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Plasmodium malariae is an overlooked malaria parasite with emerging challenges

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Plasmodium malariae (P.m.) represents the least studied of the five human-malaria-causing *Plasmodium* species, despite its widespread global distribution. Control of *P.m*. is challenging due to the parasite's unique biological features, unavailability of *P.m*.-specific diagnostic methods, chronic low-grade parasitemia, and suboptimal clinical features. Emerging evidence suggests increasing antimalarial drug resistance and reduced susceptibility to first-line antimalarials. Its capacity for chronic infection, diagnostic challenges, and emerging drug resistance threaten malaria elimination efforts. Thus, it represents a significant yet underappreciated contributor to global malaria burden. Enhanced molecular diagnostics, targeted therapeutic strategies, and improved surveillance systems are urgently needed to address this neglected pathogen and prevent its resurgence when other malaria species are under control. Here, we synthesize current knowledge on *P.m*. biology, public health impact, immune paradigm, and clinical manifestations. We discuss the research gaps, outstanding questions, and novel approaches to study *P.m*. biology.

Malaria holds a unique place in history, affecting people from the Neolithic to early Chinese, Roman, and Greek societies, infecting princes and paupers alike. Presently, it primarily afflicts people on lower income from countries including sub-Saharan Africa, Asia, the Amazon basin and other tropical regions[1]. On October 20, 1880, Charles Louis Alphonse Laveran first discovered crescent-shaped bodies in the blood of a febrile soldier, identifying the malaria parasite and naming the organism, *Oscillaria malariae*[2]. Later, Ettore Marchiafava and Angelo Celli in 1885 coined the genus name *Plasmodium* due to its resemblance to a slime mold with the same name[3,4]. Although initially met with skepticism, Laveran's findings were validated six years later by the famous Camillo Golgi, who linked the release of malaria parasites to fever cycles. Golgi demonstrated two forms of disease, one with tertian fever and another with quartan fever. In 1890, Giovanni Battista Grassi and Raimondo Filetti named the parasite causing tertian fever *Haemamoeba vivax* or *Plasmodium vivax (P.v.)* and the parasite causing quartan fever *Haemamoeba malariae* or *Plasmodium malariae (P.m.)*[5]. Due to its mild clinical symptoms, *P.m*. attracted less attention and became the least studied parasite out of the five human-infecting *Plasmodium* species, which include *P. falciparum (P.f.), P. vivax (P.v.), P. ovale (P.o.)* and more recently *P. knowlesi (P.k.)*. *P.m*. is widely present in sub-Saharan Africa, Southeast Asia, the Western Pacific, and South America[6]. In endemic regions, the microscopic *P.m*. prevalence among all malaria cases ranges between 4–20%, but there is evidence that *P.m*. infections are vastly underreported[7]. Recently, this neglected species gained attention when an unprecedentedly high *P.m*. positivity (46%, 164/356) was reported in an outbreak situation in Vietnam[8]. Widespread geographic distribution of *P.m*. includes high polymerase chain reaction (PCR) positivity in the Colombian Amazon region[9], limited infection rate in areas of the China-Myanmar border[10,11], Cambodia[12], south and north Indonesia[13,14], the Eastern and North-eastern parts of India[15–17], Brazil, and Thailand[7]. Generally, sub-Saharan Africa is the most affected region, showing a continuous increase in *P.m*. prevalence over a period of time[18,19]. *P.m*. is often misdiagnosed as *P.f*. because the ring forms of *P.f*. and *P.m*. are morphologically more similar to each other than to *P.v*. and *P.o*[5]. Such inabilities to differentiate species microscopically complicate routine diagnosis, resulting in the wrong treatment and thereby leading to untreated asymptomatic disease reservoirs, which jeopardize malaria elimination programs[20].

The technological advancements and steady increase in the frequency of *P.m*. incidence in the past two decades have attracted the attention of parasitologists towards this neglected parasite species. Therefore, some exceptional reviews have been published highlighting the important contribution of *P.m*. in disease burden[5,21–23]. Nevertheless, a few convincing reviews in the late 60s have also discussed striking features of *P.m*. and its course of infection[24,25]. Here, we have described *P.m*.'s unique biological features, ability to sustain chronic low-grade parasitemia, persistence in co-infections, clinical manifestations and polymorphic antigen variants used to escape host immunity. We have also discussed available diagnostic methods to detect *P.m*., alongside their key limitations. Lastly, we proposed the

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research gaps, current understanding, and outstanding questions, accompanied by proposed approaches to the study of *P.m.* biology.

Literature search methodology

We searched PubMed, Web of Science, and Scopus databases to retrieve articles relevant to *P.m.* The articles were searched up to 31 August 2025. We performed the literature search in PubMed using medical subject headings (MeSH) terms with Boolean operators to extract documents that are most relevant to *P.m.* malaria. The search language was restricted to English. The combination of MeSH terms and Boolean operators generated a set of search strings that retrieved 641 articles in PubMed (Supplementary Data). Similarly, *P.m.* literature search in Web of Science showed 2176 results and 1985 documents in Scopus. A list of search strings created to fetch the literature on *P.m.* is detailed in the Supplementary Data. After careful evaluation of the title and abstract of the studies, only those studies focused on *P.m.* mono-infections or *P.m.* co-infections or mixed infections were included. There was no restriction against inclusion of research or review articles, systematic reviews meta-analysis, case reports and case series, editorials, experimental animal studies, brief communication, surveillance, and drug resistance studies. However, irrelevant and duplicate studies and those concentrated exclusively on *P.f.*, *P.v., or P.o.* biology or their interactions during mixed infections were excluded.

Taxonomy and classification

P.m. belongs to the phylum *Apicomplexa*, class *Aconoidasia*, order *Haemosporida*, suborder *Haemosporidiidea*, family *Plasmodiidae* and genus *Plasmodium*. It is classified in the subgenus *Plasmodium*.

