Abstract

Plasmodium falciparum is a protozoan parasite that causes the most severe form of human malaria. Four other *Plasmodium* species can also infect humans – *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi* – but *P. falciparum* is the most prevalent *Plasmodium* species in the African region, where 90% of all malaria occurs, and it is this species that causes the great majority of malaria deaths. These were reported at 438,000 in 2015 (1) from an estimated 214 million cases; importantly, however, figures for the global burden of malaria tend to have wide margins of error due to poor and inaccurate reporting (2-4). In this Perspective, the unique features of the *P. falciparum* parasite are highlighted and current issues surrounding the control and treatment of this major human pathogen are discussed.

What is special about *Plasmodium falciparum*?

All *Plasmodium* parasites share unique and fascinating biological features, enabling them to invade, colonise, replicate and persist in diverse host environments. They have a complex and highly-evolved lifecycle that requires both an insect vector and a vertebrate host (fig 1A), and their cell and molecular biology is highly unusual. *Plasmodium* belongs to an early-diverging lineage of eukaryotes, the Apicomplexan phylum, which evolved from a free-living algal ancestor into an obligate intracellular parasite (5): the resultant cells carry a relic plastid (6) as well as other special organelles that facilitate invasion of host cells (7) (fig 1B). Inside these cells they grow via various syncytial modes of cell division rather than conventional binary fission (8). *Plasmodium* parasites are widespread throughout the animal kingdom but tend to be highly specialised for particular hosts – avian, reptilian, mammalian, etc.: *P. falciparum* infects only humans and great apes (9, 10), and although its basic biology shares all the above characteristics, it also has additional features that can cause unique and severe pathology in humans. The case fatality rate of falciparum malaria is ~0.3-0.45%, but in a subset of severe malaria cases it can exceed 20% (11) (table 1).

Table 1: Manifestations of severe falciparum malaria

Clinical manifestation	Frequency		Prognostic of poor outcome?		Linked laboratory indices
	Children	Adults	Children	Adults	
Cerebral coma or impaired consciousness	+++	++	+++	+++	Parasite sequestration in brain
Repeated convulsions	+++	+	+	++	Parasite sequestration in brain, Hypoglycaemia
Prostration	+++	+++	+	+	
Respiratory distress	+++	++	+++	+++	Metabolic acidosis/Hyperlactataemia, Severe anaemia
Pregnancy malaria	-	+++	-	+++	Parasite sequestration in placenta, Hypoglycaemia

Table shows a non-exhaustive list of key disease features in severe falciparum malaria. Adapted from data in references (4) and (11).

Firstly, in the human-pathogenic stage of its lifecycle, which consists of growth inside erythrocytes (fig 1A), *P. falciparum* can infect erythrocytes of all ages. This distinguishes it from the other major human malaria species, *P. vivax*, which is restricted to rare immature erythrocytes called reticulocytes. Accordingly, whereas *P. vivax* growth is limited by the scarcity of reticulocytes, *P. falciparum* can swiftly reach parasitaemias of 10-20%, and each infected cell can produce ~20-30 new parasites every 48 hours. This capacity to reach very high parasitaemias exacerbates malaria pathologies such as severe anaemia, metabolic acidosis and respiratory distress (11, 12) (table 1).

Secondly, *P. falciparum* has a family of major virulence genes called *var* genes which are unique to this parasite and its close relatives (ape malaria parasites in the '*Laverania*' subgenus (13)). These virulence factors play key roles in other lethal pathologies, such as cerebral malaria and pregnancy malaria (14) (table 1). *Var* genes encode an adhesive protein, 'PfEMP1', which is exported and expressed on the surface of infected erythrocytes, allowing the cells to be sequestered in capillaries as they mature. Thus they avoid clearance by the spleen, but sequestered cells obstruct blood flow and cause inflammatory responses that are particularly harmful in vessels of the brain or placenta (15). Most other malaria parasites do not adhere in this way and do not cause cerebral comas or pregnancy complications. Furthermore, the *var* gene family is variantly expressed, giving rise to antigenic variation in PfEMP1, and hence immune evasion (16), which is one reason why sterile immunity to falciparum malaria is rarely achieved in humans.

