Discovery, Development & Delivery

# IMC 2026

Driving Malaria Elimination and Beyond

## International Malaria Conference

### Discovery, Development, and Delivery

### Driving Malaria Elimination and Beyond

# Abstract Book

ICMR | NIMR
INDIAN COUNCIL OF MEDICAL RESEARCH | NATIONAL INSTITUTE OF MALARIA RESEARCH

CARAH
WOMEN FOUNDATION

**7th-9th March 2026 | Delhi**

डॉ. राजीव बहल, एमडी, पीएचडी
DR. RAJIV BAHL, MD, PhD

सत्यमेव जयते

सचिव
भारत सरकार
स्वास्थ्य अनुसंधान विभाग
स्वास्थ्य एवं परिवार कल्याण मंत्रालय एवं
महानिदेशक
भारतीय आयुर्विज्ञान अनुसंधान परिषद
Secretary
Government of India
Department of Health Research
Ministry of Health & Family Welfare and
Director-General
Indian Council of Medical Research

# Message

It is a privilege to extend my greetings on the occasion of the **International Malaria Conference 2026 (IMC 2026)**, organized by the **ICMR–National Institute of Malaria Research (ICMR-NIMR)** under the theme *"Discovery, Development, and Delivery: Driving Malaria Elimination and Beyond."*

Malaria elimination remains a national priority for India, aligned with our commitment to achieve a malaria-free nation by 2030. Over the years, sustained research, strengthened surveillance, technological innovation, and coordinated programmatic efforts have significantly reduced malaria burden across the country. However, elimination demands sustained vigilance, adaptive strategies, and scientific excellence.

**Discovery, Development, and Delivery**—reflect the continuum from generating new scientific knowledge to translating it into accessible public health solutions. IMC 2026 embodies this integrated approach by providing a platform where laboratory discoveries, field innovations, and implementation strategies converge to inform policy and practice.

This conference brings together scientists, policymakers, programme implementers, and global experts to collectively address emerging challenges such as drug resistance, vector adaptation, climate variability, and residual transmission. The deliberations and scientific contributions compiled in this Abstract Book demonstrate the depth and diversity of ongoing research dedicated to malaria elimination.

I commend ICMR-NIMR and the organizing committee for their efforts in convening this important forum. I am confident that IMC 2026 will strengthen collaborations, inspire innovation, and accelerate progress toward sustainable malaria elimination in India and beyond.

I extend my best wishes for the success of the conference.

Rajiv Bahl

**Dr. Rajiv Bahl**

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**Dr. Sanghamitra Pati, MD, MPH, Ph.D**
*FAMS, FNASc., FRSPH, FRCP-London, FRCP-Edinburgh*

## Message

I am pleased to share my message for the **International Malaria Conference 2026**, organized by **ICMR–National Institute of Malaria Research**.

The journey from malaria control to elimination requires robust scientific evidence, innovation, and effective translation of research into public health action. IMC 2026, built around the framework of **Discovery, Development, and Delivery**, underscores the importance of an integrated and multidisciplinary approach to tackling malaria and other vector-borne diseases.

India has made substantial progress in reducing malaria incidence through strategic interventions, improved diagnostics, strengthened vector control measures, and enhanced community engagement. Yet, the path toward elimination requires sustained research, operational adaptability, and continuous evaluation of emerging tools and technologies.

The abstracts presented in this volume reflect the collective commitment of researchers, scholars, and institutions contributing to malaria science. These contributions represent advancements in basic research, translational studies, field implementation, and policy- oriented solutions.

I congratulate ICMR-NIMR and the organizing committee for curating a comprehensive scientific programme and fostering a collaborative environment for knowledge exchange. I hope that the deliberations during IMC 2026 will further strengthen national and global partnerships in the shared mission of malaria elimination.

**Dr. Sanghamitra Pati**
**Additional Director General,**
**Indian Council of Medical Research**

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## MESSAGE

It gives me immense pleasure to welcome you to **"Discovery, Development, and Delivery: Driving Malaria Elimination and Beyond,"** being held from March 7–9, 2026. The theme of this meeting reflects the urgent need to bridge laboratory science with field realities.

India stands at a pivotal moment in its malaria elimination journey. Guided by the National Framework for Malaria Elimination and strengthened through the efforts of the Indian Council of Medical Research and the National Centre for Vector-Borne Diseases Control, we have made measurable progress in reducing malaria burden across diverse eco-epidemiological settings. Yet, as transmission declines, the challenges become more complex like residual transmission, zoonotic threats, asymptomatic reservoirs, drug and diagnostic resistance, and climate-linked vulnerabilities which demand sharper science and smarter strategies.

As we move toward elimination, collaboration becomes our most powerful tool. Partnerships across institutions, disciplines, and countries, particularly within South-East Asia, will determine the pace and sustainability of progress. This conference is not merely a scientific gathering; it is a strategic convergence aimed at architecting an integrated ecosystem that accelerates impact from bench to bedside to community.

I encourage all participants to actively engage, question, collaborate, and envision bold solutions. Wishing you a productive and inspiring conference.

(Dr Tanu Jain)

आई सी एम आर–राष्ट्रीय मलेरिया अनुसंधान संस्थान
स्वास्थ्य अनुसंधान विभाग, स्वास्थ्य एवं
परिवार कल्याण मंत्रालय, भारत सरकार
ICMR–National Institute of Malaria Research
Department of Health Research, Ministry of Health &
Family Welfare, Government of India

## Message

It gives me immense pleasure to welcome you to the **International Malaria Conference 2026** (IMC 2026) hosted by **ICMR-National Institute of Malaria Research.**

As the apex institute dedicated to malaria research in India, ICMR-NIMR plays a pivotal role in providing scientific support to the national malaria elimination programme. Guided by the core principles of Discovery, Development, and Delivery, our efforts span fundamental research, translational innovation, and operational implementation to address the evolving challenges of malaria.

IMC 2026 provides a dynamic platform for researchers, programme implementers, industry partners, and policymakers to deliberate on emerging evidence, innovative tools, and effective delivery strategies. The scientific contributions compiled in this Abstract Book highlight cutting-edge research and operational insights that will inform both national and global elimination efforts.

Elimination is not merely the reduction of cases; it is the establishment of sustainable systems capable of preventing resurgence. Through collaboration, scientific rigor, and shared commitment, we can collectively move closer to achieving a malaria-free India and contributing to global eradication goals.

I extend my sincere appreciation to all contributors, speakers, reviewers, and participants who have made IMC 2026 possible.

Dr. Anup Anvikar
Director,
ICMR-National Institute of Malaria Research



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# Message from the Organizing Committee

On behalf of the Organizing Committee, we are delighted to present the **Abstract Book of the International Malaria Conference 2026**.

The conference, themed *"Discovery, Development, and Delivery: Driving Malaria Elimination and Beyond,"* reflects the comprehensive approach required to achieve sustainable malaria elimination. This volume compiles the scientific abstracts submitted by researchers, scholars, and professionals representing diverse disciplines and institutions across India and the globe.

The abstracts encompass a wide spectrum of themes, including basic research, diagnostics, therapeutics, vector biology, epidemiology, surveillance, implementation research, and innovative biotechnological solutions. Each contribution represents a step forward in strengthening the scientific foundation required for elimination.

We sincerely acknowledge the efforts of the Scientific Committee, reviewers, session chairs, and contributors whose dedication ensured the quality and integrity of this publication. The collective knowledge presented herein underscores the importance of collaboration, innovation, and sustained commitment.

We hope that this Abstract Book serves not only as a record of the scientific discourse at IMC 2026 but also as a catalyst for future research partnerships and impactful public health action.

We thank all participants for their valuable contributions and wish everyone a successful and enriching conference.

with warm regards,

IMC 2026 Organizing Team

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Organizing Committee
IMC 2026

## Index of Abstracts

S-01

# Unseen Plasmodium Threats: Neglected Species in India's Malaria Landscape

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India has made measurable progress in reducing malaria burden over the past two decades through surveillance and control strategies, largely targeting *Plasmodium falciparum* and *P. vivax*. However, this programmatic focus has inadvertently masked the epidemiological relevance of neglected and emerging *Plasmodium* species, including *P. malariae*, *P. ovale*, *P. ovale*, *P. knowlesi*, *P. cynomolgi*, and occasional reports of simian parasites such as *P. inui* and *P. simiovale* infecting humans in South and Southeast Asia that could very well be imported to India due to on-going cross-border human and material transportation. In addition, mitochondrial genomic variant of *P. falciparum* such as *Pf*India* circulating in Indians and relapse-prone lineages like the *P. vivax* Chesson strain added complexity in transmission dynamics, therapeutic response, and relapse biology in India. These infections frequently remain undetected due to morphological overlap, low parasitaemia, and limitations of conventional microscopy and rapid diagnostic tests (RDTs), contributing to persistent transmission and misclassification. Molecular surveillance across forested and tribal ecosystems, suggests the occurrence of these species as mono- and mixed-species infections. Importantly, the presence of non-human primate reservoirs in hilly, forested and island settings and competent *Anopheles* vectors in India underscores the zoonotic transmission potential of malaria parasites, raising concerns about simian-to-human spillover and the emergence of novel transmission cycles. How integrating species-sensitive molecular diagnostics and genomic surveillance within the "One Health" framework is essential to address the hidden diversity of malaria parasites and to ensure sustainable malaria elimination in India by 2030, will be presented.

S-02

# A Tale of Two Parasites: A Glimpse into the RNA Methylome of Patient-derived *Plasmodium falciparum* and *Plasmodium vivax* isolates

Priyanka Roy[1A], Sukriti Gujarati[1A], Pallavi Gupta[2,6,7], Ishaan Gupta[2], Tanmaya Mahapatra[3] Dinesh Gupta[4], Sanjay Kumar Kochar[5], Dhanpat Kumar Kochar[5], Ashis Das[1*]

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**Introduction**

Understanding the molecular mechanisms governing malaria parasite biology within the host is critical for developing effective therapeutic strategies. Emerging epitranscriptomic studies highlight the importance of RNA methylation in regulating gene expression and pathogen adaptation [1]. However, comprehensive insights into RNA modification landscapes and alternative splicing in malaria parasites under severe disease conditions remain limited. This study presents the first investigation of RNA methylation signatures and alternative splicing events at single-base resolution using Nanopore Direct RNA Sequencing in *Plasmodium falciparum* and *Plasmodium vivax* clinical isolates associated with hepatic dysfunction.

**Objective**

This study aimed to characterize the RNA methylome and alternative splicing landscape of patient-derived *Plasmodium falciparum* and *Plasmodium vivax* isolates exhibiting hepatic dysfunction.

**Methods**

Nanopore Direct RNA Sequencing was performed on clinical isolates of *Plasmodium falciparum* and *Plasmodium vivax* obtained from patients with hepatic dysfunction. Transcriptome reconstruction was conducted using FLAIR, followed by transcript classification with SQANTI3. RNA methylation profiling was carried out using CHEUI and m6Anet to identify N6-methyladenosine (m6A) and 5-methylcytosine (m5C) modifications. Alternative splicing events were documented across both datasets.

**Results**

Approximately 50% of the more than 5,000 annotated genes in the *Plasmodium* reference genome were expressed in the sequenced isolates, including novel isoforms and intergenic transcripts, indicating transcriptomic diversity. Distinct m6A and m5C methylation profiles were observed across sense transcripts, natural antisense transcripts (NATs), and intergenic RNAs, suggesting species-specific regulatory mechanisms. Many transcripts exhibited dual modification events, including those derived from apicoplast and mitochondrial genomes. RNA modifications were unevenly distributed across transcript regions, potentially influencing mRNA export and translation. Multiple alternative splicing events were detected, with alternative 3′ and 5′ end splicing predominating.

**Conclusions**

The identification of modified sense transcripts, NATs, and alternatively spliced isoforms highlights multilayered post-transcriptional regulatory mechanisms shaping parasite proteome plasticity during malaria. These insights may facilitate development of novel intervention strategies [2].

**Acknowledgement**

This study was funded by the Indian Council of Medical Research (Grant No. 2019-1121). We thank Birla Institute of Technology (BITS), Pilani (Pilani campus), International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi, Indian Institute of Technology (IIT), New Delhi, India and SP Medical College, Bikaner, for providing the necessary facilities required for this work. Genotypic Technology Pvt. Ltd. provided advisory support for installation and operation of our ONT device and other related aspects. Hospital ethics approval numbers: (F29/Acad/SPMC/2020/3151, No.F. (Acad)SPMC/2003/2395). Special thanks to patients for voluntarily providing the blood samples for collection.

**Keywords:** Malaria, Epitranscriptome, RNA methylation, Natural antisense transcripts (NATs), *Plasmodium vivax*, *Plasmodium falciparum*, Direct RNA sequencing, Alternative splicing

S-03

## The Fallacy of "Non-Essential" Genes in the Malaria Parasite

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The genome of malaria parasite encodes ~5400 proteins and ~40% of them are dispensable for the asexual stages. Many of these genes are expressed only in the sexual and liver stages, and display stage-specific functions. However, there are also genes that are expressed in the asexual stages and the respective proteins are synthesized. Such proteins are often ignored because of their dispensable nature and they represent key metabolic pathways such as heme synthesis, TCA cycle, amino acid synthesis etc., and virulence factors. In my talk, I will address the significance of such "non-essential" genes and their physiological relevance in the blood stages of malaria parasite. I will highlight our research work on the role of parasite heme in disease pathogenesis and the potential of targeting it for adjunct therapy to prevent malaria mortality. Parasite heme is essential for the mosquito and liver stages, and dispensable for the blood stages. Interestingly, the blood-stage parasites synthesize heme despite having the ability to acquire host heme through hemoglobin degradation. The insights gained on the role of parasite heme in the blood-stage infections, and the attempts being made for clinical trials in humans using griseofulvin as an adjunct drug with the existing artemisinin-based combination therapies for cerebral and severe malaria will be presented. At the end, I will briefly discuss about the distinct evolution of glutamine synthetase in the malaria parasite and its species-specific essentiality, and the role of parasite HMGB1 in splenic clearance and antigenic variation. These research findings will offer a glimpse at the role of such dispensable genes in metabolic adaptations, malaria pathogenesis and host immune evasion.

S-04

## Beyond Human Parasites: The challenge of zoonotic malaria to elimination

Balbir Singh[1]
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Zoonotic malaria was thought to be extremely rare, until a large number of humans infected with Plasmodium knowlesi, a parasite typically infecting macaques in nature, were described in Malaysian Borneo in 2004. This discovery will be described followed by the early studies demonstrating human infections by blood passage and mosquito bites of P. knowlesi and P. cynomolgi. Subsequent molecular, epidemiological and entomological studies have highlighted that zoonotic malaria is widely distributed, particularly in Southeast Asia, can be potentially fatal, the hosts are predominantly long-tailed and pig-tailed macaques, and the primary vectors are outdoor feeders living in the forest and forest fringe. The reasons for the increase in zoonotic malaria are multifactorial and include increased awareness, utilisation of molecular detection methods, changes in vector populations and feeding behaviour and habitat loss of macaques due to environmental changes. Conventional methods utilised for the prevention and control of human to human transmission of malaria, are largely ineffective against zoonotic malaria. Personal protection methods are recommended for humans and since the macaque reservoir hosts are protected species, control of zoonotic malaria is challenging and innovative methods need to be developed if malaria is to be eliminated.

S-05

## From Simple Chemistry to Promising Antiparasitic Leads

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Multistage antimalarials are expected to broaden the therapeutic scope and also counteract the resistance of the frontline therapeutics. We present novel heterocyclic compounds of chemical diversity with a broad antiplasmodial activity, particularly liver stage and transmission blocking ability. A remarkable low nanomolar inhibitory activity of the compounds against the asexual blood stage was observed on *Pf*3D7 and artemisinin-resistant field strain, *Pf*C580Y. Liver stage is the most engaging stage for immunization and prophylaxis systems, and our compounds displayed a strong inhibition against the *P. berghei* liver stage with nanomolar $IC_{50}$ values in culture and in the mice model. Next, compounds treatment to the cultured mixed gametocyte stages resulted in >80% inhibition at 100 nM. A treatment of compound at 10mg/Kg dose (i.p.) to the mice having gametocyte stage, showed >65% inhibition in the number of gametocyte parasites. Compounds were administered parallelly with the gametocyte-infected blood, and the results were measured as the % reduction of oocysts in the mosquito midgut. Our results conveyed that mosquito fed with compound perceived a significant decline in the number of oocysts formed in the midgut. Intriguingly, various mosquitos could not form any oocyst and thus intimated 100% killing of the parasite. Significant reduction in the development of the ookinete (ex-vivo) and gametocyte stages leads to transmission blocking efficacy. Preliminary toxicology studies in mice indicated a safety margin in the tissues of different organs. Subacute toxicity at a dose of >400 mg/Kg was assessed in the mice that supported a normal functioning of liver and kidney organs as well, no apparent toxicity at 1000 µM in mammalian cells combined with favorable pharmacokinetics evaluated in mice.