Life cycle and biology

The identification of *P.m.* as a distinct species was based on its unique quartan fever cycle, in which fevers recur every 72 h. This periodicity differentiates it from the tertian fever cycle (48 h) of *P.f.* and *P.v.*, the two most common and fatal *Plasmodium* species. *P.m.* additionally has a smaller number of merozoites released from each schizont (~6–12) and a strict preference for mature red blood cells (RBCs)[5]. Like other *Plasmodium* species, *P.m.*'s life cycle involves both an insect vector (*Anopheles* mosquitoes) and a human host. When an infected female mosquito bites a human, the parasites, in a life stage called sporozoites, enter the bloodstream and travel to the liver, where they mature and multiply. From the liver, they re-enter the bloodstream, infecting RBCs and leading to the characteristic fever cycles and other symptoms. Some species, like *P.v.* and *P.o.*, leave dormant parasitic forms in the liver called hypnozoites. These forms can re-emerge and cause symptoms months or even years post-infection, however, it is still a subject of debate whether *P.m.* forms hypnozoites or not[26].

Evidence that supports hypnozoite formation in *P.m.* infection includes observations of prolonged, late-onset infections, sometimes re-emerging years after the initial exposure, which suggests a potential dormant stage in the liver[27,28]. Some clinical cases exhibit relapse-like patterns, similar to the behavior of hypnozoite-forming species like *P.v.* and *P.o.*, supporting the possibility of hypnozoite formation in *P.m.*[29]. However, there is no direct microscopic or molecular evidence of hypnozoites in liver biopsies or in vitro studies. The species is known for prolonged low-density blood infections, which can recrudesce without a dormant liver-stage parasite[26]. Moreover, *P.m.* does not respond to anti-hypnozoite drugs like primaquine, which are effective against *P.v.* and *P.o.* Genomic studies also lack evidence of hypnozoite-associated genes, strengthening the case against a hypnozoite stage in *P.m.*[30]. Therefore, primaquine inefficacy could be due to lack of hypnozoites, as there is no clear primaquine resistance reported in *P.m.* Overall, further research is needed, particularly focusing on liver-stage biology, to definitively resolve the question.

P.m. preferentially invades older RBCs, infecting about 0.1% of the total erythrocyte population in the human body[31]. During the early trophozoite stage, it accumulates hemoglobin breakdown products as hemozoin pigments, as well as pink-staining Ziemann's dots (small, discrete and pale refractile structures), which differentiate *P.m.* from other *Plasmodium* parasites. *P.m.* trophozoites have compact cytoplasm and a large chromatin dot. They appear occasionally as band or basket forms with a coarse, dark-brown pigment (Fig. 1). The schizont stage has 6–12 merozoites with large nuclei, clustered around a mass of the coarse dark-brown pigment. The merozoites can occasionally be arranged in a rosette shape. Merozoites synchronously rupture out of the infected cell every 72 h during asexual blood stage cycle with an accompanying fever, causing quartan malaria.

P.m. infections are often misdiagnosed due to the resemblance of ring stages between *P.m.* and *P.f.*; therefore, mature stages must be examined microscopically to differentiate the two species. While early *P.m.* trophozoites resemble *P.f.* rings, their mature stages (band forms, schizonts) may be misidentified as *P.k.*, a zoonotic parasite with 24-h cycle and higher virulence, as well as non-human primate parasites such as *P. inui* and *P. brasilianum*. Its prepatent period (the period between infection and detection of parasites in the blood) ranges between 16–59 days depending upon the strains, which are highly varied among the malaria-endemic regions[5]. The exoerythrocytic schizogony in liver cells, which is a characteristic of all *Plasmodium* species, takes a minimum of 13-15 days in *P.m.*, compared to only 6 days in *P.f.*[31]. *P.m.* also differs from *P.f.* in several other key biological aspects, such as lower parasite density, a slower growth rate, delayed gametocyte production, and a longer, often chronic duration of infection[32]. In addition to the above, a major reason for the relatively low pathogenicity of *P.m.* is presumed to be due to its inability to adhere to blood vessels, though this is not demonstrated yet. This potential inability of *P.m.* prevents it from causing cerebral, placental, or any other form of classical severe malaria, with a few exceptions of clinical severity.

The genome size of *P.m.* is 33.6 Mb, which is comparatively higher than the genomes of *P.v.* and *P.f.* It is organized into 14 chromosomes, like other malaria parasites. The *P.m.* genome has a GC content of 24.7% as compared to *P.v.* (39.7%) and *P.f.* (19.3%). Approximately 40% of the *P.m.* genome is sub-telomeric and contains only a restricted subset of *Plasmodium*

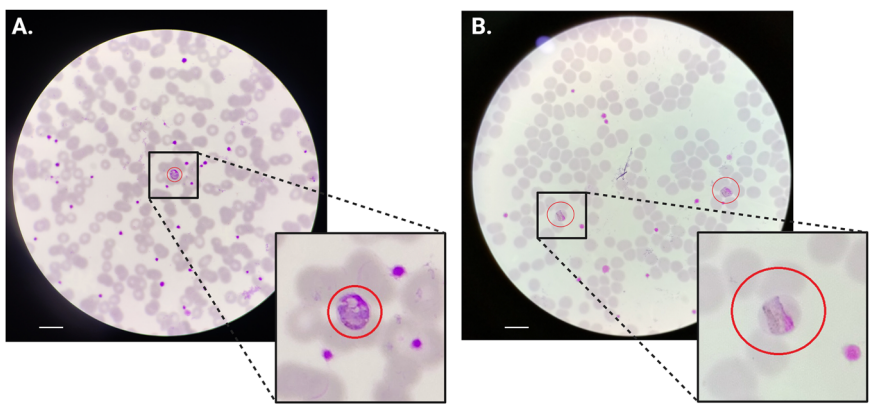

Fig. 1 | Morphological identifying features of *Plasmodium malariae (P.m.)*. Giemsa-stained asexual stages of *P.m.* showing **A** basket form trophozoite and **B** band form trophozoites in human blood. The slide was prepared during the malaria screening in Mizoram state, Northeast India. Scale bar is approximately 20 μM.

interspersed repeat (pir) gene repertoires compared to *P.o.* and *P.v.*[33,34]. The *P.m.* genome displays some unique characteristics, such as the presence of two large families, fam-l and fam-m, which have structural similarity to reticulocyte binding protein homolog 5, a protein known to have a putative role in adhesion to RBCs[35].