A third unusual feature of *P. falciparum* is its extremely biased genome, the implications of which are not yet understood. At 81% A/T, this is one of the most biased genomes ever sequenced (17). Not all human malaria parasites share this bias – the *P. vivax* genome is only ~58% A/T – and although elegant studies have recently elucidated *how* the bias is maintained at a molecular level (18), they have not established *why*. It may be that A/T-rich DNA favours permissive transcription (19) or rapid DNA replication (20, 21) – both signature features of *P. falciparum*. Certainly, this genome bias presents a severe challenge to biologists in sequencing, cloning, expressing and working with *P. falciparum* genes.

Current issues in *P. falciparum* biology

Hot topics in *P. falciparum* biology range from the molecular to the epidemiological. On the molecular and cellular level, the parasite has unusual basic biology that is both academically interesting and medically important. Understanding the parasite's unique features will help scientists to focus on new targets for antimalarial drugs and vaccines. These features include the unusual genome mentioned above; the unusual cell cycles (8)(fig 1A); the biochemical specialisations for an intracellular lifecycle (22); and the invasion pathways (7),

transport pathways (23) and capacities for host-cell remodelling (24, 25) that have evolved to facilitate life inside anucleate erythrocytes. Interestingly, a recent screen for genes that are essential for growth in the rodent malaria parasite *P. berghei* revealed an unprecedentedly high proportion of indispensable genes, which was extrapolated to be similarly true for *P. falciparum* (26). This raises conceptual questions about reductive specialisation for an intracellular parasitic lifestyle and importantly it also raises the prospect of an abundance of genetically-essential targets for antimalarial drugs.

Moving to the epidemiological level, falciparum malaria has historically been a major scourge of humans throughout the tropics and subtropics, and attempts to control the disease have a long and complex history. Excitingly and controversially, the prospect of global malaria eradication has recently returned to the fore (27), after the failure of the first in 'WHO Global Malaria Eradication Programme' in the mid-20th century. The original programme was built upon considerable success in eliminating the disease from Europe and North America in the early 1900s via large-scale environmental insecticide treatment which targeted the mosquito vector, together with drug treatment of malaria cases in humans. Reasons for its ultimate failure included the development of mosquito resistance to the insecticide DDT and parasite resistance to the antimalarial chloroquine, as well as the more challenging dynamics of disease transmission in hyperendemic tropical areas. Figure 1C illustrates the subsequent rebound in the global burden of malaria. Encouragingly, however, more modern interventions have reduced this burden once again, with a drop of ~40% in malaria in Africa over the past 15 years, attributed primarily to the use of insecticide-treated bednets (28, 29).

Whether or not malaria can actually be eradicated with current tools remains a topic of debate (30, 31), and some of these tools are now increasingly threatened, as discussed below. There is a clear historical trend for disease resurgence when control measures fail or when funding to sustain them fails (not only malaria (fig 1C) but other parasitic diseases such as sleeping sickness have illustrated this (32)). Nevertheless, striking successes have already been achieved in eliminating malaria from island nations such as Sri Lanka (33), as well as non-island nations on the margins of transmission zones, such as Morocco and Kyrgyzstan. Others including China and Malaysia, benefitting from regional cross-border collaboration, are close to the elimination goal (34).

Current challenges in *P. falciparum* biology

Key challenges in the *P. falciparum* field range from basic science to real-world intervention. At the level of basic science, the unusual biology of this parasite makes it challenging to work with, although it remains one of only two human malaria parasites that can be grown in laboratory culture at all (35) (the other is the zoonotic macaque parasite *P. knowlesi* (36)).

The ability to culture *P. falciparum* in human erythrocytes makes genetic experiments feasible (37), albeit painfully inefficient when compared to model systems. Nevertheless, in the past decade great advances have been made in developing genetic tools for gene tagging, gene knockouts, knockdowns, inducible approaches and gene editing (38), and the scope for further improvement remains substantial. Collaborative efforts to sequence hundreds of *P. falciparum* strains from around the world are fast revealing the genetic diversity of the species (39), but challenges remain in efficiently adapting field strains to *in vitro* culture (40) and there are persistent concerns about the relevance of laboratory experiments conducted exclusively in strains that have been in culture for decades.