S-06

# Recent Advances in Antigen-based Malaria Diagnostics; Sensitivity, Specificity, and field Applicability

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High-quality malaria microscopy remains limited in many endemic regions due to inadequate infrastructure, trained personnel shortages, and heavy patient burden. As a result, rapid diagnostic tests (RDTs) have become the primary tool for malaria diagnosis because they are simple, affordable, and suitable for field use. Most widely used RDTs target histidine-rich protein 2 (HRP2) produced by Plasmodium falciparum. However, the increasing emergence of parasites lacking the hrp2 and hrp3 genes has compromised the reliability of HRP2-based tests in several regions. This growing diagnostic gap highlights the urgent need to identify alternative and more robust antigen targets for malaria detection.

In our previous study, PfEXP1 (Plasmodium falciparum exported protein 1) was identified as a highly immunoreactive and consistently expressed antigen across blood-stage parasites (Vashisht et al., 2022, Translational Science). Building on this observation, the present work aimed to evaluate the diagnostic potential of PfEXP1. Recombinant PfEXP1 was successfully expressed, purified, and characterized. High-titer, highly specific polyclonal antibodies were generated and demonstrated strong reactivity against the antigen. The antibodies were able to detect PfEXP1 at very low concentrations, with analytical sensitivity reaching the picogram range. Importantly, they recognized PfEXP1 in both in vitro cultured parasites and clinical blood samples.

Different immunoassay formats confirmed the strong detection capability of PfEXP1, with sensitivity comparable to that of conventional HRP2-based detection systems. Functional and structural analyses further indicated that the protein is stable at higher temperature and retains its biological integrity, supporting its suitability as a diagnostic target. Validation using field samples from multiple endemic sites demonstrated high specificity and a strong correlation between antibody reactivity and parasitemia levels.

Overall, these findings suggest that PfEXP1 is a promising alternative diagnostic antigen that could help overcome the limitations associated with hrp2/hrp3 gene deletions and strengthen malaria detection strategies in endemic settings.

S-07

## Interrogating parasite transmission biology to inform malaria elimination

Matthias Marti[1,2]

[1] University of Glasgow, UK
[2]University of Zurich, Switzerland

Malaria remains a major global health problem and elimination of this global killer is a top priority. Parasite transmission from human to mosquito is critical for disease spread and hence a major target of interventions. Between-host transmission requires the formation of gametocytes from asexual blood stage forms, as gametocytes are the only parasite stage to progress the cycle in the mosquito. Here I will discuss ongoing work from our lab interrogating i) evolutionary and mechanistic drivers that determine the switch from asexual replication to sexual reproduction, ii) tissue tropism of gametocyte formation and development in human and animal models and iii) natural immunity against malaria transmission stages. I will also discuss the translational implications of these discovery and mechanistic studies for the malaria elimination agenda.

S-08

## The Nature of "*Plasmodium ovale*", with Reference to Biology and Elimination

Miles B. Markus[1]

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This will be a state-of-the-art-cum-historical update. What "*Plasmodium ovale*" is, will first of all be dealt with, involving consideration of taxonomic matters, as requested by the conference organizers. There are things to do and not do when referring to "*P. ovale*". That aside, "*P. ovale*" and *P. vivax* have, conventionally, both been considered to be relapsing malaria parasites and are always spoken about in the same breath in relation to hypnozoites. However, a specific detail in the literature regarding "*P. ovale*" has sometimes (if not largely) been misinterpreted or overlooked. This matter will be elaborated upon. Furthermore, a non-hypnozoite biological hindrance to eliminating "*P. ovale*" became apparent this year (2026), unsurprisingly, and will be pointed out. Historically, the speaker coined the term "hypnozoite" in 1978, after predicting in 1976 (on the basis of his research at Imperial College London concerning a parasite related to *Plasmodium*) the existence of such a plasmodial life cycle stage (Table 1 in PMID 29564998). A particular sequence of events followed. Firstly, an account by other authors was published in 1980, describing the microscopic recognition of the malarial hypnozoite. Then in 2011 and 2012, it was explained that some non-reinfection recurrences of malaria probably have hidden merozoites rather than hypnozoites as their source (PMID 22329013, 22696499 and 22118814). This idea was reinforced and expanded upon in 2017 (PMID 28366603). Something still novel in 2026 in relation to the elimination of malaria by drug administration is the potentially paradigm-shifting, biochemically based, unique (in its origin) theory that primaquine might act substantially against (in addition to inactivating liver stages) concealed extra-hepatic merozoites (PMID 31522991, 36180306 and 37235326), which are now known to occur in the life cycle of "*P. ovale*". The speaker will have elucidatory reprints of some paywalled publications available (ask him for copies, should you wish to do so).

S-09

## Closing the gaps: tackling residual transmission on the path to malaria elimination

Neil Lobo[1]

[1]University of Notre Dame, USA

Despite remarkable gains in malaria control over the past two decades, residual malaria transmission persists in many settings where long-lasting insecticidal nets and indoor residual spraying are deployed. This talk reflects on lessons learned from multiple country experiences confronting transmission that continues despite high coverage of core interventions. Residual transmission is not simply "low transmission." It is driven by gaps in protection shaped by vector behavior, human behavior, ecological heterogeneity, and operational realities. Drawing on field examples from Africa, Latin America, and Southeast Asia, this presentation highlights practical lessons: the importance of decision-focused surveillance; the central role of human behavior in defining exposure; the inevitability of heterogeneity; the adaptive capacity of vectors; the context-specific nature of larval source management; the need to move beyond reflexive attribution to insecticide resistance; the necessity of linking entomology to transmission outcomes; the value of layered tools; the constraints imposed by funding realities; and the superiority of adaptive over static strategies. Residual transmission is not a mystery, but a measurable gap in protection. Precision targeting, guided by focused surveillance and linked to clear decisions, consistently outperforms blanket coverage approaches. If elimination is the goal, strategies must evolve as rapidly as the systems they aim to control.

S-10

## From Discovery to Delivery: Operational feasibility of G6PD Testing for Radical Cure in India

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Implementation of single-dose tafenoquine and a 7-day primaquine regimen in India has the potential to substantially advance the elimination of *Plasmodium vivax* malaria; however, both therapies require prior assessment of glucose-6-phosphate dehydrogenase (G6PD) status to mitigate the risk of drug-induced haemolysis in G6PD deficient individuals. We conducted a low-interventional, multicentre implementation study between December 2024 and May 2025 across 256 health facilities in Chhattisgarh, Gujarat, Odisha, and West Bengal to evaluate the operational feasibility of semi-quantitative point-of-care G6PD testing. Healthcare providers engaged in malaria case management who provided consent received standardized training in the use of the STANDARD G6PD test (SD Biosensor). Consenting adults aged 18 years or older presenting with fever and without clinical features of severe malaria were tested for G6PD deficiency. The primary endpoint was the proportion of providers demonstrating proficiency in a post-study competency assessment. Across 256 health centres, 5,130 individuals with febrile illness were enrolled, of whom 5,102 yielded valid G6PD results. Testing was performed by laboratory technicians and trained paramedical personnel. Overall, 95% (95% CI 91–97; 243/256) of providers met predefined proficiency criteria. High levels of competency were observed in both higher-level and lower-level facilities, as well as in urban and rural settings. These findings demonstrate that semi-quantitative point-of-care G6PD testing is operationally feasible across diverse health system contexts, supporting broader deployment of radical cure strategies for *P. vivax* in India. Detailed results shall be presented in the lecture.

S-11

# Malaria Elimination in High Transmission Hard-to-Reach Areas of Odisha (India): State's Initiatives and Challenges

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**Background**

Malaria poses immense public health challenge in India's east coast state Odisha since decades. In 2011, Odisha contributed 40% of country's annual malaria cases and 91.5% falciparum malaria cases. Asymptomatic malaria poses immense problem for malaria persistence in remote villages with vast forest tracks and limited accessibility. Odisha adopted "Durgama Anchalare Malaria Nirakaran" (DAMaN) strategy for malaria elimination in hard-to-reach areas in 2017 along with implementation of large-scale Long-Lasting Insecticidal Net (LLIN) implementation and community awareness. The key components of DAMaN for malaria elimination are mass screening and treatment (MSaT), vector control and community mobilisation.

**Methods**

We conducted the evaluation study to examine the beneficial outcomes of the DAMaN program, explored the DAMaN's implementation challenges and potential strength for the enhancement of malaria elimination efforts in six sampled districts representing different physiographical regions of the state. Data from two biennial surveys, state's routine malaria programme and conducted community-based interviews and analysed using appropriate tools and methods.

**Results**

Within year of DAMaN implementation in 2017, malaria cases started declining with 80.9% reduction. The DAMaN villages showed remarkable reduction. Overall decline (around 93%) continued till 2022 compared to 2017 despite the effect of COVID 19 pandemics during 2019-2021.Malaria positivity rate was significantly higher during the first biennial survey in 2019, compared to the second biennial survey in 2021.An alarming proportion of asymptomatic malaria cases (75% to 100%) was found during second biennial survey of DAMaN. Perspective of the community level health care givers and community opinion leaders on DAMaN was largely encouraging and motivational. After the huge scale decline, rise in malaria cases is observed 2023 onwards. The overall high case rise i.e. 188% after 2023 coincides with the rise of malaria cases in DAMaN target villages.

**Conclusion**

Malaria reduction was notably high in areas where DAMaN's programme was effective. The overall positive findings indicate that DAMaN being a community-based malaria elimination initiative in far-flung villages of Odisha state, serves as a suitable supplement to the routine malaria control and elimination programme and is replicable. The rise of malaria cases since 2023 needs micro level assessment of the programme implementation and address the implementation gaps based on the evidences.

S-12

## Probing organelle protein targets and development of a quinoline to counter drug resistance in malaria

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Organelle genome stability in the malaria parasite has implications in development of resistance to drugs which target organelle-encoded protein(s). To examine DNA repair mechanisms in *Plasmodium*, we identified apicoplast- and mitochondrion-targeted enzymes and investigated their biochemical function and substrate specificities, with genetic knockout/knockdown conducted in collaboration with Dr. Satish Mishra at CSIR-CDRI. Targeting of major base excision repair (BER) proteins to the *P. falciparum* mitochondrion indicated that the organelle is the major site for BER. In addition to a unique apicoplast FEN/Exo with a functional LCR insertion (1), two 'essential' mitochondrion-targeted exonucleases, *Pf*Exo$_{mit1}$ and *Pf*Exo$_{mit2}$, were identified as highly diverged proteins conserved only in certain alveolates. They differed from each other in their DNA substrate specificities. *Pf*Exo$_{mit2}$ cleaved dsDNA bidirectionally; pull-down assays indicated its possible role in DSBR and MMR pathways. The specificity of *Pf*Exo$_{mit1}$ for ssDNA suggested its role in clearance of ssDNA "puddles" reported to accumulate during mtDNA rolling circle replication (2). Our results map the limited set of *P. falciparum* organellar exonucleases to specific DNA transactions in the apicoplast and mitochondrion.

CSIR-CDRI is working on a novel antimalarial, S-011-1793 (3), which originated from side-chain modifications of chloroquine (CQ). It was potent against CQ-resistant *P. falciparum*, against a resistance panel (with artemisinin, atovaquone, piperaquine, pyrimethamine resistant strains), and *P. vivax* and *P. falciparum* field isolates tested in Brazil and Uganda. In vivo efficacy in murine, humanized mouse, and macaque infection models for malaria was established. DMPK, PRR/PCT, dose-dependent PK, safety pharmacology and toxicity evaluations in rodent and PK in non-rodent (dog) have been completed and prediction of human dose made. If all late pre-clinical studies safety studies are cleared, it would lay a path towards progressing with clinical evaluation of a candidate drug with once daily x 3-day oral dosing to counter drug resistance in malaria infections.

S-13

# The *Plasmodium falciparum* Kelch13 protein uses heme to KEAP(1) up a deceptive appearance for altering sensitivity to artemisinins

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Artemisinins remain critically important anti-malarial drugs. Emergence and spread of resistance to them threatens global control of malaria. Mutations in *Plasmodium falciparum* Kelch13 (K13) confer artemisinin resistance (ART-R), but known K13 functions fail to account for clinical ART-R. Moreover, ART is known to kill by oxidative radicals, and we have reported that K13 binds the oxidant heme *in vitro* (Rahman *et al.,* 2024), but K13 mechanisms of redox-stress, cell survival and death remain unknown. Since taut control of free heme is not feasible in infected erythrocytes, we utilized a non-erythroid cell model to show that K13 directly and specifically binds to heme and is stabilized by nanomolar concentrations of heme. K13 also localizes to late endosomal membranes, where its levels are regulated by chaperone mediated autophagy. Furthermore, K13 binds and suppresses a major redox transcription factor, which is displaced by heme, resulting in its dysregulation, and concentration in the nucleus. The ensuing elevated redox-stress responses are suppressed during artemisinin-induced death (ART-death), revealing an unexpected mechanism of K13 action. We find that K13's evolutionarily conserved kelch domain confers heme-binding and ART-death characteristics to its mammalian orthologue KEAP1, showing that it is necessary and sufficient to effect the parasite redox mechanism. Furthermore, both chemical and genetic elevation of K13, fuels ART-death proportionate to K13 levels even in vast excess of heme (known to be pervasive in the parasite's physiological niche). Together these data suggest a novel endosomal plasmodial redox-survival mechanism licenses ART-death and a new model of clinical ART-R.

S-14

## From Burden to Elimination and Beyond: Architecting an Integrated Discovery–Development–Delivery Ecosystem for a Malaria-Free

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South-East Asia Region has made remarkable progress, reducing its malaria burden by over 88% since the turn of the century. It has reached a decisive crossroads where the remaining journey is becoming increasingly difficult. To finish the task, we must confront two equally difficult challenges that stand in our way.

The first is the "last mile paradox", the reality that as malaria disappears from the map, it retreats into hard-to-reach settings and populations that traditional health systems struggle to serve. The second, and equally important, is the need to understand the drivers and determinants of transmission in high-burden pockets that have remained resistant to progress while other areas succeeded. We must identify the biological, socio-demographic, epidemiological, and environmental factors keeping the disease alive in these settings to move from broad control to eventual elimination.

A laboratory breakthrough (Discovery) is truly valuable only if it leads to a practical, refined tool (Development) that effectively reaches the person in a remote community (Delivery). This is where implementation research becomes one of our most vital tools.

We can no longer afford to work in silos; instead, we must build a living loop where real-world experiences and gaps in delivery directly shape what research focuses on.

By drawing on the strengths of institutions like ICMR-NIMR, which acts as a bridge between foundational science and national programmes, and the global stewardship of the World Health Organization, together with the partners we can bridge these gaps. This presentation is a call to move beyond aspirations and build the practical architecture needed to deliver a malaria-free South-East Asia.

S-15

# An indigenous advanced vaccine against P. falciparum: Dual protection against both infection and community transmission

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Aligned with the Government of India's 'Make-in-India' initiative, a consortium of Indian researchers, led by ICMR-RMRC, Bhubaneswar, in collaboration with the National Institute of Immunology and ICMR-NIMR New Delhi, has developed an advanced second-generation malaria vaccine. This novel subunit vaccine employs a chimeric recombinant protein expressed in *Lactococcus lactis*, combining full-length PfCSP to elicit protective immunity against *Plasmodium falciparum* infection with Pro6C a fusion of Pfs230 and Pfs48/45 subdomains to induce potent transmission-blocking antibodies. By targeting two critical developmental bottlenecks, it promises superior reduction of malaria burden compared to licensed vaccines RTS,S and R21, both of which consist of a small and near-identical PfCSP subdomain.

Preclinical studies demonstrate robust and durable protection in mice immunized with safer alum/alum-microparticle formulations. This vaccine has been found to generate >90% protection in preclinical models against a high challenge of 10,000 dual-transgenic P. berghei parasites expressing *P. falciparum* antigens with an impressive protective efficacy persisting over four months of challenge, as well as transmission-blocking antibodies in the Standard Membrane Feeding Assay (SMFA The formulation exhibits exceptional stability, remaining potent for more than nine months at room temperature, and is projected to cost approximately Rs. 50 per dose—dramatically more economical than existing alternatives. Plans include integration with a complementary *P. vivax* subunit vaccine, broadening its impact toward malaria elimination across India.

S-16

## Next-Generation Malaria Diagnostics: The FIKK Kinase Paradigm

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Accurate and early diagnosis of malaria is essential for effective disease management and elimination, particularly because infections with Plasmodium falciparum and Plasmodium vivax require species-specific treatment regimens. However, currently used rapid diagnostic tests (RDTs) and molecular assays primarily target conserved antigens such as 18S rRNA, Pf-LDH, Pf-hrp-2, and aldolase, which are limited in sensitivity and specificity due to homology with human counterparts. In this study, we explored the unique FIKK kinase family of P. falciparum as a novel diagnostic target. A chimeric FIKK (C-FIKK) antigen was designed by selecting highly antigenic, species-specific B- and T-cell epitopes from six FIKK kinases using IEDB tools, focusing on N-terminal regions with no homology to human proteins. Polyclonal antibodies generated against C-FIKK demonstrated high sensitivity, with a detection limit of 3 μM for FIKK9.1, and exhibited strong specificity without cross-reactivity to host blood components or non-Plasmodium bacterial, viral, fungal, or algal organisms. Validation using mock patient samples showed semi-quantitative parasite detection with an average accuracy of 95.45%. In parallel, exclusive regions of FIKK kinases were identified by in silico PCR and validated in vitro, yielding primers capable of detecting parasite DNA at levels as low as $10^{-5}$ ng and 0.0003% parasitaemia. These primers selectively identified P. falciparum even in the presence of P. vivax and other pathogens, outperforming existing diagnostic methods. Collectively, our findings establish FIKK kinases as robust and highly specific biomarkers, offering a promising platform for the development of next-generation malaria diagnostic systems with enhanced sensitivity and specificity.