Diagnosis

The major burden of malaria in endemic areas is due to *P.f.* infections, although *P.v.* also contributes substantially to Southeast Asian and South American regions. However, misdiagnosis of non-*falciparum* infections by light microscopy also contributes to *P.f.* counts in endemic regions[31]. Contributing reasons include non-experienced microscopists failing to distinguish between *Plasmodium* species, lack of high-quality microscopes, asymptomatic and low-density *Plasmodium* infections, and the tendency of *P.m.* parasites to remain low-profile, particularly at early stages, becoming undetected by light microscopy[36]. *P.m.* often co-exists with other *Plasmodium* species in endemic regions, especially *P.f.*, and therefore it is often misdiagnosed in mixed or asymptomatic infections[22]. Moreover, rapid diagnostic tests (RDTs) are inefficient, showing extreme variability in sensitivity (true positive) and specificity (true negative) in the detection of mono-*P.m.* infections[37] (Table 1). Variability in detecting true positive and true negative *P.m.* cases or non-*falciparum* malaria greatly varies among the types of RDTs used for the diagnosis. Systematic reviews and meta-analysis studies on diagnostic capabilities of RDTs to detect *P.m.* or non-*falciparum* infections revealed that Type 2 RDTs (*P.f.*-histidine rich protein-2 (HRP2) + pan-aldolase) could detect nearly 80% of PCR-confirmed *P.m.*-mono-infections[37] or non-*falciparum* malaria[38]. Similarly, other RDT types such as Type 3 RDTs (*P.f.*-HRP2 + pan-*Plasmodium* lactate dehydrogenase (pLDH)), Type 4 RDT (*P.f.*-LDH + pan-pLDH) and Type 6 RDTs (*P.f.*-HRP2 + *P.v.*-pLDH + pan-pLDH) also showed variable sensitivity and specificity, with poor performance (sensitivity: 32–67%) seen in Type 6 RDTs for the detection of *P.m.*-mono-infections (Table 1). All these factors contribute to underestimation of *P.m.* infection rates. Thus, better quality microscopy operated by trained personnel should be employed in order to accurately diagnose all human malaria parasite species (particularly *P.m.* and *P.o.*), until point-of-care molecular tools are deployed to achieve malaria elimination.

Recent advancements in molecular or enzymatic assays have led to better diagnosis of *P.m.* infections (Table 1). Reports suggest that microscopy negative and PCR positive *P.m.*-infection rate varies between 12–71% in the global South region. Lo et al.[39] reported that 71% (32/45) of *P.m.*–positive samples (mono-infections) detected by PCR were undetected by microscopy in Kenya. Similarly, in the Yaoundé region of Cameroon, *P.m.* mono-infections in asymptomatic individuals remained undetected by microscopic examination of blood smears, whereas PCR-based diagnosis revealed a 12% infection rate[40]. Another region of Cameroon, the Adamawa Region, reported 17% PCR confirmed *P.f.* + *P.m.* coinfections with a sporozoite infection rate of 8.4%, highlighting a significant contribution of *P.m.*, in addition to *P.f.*, to sustained high malaria transmission rate in the region[41]. A recent outbreak of *P.m.* with nearly 46% positivity was recorded in Vietnam. All the *P.m.* cases were microscopy and PCR positive, but were negative by RDT. Almost 90% of *P.m.* positive samples in this outbreak had gametocytes with a median density of 52 per microliter, suggesting a high probability of transmission[8]. Evidence for widespread human exposure to *P.m.* is demonstrated by its detection at a high frequency (~44% PCR positivity) in the Colombian Amazon region[9], in contrast to much lower detection rates along the China–Myanmar border (less than 1%)[10,11]. A prevalence of 1.3% was identified through restriction fragment length polymorphism–denaturing high-performance liquid chromatography in Cambodia, where microscopy failed to detect any *P.m.* cases[12]. Some other Southeast Asian countries reported heterogeneous prevalence of *P.m.* ranging from as low as one microscopic case in North Sumatra province, Indonesia[14] to 37% (47/127) *P.m.*-positive smears in South Sumatra province, Indonesia[13]. The Indian state Odisha, which alone contributes nearly 40% of India's total malaria cases, indicated significant *P.m.*-positivity of around 9–10% using PCR[15,16]. However, our group observed a meager number of microscopic *P.m.* cases in a Northeastern Indian state, which were found negative by RDTs[17].

Relatively little is known about the dynamics of *P.m.* gametocyte commitment and transmission, but studies suggest that *P.m.* co-infection has an impact on *P.f.* gametocyte densities[18,42]. It is becoming evident that *P.m.* gains advantage from co-infections with other *Plasmodium* species, demonstrating a profound increase in both asexual stage and gametocyte densities in these instances. This highlights the pronounced impact of co-infections on the biological proliferation of *P.m.*, where it not only thrives alongside but sometimes outcompetes co-infecting species[43]. An exception for these observations is one study which reports a decrease in asexual and gametocyte forms of *P.m.* in the presence of *P.f.* co-infection[18], linking this inhibitory effect on *P.m.* to the development of severe disease. Therefore, future studies involving the role of host immunity, erythrocyte ecology, and parasite genetic diversity will be essential to explain the suppression or advantage of *P.m.* in mixed infections. However, elimination or significant reduction in *P.f.* infections (for example, through recent vaccine development efforts) may lead to aggravated *P.m.* parasitemia and severe malaria infection, which otherwise remains low-profile[18]. All this evidence suggests that *P.m.* species is far more prevalent than previously thought[23].

