and approved the final version, and agree to be accountable for all aspects of the work.

### Generative AI and AI-assisted technology statement

During the preparation of this manuscript, the authors used generative artificial intelligence (AI) solely for language editing, grammar improvement, and enhancement of manuscript readability. All scientific concepts, literature selection, critical evaluation, interpretation of evidence, conclusions, and final editorial decisions were performed and verified by the authors. The authors take full responsibility for the accuracy, originality, and integrity of the content of this manuscript.

### Conflict of interest

The authors have declared no conflict of interest.

## REFERENCES


Acheng S (2024). Harnessing gut microbiota for malaria prevention and control. Trends Parasitol., 40(6): 456–468.

Adeltye A, Mensah PK, Owusu D (2023). Avian malaria parasites modulate gut microbiome assembly and host immune responses. Parasit. Vectors, 16(1): 311.

Ankrah NYD, Wilks C, Lawniczak MKN, Dimopoulos G (2021). Multi-omic integration reveals the metabolic basis of host–microbiota–parasite interactions. Nat. Microbiol., 6(8): 1046–1060.

Asare KK, Duntu S (2024). The role of microbiome in malaria transmission and severity. Front. Cell. Infect. Microbiol., 14: 1298743.

Bah MT, Dabire KR, Gnankine O, Diabaté A (2022). Field evaluation of symbiont-based bioaugmentation for malaria vector control. Parasites Vectors, 15(1): 214.

Bednarsky AJ, Muturi EJ, Killeen GF, McCann RS (2025). Gut bacterial dysbiosis in pediatric severe malaria associates with post-discharge mortality. Lancet Microbe, 6(2): e123–e134.

Ben Ami R, Cohen T, Levi O, Sharon G (2023). CRISPR-engineered *Lactobacillus casei* enhances redox balance and immunoregulation. Appl. Environ. Microbiol., 89(2): e01544-22.

Cansado-Utrilla C, Zhao SY, McCall PJ, Coon KL, Hughes GL (2021). The microbiome and mosquito vectorial capacity. Microbiome, 9(1): 111. https://doi.org/10.1186/s40168-021-01073-2

Caragata EP, Shorts S (2022). Vector microbiota and immunity. Curr. Opin. Insect Sci., 50: 100881. https://doi.org/10.1016/j.cois.2022.100875

Carlos AR, Mendes AM, Silva JP, Gomes AR (2025). Metabolic reprogramming and gut microbiota ecology drive divergent *Plasmodium vivax* outcomes. Cell Host Microbe, 33(1): 78–92.

Chen Y, Zhang Y, Wang Y, Li H (2022). Microbiome analyst 2.0. Nucl. Acids Res., 50(W1): W552–W560. https://doi.org/10.1186/s40168-022-01355-3

Dong Y, Manfredini F, Dimopoulos G (2021). Metatranscriptomic analysis of mosquito midgut bacteria during Plasmodium infection. Front. Microbiol., 12: 679021.

Frosch AE, Kolmeder CA, Stewart CJ, Glynn C, Yolken RH, Schmidt NW (2022). Metagenomic signatures of severe malaria. Microbiome, 10(1): 88.

Gebreslassie M, Abebe A, Tadesse G, Dimopoulos G (2023). Symbiont co-infection networks define vector competence. ISME J., 17(2): 405–417.

Guo W, Li Y, Wang Y, Zhang J, Han X, Liu Z, Chen Y, Zhang H (2022). Probiotic modulation of IL-6 and IL-10. Foods, 11(9): 1551.

He S, Qi Z (2025). Severe malaria-induced dysbiosis and bacteremia risk. Front. Microbiol., 16: 1209.

Hwang J, Taylor JJ, Dodd D, Donohoe DR, Yu J, Wang X, Thompson JA, Lee JH, Iliev ID (2022). Gut microbial tryptophan–kynurenine pathway. Cell Host Microbe, 30(4): 550–564.

Kumar G, Gupta S, Kaur J, Pasi S, Baharia R, Mohanty AK, 2024. Mapping malaria vectors and insecticide resistance in a high-endemic district of Haryana, India: implications for vector control strategies. Malaria Journal. 2024;23:107. doi:10.1186/s12936-023-04797-8.

Lee JW, Kim JH, Park SH, Kim HY, Lee SM, Kim MJ, Cho YS (2023). Probiotic supplementation and malaria recurrence. Front. Nutr., 10: 1149392.