P-01

# Exploring Reticulocyte-Specific Host Receptors Underlying *Plasmodium vivax* Merozoite Invasion

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## Introduction

*Plasmodium vivax*, the predominant cause of malaria in Asia, exhibits strict tropism for reticulocytes, likely mediated by reticulocyte-specific surface receptors. Merozoite invasion of host cells is initiated through precise ligand–receptor interactions between parasite proteins and erythrocyte membrane components. While the Duffy antigen receptor for chemokines (DARC), transferrin receptor 1 (CD71), and CD98 have been identified as *P. vivax* receptors(2), reports of DARC- and CD71-independent invasion pathways indicate the involvement of additional, yet unidentified, host receptors. A comprehensive understanding of these invasion mechanisms is essential for elucidating *vivax*-specific tropism and for the development of therapeutic strategies to block parasite entry.

## Objective

To identify novel *Plasmodium vivax* invasion receptors among reticulocyte-enriched membrane proteins.

## Methods

Reticulocyte-enriched membrane proteins were profiled using mass spectrometry to identify candidate receptors(1). A recombinant full-length His-tagged candidate protein was expressed in a bacterial system, and a synthetic peptide corresponding to its extracellular domain was generated for functional assays and antibody production. Functional relevance was assessed using *Plasmodium falciparum* (Pf3D7) merozoite invasion inhibition assays(2), in which recombinant protein, extracellular peptides, and antibodies (against known *Plasmodium* receptors) were tested across multiple concentrations.

## Results and Conclusion

Proteomic analysis identified some interesting reticulocyte-enriched proteins with features resembling known invasion receptors. However, neither the candidate recombinant protein nor its extracellular peptide significantly inhibited *P. falciparum* erythrocyte invasion, suggesting a limited role for this protein in *P. falciparum* invasion. Given its reticulocyte-specific expression, the protein warrants further investigation in *P. vivax* reticulocyte invasion models to evaluate potential species-specific roles. These findings highlight the complexity of *P. vivax* invasion and the need for broader receptor screening to fully define the molecular basis of *vivax* malaria tropism.

P-02

# Recombinant Production of Structurally Complex Malaria Antigens in *Lactococcus lactis* for Diagnostic and Vaccine Applications

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Malaria, caused by *Plasmodium* species and transmitted by infected mosquitoes, remains a major global health challenge, particularly in endemic regions. Among human parasites, *P. falciparum* and *P. vivax* are the most prevalent species. This study aims to generate a panel of recombinant antigens from both these species to (i) evaluate combinations for deriving a differential diagnostic kit with high specificity and sensitivity, and (ii) use as exposure markers in serological analyses for vaccine trials.

Production of recombinant *Plasmodium* antigens, particularly disulfide-rich proteins, is difficult due to issues of misfolding, low yields, and improper post-translational modifications in common expression hosts such as *E. coli* or eukaryotic systems. *Lactococcus lactis*, a GRAS (Generally Recognized as Safe) Gram-positive bacterium, offers an efficient platform for expressing structurally complex malaria antigens. With optimized secretion signals and tightly regulated expression systems, *L. lactis* enables consistent, high-quality expression of diverse recombinant antigens which exhibit varying degrees of predicted structural complexity and are typically considered "difficult-to-produce".

A panel of *P. falciparum* and *P. vivax* antigens (ranging in size from 9 to 150 kDa), including PfsCSP, Pfs230, Pfs48/45, PfMSP1, PfMSP2, PfMSP6, PfMSP3.3, PfEBA140RIII-V, PfRh2b, PfRAMA, PfRON2, PvMSP9, PvMSP3α, PvAMA1, PvRAMA, PvEBPII, PvRBPIIb, PvDBPII, Pv-fam-a, and Pvs25, were successfully expressed in *L. lactis* as secreted, soluble and stable with properly folded secondary structures. These recombinant antigens were purified and characterized using SDS-PAGE and immunoblot analyses. Antigenicity of these purified recombinant proteins was confirmed by ELISA using sera from individuals from endemic regions in Odisha with dual transmission of both *P. falciparum* and *P. vivax*, demonstrating their diagnostic potential.

Overall, *L. lactis* represents a promising, scalable, and cost-effective platform for recombinant malaria antigen production. Future work will focus on scale-up and combination testing to develop highly sensitive and specific diagnostic kits, along with applications in serological analysis for malaria vaccine trials.

**O-03**

## Functional Phenotyping of MMV Pandemic Response Box Identifies Stage and Mechanism-Specific Inhibitors Against Blood Stage *Plasmodium falciparum.*

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**Introduction:**

The rapid emergence of resistance to frontline antimalarials poses a significant threat to global efforts to control malaria. Phenotype-driven discovery provides a powerful route in identifying compounds with novel, stage-restricted mechanisms that are often overlooked by target-based screens.

**Objectives:**

To identify potent, stage-specific inhibitors of *Plasmodium falciparum* blood-stage development and delineate their mechanistic signatures using systematic functional phenotyping.

**Methods:**

A comprehensive phenotypic screen of the MMV Pandemic Response Box was conducted against drug-sensitive (3D7) and chloroquine-resistant (Dd2) *P. falciparum*. Active compounds were prioritized. based on sub-micromolar potency and profiled across parasite developmental stages using microscopy, flow cytometry, recrudescence assays, drug interaction studies, reactive oxygen species analysis, and confocal imaging of infected red blood cells. To elucidate their mechanisms of action, we employed an integrated strategy that combined the selection of resistant mutants, molecular docking, untargeted metabolomics, and various cell-based assays.

**Results:**

Sixty compounds were initially identified with activity at 10 µM, of which 28 compounds exhibited potency below 3 µM. Distinct stage-specific inhibitors were uncovered targeting ring (MMV001014), trophozoite (MMV1593540, MMV1634402), and schizont stages (MMV1580844, MMV1580496, MMV1580173, MMV1580483). The ring-stage inhibitor MMV001014 exhibited irreversible activity, resulting in complete suppression of recrudescence and antagonism with artemisinin, suggesting mechanistic convergence. Trophozoite inhibitors demonstrated nanomolar efficacy with differential interactions with artemisinin and chloroquine. Selected schizont inhibitors induced oxidative stress and caused compound-specific disruption of infected red blood cell membrane integrity. Resistant selection studies, genome sequencing and metabolome analysis revealed possible targets of the compounds.

**Conclusion:**

This study reveals multiple chemically diverse, stage-restricted antimalarial candidates with distinct mechanistic fingerprints, underscoring the value of functional phenotyping in accelerating the discovery of first-in-class antimalarials and rational drug repurposing. The study also elucidates stage-specific parasite vulnerabilities, provides mechanistic insights relevant to resistance monitoring, and their strategic combination with existing drugs.

P-04

# Shifting of malaria parasite paradigm: Reinventing pathway towards elimination drive in India

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## Introduction

Malaria elimination has substantially progressed over the last two decades in India, yet persistent transmission undergoes to consider current challenges for elimination drive also need to review the paradigm of parasite in the country. The shifting of paradigm reflects in parasite biology and behaviors influence in the treatment outcome as well as disease transmission.

## Methods

A retrospective, study design was adopted to evaluate the incidence of malaria parasite during and post COVID-19 in highly endemic states in India. Temporal trends in malaria incidence were analysed using descriptive statistics and time-series analysis to assess variations across the two phases.

## Results

Analysis of incidence data from the highly endemic states revealed a distinct variation in cases incidence, treatment outcome and drug resistance pattern during and post COVID-19. There is shifting of parasite paradigm has been observed from Maharashtra, Utter Pradesh, Mizoram from 10% to 13% respective to *P. vivax* & *P. falciparum*. The highly endemic states Odisha, Jharkhand and Chhattisgarh reflect this shifting from 17 %, 10% and 11% as well as persistence of artemisinin combination therapy partial resistance markers.

## Conclusion

These results indicate that the change in parasite dynamics impacted on the efforts in elimination drive also underscore the evolving complexity of malaria control in India. The coexistence of changing species dominance and emerging drug resistance highlights the need for region-specific strategies, strengthened the research activity as well as other important intervention.

P-05

## Smear Grade and Hospital Bill: Parasite Density and Cost of Illness in *Vivax* Malaria at a Tertiary Care Hospital in Karnataka

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**Introduction**
*Plasmodium vivax* malaria is a common cause of admission in coastal India, but the relationship between parasite density and hospital costs is unclear.

**Objectives**
To compare the cost of illness between low and high parasite-density groups in *P. vivax* malaria.

**Methods**
The retrospective study included 48 adults admitted to a tertiary care hospital in coastal Karnataka with QBC-smear confirmed *P. vivax* malaria. Parasite density on quantitative buffy coat smear was graded as 1+, 2+, 3+ or 4+ and grouped as low density (1+/2+) or high density (3+/4+). Direct inpatient costs in Indian Rupees (INR) were obtained from hospital billing and divided into total hospital costs, the amount paid by the insurer, and out-of-pocket expenditures; length of stay was calculated from admission and discharge dates. Continuous variables were summarised as median (interquartile range, IQR) and compared using the Mann–Whitney U test; categorical variables were compared using the chi-square test.

**Results**
The low- and high-parasite-density groups each included 24 patients. Median age was 30 years (IQR 22–39), and 41/48 (85.4%) were male, with no significant differences in age or sex between groups (p=0.54 and p=1.00, respectively). Median length of stay was similar (low 4.1 days [3.5–4.8] vs high 4.3 days [3.1–4.8]; p=0.84). Median total hospital cost was significantly higher in the high-density group (INR 18,012 [IQR 14,019–26,594]) than in the low-density group (INR 9,538 [6,563–18,274]; p=0.007). High-density episodes were more often associated with any insurer payment (79.2% vs 25.0%; p<0.001) and had higher median insurer contributions (INR 16,612 vs 0; p=0.001). In contrast, median out-of-pocket expenditure was higher in low-density infections (INR 6,932 [4,835–10,085]) than in high-density infections (INR 2,000 [0–6,640]; p=0.014), with patients in the low-density group paying a median 100% of their hospital bill out-of-pocket versus 11% in the high-density group (p=0.001).

**Conclusions**
Higher parasite density in *P. vivax* malaria was associated with greater inpatient costs, largely absorbed by insurers. At the same time, patients with lower-density infections bore a higher out-of-pocket burden.

**Acknowledgements/Funding:** None

**Keywords:** *Plasmodium vivax*; parasite density; cost of illness; health economics; India

P-06

## Probing the Functional Properties of Human Malarial Parasite Porin

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**Introduction**

Drug resistance is one of the factors that causes a burden in decreasing the malaria cases, there is a need to unravel new potential drug targets in the malaria parasite. Survival of the parasite in humans is dependent on the proteins and metabolites that it acquires from the host, and also shuttling of metabolites across various organelles. The transportome of malaria parasites is comparatively less. So it is important to characterize such proteins, which may have a major role in drug efflux and so on. Since some reports are suggesting that many of the proteins that may help in the transport of nutrients and metabolites are devoid of human orthologs, they can be exploited as a novel drug target.

**Objective**

Our study aims to functionally and biophysically characterize one of the putative porins present in the human malaria parasite.

**Methods**

Recombinant Pfporin was expressed, purified, and tested for channel activity using high-resolution single-channel electrical recordings in artificial lipid bilayers.

**Results**

Purified recombinant porin shows channel activity in high-resolution single-channel electrical recordings. Further studies will be conducted to elucidate the functional relevance of the porin in the malarial parasite.

**Conclusions**

The putative porin forms functional channels in artificial bilayers, suggesting a potential role in metabolite or drug transport. It shows high sequence divergence from human homologs, making it a parasite-specific target necessitating further investigation.

**Funding:** ICMR

**Keywords:** porin, drug target, mitochondria, transportome, single-channel electrical recordings

P-07

## Modalysis: DNA Methylation Analysis from Long Read Oxford-Nanopore Sequencing Data

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**Introduction**

DNA methylation is central to regulating gene expression in malaria parasites [1]. However, extracting biologically interpretable signals from long-read sequencing data remains challenging because existing workflows are fragmented and poorly reproducible.

**Objective**

This study aims to develop an automated tool for methylation analysis from Oxford Nanopore sequencing data and to characterize epigenetic patterns in *Plasmodium vivax* clinical isolates.

**Methods**

We developed Modalysis, an end-to-end analytical workflow designed for gene and putative regulatory region–resolved DNA methylation profiling using Oxford Nanopore sequencing data. *Plasmodium vivax* infected clinical isolates representing hepatic dysfunction, cerebral malaria, and uncomplicated malaria were sequenced on the MinION Mk1B platform. Raw reads were modified basecalled using the MinKNOW-integrated Guppy basecaller, generating BAM files. Modalysis integrates SAMtools for alignment processing, Modkit for bedMethyl generation and differential methylation region (DMR) analysis, and GFF-based genome annotations to systematically quantify methylation patterns across putative regulatory regions and gene bodies. The pipeline enables comprehensive summarization and visualization of 5-methylcytosine (5mC) and combined 5mC/5-hydroxymethylcytosine (5mC_5hmC) signals, facilitating regulatory context-aware interpretation of epigenetic variation across clinical disease states.

**Results**

This analysis generated a comprehensive DNA methylation signature encompassing 5,190 *Plasmodium vivax* genes, uncovering severity-associated and modification-specific methylation signatures that distinguish uncomplicated from severe malaria patient cohorts. Chromosome-level and putative regulatory region visualizations delineated distinct epigenomic landscapes across clinical severities, highlighting methylation hotspots in promoter and enhancer regions. High-confidence differentially methylated regions (DMRs) demonstrated pronounced methylation divergence between severe and uncomplicated cases. These multi-omics signatures underscore dynamic gene expression modulation underpinning clinical severity outcomes in *P. vivax* infections.

**Conclusions**

Although developed in the context of *P. vivax* epigenomic analysis, the framework is generalizable to any long-read sequencing dataset (ONT) requiring reproducible processing. By unifying analytical steps into a single pipeline and producing interpretable outputs, this work lowers the barrier to long-read methylation analysis.

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**Keywords:** Malaria, *Plasmodium vivax*, Oxford Nanopore Sequencing, DNA Methylation, Bioinformatics Pipeline.

O-08

## Cohort Profile of a Multicentric Longitudinal Study on Malaria Burden, Vector Bionomics, and Health System Assessment across Diverse Eco-Epidemiological Settings in India

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**Introduction**

India continues to face heterogeneous malaria transmission driven by ecological diversity, coexistence of *Plasmodium falciparum* and *P. vivax*, and persistent social and economic inequities. This cohort profile describes a multi-centric longitudinal study led by ICMR-Vector Control Research Centre, designed to characterize malaria burden across urban, peri-urban, rural, forest-foothill, and coastal settings in India.

**Objectives**

The study aims to estimate malaria prevalence and longitudinally track incidence across diverse eco-epidemiological zones, and to identify epidemiological and socio-behavioural drivers of transmission. Additional objectives include assessing malaria-related health system performance and equity gaps, vector bionomics, insecticide resistance, and spatio-temporal factors to inform context-specific interventions.

**Methods**

The study is being conducted in 12 districts across 10 Indian states: South Andaman (A&N Islands), Tinsukia (Assam), Nawada (Bihar), Bengaluru Urban (Karnataka), Tiruchirappalli (Tamil Nadu), Sonbhadra (Uttar Pradesh), Gariyaband and Bastar (Chhattisgarh), Boudh and Koraput (Odisha), Pakur (Jharkhand), and Lunglei (Mizoram). Two clusters of ~1,000–1,500 individuals were selected per district. Baseline surveys enumerated households, recruited participants, and measured prevalence through mass testing by bivalent RDTs and dried blood spots for PCR detection of low-density infections. Consented participants undergo 12-month follow-up for incident infections, treatment adherence, relapses, socio-behavioural, health system, and monthly entomological assessments.

**Results**

The baseline survey enumerated 29,789 individuals, yielding 25,509 consenting participants (89.0% of eligible). The cohort has a median age 27 years (range:0.2-108), 58.4% female, 38.1% illiterate, and 71.0% belonged to upper-lower/lower socioeconomic classes. Reported comorbidities were infrequent (diabetes 3.1%, hypertension 3.3%), 34.0% were underweight and 20.1% were obese. Past history of malaria was reported by 8.4%. Malaria symptoms were present in 733 (2.9%) of the participants, of whom 263 (35.9%) sought care; 60 (22.8%) were diagnosed by the health system (*P. falciparum*-8.3%, *P. vivax*-3.3%, mixed infections-5.0%).