Nevertheless, upcoming diagnostic tools have demonstrated the ability to discriminate *P.f.* from *P.m./P.o.* infections. These newer RDTs are based on the pan- pLDH-to-*P.f.*-LDH ratio using malaria immunoassay, and are specifically useful in regions with *P.f.*- HRP2 deletions[44]. Due to the remarkable conservation of pLDH across all major human-infecting *Plasmodium* species (higher than 90% identity), it is a potential target for malaria detection using aptamer-based diagnostic methods[45]; however, until now, this approach is only confined to *P.f.* and *P.v.* diagnosis[46,47]. A large-scale sero-surveillance study detected the presence of immunoglobulin-G antibodies against *P.m.* merozoite surface protein 1 (*Pm*MSP1) antigen in 278 out of 21,719 school children. Sero-positivity in children aged 6 to 7 years indicates that exposure to the infection in this region has occurred frequently, as such sero-positivity can only be attributed to natural infection[48]. In addition to the above diagnostic methods, several new advanced tools promise improved diagnosis and surveillance of *P.m.* These include a CRISPR-based nucleic acid detection platform SHERLOCK (specific high-sensitivity enzymatic reporter unlocking)[49,50], recombinant *Pm*MSP1[51], recombinant apical membrane antigen-1 (AMA1)[52], and cooperative primer-based RT-PCR[53]. Recently, a variety of field-deployable tests have been developed based on CRISPR, recombinase-aided amplification, LAMP, etc. Some of these utilize rapid, isothermal recombinase polymerase amplification (RPA) and lateral flow detection to detect *P.m.* with a detection limit ~10–100 times better than the existing pLDH tests[54], with an analytical sensitivity of 100 pg per microliter of blood infected with *P.m.*[55]. These methods combine microfluidic paper-based analytical devices with specific primers and probes against *Plasmodium* 18S ribosomal RNA and an integrated RPA on the paper substrate for the detection of *Plasmodium* species as low as 0.028 parasites per microliter of blood[56]. Also, an optimized, cost-effective RT-PCR method has been reported to detect as low as 0.32 parasites of *P.m.* per microliter of blood[57]. The above-mentioned diagnostic methods with varying accuracy and limitations to detect *P.m.* infection are summarized in Table 1. These recent engineered solutions are of utmost importance to improve the detection of *P.m.* infections and support more accurate malaria surveillance and elimination efforts.

Clinical manifestations and public health impact

Historically, during the ancient Greek and Roman periods, quartan fever was believed to have a potentially beneficial effect on neuropsychiatric patients for many centuries[58]. Nevertheless, modern research has failed to demonstrate any neuro-immunological connection between malarial fever and mental health. In contrast to *P.f.*, *P.m.* infections are generally less severe; however, they can persist for extended periods. If left untreated, these infections may result in severe anemia, renal syndrome, and convulsions[59]. In a study conducted in southern Papua, Indonesia, patients with *P.m.*

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Table 1 | Diagnostic methods used for the detection of *Plasmodium malariae* infection

Diagnostic method	Target species	Sensitivity/LOD*	Specificity	Key limitations	References
Rapid diagnostic tests (RDT)					
Type 2 RDT (*P.f.*-HRP2+ pan-aldolase) vs. PCR	*P.m.*	0–80% (highly variable)	42–99% (Highly variable)	Small number of *P.m.* mono-infection cases were evaluated (median 5 cases per study); low statistical power due to meta-analysis study type; varying reference standard used (mostly PCR and in a few studies, microscopy).	37
Type 3 RDT (*P.f.*-HRP2+ pan-pLDH) vs. microscopy	*P.m.*/mixed *Plasmodium* infection or non-*falciparum* species	14–100% (highly variable)	65–100%	Lack of *P.m.*-specific data, rather diagnosis reported for non-*falciparum* malaria in Cochrane review; heterogeneity across study types and the reference methods used in the studies selected in meta-analysis.	37,38
Type 4 RDT (*P.f.*-LDH + pan-pLDH) vs. microscopy	*P.m.*/non-*falciparum*	0–100% (highly variable)	72–91%	Unable to distinguish *P.m.* from *P.o.*; Unclear study design in many studies; a smaller number of *P.m.* cases in the selected studies.	37,38
Type 6 RDT (*P.f.*-HRP2 + *P.v.*-pLDH + pan-pLDH) vs. microscopy	*P.m.*	32–67%	99%	Extremely less number of studies included; different brands used for RDT evaluation.	37
Light microscopy					
Microscopy vs. nested PCR	*P.m.*	Missed ~71% of PCR-positive cases (extremely low)	Not reported	Less number of samples; Microscopy found highly insensitive to detect asymptomatic *P.m.* cases.	39
Molecular methods					
Optimized Real-time qPCR vs. microscopy	*P.m.*	95%; LOD: 0.32 parasite/µl (analytical sensitivity)*	95%; Excellent (no cross-reactivity to other species)*	Unable to detect mixed *Plasmodium* infections as *P.m.* is often present in mixed infections.	57
CRISPR-SHERLOCK	*P.m.*; *P.f.*; *P.v.*; *P.o.*; *P.k.*	94% for *P.f.*; Not reported for non-falciparum species	94% for *P.f.*; Not reported for non-falciparum species	Used cultured parasites & not field-collected or diverse clinical samples; CRISPR-RNA design may not account for all circulating variants of non-*falciparum* species.	49
Cooperative primers	*P.m.*	Analytical sensitivity rose 10-times than conventional primers	Not reported	No clinical validation; Absence of diagnostic benchmark i.e., no comparison with gold standard tests.	53
Molecular methods—Field deployable					
Rapid isothermal amplification lateral flow assay vs qPCR	*P.m.*	95%	88%	False positivity in a *P.v.* sample; visual interpretation of lateral flow (faint band in low-endemic settings) could create bias.	54
Ultrasensitive CRISPR-SHERLOCK	*P.m.*; *P.f.*; *P.v.*; *P.o.*	100% for *P.f.* and *P.v.*; LOD: 0.36–2.4 parasites/µL for *P.f.* and *P.v.*, but for *P.m.* is 1.9 parasites/µL*	100% for *P.f.* and *P.v.*; LOD: 0.36–2.4 parasites/µL, including *P.m.* at 1.9 parasites/µL*	Very low sample size; No clinical performance check on *P.m.* samples.	50
Recombinase-aided amplification with lateral flow dipstick assay	*P.f.*; *P.v.*; *P.m.*; *P.o.*	100 pg/µl—Analytical sensitivity (LOD) for *P.m.**	Not reported	Less sensitive for *P.m.* compared to *P.f.* (0.1 pg/µl); not good in low parasitemia settings.	55
Paper-based microfluidic LAMP device	*P.f.*; possibly other species	98%	High as seen by comparison with gold standards, but not stated explicitly	*Plasmodium* species-specific data not shown; not clear about the robustness of *P.m.* diagnosis.	56
Immunoassays					
Recombinant proteins of *P.m.* merozoite surface protein 1 (*P.m.*-MSP1)	*P.m.*	Not reported	High-human anti-*P.m.* sera reactivity; non-reactive to *P.f./ P.v.* sera	Small sample size; immunogenicity tested on mouse model; not checked diagnostic performance on clinical samples/cohorts; limited cross-reactivity testing (tested for *P.f.* & *P.v.*).	51
5-plex malaria immunoassay vs. PCR	*P.m.*	96.2–100%	96.1–97.1% against malaria-negative controls	Significant cross-reactivity of *P.f.*-LDH in *P.m.* infections with specificity of 3.9%.	44
Aptamer-linked immobilized sorbent assay (ALISA)	*P.v.*	96%	100%	No aptamer-based testing for *P.m.* exists; data are lacking.	46
Aptamer-tethered enzyme capture bead-based assay	*P.f.*	~90%	Not reported	No aptamer-based testing for *P.m.* exists; data are lacking.	47

