University Library



A gateway to Melbourne's research publications

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:
Roe, MS;O'Flaherty, K;Fowkes, FJI

Title:
Can malaria parasites be spontaneously cleared?

Date:
2022-05-01

Citation:
Roe, M. S., O'Flaherty, K. & Fowkes, F. J. I. (2022). Can malaria parasites be spontaneously cleared?. Trends in Parasitology, 38 (5), pp.356-364. https://doi.org/10.1016/j.pt.2022.02.005.

Persistent Link:
https://hdl.handle.net/11343/308734

License:
CC BY-NC-ND

CellPress
OPEN ACCESS

Trends in
Parasitology

Opinion

Can malaria parasites be spontaneously cleared?

Merryn S. Roe [1,2,@] Katherine O'Flaherty [1] and Freya J.I. Fowkes [1,2,3,4,*,@]

A large body of evidence demonstrates that *Plasmodium falciparum* infections are chronic in malaria endemic areas; however, the notion of spontaneous clearance in the absence of antimalarial drug treatment is rarely discussed. In this opinion article, we review and reinterpret data to postulate that spontaneous clearance of *P. falciparum* infections occurs frequently, has been demonstrated in a range of transmission settings, and confirmed by the most sensitive malaria diagnostic techniques. We also discuss factors which may influence the likelihood, measurement, and conclusions of spontaneous clearance. A greater understanding of the phenomenon of spontaneous clearance will advance our knowledge of malaria epidemiology, transmission potential of malaria parasites, as well as inform interventions for malaria control and elimination.

Highlights

Spontaneous clearance of *P. falciparum* infections in the absence of antimalarial drug treatment has been demonstrated in a range of transmission settings and confirmed by sensitive molecular diagnostics.

Most infections are spontaneously cleared within months and almost all within a year.

Many studies in demonstrating evidence for 'chronic' infections do not consider multiclonal infections and likely miss clearance events of single clones.

Insensitive diagnosis methods and sequestered parasites may confound our interpretation of spontaneous clearance events.

Epidemiological data infer a role of host immunity in spontaneous clearance and infection duration, but there is a paucity of laboratory data defining their explicit role.

Advancing our understanding of the occurrence of, and factors that underpin, spontaneous clearance of *P. falciparum* infections will inform interventions to eliminate malaria.

Are *Plasmodium* spp. infections chronic?

Explorations of **spontaneous clearance** (see Glossary) of *Plasmodium* spp. parasites, the causative agent of malaria, in the absence of antimalarial drug treatment are not widely discussed in the literature. To date, the literature has focused on exploring **chronic infections** in a range of populations, including immigrants and donor recipients, as well as longitudinal studies in malaria-endemic areas, predominantly in Africa (e.g., [1–7]). These investigations have all demonstrated sustained asymptomatic *Plasmodium* spp. infections, also known as **subclinical** infections, over time in the absence of treatment. While there is a large body of evidence demonstrating prolonged infections, clearance of *Plasmodium* spp. infection in the absence of treatment has also been frequently reported, yet rarely deliberated.

Here we discuss the evidence for and against the occurrence of spontaneous clearance of malaria parasites in the absence of antimalaria drug treatment. We focus on untreated *P. falciparum* infections for which evaluations of clearance are not confounded by relapses from dormant hypnozoites, as is the case for *Plasmodium vivax* and *Plasmodium ovale* infections. Our opinion piece presents evidence of spontaneous clearance as well as re-evaluating the evidence supporting long-lasting *P. falciparum* infections from clinical reports and cohorts of nontreated clinical and subclinical infections. The mechanism(s) behind observations of spontaneous clearance are yet to be elucidated but may involve naturally acquired immunity or immune-independent mechanisms involving the spleen. Finally, we highlight that measurement of spontaneous clearance relies on counting circulating parasites and that insensitive diagnosis methods and sequestered parasites may confound our interpretation of spontaneous clearance events.

The aim of this article is to highlight that spontaneous clearance of parasites from the circulation can occur. We hope to encourage further investigation into the phenomenon of spontaneous clearance of *P. falciparum* in order to gain a deeper understanding of how the duration of infections (and therefore infectiousness) underpins malaria epidemiology across a range of malaria transmission settings.