**Conclusions**

This ongoing study will profile the malaria burden including incidence trends, relapse/reinfection patterns, socio-behavioural patterns, vector bionomics and resistance, and health system preparedness to guide policies for India's malaria elimination.

P-09

# Harmony in the Heat: Unraveling the Symphony of Heat Shock Protein 70 in Indian Malarial vector- A Molecular Ballet Shaping Mosquito Development and Influencing *Plasmodium* Transmission

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**Introduction**

Heat Shock Proteins (HSPs) are critical molecular chaperones, categorized into various classes based on their molecular mass, which assist in protein folding and support organisms in adapting to extreme environmental conditions. Despite their importance, the role of HSPs in the stress response of mosquitoes remains insufficiently understood. This study investigates Heat Shock Protein 70 (HSP-70) in two primary Indian malaria vectors, Anopheles culicifacies and Anopheles stephensi, aiming to elucidate its role in stress response mechanisms and its potential application in vector control.

**Materials and methods**

The investigation involved a comprehensive bioinformatics analysis of HSP-70 in An. culicifacies and An. stephensi using tools such as BLASTP, tBLASTn, and Pfam for sequence analysis, and phylogenetic and syntenic analyses to explore evolutionary relationships. Experimental validation included qPCR to examine HSP70 expression across developmental stages and under various stress conditions, alongside recombinant protein expression and characterization. Functional studies involved RNAi-mediated silencing of HSP70-1 in An. culicifacies larvae, coupled with selective inhibition of HSP70 to evaluate effects on developmental cycle of mosquito and *Plasmodium* development inside mosquito.

**Results**

Seven distinct subcellular localizations of HSP70 isoforms were identified. Notably, HSP70-1 exhibited increased expression at the L3 larval stage, underscoring its role in larval development. Silencing HSP70-1 led to significant upregulation of other HSP70 isoforms indicating compensation of stress. In *P. berghei*-infected mosquitoes, HSP70 inhibition markedly impaired oocyst development, suggesting its critical role in parasite survival within the mosquito host.

**Conclusion**

This study provides novel insights into the function of HSP70 in malaria vectors, particularly how its inhibition impacts mosquito development and malaria parasite progression. The findings suggest that targeting HSP70 could serve as a potential vector control strategy. By examining the interactions between HSP70 and malaria parasites, this research identifies exploitable vulnerabilities that may aid in reducing malaria transmission.

**Funding**

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O-10

# Hsp70 Interaction with D7 Class of Salivary Gland Proteins: A Facilitator of Mosquito Blood Feeding?

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**Introduction**

The salivary glands of hematophagous Nematocera express abundant D7 family proteins that facilitate blood feeding by counteracting host inflammatory, hemostatic, and immune responses. Although several short and long D7 proteins from *Anopheles* species have been structurally characterized, their recombinant expression frequently results in inclusion body formation, indicating a possible requirement for molecular chaperones for maintaining protein stability. Given the importance of D7 protein integrity for successful blood feeding, we investigated the interaction of the molecular chaperone HSP70 with D7 proteins in the Indian malaria vector *Anopheles culicifacies*.

**Objective**

- Determine the expression of D7L3 and D7R5 in *An. culicifacies*
- Examine the interaction between D7 proteins and HSP70
- Assess colocalization of D7 proteins and HSP70 in the salivary gland

**Methodology**

- Recombinant expression and purification of D7L3, D7R5, and HSP70
- Generation of polyclonal antibodies against D7 proteins in mice and HSP70 in rabbit
- Immunoblot analysis of *An. culicifacies* head lysate
- Protein–protein interaction studies using dot blot, ELISA, and microscale thermophoresis (MST)
- Immunofluorescence-based colocalization analysis

**Results**

- ✓ Expression of D7L3 and D7R5 was confirmed in *An. culicifacies*
- ✓ Both D7 proteins showed specific interaction with HSP70 in dot blot and ELISA assays
- ✓ MST revealed moderate binding affinity between D7 proteins and HSP70
- ✓ D7L3, D7R5, and HSP70 colocalized in the salivary glands

**Conclusion**

We hypothesize that HSP70, alone or in association with other molecular chaperones, contributes to the stability and functional maintenance of D7 proteins in mosquito salivary glands, thereby facilitating efficient blood feeding. Furthermore, considering the high sequence identity and structural conservation of cytosolic HSP70 between *An. culicifacies* and *Homo sapiens*, we propose a potential host–vector molecular cross-talk in which human HSP70 at the bite site may transiently stabilize mosquito D7 proteins, enhancing their anti-inflammatory activity.

**Keywords:** D7L3, D7R5, HSP70, *Anopheles culicifacies*, binding affinity, colocalization

P-11

## Profiling of *Plasmodium vivax* Malaria Among Children

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### Introduction

*Plasmodium vivax* accounts for approximately a more than one third of all Malaria cases in India. Around 30% of all *P. vivax* cases in India occur in children.

### Objectives

To determine incidence of *Plasmodium vivax* malaria cases in children aged 0-18 years of age. To determine association of laboratory and clinico-demographic profile with the severity of infection.

### Methodology

A Prospective observational study was conducted in Tertiary care pediatric centre after Institutional ethical approval. Blood sample were collected in EDTA vial and subjected for Rapid malaria antigen test and peripheral blood smear examination and polymerase chain reaction (PCR) for detection of malarial parasite. Clinical history taken from patient clinical records.

### Result

A Total of 895 blood samples were collected during study period (July 2025 to august 2025) from clinically suspected malaria cases. 27 samples were positive for *Plasmodium vivax* (27/895, 3.01%). Out of 27 positive cases 7 (7/27,25.9%) had severe infection. Median value of parasitemia load was higher (0.41%) in severe cases compared to non-severe ones (0.20%). Fever was present in all positive cases of *Plasmodium vivax*. 51% cases fever was associated with chills and rigor. Out of all 59.2% cases had hepatomegaly, 51.8 % had spleenomegaly, pallor in 29.6% cases, jaundice in 25.9% cases, bleeding manifestations in 11.1% and 6 cases had co-infection with dengue. PCR done for 20 cases out of total 27 positive cases. All cases were positive for *Plasmodium vivax* while 3 had mixed infection (*Plasmodium vivax* and *Plasmodium falciparum*). Out of these 3 mixed infection cases 2 cases had severe malaria and 1 case had coinfection with dengue.

### Conclusion

*Plasmodium vivax* once considered 'benign' now recognized as potentially severe malaria in cases with high parasitemia level and other co- morbid conditions.

P-12

## Reticulocytes: A Favoured Home for *Plasmodium falciparum*

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### Introduction

Malaria remains a global health challenge, resulting in half million deaths annually. *Plasmodium falciparum*, the most virulent human malaria parasite, can invade all erythroid sub-populations, a feature that contribute to pathophysiological manifestations. Parasite growth kinetics are modulated not only by the intrinsic parasitic factors but also by the characteristics of their host erythrocytes. Erythroblasts and reticulocytes, enriched within erythropoietic niches such the spleen and bone marrow, where the parasite growth kinetics were found distinct from that observed in the peripheral blood. In this study, we investigated how reticulocytes shape the *P. falciparum* development and survival using in vitro culture model.

### Objectives

To investigate the impact of reticulocyte infection on *P. falciparum* growth kinetics and antimalarial drug tolerance.

### Methods

Reticulocytes (CD71+ve) and normocytes (CD71-ve) were separated via immunomagnetic separation using anti-CD71 antibodies. Invasion, growth, and drug sensitivities were compared through standard in vitro assays. Untargeted Liquid Chromatography (LC)-Mass Spectrometry (MS) performed for proteome profiling. Statistical significance was determined using Student's T-test.

### Results

Reticulocytes are the preferred host erythrocytes, exhibiting higher abundance of metabolic intermediates and enzymes essential for *P. falciparum* development. Parasites cultured in reticulocytes displayed accelerated asexual cycles than those in normocytes. Notably, intra-reticulocytic parasites exhibited enhanced tolerance to artemisinin and reactive oxygen species (ROS), mediated by utilization of host-derived metabolic intermediates and proteins involved in redox homeostasis. Reticulocytes maintain elevated basal levels of antioxidants and ROS-modulating enzymes compared to normocytes, thereby providing parasites with superior protection against oxidative stress and artemisinin.

### Conclusions

Our findings identify reticulocytes as a preferred host cell for *Plasmodium* parasites, offering both metabolic support and protection from drug-induced oxidative stress. Future studies are needed to explore how preferential growth in reticulocytes influences parasite adaptation, drug resistance, and disease progression.

**Keywords:** Reticulocytes, *P. falciparum*, Artemisinin, Reactive Oxygen Species.

**Funding support:** Department of Biotechnology (DBT), India.

**P-13**

## Biological Screening and Molecular Docking Analysis of Zn(II) Compounds as Antiplasmodial Agents

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Malaria remains a critical global health concern, with sub-Saharan Africa bearing the highest disease burden. Despite significant advances in diagnostics, treatment, and prevention, the rapid emergence of drug-resistant *Plasmodium falciparum* strains continues to undermine existing control strategies, highlighting the urgent need for novel antimalarial agents. In this study, a rational drug discovery approach integrating chemical synthesis, computational analysis, and biological evaluation was employed. To overcome resistance associated with classical antimalarial drugs, two novel Zn(II) compounds were synthesized and comprehensively characterized using physicochemical and spectroscopic techniques. Density Functional Theory (DFT) calculations revealed substantial kinetic stability, with HOMO–LUMO energy gaps of 3.90 eV for compound-1 and 3.98 eV for compound-2. Antiplasmodial activity was evaluated using a high-throughput MTT assay against the chloroquine-sensitive *P. falciparum* 3D7 strain. Both compounds exhibited moderate antiplasmodial activity, with $IC_{50}$ values of 5.64 ± 1.07 µg/mL (compound-1) and 6.56 ± 0.29 µg/mL (compound-2), accompanied by low hemolytic activity (<3%). Molecular docking studies using AutoDock Vina demonstrated stable binding of the synthesised compounds within the active cavity of the target plasmepsin protein. These findings suggest that the Zn(II) compounds possess promising antiplasmodial potential. Future studies involving structural optimization, rational design, and synthesis of Zn-based compounds may facilitate the development of potent lead candidates for antimalarial drug discovery.

**Key words:** Antimalarial activity, Molecular Docking, Zn(II) compounds, HOMO–LUMO

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**P-14**

## Persistence of malaria in malaria-endemic regions with a focus on asymptomatic and submicroscopic malaria among construction workers and natives: A multicentric cross-sectional study

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**Introduction**

Malaria remains a major public health problem in the Southeast Asia region (1)**.** Asymptomatic malaria is widely considered a significant contributor to sustained transmission in India (2). However, the evidence remains limited as it is often undetected by routine diagnostics such as Microscopy and RDTs and is rarely treated. As India moves toward malaria elimination by 2030, understanding the role of asymptomatic infections is crucial (2). This study was undertaken to generate evidence on their burden in an elimination setting.

**Objectives:**

**1.** To estimate the prevalence of asymptomatic and sub-microscopic malaria among construction workers and natives in two coastal districts of Karnataka. **2.** To determine the receptivity to malaria transmission in Mangalore and Udupi districts

**Methods**

A community-based surveillance was conducted in Udupi and the Mangalore district from 2024 to 2025. Healthy individuals aged 12 years and above were recruited with written consent. Finger-prick blood samples, along with socio-demographic details and exposure history, were collected. Blood samples were screened using microscopy, rapid diagnostic test, and nested PCR (targeting 18S rRNA gene). Entomological surveillance was concurrently performed to assess receptivity.

**Results**

A total of 2207 participants were recruited, of whom 55.1% were construction workers and 44.9% were residents. Of the enrolled participants, 32 (1.4%) were in the 12-17 years age group, while 2175 (98.6%) were 18 years and older. The gender distribution revealed that 72% of the participants were male, and 28% were female. A large proportion of construction workers were migrants (80.8%) from malaria-endemic states outside Karnataka. No malaria infections were detected via microscopy, RDT, and nested PCR, except for a single RDT-positive case in a resident who tested negative on PCR and microscopy. An adult mosquito survey yielded 394 *Anopheles* mosquitoes, comprising 11 species, all of which tested negative for *Plasmodium* DNA. *Anopheles stephensi*, a known malaria vector species, was found in low density (2%) in this study.

**Conclusions**

No detectable asymptomatic or submicroscopic malaria burden was found among the surveyed populations in the study sites, despite significant in-migration of labourers from Malaria-endemic regions. Moreover, mosquito specimens were tested negative for *Plasmodium* DNA. This could be due to the successful administration of the malaria control program, particularly active case detection, in these districts in recent years.

**Keywords**: Malaria, Asymptomatic, nPCR, Migrants, Karnataka.

**Funding:** This study was supported by ICMR Ad Hoc Grant

O-15

## Carbon Quantum Dot–Enhanced Glycyrrhizin as a Dual Inhibitor of β-Hematin Formation and Protease Targets: A Repurposing Strategy Towards Antiplasmodial Activity

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### Introduction

Hemozoin formation is an essential detoxification pathway in *Plasmodium falciparum*, allowing the parasite to neutralize toxic free heme released during hemoglobin degradation (1). Inhibiting this pathway remains a validated strategy for antimalarial drug development. Glycyrrhizin, an anti-inflammatory triterpenoid, has shown potential biological activity but limited bioavailability (2). This study investigates both free glycyrrhizin and its nano-formulated form to enhance antiplasmodial efficiency and understand the kinetics of hemozoin inhibition (3).

### Objectives

- To Evaluate β-hematin inhibition and kinetics of glycyrrhizin.
- Synthesize glycyrrhizin-doped yeast-derived carbon quantum dots
- Assess CQD-enhanced interactions via molecular docking.

### Methods

β-Hematin inhibition was assessed using a cell-free hemozoin formation assay by measuring free heme absorbance at 405 nm, with time-dependent kinetic studies evaluating inhibition rates. Y-CQDs were synthesized from Saccharomyces cerevisiae via microwave-assisted carbonization and doped with glycyrrhizin. UV–Vis, photoluminescence, and FT-IR confirmed conjugation. Blind molecular docking of glycyrrhizin, coronene, and their conjugate was performed against 16 *Plasmodium falciparum* targets.

### Results

Microwave-assisted synthesis of Y-CQDs from S. cerevisiae produced blue-fluorescent nanoparticles with a UV–Vis peak at 270 nm. Glycyrrhizin showed strong β-hematin inhibition (61.03%, $IC_{50}$ = 11.384 nM) and was subsequently doped onto Y-CQDs. Photoluminescence shifts and FT-IR features—including O–H/N–H (3336 $cm^{-1}$) and C=O (1748 $cm^{-1}$)—confirmed successful Y-CQD-Gly formation. The doped nanoconjugate displayed enhanced β-hematin inhibition (68.45%) with an $IC_{50}$ of 9.52 nM and demonstrated slower β-hematin formation rates, indicating improved inhibitory kinetics. Molecular docking against 16 *P. falciparum* targets showed the glycyrrhizin–coronene conjugate (Y-CQD mimic) achieved the strongest interactions, with a best score of −11.4 kcal/mol and up to 10 hydrogen bonds, exceeding free glycyrrhizin.

### Conclusion

Glycyrrhizin shows strong β-hematin inhibition, favourable inhibition kinetics, and multi-target binding. Carbon quantum dot doping significantly enhances hemozoin inhibition and molecular interactions, positioning the Y-CQD-Gly nanoconjugate as a promising dual-action, nano-enabled platform for next-generation antiplasmodial drug development.

P-16

## Mapping The Resistance Profiles of Priority Pathogens for Small Molecule Interventions to Circumvent AMR

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### Introduction

Antimicrobial resistance (AMR) poses a major global health threat as microorganisms evolve resistance to existing medications. Among protozoan parasites, *Plasmodium* spP., the causative agents of malaria, remain a significant concern. According to the World Health Organization, India accounts for 73.3% of malaria cases in the South-East Asia region. The widespread resistance to frontline antimalarials, including chloroquine, sulfadoxine–pyrimethamine, and even artemisinin, underscores the urgent need for new therapeutic strategies.

### Objective

Understanding the genetic and phenotypic basis of drug resistance in *Plasmodium* sp*P.* is critical to combating malaria. Repurposing existing molecules can significantly reduce drug development timelines by leveraging known pharmacokinetics and mechanisms. This project aims to identify and repurpose clinically relevant compounds active against *Plasmodium falciparum* to combat AMR

### Methods

The development of small-molecule inhibitors begins with phenotype-based screening followed by target identification. We started with the *in vitro* screening of a chemical library, COVID box, launched by Medicines for Malaria Venture (MMV), and a set of antibiotics shortlisted from the literature. The chemical library consists of 160 compounds with known or predicted activity against COVID-19. *Plasmodium falciparum* 3D7 parasites were incubated with these compounds for 48 hours at a concentration of 10 µM. After the incubation, parasitemia was estimated using Giemsa-stained smears to assess the activity of the tested compounds.

### Results and Conclusion

Our screening identified 38 novel compounds with potential activity against the *P. falciparum* 3D7 strain. Future work will focus on elucidating their modes of action. By correlating laboratory outcomes with real-world clinical evidence and utilizing advanced methodologies for drug design and target validation, the research aims to accelerate the discovery of effective small-molecule therapeutics that are adaptable to resistance.