P.m. Plasmodium malariae, *P.f. Plasmodium falciparum*, *P.v. Plasmodium vivax*, *P.o. Plasmodium ovale*, *P.k. Plasmodium knowlesi*, HRP2 *histidine rich protein-2*, *PCR polymerase chain reaction*, pLDH *Plasmodium lactate dehydrogenase*, qPCR *quantitative real-time PCR*, LAMP *loop-mediated isothermal amplification*, CRISPR-SHERLOCK *clustered regularly interspaced short palindromic repeats-specific high-sensitivity enzymatic reporter unlocking.*

*Limit of detection (LOD).

infections had a lower mean hemoglobin concentration than patients with other malarial parasite infections[60]. Similarly, in another study conducted in the same region, *P.m.* infected patients had the lowest average hemoglobin concentrations[61]. *P.m.* infections can also lead to chronic membranous glomerulopathy[62], nephrotic syndrome[63,64] or acute kidney injury[65]. Almost 50% of *P.m.*-associated renal complications are accompanied with proteinuria, and despite being treated with antimalarials, over a period of 3–5 years, infection may eventually result in renal failure[62]. In contrast, an experimental malaria-associated acute kidney injury (MAKI) model demonstrated that antimalarial treatment fully resolved the MAKI pathologies[66]. Moreover, in this mouse-infection model, kidneys were not the preferred sites of parasite sequestration, though renal sequestration by *P.f.* among MAKI cases is well documented in sub-Saharan African countries[67]. Overall, these studies suggest that malaria-associated renal complications vary among the infecting *Plasmodium* species.

A recent case report further complicates the clinical manifestation of *P.m.* malaria. It is a case of a congenital *P.m.* infection in a two-month-old infant born in a non-endemic country (Germany). The mother traveled to a malaria-endemic country (Nigeria) more than two years before the date of delivery, where she reportedly suffered from *P.f.* malaria and was treated with chloroquine[68]. This case study suggests that the mother either had *P.m.* infection which was misdiagnosed as *P.f.* in the past, or she had co-infection with both *P.f.* and *P.m.* but *P.m.* was not correctly identified. Strikingly, that sub-patent and chronic *P.m.* parasitemia could manage to pass through the placenta and caused neonatal complications, which is a serious public health concern in the regions endemic with *Plasmodium* co-infections. Some studies suggest that school-aged children are the most vulnerable group to *P.m.* infections[19]. However, others have shown that adults can also have long-term *P.m.* infections, often because they have been exposed to the infection more frequently as they've aged[69]. A longitudinal household survey was accompanied with health-clinic visits during the follow-up period of 34 months in Kinshasa Province, Democratic Republic of Congo (DRC)[19]. The study reported that in addition to *P.f.* infection, *P.m.* incidence increased in a stepwise manner from baseline to the follow-up sampling at around 12 months and thereafter until the end of the survey. Also, during the active screening, there were individuals who were detected with persistent *P.m.* infection four times throughout the follow-up, suggesting a long-lasting infection; though, the parasite strains were not sequenced to distinguish between recrudescence or reinfection. Similar observations were recorded in another study conducted during the malaria wet season across 4 years among children aged 3–15 years from Burkina Faso. The prevalence of *P.m.* increased drastically during this period from ~1 to 13%, with a significant rise in gametocyte prevalence, particularly after the rainy season[18].

A molecular epidemiological study in Malawi demonstrated the extent of similarities between *P.f.* and *P.m.* population structure during seasonal and perennial malaria transmission seasons[32]. The authors observed a high degree of multiple genotype infections in *P.m.* cases, despite their lower prevalence and density. Due to its low prevalence and transmission potential, *P.m.* is expected to have lower multiplicity of infection (MOI) compared to *P.f.*, but a similar MOI was observed for both species[32]. Thus, the long-lasting nature of *P.m.* infections, often with low-grade parasitemia, poses challenges for diagnosis and treatment. A recent genomic study of *P.m.* was conducted on clinical isolates collected from the four high-transmission countries of Africa: Cameroon, DRC, Nigeria, and Tanzania[70]. The study shows that *P.m.* has a surprisingly uniform genetic structure across Africa, unlike *P.f.* Its low diversity and high genetic similarity suggest that it went through a population crash in the past, but is now recovering. Some genes related to red blood cell invasion and drug resistance show signs of recent adaptation, which may influence the spread of *P.m.* in the near future[70]. However, because most surveys are based on short-term clinical samples focusing on parasite densities and patient illness, they capture only a single generation of parasites[71]. Such single-generation experiments cannot be directly used to infer how virulence evolves over evolutionary time. Mathematical models showed that mixed infections are influenced not only by resource competition, but also by plasticity and immune dynamics. Therefore, experiments on single infections can be misleading if used to predict how parasite harmfulness will evolve in the long run[71]. This may help explain how *P.m.* persists in co-infections with more virulent parasites like *P.f.*