Meanwhile, major challenges persist for *P. falciparum* control in the real world. Drug resistant parasites (as well as insecticide-resistant mosquito vectors) are a recurring problem, as are sufficiently accurate and sensitive diagnostics, while the gold-standard disease-prevention tool of a vaccine against *P. falciparum* remains elusive despite decades of scientific effort.

P. falciparum parasites have historically developed resistance to every antimalarial deployed (fig 1C). Current first-line treatments are all based on artemisinin derivatives, which are highly effective but very short-lived in the bloodstream. Therefore they are always supplied with a second longer-lasting antimalarial as a combination therapy or 'ACT'. Resistance to ACTs is now found in much of the greater Mekong region (41, 42). As yet, there is no strong evidence that resistance has spread from Asia to Africa, where it would be particularly devastating, but this has previously happened with antimalarials such as chloroquine and antifolates, and the ever-increasing global movement of people makes the transport of resistant parasites very likely. The current picture is complicated by the unusual nature of artemisinin resistance: a phenotype of 'delayed parasite clearance' in which parasites are cleared only slowly from the blood, and may go 'dormant' to survive the brief period of drug exposure before recrudescing (41, 43). This phenotype is difficult to measure *in vitro*, its genetic basis is only partially understood (44, 45), and it may be dependent on the genetic background of the parasite, perhaps explaining why it has developed in Asian but not yet in African strains (46, 47). As highlighted below, it will be imperative to preserve the effectiveness of the ACT antimalarials for as long as possible.

Developing an effective vaccine remains a huge challenge owing to the parasite's antigenic complexity, antigenic redundancy and capacity for antigenic variation (48). In 2018, the first ever vaccine for falciparum malaria, Mosquirix™, will begin to be supplied in three African countries, Ghana, Kenya and Malawi, supported by the WHO 'Malaria Vaccine Implementation Programme'. However, this programme remains exploratory and the vaccine is unlikely to be a game-changer in global malaria control because it does not offer

sterile or long-term protection. Mosquirix™ features an epitope from the invading 'sporozoite' stage of the parasite (fig 1A), and thus targets the pre-erythrocytic parasite stages in order to stimulate an immune response pre-empting symptomatic blood-stage malaria. Unfortunately, Phase-3 clinical trials revealed that the vaccine offered only ~30% protection, waning rapidly over a four-year period (49). Deployment in young children across Africa might still prevent millions of severe malaria episodes and deaths, but this must be weighed against concerns about cost, uptake, impact on other malaria control interventions, and the potential risk of shifting severe disease to older age groups (50).

Finally, in order to prevent malaria transmission it is vital to detect and then treat *P. falciparum* infections accurately, even when asymptomatic, because they may nevertheless still be transmitted by mosquitoes (51). People who have been repeatedly exposed tend to develop functional – albeit non-sterile – immunity to the parasite, suppressing infections to a level that causes few symptoms. These can be difficult to detect (because asymptomatic people do not seek treatment) and if the parasitaemia is very low they can also be difficult to diagnose without sensitive PCR-based tests or expert microscopy. Field diagnosis is often limited to antibody-based 'rapid diagnostic tests' (52), which frequently have lower sensitivity.

Future perspectives on *P. falciparum*

Arguably the most urgent current issue in the *P. falciparum* field is the threat now posed by artemisinin-resistant parasites. The community must work to understand the underlying biology of resistance, develop and deploy the right assays for it in the field – genetic, phenotypic or a combination of both – and thus track its spread across the malaria-endemic world. Retrospective studies have traced the emergence and spread of chloroquine resistance in the mid-1900s (53), but for the first time we now have the capacity to do this in real time, putting in place proactive interventions. Indeed, the genetic basis of artemisinin resistance was at least partially elucidated almost as it emerged, via a massive multicentre effort to sequence parasites and perform genome-wide association studies (44). It may be possible to prevent, or at least impede, the spread or *de novo* emergence of artemisinin-resistant parasites in Africa via in-depth surveillance, preventing the use of artemisinin monotherapy and using the right partner drugs in ACTs (since partner drugs are also at risk of resistance, invalidating the ACT approach (54)).