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- Council of Science and Industrial Research (CSIR)

P-17

## Exported Protein-1 (EXP1) of *Plasmodium falciparum* as a New Diagnostic Target for Malaria

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### Introduction

Malaria remains a major public health burden in tropical and subtropical regions, and accurate diagnosis is central to effective control and elimination strategies. Rapid diagnostic tests (RDTs), which primarily detect histidine-rich protein 2 (HRP2), are widely used due to their cost-effectiveness and ease of use. However, their performance is compromised in low-transmission settings and by the emergence of *Plasmodium falciparum* parasites harboring deletions in pfhrp2/3 genes or sequence polymorphisms. These limitations underscore the urgent need to identify alternative diagnostic targets. In our previous protein microarray study, cyclic constrained peptides derived from Exported Protein 1 (EXP-1) and Glutamate-Rich Protein (GLURP) showed strong immunoreactivity with sera from *P. falciparum*–infected individuals.

### Objective

This study aimed to characterize the diagnostic potential of *P. falciparum* Exported Protein 1 (PfEXP-1) for detecting malaria infection in patient samples.

### Methods

PfEXP-1 was heterologously expressed in Escherichia coli BL21 (DE3) Rosetta cells and purified using Ni–NTA affinity chromatography followed by dialysis. Protein identity and expression were confirmed by Western blotting using anti-His and anti-EXP1 antibodies. Size-exclusion chromatography revealed the native oligomeric state, while circular dichroism spectroscopy was employed to assess secondary structure and thermal stability. Polyclonal antibodies against recombinant PfEXP-1 were raised in rabbit. Their sensitivity and specificity were evaluated using in vitro–cultured *P. falciparum* lysates and infected patient blood samples via Western blotting and indirect ELISA.

### Results

PfEXP-1 was successfully expressed and purified to homogeneity. Biophysical analysis demonstrated that PfEXP-1 exists as a heptamer (~130 kDa) under native conditions and as a ~23 kDa monomer under denaturing conditions. Circular dichroism spectra confirmed a predominantly α-helical structure. Anti-PfEXP1 polyclonal antibodies specifically detected both recombinant and native PfEXP-1. The limit of detection in patient samples was approximately 250 parasites/µL, comparable to that of HRP2-based detection.

### Conclusion

Anti-PfEXP1 polyclonal antibodies demonstrated sensitive and specific detection of *P. falciparum* in culture lysates and patient samples, highlighting PfEXP-1 as a promising alternative diagnostic target for malaria, particularly in settings affected by HRP2/3 deletions.

**Acknowledgment:** We thank ICMR- NIMR for providing lab facilities and CSIR for SRF fellowship

**Keywords:** *Plasmodium falciparum*, EXP-1, diagnostic marker, malaria, HRP2 deletion, ELISA

**P-18**

## Exploring Novel Insecticide Resistance Mechanisms in *Anopheles stephensi*: The Role of CSDIR Protein

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The emergence of insecticide resistance in malaria vectors, including *Anopheles stephensi*, poses a significant challenge to global malaria control efforts. Our study identifies and characterizes a novel Cub and Sushi Domain-Containing Insecticide Resistance (CSDIR) protein in An. stephensi, revealing its pivotal role in mediating resistance to organophosphates and pyrethroids. Using differential proteomics profiling, the CSDIR protein was found to be overexpressed in resistant mosquitoes. A strong binding affinity of the CSDIR protein towards different classes of insecticide molecules-malathion (KD 6.43 μM) and deltamethrin (KD 46.7 μM) were demonstrated using MD simulation studies and Surface Plasmon Resonance (SPR) experiments. Functional assays revealed that the recombinant CSDIR913-1190 protein exhibited potent esterase-like activity critical for metabolizing these compounds. Structural analysis identified a catalytic triad (S956, D964, and H1011) within the CSDIR protein, essential for its esterase like activity. Mutational studies confirmed these residues are indispensable for CSDIR's enzymatic activity. Furthermore, Analytical HPLC confirmed the metabolic degradation of deltamethrin by CSDIR protein. Gene silencing of CSDIR via RNA interference significantly increased mortality (>60%) in resistant mosquitoes exposed to deltamethrin and malathion, corroborating its role in detoxification of these compounds. Overall, these findings provide novel insights into the mechanisms of metabolic resistance, positioning CSDIR as a promising target for developing next-generation insecticides or resistance-management strategies. Future exploration of CSDIR inhibitors could complement current vector control tools and mitigate resistance development.

**Keywords:**

*Malaria, Anopheles stephensi, Insecticide resistance, Esterase-like protein, Silencing*

**P-19**

## *Lactococcus lactis* as an efficient platform for expression of recombinant *P. vivax* antigens

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*Plasmodium vivax* is the major causative agent of malaria in several parts of the world. Unique biological features such as reticulocyte preference, early gametocyte formation, low parasitaemia and the presence of dormant liver-stage hypnozoites, make *P. vivax* challenging to study and develop accurate diagnostics and vaccines. This study explored production of twelve recombinant antigens of *P. vivax* (PvCSP, PvCelTos, PvMSP3α, PvMSP9, PvMSP1-19, PvAMA1, PvRAMA, PvEBPII, PvRBPIIb, PvDBPII, Pv-fam-a and Pvs25) covering all three stages of parasite development in *Lactococcus lactis*. As a GRAS, lacking endotoxin and unwanted glycosylation, *L. lactis* is an efficient platform for recombinant production of structurally complex, disulfide-rich proteins where correct folding and secretion are crucial. Further, *L. lactis* ensures post-translational modifications similar to native malaria proteins and simplifies purification. The Sec-dependent secretion pathway of *L. lactis* efficiently exports proteins into the medium with low host contaminants, while an oxidizing extracellular environment supports disulfide bond formation. Our previous studies have shown successful expression of challenging *P. falciparum* antigens in *L. lactis* with high yields of previously intractable targets using fusion partners. In this study, recombinant *P. vivax* antigens were optimized for expression and purification in *L. lactis*. The expression and purity of *P. vivax* recombinant antigens were confirmed using SDS-PAGE and immunoblot analysis. Antigenicity of these *L. lactis*-derived recombinant *P. vivax* antigens was determined using human sera collected from high malaria transmission areas in Odisha by enzyme-linked immunosorbent assay (ELISA). Most of these recombinant *P. vivax* antigens showed significant seropositivity rates, minimal cross-reactivity and strong and persistent seroreactivity with sera collected from malaria endemic regions of Odisha. These findings suggest that *P. vivax* antigens produced from *L. lactis* potentially maintain their structural and immunological integrity needed for serodiagnostic applications. In future, these *L. lactis*-derived recombinant *P. vivax* antigens could be used as serodiagnostic markers and vaccine candidates.

**P-20**

## Genetic Variation in Host Factors Involved in Primaquine Metabolism in Surat, India

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From a malaria elimination perspective, inter-individual variability in anti-malarial drug response poses a significant challenge. *Primaquine* (PQ), a potent WHO-approved anti-malarial drug, is widely used in malaria-endemic countries as it plays a crucial role in preventing relapse of *Plasmodium vivax* and *Plasmodium ovale*, thereby making it integral to malaria elimination programs, however, its efficacy depends on host-mediated bioactivation. Cytochrome P450 2D6 (*CYP2D6*) converts PQ into its active metabolite, hydroxy-primaquine($H_2O_2$), in coordination with cytochrome NADPH oxidoreductase (CPR) which act as redox partner. Whereas Monoamine Oxidase A (*MAO-A*) produces an inactive carboxy metabolite, which requires further conversion into the active hydroxy-primaquine for therapeutic effectiveness. Variations in these genes can therefore compromise PQ metabolism and reduce its therapeutic efficacy. This study aims to investigate genetic polymorphisms of Cytochrome P450 2D6 (*CYP2D6*), Monoamine Oxidase A (*MAO-A*), and Cytochrome P450 oxidoreductase *(POR/CPR)* in malaria endemic Surat District. In addition to genetic variation, malaria infection is characterized by heightened inflammation, oxidative stress, hemolysis, and host metabolic reprogramming, all of which may modulate *MAO-A* expression as a stress-adaptive response. Such infection-driven *MAO-A* upregulation and reduced *CYP2D6* activity may shift cellular redox balance, collectively diminishing the generation of effective radical species and may compromise radical cure efficacy and increase relapse risk.

P-21

# DEVELOPMENT OF A TRANSDERMAL PRIMAQUINE DELIVERY SYSTEM FOR THE RADICAL CURE OF MALARIA

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**Introduction**

Among available antimalarial drugs, primaquine is the only widely used drug that effectively targets both blood-stage and liver-stage. The antimalarial drug primaquine has broader spectrum of action, including prophylactic activity against various *Plasmodium* species and eliminates mature gametocytes of *P. falciparum* which helps prevent malaria transmission. Clinical use of Primaquine has various limitations including its short half-life, first-pass hepatic metabolism resulting low systemic bioavailability, poor patient compliance due to the required 14-day oral administration and gastrointestinal side effects. So, the development of effective and patient-friendly drug delivery system is required to maintain the sustained plasma concentrations required for therapeutic action**.**

**Objectives**

To perform pre-formulation studies to assess physicochemical properties and excipient compatibility. To fabricate and optimize a transdermal drug delivery patch containing primaquine.

**Methods**

Pre-formulation experiments were performed for comprehensive physicochemical characterization of primaquine and excipients. Transdermal patches were fabricated by solvent casting using different excipients and a design experiment approach was employed to generate the formulation design.

**Results**

Pre-formulation studies were carried out to comprehensively characterize primaquine and to evaluate its physicochemical properties, including drug–excipient compatibility with various formulation components. Based on these studies, multiple transdermal patch formulations were prepared using different polymer combinations. The formulations were subsequently optimized using statistical approaches to achieve desirable performance characteristics. The fabricated transdermal patches exhibited good uniformity and smooth surface characteristics, indicating the formation of a stable and homogeneous polymeric matrix suitable for primaquine transdermal delivery.

**Conclusions**

So, development of transdermal primaquine delivery system would allow non-invasive, controlled and sustained drug release which might potentially improve therapeutic outcomes.

**Keywords:** Malaria, Primaquine, Transdermal, Delivery system

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P-22

## Prognostic Significance of C-Reactive Protein in Malaria Patients at a Tertiary Care Hospital

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**Background**

Malaria remains a significant public health problem in tropical regions, contributing substantially to morbidity and mortality. Early identification of severe disease is essential for timely intervention. C-reactive protein (CRP), an acute-phase reactant, may serve as a simple and cost-effective marker for assessing disease severity and prognosis in malaria.

**Methods**:

A prospective observational study was conducted from October 2024 to March 2025 at a tertiary care centre. Patients with laboratory-confirmed malaria by peripheral smear examination or rapid antigen testing were included. Serum CRP levels were measured and correlated with *Plasmodium* species, presence of complications, requirement for hospital admission, and duration of hospital stay. Statistical analysis was performed using SPSS version 14.

**Results**:

A total of 113 malaria patients were enrolled, of whom 97% were infected with *Plasmodium vivax* and 3% with *Plasmodium falciparum*. Mean CRP levels were significantly higher in patients with complications (59.01 ± 15.56 mg/L) compared to those with single or no complications. Patients requiring hospital admission had higher mean CRP levels (48.11 ± 19.82 mg/L) than those managed on an outpatient basis. Elevated CRP levels showed a statistically significant association with prolonged duration of hospital stay.

**Conclusion**:

Serum CRP levels correlate positively with disease severity, complications, and length of hospitalization in malaria. CRP is a simple, affordable, and reliable biomarker for assessing malaria severity and may aid in prognostication. Further studies incorporating additional inflammatory markers are recommended to strengthen its role in severity assessment.

P-23

# Deciphering the Dynamics of Reticulocyte Maturation and Its Role in *Plasmodium* Infection

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## Introduction

Reticulocytes are the penultimate stage of erythroid maturation and retain residual organelles, RNA, and membrane proteins that are progressively expelled during their 72-hour maturation into erythrocytes. During *Plasmodium* infection, despite the parasite's preferred tropism toward reticulocytes, maturation is markedly accelerated following invasion, within hours in *P. vivax*, and a similar trend has been observed in *P. falciparum*, suggesting parasite-driven host cell remodelling to create a permissive intracellular niche for development. The mechanism and significance of this accelerated transition remain poorly understood, but it is central to understanding host-parasite interactions and can reveal exploitable vulnerabilities in host-parasite crosstalk.

## Objectives

1. Elucidating human reticulocyte maturation and the role of vesicle-mediated exocytosis during erythrocyte development.
2. Understanding the interplay of rapid reticulocyte maturation and *P. falciparum* infection and establishment within.

## Methods

Reticulocyte maturation under healthy and infected conditions was evaluated through exosome isolation and multi-omics analysis (transcriptomics and proteomics).

## Results

Transcriptomic profiling of healthy reticulocytes revealed progressive loss of organelle-associated transcripts and activation of vesicular trafficking, protein-turnover, and cytoskeletal remodelling pathways, indicating active cellular clearance during maturation. In contrast, *P. falciparum* infection triggered accelerated maturation, with CD71 and reticular matter lost by ~20 h post-invasion, mirroring rapid maturation seen in *P. vivax*. Proteomic analysis revealed that ring-stage exosomes from infected reticulocytes were enriched in reticulocyte-specific proteins, which were also detected in the parasite fraction, indicating internal uptake and subsequent degradation of these proteins. These findings reveal a dual-clearance mechanism - export of host components via exosomes, coupled with parasite-linked proteasomal turnover, suggesting that the parasite rapidly remodels the host cell to create an erythrocyte-like niche optimal for development.

## Conclusions

Our findings suggest that *P. falciparum* does not merely prefer reticulocytes but hijacks maturation machinery to engineer its niche, and targeting these vesicle-proteasome mechanisms may offer new strategies to prevent parasite establishment.

P-24

# Evaluation of Atovaquone for oocyst-Stage Inhibition through contact exposure in *Plasmodium*-infected Mosquitoes

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**Introduction**

*Anopheles stephensi,* the primary urban malaria vector in India, has expanded its geographic range into parts of Africa, posing an increased risk of malaria transmission. As the widespread emergence of insecticide resistance increasingly compromises the effectiveness of current vector control tools, there is an urgent need for novel tools and strategies. Transmission-blocking interventions that target *Plasmodium* development within the mosquito vector are emerging as a promising complementary approach to existing control measures.

**Aim**

To determine if the lipophilic compound atovaquone can serve as a contact-based transmission blocker by interrupting *Plasmodium berghei* development in *Anopheles stephensi*.

**Method**

*In silico* analyses were conducted by docking the atovaquone with three ookinete surface proteins, and the resulting complexes were further evaluated using 100-ns molecular dynamics simulations. Further, the atovaquone was evaluated for its ability to inhibit *Plasmodium berghei* development by feeding *Anopheles stephensi* on infected BALB/c mice. Mosquitoes were subsequently exposed to the atovaquone using a contact-based bottle bioassay, and midguts were dissected 8–10 days post-infection to determine oocyst counts microscopically.

**Result**

In our study, the atovaquone exhibited stable binding with three ookinete surface proteins, with docking scores greater than −6.0 (kcal/mol). Ramachandran plots of all protein structures showed that more than 80% of residues were in the favored regions. Subsequent 100-ns molecular dynamics simulations further confirmed stable protein–ligand interactions. Infection studies demonstrated that atovaquone exhibited strong transmission-blocking activity, resulting in a mean oocyst inhibition of 100% at 1.5 mM in *Anopheles stephensi* mosquitoes.

**Conclusion**

Atovaquone compound showed strong potential as a contact-based transmission-blocking agent by significantly impairing *Plasmodium* sexual development inside *Anopheles stephensi* upon contact exposure.

**Keywords:** transmission blocking, *Plasmodium berghei,* oocyst, infection, bottle assay, *An. stephensi*

**Acknowledgement:** Authors are thankful to ICMR-NIMR and AcSIR for providing institutional support. The work has been carried through Senior Research Fellowship from ICMR.

P-25

# Hub Genes in Heme Biosynthesis: Potential Host-Directed Targets for Severe Malarial Anaemia

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**Introduction**
Severe malarial anaemia (SMA) continues to be a significant cause of malaria-related morbidity and mortality, especially in endemic areas, highlighting the necessity for enhanced molecular understanding to inform development and implementation of effective intervention strategies. Systems-level transcriptome analysis is a powerful means to uncover host determinants of disease severity and therapeutic susceptibility.

**Objective**
This study aimed to identify host hub genes in severe malarial anaemia via transcriptome, protein–protein interaction (PPI) network analysis, and docking to elucidate their roles in erythropoiesis and heme pathways, and propose them as targets.

**Methods**
We analyzed publicly available gene expression data (GSE255403) from pediatric severe malarial anaemia cases to find host regulatory factors. Differentially expressed genes (DEGs; $|\log_2 \text{fold change}| \geq 1$) were identified (n=969). These genes were then uploaded onto STRING to form the high-confidence PPI network. Network topology was analyzed by Cytoscape with the application of a plugin-cytoHubba, and hub genes were screened with multiple algorithms like MCC (maximal clique centrality), MNC (maximum neighbourhood component), and degree centrality.