Investigation of the molecular mechanisms employed by *P.m.* to invade RBCs showed homology to other *Plasmodium* species[72]. Specifically, orthologues of known *P.f.* ligands such as *P.f.* reticulocyte homology protein-2b (PfRh2b) and *P.f.* Rh5-interacting protein are natural candidates, in addition to others yet to be identified. However, antibody-mediated blocking of RBC receptors known for *P.f.* ligand-receptor interactions did not result in *P.m.* invasion deficit. This suggests that *P.m.* merozoites invade RBCs by additional mechanisms. Despite the well-known fact that *P.m.* preferentially infects mature RBCs, this recent report demonstrated its invasion capabilities in reticulocytes, which needs further validation[72]. Together, these results highlight that *P.m.* is genomically unusual, still adapting, and uses invasion mechanisms distinct from *P.f.*, possibly allowing itself to persist in mixed infections and contribute more significantly to malaria than previously recognized. Studies performing mathematical modeling predictions of species replacement dynamics could provide valuable insights. Additionally, it would be interesting to monitor a trend of *P.m.* cases in areas with declining *P.f.* transmission.

There is currently no specific antimalarial available to target *P.m.* infection. The World Health Organization (WHO) has recommended that, in cases of ambiguity, the treatment for *P.m.* should follow the protocol used for uncomplicated *P.f.* malaria[73]. The WHO advocacy for the treatment of *P.m.* is based upon region-specific susceptibility to chloroquine. Areas in which chloroquine-susceptible parasite strains circulate have a choice of using Artemisinin Combination Therapy (ACT) or chloroquine. However, areas infested with chloroquine-resistant parasites should use ACT for the treatment of *P.m.* Nevertheless, the US Center for Disease Control and Prevention widely recommends the use of chloroquine to treat *P.m.* infections due to the very sporadic evidence for chloroquine-resistant *P.m.* Indeed, only a single study has reported chloroquine-resistance in *P.m.*[13]. Despite that, efficacy studies to use alternative antimalarials against *P.m.* are being performed. A recent clinical trial from central Africa evidenced high efficacy of pyronaridine-artesunate treatment against *P.m.* mono-infection cases with a cure rate of 99·2% (95% CI: 95·7–100)[74] showing promising treatment alternatives.

Recently, an ex vivo growth assay was performed in *P.m.* field isolates obtained from Mali, demonstrating that nearly half of the isolates had high inhibitory concentration (IC_{50}) towards Artemether or Lumefantrine. Furthermore, they found that only half of the tested clinical isolates were susceptible to chloroquine[75], suggesting emerging drug resistance against currently used treatments. Nevertheless, piperaquine was found effective at much lower IC_{50} against *P.m.* Another contemporary and unique study has developed an induced blood-stage malaria model of *P.m.* in humans, where they report this parasite's clearance half-life after artemisinin treatment was 6.7 h, which is longer than the cut-off value of 5 h for *P.f.*, thereby showing less susceptibility to artemether-lumefantrine than *P.f.* This research opens new avenues to study this parasite's biology (which otherwise is not cultivable in the laboratory), diagnosis, and treatments[76]. These findings could plausibly explain the reasons for artemether/ lumefantrine treatment failure. It is likely that the standard three-day regime may not be sufficient to tackle *P.m.*, which has a longer intra-erythrocytic asexual life cycle than *P.f.* (72 h instead of 48 h). Another major observation was the high frequency of *P.m.* infections in asymptomatic individuals. The studies suggest that asymptomatic *P.m.* infections contribute to chronic infections that may develop into malarial splenomegaly. When left undetected and untreated, this condition is associated with high morbidity and mortality [75]. Considering the low susceptibility of *P.m.* to standard antimalarials, the data support some promising treatment alternatives such as piperaquine/ di-hydropiperaquine and pyronaridine-artesunate, which are reported to have low IC_{50} and high cure rates, respectively. Additionally, some newly discovered compounds, such as *Plasmodium* phosphatidylinositol-4 kinase-specific inhibitor KDU691and imidazolopiperazine GNF179, have been tested and proven to

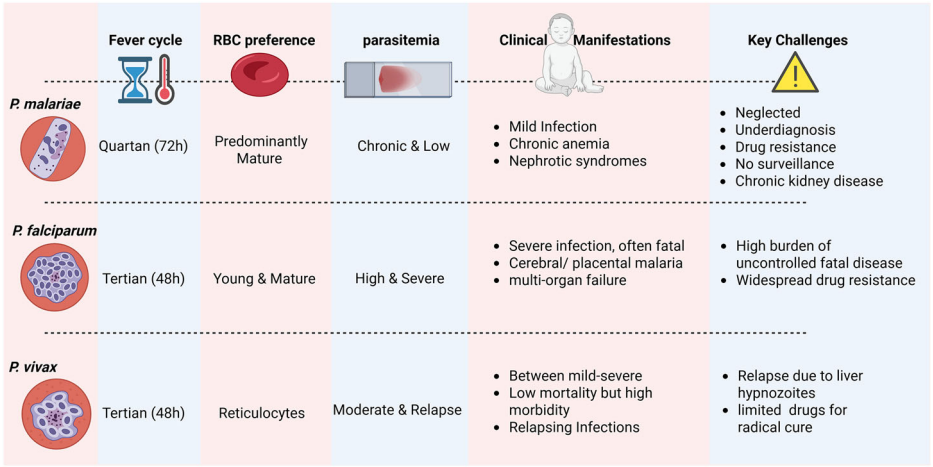

Fig. 2 | Comparison of *Plasmodium malariae* with other two predominant human-infecting *Plasmodium* species i.e., *Plasmodium falciparum* and *Plasmodium vivax* across key biological features, clinical and epidemiological characteristics, and key challenges.