In parallel with this effort, since artemisinin will inevitably be lost sooner or later as an effective first-line drug, it is crucial to develop new drugs with different modes of action, and to improve their transit through the drug development pipeline (55). Product Development Partnerships such as the Medicines for Malaria Venture (MMV) are key players here.

Finally, returning to the eradication agenda, there are strong advocates for an audacious plan which was backed by the WHO in 2015 to halt the spread of artemisinin resistance by entirely eliminating *P. falciparum* from the Mekong region, where resistant parasites currently reside (56). This would probably require the unprecedented use of mass drug administration in complete populations – a logistical and ethical challenge – but the concept merits serious consideration if the WHO target of reducing malaria cases and deaths by 90% by 2030 is to be met. If successful, it could set a template for other regional elimination programmes.

P. falciparum is a fascinating and sophisticated parasite that has co-evolved with humans for thousands of years, shaping human genetics (57) and remaining a major public health problem to this day. There has never been a better moment for a concerted effort at the elimination, and eventually the global eradication, of this parasite.

Summary points

- *Plasmodium falciparum* is responsible for most of the global burden of death from malaria – approximately half a million per annum.
- The *P. falciparum* parasite is an early-diverging eukaryote with many unusual and interesting biological features.
- Studying this parasite in the laboratory is challenging but great advances have been made in recent decades.
- Control of falciparum malaria has improved greatly in the past 15 years but is threatened by the repeated emergence of drug resistant parasites.

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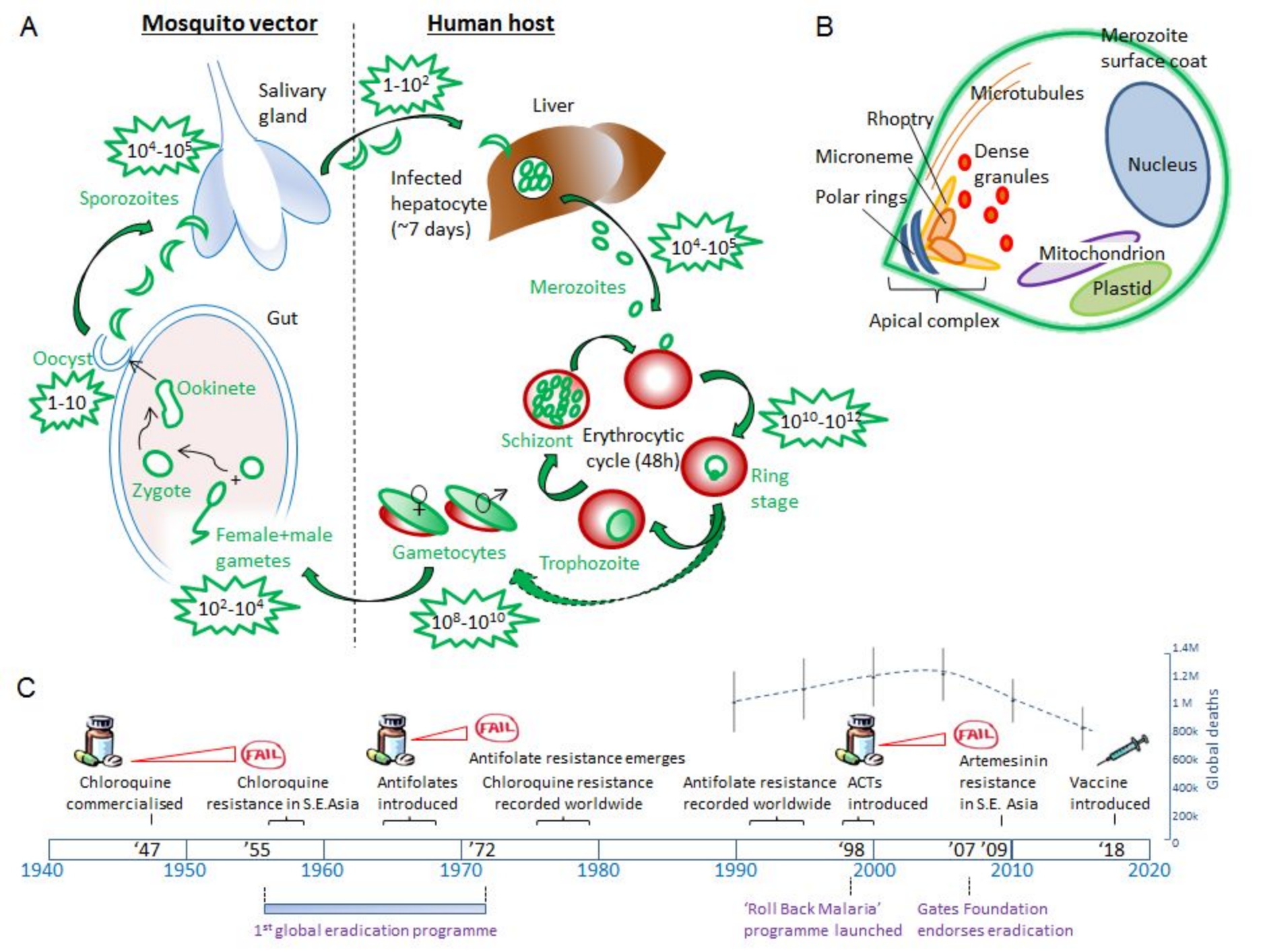
Figure legend