**Results**
Integrated-network analysis consistently suggested that FECH, GATA1, SLC4A1, ALAS2 and EPB42 constituted central nodes in the disease-associated interactome of SMA. These genes are major players in erythropoiesis, heme biosynthesis, iron homeostasis and red cell membrane integrity—all of which are affected during SMA.

**Conclusion**
These hub genes driving erythroid and heme pathway are central to understanding the pathways of the host that lead to severe malarial anaemia, and hence serve as potential drug targets for therapeutic intervention. From the Discovery–Development perspective, these insights advocate for including host-directed approaches in strategies to enhance diagnosis, risk stratification, and intervention design to accelerate malaria control, elimination, and prevention of resurgence.

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P-26

# Allele Specific Distribution of Duffy Genotyping Among Malaria Positive and Healthy Individuals

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**Background**
Blood group variants are characteristic of population groups, and can show conspicuous geographic patterns. Interest in the global prevalence of the Duffy blood group variants is multidisciplinary but particularly important to malariologists due to the resistance generally conferred by the Duffy-negative phenotype against *Plasmodium vivax* infection.

**Objective**
Duffy genotyping among malaria positive and healthy individuals' in malaria endemic states of India.

**Methods**
To check most of the FY gene polymorphisms, we have received a retrospective sample from another study from 5 different malaria endemic states India. DNA was extracted from dried blood spots (DBS) collected from 481 subjects (238 malaria-positive patients and 243 malaria-negative persons) from five locations in the states. *Plasmodium* species identification was done by nested polymerase chain reaction (PCR) amplification of the small-subunit (SSU) rRNA genes **(1).** Duffy blood group genotyping was performed by allele-specific PCR to detect the presence of the genotypic pattern in the different population group **(2).**

**Results**
Out of 238 malaria positive subjects, 4 were found to be Duffy negative in *Plasmodium vivax* infected individuals. A preliminary result of Duffy genotyping shows 50% distribution of FYA+/FYB+ alleles, 2% of FYA+/FYB-, 19% of FYA-/FYB+ and 14% of FYA-/FYB- respectively.

**Conclusion**
Given the importance of the Duffy blood group system in clinical medicine, further studies utilizing molecular biology approaches must be developed to elucidate and characterize new sequence variants. Such molecular typing can help resolve and clarify the equivocal and discrepant results that arise in erythrocyte phenotyping performed by haemagglutination assays.

**Keywords:** Duffy, PCR, DARC, ACKR1, PvDBP-II

P-27

# Trehalose Transport Links Parasite-Induced Metabolic Reprogramming to Fitness Traits in *Anopheles stephensi*

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**Introduction**

Trehalose is the principal circulating sugar in mosquitoes and is central to survival, reproduction, and stress tolerance. Malaria parasite infection can reprogram vector metabolism, but the functional significance of infection-driven changes in trehalose pathways remains unclear.

**Objectives**

To determine whether *Plasmodium vivax* infection alters trehalose metabolism in female *Anopheles stephensi* and to test whether trehalose transport via trehalose transporter 1 (TRET1) is required to sustain mosquito survival and reproductive fitness.

**Methods**

RNA sequencing was performed on *P. vivax*–infected and uninfected adult females to identify differentially expressed metabolic pathways. Independently, *Tret1* expression was silenced by RNA interference in adult mosquitoes maintained on defined sugar diets; sugar ingestion was confirmed with dyed feeding assays. Survival was monitored daily. Reproductive fitness was assessed by measuring male mating success and mean egg number per female after blood feeding. Statistical comparisons used parametric tests with significance set at $p < 0.05$.

**Results**

Transcriptomic analysis showed significant upregulation of trehalose metabolism–associated genes in *P. vivax*–infected females, with several transcripts exhibiting >2-fold increases versus controls ($p < 0.01$). Functional assays revealed that *Tret1* knockdown reduced median survival by ~30% under sugar-feeding conditions ($p < 0.05$) despite measurable circulating trehalose, indicating impaired tissue-level sugar availability. *Tret1*-suppressed males exhibited reduced mating success, which translated into a ~40% decrease in mean female egg output ($p < 0.01$).

**Conclusions**

These combined transcriptomic and functional data indicate that *P. vivax* infection elevates reliance on trehalose pathways and that efficient trehalose transport via TRET1 is essential to meet that demand and maintain mosquito survival and reproductive competence. Targeting trehalose transport may represent a novel metabolic approach for malaria vector control.

**Keyword**

Trehalose metabolism; trehalose transporter; *Anopheles stephensi*; *Plasmodium vivax*; mosquito fitness; RNA sequencing

P-28

# Exploring the possible role of gut microbiota in insecticide resistance in Indian malaria vector *Anopheles stephensi*

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*Anopheles stephensi*, a dominant urban malaria vector in India, has exhibited increasing levels of insecticide resistance, threatening vector control interventions. While genetic mechanisms such as knockdown resistance (*kdr*) mutations and metabolic enzyme overexpression are well recognized, the contribution of the mosquito gut microbiome to resistance remains underexplored. This study examined the role of midgut microbiota in mediating insecticide resistance in laboratory-maintained susceptible and resistant strains of *Anopheles stephensi.* Adult susceptibility assays were conducted following WHO guidelines to categorize susceptible and resistant strains. Esterase, glutathione S-transferase (GST), and cytochrome P450 enzyme activity was assessed by biochemical assays. *kdr* genotyping was performed to detect target-site mutations associated with pyrethroid resistance. Shotgun metagenomic sequencing was carried out to analyze gut microbial composition, alpha and beta diversity patterns, and functional pathways associated with detoxification and xenobiotic degradation. Metagenomic analysis of adult midgut microbiota of *Anopheles stephensi* identified dominant phyla such as *Pseudomonadota, Actinomycetota, Bacteriodota* and *Bacillota*. In resistant strains, *Klebsiella pneumoniae* and *Saccharopolyspora sP.* were predominant, while in susceptible strains *Thorsellia anophelis* and *Escherichia coli* were most frequent. *Kdr* genotyping confirmed the presence of the pyrethroid-associated homozygous L1014S mutation in resistant mosquitoes indicating target-site insensitivity. WHO biochemical assays further demonstrated significantly elevated detoxification enzyme activity in resistant individuals compared to susceptible strains. KEGG pathway analysis corroborated these results, revealing elevated abundance of oxidative phosphorylation–related genes, including NADH-ubiquinone oxidoreductase and cytochrome c oxidase subunits, in resistant populations, indicating enhanced microbial contributions to energy production and metabolic detoxification. The integration of biochemical, genetic, and metagenomic analyses suggests that the gut microbiome, alongside host metabolic and target-site mechanisms, may contribute to insecticide resistance in *An. stephensi*. These findings highlight the importance of incorporating microbiome-based insights into future vector control strategies.

P-29

# Integrating Village-Level Risk Stratification and Diagnosis Delay Modeling for Targeted Malaria Elimination Strategies: A Composite Framework Proposal

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**Background**

Despite significant progress in reducing malaria burden over recent decades, transmission continues to persist in geographically and socially vulnerable pockets, challenging the goal of national and global malaria elimination. Traditional programmatic approaches rely heavily on case detection and routine vector control, but often do not sufficiently prioritize areas based on underlying structural determinants such as ecological suitability, housing conditions, preventive behavior, and migration-linked exposure. At the same time, operational delays between fever onset and definitive diagnosis extend the infectious window of *Plasmodium falciparum*, enabling ongoing transmission even in areas with declining incidence. Although both structural risk and diagnostic delays are known contributors to sustained malaria transmission, they are typically assessed separately and rarely integrated into planning tools at the village or sub-district level. This highlights the need for a simple, field-applicable framework that combines these dimensions to guide targeted microplanning and optimize allocation of surveillance and vector control resources under malaria elimination settings.

**Objectives**

1. To construct a Village Malaria Risk Index (VMRI) using five domains.
2. To categorize fever-to-treatment delays using a Time-to-Diagnosis Delay Model (TDDM).
3. To integrate both into a decision-support matrix for targeted malaria intervention prioritization.

**Methods**

Conceptual exploratory framework developed using literature review and national elimination guidelines. VMRI comprised epidemiological trends, environmental breeding sources, preventive behavior, occupational exposure, and housing type (score 0–10; Low 0–3, Moderate 4–6, High 7–10). TDDM classified delays as Early (<24h), Intermediate (24–48h), and Late (>48h). Hypothetical datasets were applied to generate a combined prioritization matrix.

**Results**

Integration of VMRI and TDDM produced a two-dimensional model differentiating villages requiring routine preventive reinforcement, enhanced surveillance, or urgent rapid diagnostic outreach. Villages categorized as High VMRI + Late Delay showed highest sustained transmission risk, indicating priority for intensified ASHA-led screenings, LLIN adherence activities, and mobile diagnostic camps.

**Conclusion**

The combined VMRI–TDDM framework is simple, scalable, and usable with routine surveillance data. It provides a decision-support tool to focus malaria elimination resources where both structural vulnerability and diagnostic delays are greatest. Field validation is recommended to operationalize prioritization at PHC and block levels.

**Keywords**

malaria elimination, risk stratification, diagnostic delay, village prioritization, decision-support framework

P-30

# Delving the Molecular Complexity of Mating Behavior and Reproductive Physiology in *Anopheles culicifacies.*

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**Introduction**
The widespread reliance on chemical insecticides for mosquito control has resulted in significant negative consequences for human health, non-target organisms, and ecosystems. These limitations underscore the urgent need for innovative and sustainable vector control strategies. The Sterile Insect Technique (SIT) represents a promising alternative; however, its successful application in malaria vectors is constrained by limited understanding of male reproductive biology, particularly the molecular regulation of spermatogenesis.
**Objectives**
This study aims to identify and characterize key genes regulating spermatogenesis in the male reproductive organs of *Anopheles culicifacies*, with a specific focus on evaluating the functional relevance of the Lipopolysaccharide-Induced TNF-Alpha Factor (LITAF/LL6) transcript in reproductive physiology.
**Methods**
We initiated a pilot transcriptomic investigation using RNA-sequencing data derived from female hemocytes of *Anopheles culicifacies*. A previously unanticipated transcript, LITAF, was identified and subsequently analyzed through spatial and temporal expression profiling across developmental stages and tissues. Comparative expression analysis was conducted to assess transcript enrichment in male reproductive organs, including the testes, and RNAi to infer potential roles in spermatogenesis.
**Results**
Transcriptomic analysis revealed the unexpected presence of LITAF, a gene traditionally associated with immune signaling, suggesting pleiotropic functions beyond immunity. Spatial and temporal expression profiling demonstrated significant enrichment of LITAF in male reproductive organs, with pronounced expression in the testes. These findings indicate a potential role for LITAF in regulating male reproductive homeostasis and spermatogenic processes.
**Conclusions**
Our findings provide novel insights into the molecular crosstalk between immune-related genes and reproductive physiology in *Anopheles culicifacies*. The enrichment of LITAF in male reproductive tissues highlights its potential as a regulatory factor in spermatogenesis. Functional characterization of LL6 may uncover new molecular targets to disrupt male fertility, thereby strengthening SIT-based vector control strategies. This study lays foundational groundwork for exploiting reproductive molecular pathways in the development of next-generation, eco-friendly malaria vector control tools.
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P-31

# Decoding the Multifaceted Role of erythrocyte PMCA4b in Oxidative stress mediated Malaria Protection and Artemisinin Resistance

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The ATP2B4 gene, encoding the PMCA4b $Ca^{2+}$-ATPase in erythrocytes, has been linked to malaria protection via genome-wide association studies, though the proposed dehydration mechanism remains unclear. This study evaluates ATP2B4 genotypes and PMCA4b expression in malaria susceptibility and artemisinin sensitivity. ATP2B4 genotypes were compared across severe malaria, uncomplicated malaria, and healthy controls in Indian population. PMCA4b expression, intra-erythrocytic calcium, oxidative stress markers, Gardos channel activity, and *P. falciparum* growth dynamics were analysed. Artemisinin sensitivity was assessed using growth inhibition and ring survival assays. ATP2B4 genotypes showed no significant association with malaria protection. Regardless of genotype, low PMCA4b expression correlated with increased intra-erythrocytic calcium, oxidative stress, and reduced in-vitro parasite growth. Gardos channel activity inversely correlated with PMCA4b but did not induce dehydration. Instead, oxidative-stress regulation by PMCA4b emerged as a key factor in malaria protection. Treatment of RBCs with resveratrol, a PMCA inhibitor further validates the functional role of PMCA in regulation of intracellular calcium and oxidative stress. Notably, low PMCA4b expression also reduced *P. falciparum* artemisinin sensitivity, providing evidence that host genetic variation can influence drug efficacy. These findings suggest PMCA4b's role in oxidative stress and drug response as critical to malaria pathophysiology. We further emphasize that many redox modulating RBC polymorphisms in malaria endemic areas, could also influence artemisinin efficacy and serve as potential biomarkers for predicting therapeutic response.

P-32

# Mapping NATs-Protein Interactions to Reveal Gene Regulatory Landscapes in severe *Plasmodium vivax* clinical isolates

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## Introduction

Malaria remains a major global health burden, according to the World Malaria Report 2025, nearly half of the global population is at risk of *P. vivax* infection. Although traditionally regarded as benign, increasing clinical evidence demonstrates that *P. vivax* can cause severe complications. However, the molecular mechanisms underlying disease severity remain poorly understood. Our previous studies implicate natural antisense transcripts (NATs), a subclass of long non-coding RNAs (lncRNAs), in shaping parasite transcriptomic states associated with diverse clinical outcomes [1]. Given their limited sequence conservation and lack of protein-coding potential, lncRNAs primarily exert regulatory functions through interactions with protein partners [2], forming ribonucleoprotein complexes that modulate gene expression.

## Objectives

Identification of downstream molecules involved in NATs mediated regulation of gene expression.

## Methods

Transcriptome profiling was performed using custom-designed microarrays to identify NATs from *P. vivax* infected clinical isolates. Differential expression analysis distinguished NATs associated with complicated malaria. To complement experimental findings, catRAPID omics [3] was employed to identify high-confidence RNA-binding protein-NATs interaction pairs, RNA interaction sites, and corresponding protein RNA-binding domains.

## Results

The transcriptomic analysis revealed a subset of differentially expressed NATs in *P. vivax* clinical isolates. catRAPID predictions indicated that these NATs interact with multiple parasite proteins, including vir family members and other regulatory proteins, with interaction propensities suggestive of stable complexes. Identification of specific RNA interaction site and corresponding protein-binding domains provided mechanistic insight into NAT-mediated regulatory mechanisms, suggesting their potential involvement in modulating DNA repair, chromatin remodelling, and transcriptional processes in *P. vivax* clinical isolates, thereby fine-tuning virulence-associated gene networks and adaptive parasite responses during infection.

## Conclusions

This study uncovers NATs–protein interaction networks in *P. vivax*, highlighting roles in virulence regulation and disease progression. The framework advances understanding of NATs-mediated gene regulation and identifies NATs–protein complexes as targets for therapeutic strategies against *P. vivax* malaria.

## Acknowledgments

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**Keywords:** Malaria, NATs, catRAPID, Protein-RNA interaction

P-33

## Repurposing *Plasmodium falciparum* Antimalarials to Target *Plasmodium vivax* Liver-Stage Proteins: A Hypothesis

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*Plasmodium vivax* is a major obstacle for malaria eradication, as it can develop dormant liver-stage hypnozoites that may reactivate and lead to relapse. *P. falciparum*, on the other hand, is responsible for most malaria deaths and also does not possess a dormant stage, so this species has been the traditional focus of antimalarial drug development. *This repurposing strategy is biologically plausible because key metabolic, mitochondrial, and adhesive pathways are highly conserved between P. falciparum and P. vivax*, enabling Pf-directed compounds to interact effectively with homologous Pv liver-stage proteins. In silico docking studies suggest that a number of *P. falciparum* drugs have high binding affinities with essential proteins, such as thrombospondin-related anonymous protein (TRAP), circumsporozoite protein (CSP) and apical asparagine-rich protein (AARP) of *P. vivax* which play an important role in the invasion of sporozoites into liver. While considered vaccine antigens, these proteins could be ideal small-molecule therapeutic targets. However, no current therapeutic approach exploits this cross-species molecular conservation to target *P. vivax* liver-stage proteins, highlighting a critical gap that we address with the hypothesis that clinically approved *P. falciparum* antimalarials can be repurposed to inhibit key Pv pre-erythrocytic targets and disrupt hypnozoite formation. This transspecies drug repositioning approach could potentially facilitate the discovery of a radical cure, cut development expenditure and aid substantially in malaria elimination.