Table 2 | Characteristics, immune evasion strategies, host immune responses, and clinical outcomes of *Plasmodium malariae* in comparison with *Plasmodium falciparum* and *Plasmodium vivax*

Features	*Plasmodium malariae* (*P.m.*)	*Plasmodium falciparum* (*P.f.*)	*Plasmodium vivax* (*P.v.*)
Parasite biology	72-h lifecycle, low parasitemia, chronic infections	48-h lifecycle, high parasitemia, acute/severe infections	48-h lifecycle, preferential invasion of reticulocytes, relapses due to hypnozoites
RBC tropism	Mature RBCs mainly; some evidence for reticulocyte invasion	Mainly young and mature RBCs	Preferentially reticulocytes
Antigenic variation	Polymorphic MSP1, pir-like variant surface antigens; immune evasion via variant expression and switching	Strong *P.f.*-EMP1-mediated variation; highly antigenic, complex evasion via cytoadherence and sequestration	Moderate antigenic variation; CSP and other surface proteins contribute to immune escape
Immune evasion mechanisms	Antigenic variation; complement inhibition; immune complexes causing kidney pathology (glomerulonephritis); possible splenic DC reservoirs	Cytoadherence or sequestration causing microvasculature occlusion; antigenic variation; immune modulation and destruction of dendritic cells	Hypnozoite formation enabling relapse; antigenic variation; moderate immune evasion
Immune response strength	Generally weaker antibody responses than *P.f.*; chronic exposure drives complement activation and immune complex deposition	Strong antibody responses and pro-inflammatory cytokines; immune exhaustion possible	Intermediate antibody responses; immunity affected by relapses and liver stages
Clinical severity and outcomes	Generally mild or chronic; renal complications from immune complexes; anemia; long-lasting asymptomatic infections	Severe malaria, including cerebral malaria and multi-organ failure	Generally less severe than *P.f.*; relapse and anemia common
Chronicity and persistence	Marked chronicity; ability to persist asymptomatically for years; immune modulation allows low-level persistence	Acute infections often cleared; immunity develops but reinfections common	Relapses cause chronic infection via hypnozoites; immune response partially protective
Immune complexes and kidney impact	Prominent immune complex deposition causing membranoproliferative glomerulonephritis	Less prominent in kidney; primarily vascular sequestration pathology	Less commonly associated with nephropathy

MSP1 *merozoite surface protein-1*, *P.f.*-EMP1 *Plasmodium falciparum-erythrocyte membrane protein1*, CSP *circumsporozoite protein*.

efficiently inhibit *P.m.* isolates at a very low IC_{50}, suggesting potentially strong potency of these compounds in field conditions[75].

Despite being overshadowed by more virulent malaria species, *P.m.* still plays a significant role in the global malaria burden. Emerging evidence suggests a nearly two-fold increase in *P.m.* infections between 2010–2016[20]. There are differences in the clinical presentation and epidemiology of this species among different endemic regions that could be due to the interactions between co-infected species under varying transmission intensities[77]. The ability of *P.m.* to cause chronic infections means it can persist in the population even when other malaria species are brought under control, thereby complicating eradication efforts. A comparison of *P.m.* with two other predominant *Plasmodium* species across key biological, clinical, and epidemiological characteristics is shown in Fig. 2.

Immune paradigm during *P.m.* infection

The parasite's sophisticated evasion tactics against human and mosquito host immune responses are largely based on the limitation of strain-specific immunity[78]. Like many other infections countered by the immune system, *P.m.* exposed antigens (MSP1/ AMA1 or RBP) become highly polymorphic over time due to immune pressure[79]. This enables the parasites to escape from antibodies developed against the encountered parasite strain. This might be the reason for the limited efficacy seen for AMA1-based vaccine approaches, in which natural antigenic diversity of the parasites is not completely covered[79]. Further, genome diversification of *P.m.* antigens indicates immune-mediated variation[30]. *P.m.* has an expanded family of pir-like proteins, which are known as variant surface antigens, and which are likely to be involved in antigenic switching and sequestration. These events result in the generation of highly polymorphic *P.m.* strains which are capable of evading host immunity. Further, amino acid variations with low sequence similarity in circumsporozoite proteins of human-infecting *Plasmodium* species suggest that exposure to one species does not necessarily elicit an immune response sufficient to recognize secondary infection with a different species[80]. However, contrary to this, previous exposure to *P.m.* has been shown to induce protection against *P.f.* by reducing the frequency of fever episodes and parasitemia levels, suggesting both species share common antigens, and that the observed parasitological and clinical protection

was due to *P.m.*-evoked immunity[81]. The chronic and low-grade parasitemia frequently observed in *P.m.* infection might also be attributed to its strategic infiltration of splenic dendritic cells, thereby escaping from the host immune system, as seen in *P. berghei*[82]. Nonetheless, it is not yet clear whether these dendritic cells harbor human *Plasmodium* species, but a case report on the appearance of *P.m.* parasites in the blood film of a man who underwent splenectomy after 36 years of malaria infection suggests the possible involvement of splenic dendritic cells acting as a reservoir of infection[83].

Though the immune response to *P.m.* is distinct, it still shares some common features with responses to other *Plasmodium* species, especially in terms of antibody specificity and cross-reactivity. A Brazilian sero-surveillance study compared the influence of *P.m.* and *P.f.* antigens on host immune response[84]. This sero-epidemiological survey used recombinant *P.m.* proteins to perform antibody profiling against *P.m.*-MSP1 in human sera obtained from two different Brazilian populations. The study detected a significant proportion (~70%) of samples that recognized at least one out of five *P.m.*-MSP1 recombinant proteins used in the assay. However, the reactivity index of *P.m.* was lower than *P.f.*, suggesting a comparatively weaker immune response elicited by the host against *P.m.* antigens[84]. It is evident that chronic exposure to *P.m.* produces a high antibody titer and strongly activates the complement system, leading to accumulation of immune complexes in the kidney glomeruli[62]. This causes membrano-proliferative glomerulonephritis, which not only causes kidney disease, but is also a known parasite strategy to distract the immune system and subvert host immunity from a protective to a damaging response[85]. An exhaustive comparison of key characteristics, host immunity, immune evasion strategies by the parasite, and clinical outcomes between *P.m.*, *P.f.*, and *P.v.* is summarized in Table 2.