Liu Y, Wang S, Dong Y, Dimopoulos G (2024). Mosquito gut microbiota. Curr. Opin. Insect Sci., 58: 101062.

Lo K, Chan KY (2022). Ethics of microbial interventions in

vector control. Bioethics, 36(5): 489–499.
Mahajan E, Kaur R, Kaur M, Singh S, Kaur S (2021). Probiotic therapy in malaria-induced mice. J. Pharm. Pharmacol., 73(7): 949–957.
Mancini MV, Favia G. 2022. Asaia paratransgenesis in mosquitoes. In: Benedict MQ, editor. Transgenic Insects: Techniques and Applications. Wallingford, UK: CABI. p. 308–319. https://doi.org/10.1079/9781800621176.0015
Mandal RK, Denny JE, Namazzi R, Opoka RO, Datta D, John CC, Schmidt NW. 2021. Dynamic modulation of spleen germinal center reactions by gut bacteria during Plasmodium infection. Cell Reports, 35(6):109094. https://doi.org/10.1016/j.celrep.2021.109094
Mukherjee A, Dutta S, Saha P, Bhattacharya S, Mandal S (2021). Gut microbiota diversity and malaria severity. Front. Cell. Infect. Microbiol., 11: 640898.
Naidoo N, Oliver SV (2025). Gene drives and malaria control. Trends Parasitol., 41(2): 150–162.
Oluwafemi O, Azuaba O (2021). Hygiene and malaria transmission. Infect. Dis. Model., 6: 421–433.
Ouattara SM, Ouattara D, Badoum ES, Diarra A, Hien D, Ouedraogo AZ, Nebie I, Ouedraogo A, Tiono AB, Sirima SB. 2023. Malariometric indices in the context of seasonal malaria chemoprevention in children aged 1.5 to 12 years during the period of high malaria transmission in the suburban area of Banfora, Burkina Faso. Tropical Medicine and Infectious Disease, 8(9):442. https://doi.org/10.3390/tropicalmed8090442
Palinauskas V, Abdelali SK, Cabezas-Cruz A (2024). The mosquito microbiota. Pathogens, 13(12): 1101. https://doi.org/10.3390/pathogens13121101
Patel H, Minkah NK, Kumar S, Zanghi G, Schepis A, Goswami D, 2024. Malaria blood stage infection suppresses liver stage infection via host-induced interferons but not hepcidin. Nature Communications, 15:2022. https://doi.org/10.1038/s41467-024-46270-3
Riithi MK, Muturi EJ, Dimopoulos G, Killeen GF (2025). Microbial regulation of malaria rhythms. PLoS Pathog., 21(1): e1011912.
Salgado JFM, Premkrishnan BNV, Oliveira EL, Vettath VK, Goh FG, Hou X, Junqueira ACM (2024). Midgut microbiome dynamics. PNAS Nexus, 3: 317. https://doi.org/10.1186/s40168-024-01917-7
Schmidt NW, Jane SR (2023). Gut microbiota and human malaria. Front. Immunol., 14: 1145632.
Singh P, Verma A, Sharma R, Pandey S, Gupta R, Singh S (2023). Machine-learning microbiome prediction. Sci. Rep., 13: 12454.
Tseng M, Chen Y, Yang C, Liu C, Wang S, Lin Y, Hsu T (2023). Delftia tsuruhatensis inhibits Plasmodium. Nat. Commun., 14(1): 344.
Van den Ham HJ, Olotu A, Wambua J, Kimani D, Bejon P, Crompton PD, Schmidt NW (2024). Gut microbiome and malaria susceptibility. Nat. Commun., 15(1): 1127. https://doi.org/10.1038/s41467-024-52953-8
Verma R, Ganti K, Chakravarty S. 2021. Anti-malarial humoral immunity: the long and short of it. Microbes and Infection, 23(4–5):104807. https://doi.org/10.1016/j.micinf.2021.104807
Wilmanski T, Rappaport N, Earls JC, 2019. Blood metabolome predicts gut microbiome α-diversity. Nature Biotechnology. 37:1217–1228.
World Health Organization (2025). World malaria report 2025. WHO, Geneva.
Yawen Z, Li X, Wang Y, Zhang H, Liu J, Chen Q, Zhou L, Sun B (2022). Parasitemia-dependent microbiota shifts. Front. Cell. Infect. Microbiol., 12: 873214.