**Keywords:** *Plasmodium vivax*, drug repurposing, liver stage, TRAP, CSP, AARP, hypnozoite, malaria elimination

**O-34**

## A Moonlighting Nuclear Role for PfPGM1 in Metabolic Feedback Regulation in *Plasmodium falciparum*

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Phosphoglycerate mutase (PGM) enzyme is involved in multiple metabolic pathways including glycolysis and pentose phosphate pathway. *Plasmodium falciparum* PGM1 (PfPGM1) is a tetrameric enzyme expressed in the different parasitic life stages, and its oligomerization is important for its activity both *in vitro* and *in vivo*. Moreover, a fraction of PfPGM1 is found in the nucleus. The presence of a glycolytic enzyme in the nucleus is intriguing and therefore, analysing its role in nucleus is necessary and this laid the foundation of our study. Our sub-cellular fractionation studies showed that PfPGM1 is mostly localized to the cytoplasm but small fraction also goes to the nucleus, in each stage in the asexual cycle of *P. falciparum*. This is also corroborated by our indirect immunofluorescence assays and live cell imaging. Surprisingly, over-expression of wild type PfPGM1 but not an oligomerization mutant form of PfPGM1 as GFP fusion protein, showed down regulation of the endogenous PfPGM1 protein. Further, the transcripts of endogenous PfPGM1 were also downregulated when PfPGM1 was overexpressed as GFP fusion protein as above. ChIP-qPCR analysis showed that affinity of PfPGM1 with its own promoter is significantly higher in wild type overexpression line compare to its mutants. In conclusion, we may suggest the possibility of autoregulation mechanism for PfPGM1 expression in *P. falciparum*.

**P-35**

**Pacifastin, a protease inhibitor controlling the physiology of Indian malaria vector *Anopheles stephensi*.**

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*Anopheles stephensi* and *Anopheles culicifacies* are major malaria vectors in India, equipped with serine proteases that act as regulators and catalysts. Pacifastin (Pac), an endogenous serine protease inhibitor in arthropods, regulates serine protease activity but its role in malaria vectors remains unclear. In insects, Pac regulates trypsin- and chymotrypsin-mediated prophenoloxidase pathways and digestion. To investigate the potential role of Pacifastin in regulating mosquito's digestive physiology. Basal transcription levels and localization of Pacifastin in sugar-fed and blood-fed mosquitoes were analyzed via confocal microscopy. rPac was expressed and purified from *E. coli*, with its functional form confirmed through caesinolytic plate assay, circular dichroism (CD), and kinetic studies. Interaction studies with serine protease included molecular docking, dynamic simulations, dot-blot, and ELISA. Transcription and confocal microscopy reveal higher Pacifastin levels in sugar-fed than blood-fed females. Recombinant Pacifastin (rPac), expressed in *E. coli* and refolded to its native state, showed functional activity via caesinolytic plate assay and circular dichroism (CD) analysis. Enzyme inhibition kinetics demonstrated rPac as a stronger trypsin inhibitor than commercial alternatives. Molecular docking and dynamic simulations indicated higher Pacifastin affinity for chymotrypsin over trypsin, supported by dot-blot and ELISA results. Pac knockdown study in the sugar fed adult females' midgut clearly indicate the reciprocal relation of Pac with the serine proteases. These findings position Pacifastin as a key regulator of protease-mediated processes in mosquito physiology, warranting further exploration of it as a potential novel target for vector-control strategies.

P-36

## Interrelationship between vitamin D polymorphisms and malaria.

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### Introduction

The vitamin D receptor (VDR) plays an important role in regulating host immune responses, and emerging evidence suggests that VDR-mediated pathways influence susceptibility to malaria infection and disease severity.

### Objective

Investigating the association between malaria susceptibility and VDR variants.

### Methodology

A total n=463 samples were collected in the form of dried blood spots from the Gujarat, India followed by genomic DNA isolation. Out of these collected samples, n=252 samples were used as control and 211 malaria-positive samples were taken. Following the amplification of three VDR gene variants, Apa1, Fok1, Taq1 were subjected to perform restriction fragment length polymorphism on these amplified amplicons.

### Results

The analysis suggests the wild type allele (G) of apa1 as a risk allele however, the heterozygous advantage has been observed. In the dominant model for Taq1 (TT vs TC+CC), suggest that the TT genotype is protective against the disease (B = –0.382, OR = 0.630, p = 0.019). However, the TC vs TT comparison revealed a significant protective effect (OR = 0.602, p = 0.013; adjusted OR = 0.567, p = 0.007). The analysis indicating the heterozygous genotype TC as protective genotype. For Fok1 SNP the recessive model (TT+TG/GG) suggest that the GG genotype showed the risk; however, the B coefficient is 0.542 (odds ratio (1.702 (1.177–2.462, p = 0.0.005). The haplotype CTT haplotype has highest frequency, where C is mutant allele of Apa 1, T represents wild allele of Fok1 and Taq 1 polymorphism.

### Conclusion

Here in this study, heterozygous advantage is found to have a major importance against malaria. The significant protective associations were seen between VDR variants and malaria outcomes which strengthens the evidence of the possible interrelationship of VDR pathway and malaria outcomes.

P-37

# Essential Oil-Based Long-Lasting Nano-Formulations for Malaria Vector Control

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## Introduction

Malaria remains a key global public health challenge, particularly in tropical and subtropical regions. Control of *Anopheles* mosquito vectors is a cornerstone of malaria prevention strategies. Although synthetic mosquito repellents such as N, N-diethyl-m-toluamide (DEET) are effective, concerns related to toxicity, skin penetration, and environmental persistence limit their long-term acceptability. Safer, eco-friendly alternatives are therefore required. Essential oils have demonstrated repellent activity against malaria vectors; however, their high volatility limits prolonged effectiveness.

## Objectives

To develop and evaluate an essential oil-based nano-formulation with improved stability and sustained repellent activity against malaria vectors.

## Methods

An essential oil-based preconcentrate was developed using simple and conventional formulation techniques. Selected essential oils were mixed with suitable surfactants and co-surfactants under continuous stirring at ambient conditions until a homogeneous and stable system was obtained. The prepared preconcentrate was evaluated for physicochemical stability and repellency against mosquito vectors.

## Results

A stable and homogeneous preconcentrate was successfully developed through conventional mixing of essential oils, surfactants, and co-surfactants. The formulation exhibited improved dispersion, reduced volatility, and controlled release of essential oils, indicating prolonged repellent activity against mosquito vectors.

## Conclusion

The developed essential oil-based nano-formulation provides a simple, cost-effective, and environmentally sustainable approach for malaria vector control. The formulation shows potential for integration into malaria prevention programs and for reducing reliance on synthetic chemical repellents.

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P-38

# Extra Domiciliary Malaria Transmission in Forested Tribal Areas of Odisha, a challenge for Malaria elimination in India: Entomological and Epidemiological Evidences

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**Introduction**

Malaria transmission persists in forested tribal regions of Odisha, India, despite long-standing vector control interventions. In Bandhugaon Community Health Centre (CHC), Koraput district, a substantial proportion of the population spends nights in *kudias* extra domiciliary agricultural huts located in forest fringes potentially increases the risk of human-vector contact sustaining residual malaria transmission beyond the community-based vector control strategies.

**Objectives**

To assess the contribution of extra domiciliary (*kudia*) settings to malaria transmission through integrated entomological and epidemiological investigations.

**Methods**

A longitudinal study (July 2022–June 2023) was conducted in six high endemic tribal villages and associated *kudia* sites. Fortnightly entomological collections were performed using indoor and outdoor resting catches and light traps collections across three seasons. Vector density, parity, blood feeding behavior, insecticide susceptibility, and *Plasmodium* infection/infectivity rates were assessed for *Anopheles culicifacies* and *An. fluviatilis*. Active fever surveillance was carried out to determine malaria incidence. Generalized mixed effects Poisson and negative binomial regression models were used to identify determinants of vector density.

**Results**

Of the 11,161 mosquitoes collected, 71.5% were anophelines, with *An. culicifacies* (8.2%) and *An. fluviatilis* (2.3%) as principal vectors. The per-man-hour density of *An. fluviatilis* was significantly higher in *kudia* sites than villages (0.112 vs. 0.036; $P<0.001$), while *An. culicifacies* density was similar across sites. Parity rates were comparable in *kudia* and villages, indicating sustained vector survival. Human blood index was higher in *An. fluviatilis* (5.4%) than *An. culicifacies* (0.3%). Minimum infection rates reached 10.2 for *An. culicifacies* and 5.3 for *An. fluviatilis*. Malaria prevalence was significantly higher in *kudia* populations than villages (26.6% vs. 12.5%; $P<0.001$), with *Plasmodium falciparum* accounting for 94.7% of cases. *An. culicifacies* exhibited resistance to commonly used public health insecticides, whereas *An. fluviatilis* remained fully susceptible.

**Conclusions**

This study highlights the complexity of extra domiciliary malaria transmission substantially contributes towards the persistence of malaria burden in forested tribal areas. To eliminate malaria in Koraput, elimination strategies must prioritize enhanced entomological surveillance and behavior-responsive, targeted vector control beyond village communities to mitigate residual transmission.

**Keywords:** Extra domiciliary Malaria, Tribal Malaria, Malaria Elimination, Koraput, Odisha

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2. Medical Officer, Bandhugaon CHC and District Vector Control Program unit (NCVBDC), Koraput District.

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## O-39

**Evolving Malaria Diagnosis: Gold standard Smear, Rapid Tests with novel Pf Markers and POCT based Molecular Testing**

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Malaria continues to be a common and life-threatening infection, and it needs to diagnosed fast and reliable especially for children. The world has used malaria microscopy, rapid tests and molecular tests depending upon accessibility and affordability. While microscopy is reliable but is slow and tedious, the rapid tests are showing comparative lower sensitivity and are increasingly affected by gene deletions for HRP-II and molecular RT-PCR based test are expensive and difficult to deploy.

**Method**

Review of published literature from India and other countries, scooping information from incubators across country, interviewing IVD industry, and summarizing the information from Government reports and WHO reports. We covered a period of 2020 to 2025 and compiling the data for presentation at the conference.

**Results**

**Microscopy**: At least one automated hematology analyzer Sysmex XN-31 (Sysmex, Kobe, Japan) demonstrated rapid detection and quantification of malaria-infected red blood cells (Mi-RBCs) by using fluorescence flow cytometry[1]. However, fully automating the movement of the microscope slide, image autofocusing of the samples by hardware implementation would along with AI based image analysis awaits design freeze and multisite validations.

**RDT**: World has witnessed improvements in the sensitivity and specificity of RDTs for *Plasmodium falciparum* diagnosis, making them comparable to expert microscopic examination. In addition, there is a global push towards assessing and confronting the growing concerns of widespread pfhrp2 gene deletions by developing multiple test lines in several combinations either using antibodies against Pf LDH or using conserved markers like GDH and others.[2]

**Molecular Test**: Molecular tools like Polymerase Chain Reaction (PCR), Loop-mediated isothermal amplification (LAMP) and other platforms continue to remain expensive for deployment at large scale.[3]

**P-40**

# Rational Design of Novel 2-aminopyrido[3,4-*d*]pyrimidin-4-one derivatives as *Plasmodium falciparum* Dihydroorotate Dehydrogenase *(Pf*DHODH*)* Inhibitors Using 3D-QSAR, Docking, Molecular Dynamics Simulation Approaches.

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*Plasmodium falciparum* dihydroorotate dehydrogenase (*Pf*DHODH) is an essential enzyme in the de novo pyrimidine biosynthesis pathway and a validated antimalarial drug target. In this study, a field-based 3D-QSAR model was developed using 75 *Pf*DHODH inhibitors to identify key features interacting with the enzyme's active site. The PLS-5 model showed good validation with $R^2$ value of 0.899 and $Q^2$ value of 0.805, and the contour map generated was used to develop new analogues. These analogues showed moderate predictive activity, pIC50 in the range of 7.3 to 7.741 using the PLS-5 model. ADMET evaluation showed that the designed molecule has acceptable drug-likeness and pharmacokinetic profiles. The newly designed compounds were docked into the *PfDHODH* active site (PDB ID: 6I55) using AutoDock Vina 1.2.7. Compound 2d exhibited the strongest affinity (-9.541 kcal/mol) and showed interactions comparable to the reference (DSM265) *Pf*DHODH inhibitor (-9.326 kcal/mol). Based on good binding affinity, favourable interactions, compound 2d was selected for molecular dynamics simulations. Compound 2d showed good stability during 200ns simulations. From this study, the most promising compounds can be synthesized and further evaluated for their biological activity as a potential *Pf*DHODH inhibitor.

**Keywords:** *Pf*DHODH, 3D QSAR, Molecular Docking, Molecular dynamic simulations

O-41

## Targeted Nanoparticle Delivery for Selective Metabolic Pathway Disruption in *Plasmodium falciparum*

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The swift rise of drug-resistant *Plasmodium falciparum* is a significant obstacle to efficient malaria management and underscores the pressing necessity for innovative treatment approaches. Targeting specific critical metabolic pathways in the parasite offers a viable strategy to combat resistance and enhance treatment success. This study devised a tailored nanoparticle-based medication delivery method to selectively disrupt essential metabolic pathways vital for the survival and proliferation of *P. falciparum*. Biocompatible nanoparticles were designed to encapsulate antiplasmodial medicines and functionalized with targeting ligands to improve selective uptake by infected erythrocytes. The nanoformulations were engineered to disrupt essential metabolic processes, such as glycolysis, heme detoxification, and mitochondrial electron transport. In vitro assessment revealed increased cellular uptake, prolonged drug release, and markedly superior antiplasmodial efficacy relative to unencapsulated medicines. Mechanistic investigations demonstrated significant inhibition of essential metabolic enzymes, resulting in disrupted energy production and cessation of parasite growth. Moreover, targeted nanoparticle delivery diminished off-target toxicity and enhanced selectivity for infected cells, underscoring its therapeutic benefit. Initial safety evaluations demonstrated positive biocompatibility with few negative impacts on host erythrocytes. This study highlights the efficacy of targeted nanoparticle distribution as an approach for selectively disrupting metabolic pathways in *Plasmodium falciparum*, providing a solid foundation for the advancement of next-generation antimalarial medicines to address drug resistance.

**Keywords**: Antimalarial drugs, drug resistance genetic markers, malaria, Artemisinin, *Plasmodium falciparum, Plasmodium vivax*

P-42

## Mining Malaria Patient Biofluids for Extracellular Vesicles Based Biomarker Candidates

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Malaria continues to exert a major global health burden, with high mortality rates. Limitations in early diagnosis intensify disease severity, underscoring the need for accurate diagnostic biomarkers. Emerging studies show that Extracellular Vesicles (EVs) released from *Plasmodium*-infected and host red blood cells facilitate intercellular communication and may serve as promising biofluid-based diagnostic tools. EVs are membrane-bound nanovesicles carrying cell-specific proteins, nucleic acids, and metabolites. Given the cross reactivity of existing markers such as Lactate dehydrogenase, identifying novel, species-specific biomarkers remain crucial for improved malaria diagnostics.

### Objectives

1. Isolation and characterization of Extracellular vesicles from malaria infected patient plasma samples.
2. Analysis of the proteomic landscape of isolated Extracellular vesicles in Severe and Non severe infection.
3. Defining candidate biomarkers based on statistical analysis to devise detection platforms.

### Methods

EVs were isolated from plasma of healthy controls (n=4) and *Plasmodium vivax*–infected patients (n=20), including severe and non-severe cases, using differential centrifugation followed by size-exclusion chromatography. Biophysical characterization involved particle sizing and electron microscopy, while Western blotting confirmed EV markers and purity. Differential proteomic profiling was then conducted using mass spectrometry.

### Results

Preliminary mass spectrometry of *P. vivax* plasma EVs identified 1339 proteins (1239 human, 100 parasite), with species-specific proteins present in >70% of clinical isolates prioritized for analysis. Most parasite proteins were hypothetical or associated with core cellular functions. Functional annotation revealed enrichment of mitochondrial transport, heat-shock response, adhesion, and nuclear pathways. Distinct host protein alterations between severe and non-severe cases were also noted, supporting improved diagnostic precision.

### Conclusion

Future work will expand sample size and implement targeted proteomics in larger EV cohorts to validate diagnostic value. Antibodies or peptides against key biomarker candidates will support development of a diagnostic–prognostic panel for malaria, alongside efforts to establish minimally invasive, finger-prick–compatible detection platforms.

Study has been funded by BITS BioCyTiH foundation.

P-43

# Bridging the Indian Genomic Gap: Whole-Genome Population Genomics of Indian *Plasmodium vivax* in the Global Landscape

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## Introduction

India contributes nearly half of the global *Plasmodium vivax (Pv)* burden, yet its population genomic structure and diversity remain poorly characterized despite its major public health impact.

## Methods

Whole-genome sequencing was performed using the Illumina platform on five *Pv* field isolates from previously unexplored *Pv* endemic regions of India. These were integrated with 47 underexplored publicly available Indian genomes and global *Pv* genomes, totalling 2,189 samples, of which 1,117 passed QC (Africa 210, Latin America 217, East Southeast Asia 284, West Southeast Asia 77, West Asia 165 including India, Oceania 33, Middle East 5, Maritime Southeast Asia 126). Analyses using LD-pruned variants ($r^2$>0.2) included Principal Component Analysis (PCA), ADMIXTURE (K = 8), pairwise $F_{ST}$ estimation, and scans for genomic regions under selection.