The host immune system is compromised when it encounters multiple co-infected parasite species or different strains of the same species. This complex infection environment drives the evolution of parasites to evade host immunity[86], and that compromised immunity is incapable of mitigating parasite load. Under such circumstances, the human immune system shifts towards a broad, less-targeted response to balance and regulate parasitemia[86]. Such immune modulation may represent a propagation strategy of *P.m.*, which is mostly present in co-infections with other *Plasmodium* species. Importantly, the interplay between parasite species-specific and non-specific (density-dependent) immunity during co-infections maintains overall parasitemia around a threshold limit[87]. This implies that during co-infections, when one species of parasite expands to a high density, the density-dependent immune regulation is triggered, which suppresses its growth and therefore potentially favors the establishment of another species. Therefore, understanding cross-species regulation during co-infections has implications for malaria control, particularly when research is focused on single-species vaccines to reduce the prevalence of dominant species like *P.f.* Focusing on one species could lead to an increase in infection rate by other species that normally remain at low levels (such as *P.m.*), potentially leading to a shift in the dominant malaria species.

Although the literature on *P.m.*-mediated host immune regulation is sparse, evidence still exists reporting immune evasion strategies adopted by *P.m.*, such as antigenic variation via polymorphism and switching[30,79], inhibition of the complement system and dendritic cell function[79], and activation of immune complexes[62]. These mechanisms allow *P.m.* to persist at low levels and evade sterilizing immunity.

Conclusions

Modern research on *P.m.* has focused on improving diagnostic techniques, understanding its unique biology, and developing effective treatments. *P.m.* displays strict (but not exclusive) tropism for mature RBCs, but is also capable of invading reticulocytes. It maintains slow asexual cycling (72-h), and causes less acute but more chronic morbidity, including renal complications, chronic anemia, and congenital infections. *P.m.* is far more prevalent than appreciated before, with substantial underreporting across sub-Saharan Africa, Asia, Western Pacific and South America due to non-availability of *P.m.*-specific RDTs and frequent misclassification as other

Table 3 | Research gaps, current understanding and priorities for *Plasmodium malariae* research

Research area	Current understanding	Outstanding questions	Research priority	Proposed approach
Epidemiological burden	Underestimated	What is true global prevalence and burden?	Critical	Large-scale PCR surveys, mathematical modeling
Diagnostic tools	Improving but insufficient	How to develop *P.m.*-specific RDTs for field use?	Critical	*P.m.*-specific antigen development, field validation
Hypnozoite formation	Controversial/limited	Do *P.m.* form dormant liver stages? Evidence is contradictory	High	Liver biopsy studies, search for molecular markers, primaquine response studies
Drug resistance mechanisms	Emerging/limited	What are molecular mechanisms of artemether-lumefantrine/chloroquine resistance?	High	Ex vivo studies, molecular surveillance, genomics
Clinical manifestations	Well-characterized	What determines chronic versus acute manifestations?	Medium	Longitudinal cohort studies, biomarker development
Transmission dynamics	Limited	How does *P.m.* transmission differ across regions?	High	Vector competence studies, seasonal dynamics
Co-infection effects	Growing understanding	How do co-infections affect *P.m.* dynamics in long-term?	High	Multi-species infection models, competitive dynamics
Immune evasion	Basic understanding	What are specific mechanisms beyond antigenic variation?	Medium	Functional immunology, host-parasite interaction studies
Vaccine development	Minimal	Why no *P.m.* vaccine candidates in clinical trials?	High	MSP1/AMA1 candidate development, funding allocation
Elimination strategies	Early stage	Will *P.m.* fill ecological niche after *P.f.* elimination?	Critical	Mathematical modeling, surveillance strengthening

P.m. Plasmodium malariae, *P.f. Plasmodium falciparum*, PCR *polymerase chain reaction*, RDT *rapid diagnostic tests*, MSP *merozoite surface protein*, AMA *apical membrane antigen*.

species using light microscopy. Molecular methods and serological surveillance have unmasked extensive reservoirs of chronic, low-density parasitemia infections, highlighting *P.m.*'s ability to sustain in both symptomatic and asymptomatic hosts. Its reduced susceptibility to artemether/lumefantrine and infrequent chloroquine resistance raise concerns regarding effective treatment of *P.m.* infections. Moreover, *P.m.*'s host immune evasion tactics—including antigenic variation (notably MSP1 polymorphism), complement inhibition, and immune complex formation—that support its chronicity and long-term persistence behavior, making it unique from other *Plasmodium* species.

Having said that, true epidemiological burden and transmission dynamics of *P.m.* remain underestimated due to underdiagnosis, lack of clinical awareness, meager species-specific reporting by health agencies, and lack of field deployable rapid tests. There is a great void of vaccine research for *P.m.*, and no apparent vaccine candidate in the pipeline. Thus, the interventions targeting *P.f.* and *P.v.* may potentially result in the expansion of *P.m.*'s ecological niche, which may threaten malaria elimination efforts. Furthermore, some unresolved mysteries of this parasite, such as potential for hypnozoite formation, mechanisms of sustained chronicity, and reticulocytes versus normocytes invasion, require further research. Owing to these, we have identified the research gaps in our present understanding on *P.m.* biology. Together, these gaps prompt some outstanding questions, for which research should be prioritized following the proposed approaches that we have summarized in Table 3.

We propose to incorporate malaria species-specific reporting on regional, national, and global malaria data portals. There is a need to develop and deploy *P.m.*-specific diagnostics capable of detecting low-density and mixed-species infections in endemic and elimination settings. Investing in research to explore hepatic stages of *P.m.*, liver biopsy studies, primaquine dose response studies (especially to resolve hypnozoite controversy), invasion biology, immune modulation, and renal pathology using experimental models and longitudinal cohorts could have positive effects on this species' eradication. In parallel, therapeutic efficacy studies targeting *P.m.* to monitor and prevent drug resistance and search for alternative rescue therapies should be implemented. Finally, *P.m.* vaccine research that at least includes *P.m.* antigens in preclinical screening or multi-antigen vaccine initiatives should be prioritized.

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Author contributions

H.S., G.K., N.A., and A.F. together wrote the manuscript. H.S. and A.F. conceived the idea, G.K. with H.S. wrote the first draft, N.A. is a WHO Level 1 certified microscopist provided the Giemsa-stained *P.m*. light microscopy image and its identifying features. AF created the images and edited the manuscript. All authors read and approved the final submitted version.

Competing interests

The authors declare no competing interests.

Additional information

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