Fig. 1: *P. falciparum* lifecycle, parasite structure and disease-control timeline

A) Schematic showing the lifecycle of *P. falciparum.* Approximate parasite numbers shown at each stage highlight the severe bottlenecks and massive expansions at various stages. In the mosquito vector the sexual cycle occurs: pre-gametes called gametocytes are taken up in a blood meal from an infected human; these mature into gametes, mate and form a motile zygote called an ookinete which crosses the gut wall and encysts to form an oocyst. In the oocyst, asexual replication occurs and sporozoites are released to migrate to the salivary glands, whence they are injected into another human host during a mosquito bite. Sporozoites migrate from the bite site to the liver, where they multiply asexually inside hepatocytes over a period of ~7 days and then release merozoites which infect erythrocytes. In erythrocytes, 48-hour cycles of asexual replication, cell lysis and reinvasion occur, causing all the symptoms of malaria. A small subset of these parasites differentiates into gametocytes ready for mosquito transmission.

B) Structure and organelles of *P. falciparum*. The apical complex that facilitates host cell invasion includes rhoptries, micronemes and dense granules, all containing proteins that are released during host cell invasion. The merozoite surface is densely coated with proteins that aid host cell attachment and are cleaved and shed during invasion. The two endosymbiont-derived organelles, mitochondrion and plastid, are also shown.

C) Timeline showing malaria control interventions and the global burden of malaria deaths from 1990 to 2015 (+/- 95% confidence intervals). Data are from the Global Burden of Disease study (58), which records data from 1990 onwards; comparable global data prior to 1990 are lacking.


A
Mosquito vector
Human host
Salivary gland
10^4-10^5
Sporozoites
1-10^2
Liver
Infected hepatocyte (~7 days)
10^4-10^5
Merozoites
Gut
Oocyst
1-10
Ookinete
Zygote
Female+male gametes
10^2-10^4
Gametocytes
10^8-10^{10}
Schizont
Erythrocytic cycle (48h)
Ring stage
Trophozoite
10^{10}-10^{12}
B
Merozoite surface coat
Microtubules
Rhoptry
Microneme
Dense granules
Polar rings
Nucleus
Mitochondrion
Plastid
Apical complex
C
Chloroquine commercialised
Chloroquine resistance in S.E.Asia
Antifolates introduced
Antifolate resistance emerges
Chloroquine resistance recorded worldwide
Antifolate resistance recorded worldwide
ACTs introduced
Artemesinin resistance in S.E. Asia
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2020
1st global eradication programme
'Roll Back Malaria' programme launched
Gates Foundation endorses eradication
Global deaths
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1.2M
1 M
800k
600k
400k
200k
0