## Results & Discussions

PCA showed Indian and West Asian isolates clustering closer to Africa than East or West Southeast Asia, suggesting ancient gene flow or shared ancestry predating major Asian diversification events. The consolidated Asia-Pacific cluster aligns with documented Southeast Asian/Oceanian expansions, while Latin America's isolation reflects bottleneck effects from colonial-era introductions. Admixture proportions (~30-50% African-like) support hybridization models, positioning Indian *Pv* as a genetic bridge between continents. Pairwise $F_{ST}$ values supported these patterns, showing lower differentiation between West Asia/India and Africa (0.05–0.10) than with Southeast Asia (~0.15). Selection scans identified recent positive selection in antimalarial drug-resistance genes (*mrp1, dhfr, dhps*), highlighting ongoing local adaptation. These findings emphasize that Indian genomes are crucial for resolving global *Pv* population structure and understanding evolutionary dynamics, while also revealing adaptive pressures relevant for malaria control.

## Conclusion

Indian *Pv* occupies an intermediate genetic position between African and Southeast Asian populations, reflecting shared ancestry, historical gene flow, and India's role as a genetic bridge in global *Pv* evolution. Evidence of recent positive selection at key antimalarial drug-resistance loci (*mrp1, dhfr, dhps*) highlights ongoing local adaptation, underscoring the critical importance of Indian genomes for understanding global *Pv* population structure, evolutionary dynamics, and adaptive responses relevant to malaria control.

P-44

# Insecticide resistance status in *Anopheles stephensi,* an urban malaria vector in Chennai (Urban) and Bengaluru (Rural)

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## Introduction

Malaria is a major public health problem in India, and *An. stephensi* is responsible for urban malaria transmission (World Malaria Report, 2024). In India, the current situation regarding resistance in malaria vectors to conventional insecticides requires strategies to manage vector resistance. Currently, synthetic pyrethroid insecticides are the only class of compounds available for IRS in areas where vectors have developed resistance to other insecticides, and for the treatment of mosquito nets (Raghavendra et al. 2022).

## Objectives

1. To understand the larvicide (Temephos) susceptibility of *Anopheles stephensi* against diagnostic dose and differential dosages of operational larvicide and to determine the $LC_{50}$ and $LC_{90}$ values.
2. To find out the insecticide (adult) susceptibility status of *Anopheles stephensi* and to determine the $KDT_{50}$ and $KDT_{90}$ values in selected districts of Tamil Nadu and Karnataka, India.

## Methods

The immature and adult anopheles mosquitoes were collected in selected study sites in Chennai and Bengaluru. Immature collections were done in overhead tanks, wells, underground tanks and other clear water storage breeding habitats. Adult collections were undertaken in cattle sheds and human dwellings. The samples collected from the selected areas were brought to the laboratory, identified to the species level. *An. stephensi* were reared to the $F_1$ generation under standard laboratory conditions (-27°C and 80% RH) to perform larval susceptibility tests against the diagnostic dosage of Temephos and operational dosage. Adult susceptibility tests were performed against deltamethrin 0.05% and alphacypermethrin 0.3% following the procedure mentioned in the WHO protocol. Knockdown (1 hour during exposure) and mortality (24 hours) were recorded. In larval susceptibility, 24-hour mortality was recorded. $LC_{50,}$ $LC_{90,}$ $KDT_{50}$ and $KDT_{90}$ values were obtained using SPSS.

## Results

The larval susceptibility test results indicated susceptibility against *An. stephensi* to Temephos diagnostic dose at 0.25ppm. However, the susceptibility status against operational dose was at 1.5ppm and 2ppm in the Chennai urban area and 1ppm and 1.5ppm in the Bengaluru rural area. Deltamethrin 0.05% and Alphacypermethrin 0.3% indicated resistance in the Chennai population but were susceptible in the Bengaluru rural area.

## Conclusions

The current study proves that resistance has developed in *An. stephensi* in Chennai urban unlike rural areas in Bengaluru. Further studies are imperative to elucidate the resistance mechanisms and identify the genotype and metabolic activity responsible for resistance in *An.stephensi*.

## Keywords

Insecticide resistance, Malaria, *Anopheles stephensi* and the WHO tube test.

P-45

# Potential of Secondary malaria vectors in malaria transmission dynamics across North-eastern states of India

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**Background**

Malaria is endemic in the North eastern regions of India, in which a variety of ecological variables promote diverse vector species. The major primary malaria vector in this region has long been considered to be *Anopheles minimus* and *Anopheles baimaii,* both closely associated with forest ecosystems. Despite the reduced abundance of these primary anopheline vectors, the persistence of malaria suggests the involvement of additional anopheline species. Secondary malaria vectors may also play a role in transmitting malaria, along with primary malaria vectors, and are widely distributed across northeastern India. These secondary vectors have significantly lower sporozoite rates compared to primary vectors, yet are capable of sustaining malaria transmission in a specific region. This study aimed to investigate the sporozoite positivity of secondary anopheline species in the high-malaria-endemic district of Kokrajhar, Assam, in northeastern India.

**Methods**

During the study period, 1,794 female mosquitoes representing five genera in *Anopheles*, *Culex*, *Aedes*, *Mansonia*, and *Armigeres* were collected from Kochugaon PHC in Kokrajhar, Assam. The three methods of collection were using: CDC light trap collection, Indoor resting collection using the mouth aspiration method, and pyrethrum spray collection.

**Results**

The *Plasmodium* positivity (Percent, number/total number) was highest in *An. maculatus* (4%; 5/80), followed by *An. minimus* (4.8%; 1/21), and *Anopheles kochi* (4.6%; 1/22). All the *Plasmodium*- positive samples of mosquito were identified as *P. vivax*. The highest human blood index form in *An. minimus* and then *An. maculatus* is 0.86 and 0.42 respectively. These results suggest that anopheline species beyond the traditionally recognized primary vectors, may play a role in sustaining malaria transmission in endemic areas of northeastern India.

**Conclusion**

The study highlights the spatiotemporal variability in the density of primary malaria vectors and reconfirms that multiple *Anopheles* species carry malaria parasites in the international border areas of Kokrajhar (Assam, India). Recognizing and integrating the behavior and ecology of secondary vectors into malaria control programs is essential for the development and deployment of more targeted and sustainable vector control strategies. A reassessment of vector control priorities is essential for achieving malaria elimination in the northeastern region of India.

P-46

# Persistent malaria in the Nicobar Islands: Exploring the underlying cause from the source of the blood meal

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## Introduction

Andaman and Nicobar Islands are characterized by unique ecological settings, including substantial tribal populations, and have historically been endemic for several vector-borne diseases, particularly malaria. As India advances towards its national goal of malaria elimination by 2030, understanding residual transmission dynamics becomes critical, since even sporadic malaria cases can threaten elimination efforts. *Anopheles sundaicus* is the principal malaria vector in the Andaman and Nicobar Islands, with *Plasmodium falciparum* and *Plasmodium vivax* being the predominant malaria parasites circulating in the region.

## Objectives

To better understand vector-host interactions and their implications for malaria transmission, an entomological survey was conducted to assess the feeding behaviour of anopheline mosquitoes, including *An. sundaicus*, across the three districts of the Andaman and Nicobar Islands: South Andaman, North & Middle Andaman, and Nicobar.

## Methods

Adult anophelines were collected from different villages in each of the three districts of Andaman & Nicobar viz, South Andaman, North & Middle Andaman and Nicobar. Adult mosquito specimens were collected by CDC light trap and Indoor Resting Collection (IRC) methods. Freshly blood-engorged specimens were subjected to blood meal identification using specific antisera through the agarose gel diffusion technique.

## Results

A total of 503 anophelines, belonging to 12 species, were collected and analysed for their blood meal source. Blood meal analysis revealed that all twelve anopheline species exhibited predominantly zoophagic behaviour. The Human Blood Index (HBI) of *An. sundaicus* was 0.35 in the Nicobar and 0.19 in the Andaman districts, indicating limited but significant human feeding.

## Conclusion

The findings highlight distinct anopheline feeding patterns across the Andaman and Nicobar Islands, with *An. sundaicus* represents a higher HBI in the Nicobar district. The high HBI in this malaria vector, along with its abundance, are likely contributing factors for the continued transmission of malaria in the Nicobar district. These results emphasize the need for targeted vector surveillance and control strategies focusing on this species to sustain malaria elimination efforts and prevent disease resurgence in the islands.

## Keywords

*Anopheles sundaicus*; Blood meal; Nicobar; Malaria; Mosquito; Host preference

P-47

# Monitoring genetic markers to antimalarial resistance in *Plasmodium vivax* at Bareilly district of Uttar Pradesh, India

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## Introduction

Emergence of antimalarial resistance in malaria parasite may impede the efforts of malaria elimination. Chloroquine as first line treatment for *Plasmodium vivax* is used since past six decades throughout India. Single nucleotide polymorphisms as genetic markers are associated with resistance against chloroquine, sulfadoxine and pyrimethamine in *P. vivax*.

## Objective

Investigation of the genetic markers of antimalarial resistance within *Plasmodium vivax* isolates collected from Bareilly district.

## Methods

A total of n=249 Rapid diagnostic test positive *P. vivax* isolates were collected from eight community health centres across Bareilly district during July-September in year 2022-25. Molecular diagnosis was performed on DNA isolated from dried blood spots (n=161) and followed by PCR amplification (n=155) of antimalarial resistance markers *pvcrt-o, pvmdr-1, pvdhfr* and *pvdhps* genes and subsequently Sanger sequenced for variant calling.

## Results

In total (n=155) sequenced isolates showed nonsynonymous mutants *viz.* T958M, Y1028C and F1076L in *pvmdr-1* gene. The double mutants T958M+Y1028C, T958M+F1076L and triple mutant T958M+Y1028C+F1076L, were observed in 23.48% (31/132), 76.5% (101/132) and 3.03% (4/132) respectively. The K10 insertion in *pvcrt-o* gene was observed in 10.88% (16/147) isolates. Double mutant S58R+S117N, were observed in 12.5% (13/104) of the *pvdhfr* gene. Synonymous single mutants Y69, N103 and nonsynonymous S93H were also observed in 14.4% (15/104), 1.92% (2/104) and 0.96% (1/104) respectively. Single mutant D417A in *pvdhps* gene was observed in 5.77% (6/104).

## Conclusion

Circulation of double and triple mutants in *pvmdr-1* gene and K10 insertion in *pvcrt-o* gene infers possibility of emergence of chloroquine resistance within *P. vivax* population at Bareilly district. The presence of the double mutant along with few single mutations in *pvdhfr* and *pvdhps* genes also suggests early signs of emerging antifolate drug resistance and highlights the need of regular molecular monitoring.

P-48

# High *Plasmodium ovalecurtisi* and mixed-species infections during malaria outbreak in Udalguri, Assam, India in 2024

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**Introduction and objectives**: India is committed to malaria elimination by 2030. However, Assam is one of the states with its three districts (Udalguri, Chirang and Kokrajhar) contributing to the recent rising burden of malaria. This study reports unusual insights stemming from a malaria outbreak investigation in Udalguri district in August 2024.

**Methods**: The outbreak investigation team conducted limited house-to-house surveys, collected finger-prick blood samples from suspected malaria patients, and collected mosquitoes (adult and larvae) from their surroundings. Blood samples were analysed for *Plasmodium* infection using rapid diagnostic tests (RDTs), microscopy and realtime qPCR. Mosquitoes were morphologically classified into genus/species and analysed for presence of *Plasmodium* using qPCR.

**Results**: Out of 68 blood samples, RDT, microscopy and qPCR were done on 68, 67 and 22 samples, respectively. The prevalence of *Plasmodium* infection varied between RDT (5/68;7%), microscopy (4/67;6%) and qPCR (20/22;91%). Majority of qPCR-positive samples were mixed-species infections comprising *P. falciparum*, *P. vivax* and *P. ovalecurtisi*. Presence of *P. ovalecurtisi* in 23% cases (5/22) was unusual. Adult mosquitoes (n=4) were *Anopheles vagus* (with no *Plasmodium* infection) whereas the larvae were *Culex* (n=329) and *Aedes* (n=16).

**Conclusions**: Although limited by sample size, this outbreak investigation highlighted that a large proportion of *Plasmodium* infection remains undetected by RDT and microscopy. Further, an unexpected presence of *P. ovalecurtisi* in almost one-fourth of qPCR-positive cases raises a flag. This hidden burden of malaria parasites might be related with a sustained increase in the number of malaria cases in the area.

**Key words**
Outbreak, malaria, *P. ovalecurtisi*, mixed infection, Assam, India

P-49

# Comparative Genomic and Evolutionary analysis of cuticular proteins in *Anopheles stephensi* and *Anopheles culicifacies*

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**Background**

*Anopheles culicifacies* and *An. stephensi* are the key vectors of malaria in India. However, the increasing incidence of insecticide resistance in these species poses a major challenge to vector control strategies. Thickening of the cuticle layers made up of cuticle proteins (CPs) and chitin, can reduce insecticide penetration.

**Aim**

To determine and annotate cuticular proteins in major malaria vectors in India, *An. stephensi* and *An. culicifacies.*

**Methodology**

To predict the possible orthologous CPs in *An. culicifacies* and *An. stephensi* were identified through comparative analysis with *An. gambiae* sequences using VectorBase. The chromosome alignment was created by using map2gene software. For conserved domain structure analysis, we used the SwissModel, GalaxyWeb and PyMol for all sub-families of CPs from *An. gambiae* and *An. culicifacies,* Phylogenetic relationships among CPs were reconstructed using Mega11.

**Results**

*Anopheles stephensi* has 166 identified cuticle proteins (CPs), while *Anopheles culicifacies* has 119, and *Anopheles gambiae* possesses 314 CPs. Phylogenetic analysis suggests CPAP family evolved much earlier and have since remained relatively conserved as inferred from their fewer branching nodes in the phylogenetic tree compared to other CPs, whereas the CPR family, appears to be the most ancient but more diverged in the phylogenetic tree. A few uncharacterised CPs appeared to have diverged from the CPR lineage. Additionally, several unclassified proteins are found between the CPLCG, CPCFC, and CPCT protein families.

**Conclusion**

Cuticle proteins have diverged over time to support survival throughout the evolution. Leveraging bioinformatics tools may help identify key molecular targets for developing effective strategies to manage vector populations and the rising threat of insecticide resistance.

**Acknowledgement**

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P-50

# Automated Parasite Density Estimation Using YOLO-Based Object Detection in Microscopy Images

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## Introduction

Accurate parasite density estimation is essential for malaria diagnosis, clinical decision-making, and monitoring treatment response. Conventional microscopy-based counting of *Plasmodium vivax* and *Plasmodium falciparum* parasites and white blood cells (WBCs) is time-consuming and subject to human variation. Automated detection systems offer a potential solution, particularly in low-resource settings where trained microscopists may be limited.

## Objectives

This study aimed to develop and validate a YOLO (You Only Look Once)-based object detection model capable of identifying malaria parasites and WBCs in stained blood smear images, and to compute parasite density automatically using standard clinical formulas.[1]

## Methods

The dataset used in this study [2] was annotated with bounding boxes for parasites and WBCs and used to train YOLO for two-class detection.[3] YOLO was selected for its fast real-time detection and suitability for mobile deployment. The model was trained and validated on annotated clinical samples, using performance metrics such as precision, recall, and mean Average Precision (mAP). An automated pipeline was implemented to count detected parasites and WBCs and calculate parasite density.

## Results

The trained model achieved high detection accuracy for both parasites and WBCs, with strong mAP values across classes. In addition to evaluation on the annotated dataset, the trained model was further validated using real-life clinical blood smear images. These images were not part of the original training set. Parasite density for these clinical samples was calculated manually by an experienced microscopist and used as a reference standard. The trained YOLO-based model was then applied to these clinical images, and the automatically estimated parasite densities were compared against the manual counts. The model demonstrated good agreement with expert derived parasite density values, confirming its ability to generalize beyond the training dataset. This also prompted us to integrate the model into a lightweight mobile compatible framework for rapid field level detection.

## Conclusions

This study presents an effective approach for automated parasite density estimation using YOLO-based object detection. The system offers rapid, accurate, and low-cost detection support, with strong potential for deployment in resource-limited settings. Further validation on larger and more diverse clinical datasets is required.

## Acknowledgments

We gratefully acknowledge Ugrasen Bharti (ICMR-NIMR, Dwarka, New Delhi) for acquiring the microscopic images from blood smear slides and for performing manual parasite density calculations used for validation in this study.

**Keywords:** Malaria, Parasite Density, YOLO, Microscopy, Deep Learning

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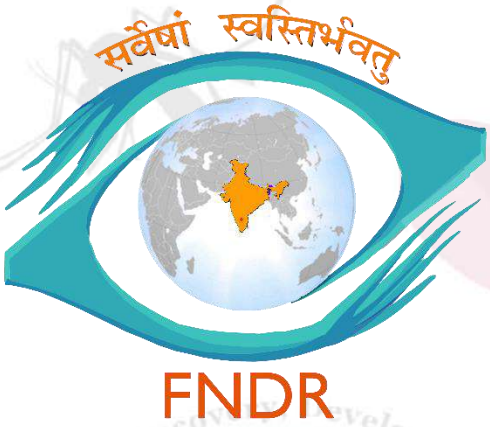


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