[1]Disease Elimination Program, Burnet Institute, Melbourne, Australia
[2]School of Public Health and Preventive Medicine, Monash University, Melbourne, Australia
[3]Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, Australia
[4]Department of Infectious Diseases, Monash University, Melbourne, Australia

© 2022 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

CellPress
OPEN ACCESS

*Correspondence:
freya.fowkes@burnet.edu.au
(F.J.I. Fowkes).
@Twitter: @MerrynRoe (M.S. Roe) and
@FreyaFowkes (F.J.I. Fowkes).

Reports of spontaneous clearance in untreated malaria cohorts

Some of the first evidence supporting the notion of spontaneous clearance came from a handful of historical longitudinal studies examining the course of untreated clinical malaria, which are considered unethical by contemporary standards (mainly because they withheld treatment and/or involved unconsenting vulnerable populations). Two studies published in the 1950s and 1960s focused on *P. falciparum,* providing some of the first observations of spontaneous clearance of *P. falciparum* infections [8,9]. The first study involved the artificial malaria infection of 38 African American men to 'treat' neurosyphilis [8]. In these artificial infections, microscopy-detectable *P. falciparum* infections were reported to last 121 days on average, with the maximum infection duration recorded at 480 days. Spontaneous clearance events were reported through a number of patient examples, but the number of spontaneous clearance events was not quantified and there is no stratification between 'untreated' and 'inadequately treated' individuals [8]. The second study involved 74 Nigerian psychiatric inmates with 'induced' *P. falciparum* infections. In this cohort 80% of (microscopy-detectable) infections were found to be spontaneously cleared within 56 days, with an average duration of 21 days (range 11 to ≥56) [9]. The shorter duration of detectable parasitaemia observed in this study may be due to some level of naturally acquired immunity as Nigerian individuals may have had a prior history of malaria infection unlike the American neurosyphilis subjects who were presumably nonimmune.

While these studies present early observations of spontaneous clearance of high-density *P. falciparum* infections and resolution of clinical symptoms in the absence of treatment, they are not representative of malaria-endemic populations in which, due to acquisition of naturally acquired immunity, infections can be subclinical and therefore remain untreated. The consensus from observational studies following the natural course of individual-level subclinical *P. falciparum* infections in the absence of treatment, across malaria transmission settings, is that *P. falciparum* infections are chronic [1]. Often cited as compelling evidence for chronic infections comes from longitudinal studies of untreated individuals living in high seasonal transmission settings in Africa where individuals can harbour subclinical infections from the rainy season throughout the dry season (when mosquito densities and transmission is greatly reduced) [7,10–13]. While it is common for these studies to report that a proportion of participants harboured infections throughout the dry season (20–47%), the inverse is not acknowledged – that the majority of participants 'cleared' their infections (53–80%) [7,10,14,15]. However, the relative contribution of spontaneous clearance to these observations is unclear, particularly as clearance and reinfection events may be missed in the 5 to 6 months between seasonal surveys.

Cohort studies with more 'intensive' sampling (varying from multiple samples a day to one every month) of the natural course of untreated subclinical infections can provide more accurate assessments of spontaneous clearance. Studies which have used microscopy for the detection of *P. falciparum* parasites during the natural course of untreated subclinical infections have all found evidence of spontaneous clearance, defined here as a negative smear after a positive smear [12,14,16–21]. Of those which quantified the proportions of infections spontaneously cleared, two studies undertaken in high-transmission settings reported proportions cleared: 22% of infections in newborns within 15 months [18] and 96% of infections in young adults within 1 year [12]. There was only one study in a moderate–low transmission setting which reported proportions of infections cleared; it found that 53% of subclinical infections were cleared within a week [21]. However, the robustness of this evidence as 'true' spontaneous clearance is debateable. In particular, the use of light microscopy for the detection of parasites, which may not detect **submicroscopic** parasitaemia which occurs at densities below the limit of detection of microscopy (50–100 parasites/μl [22]), and which can be detected only by molecular methods

such as PCR (the limit of detection for standard PCRs is 0.5 parasites/μl [23]), means that assessments of spontaneous clearance events are compromised.

Studies which have utilised more sensitive molecular diagnostics in following the natural course of untreated submicroscopic *P. falciparum* infections can provide more robust evidence for spontaneous clearance events, as well as more accurate estimates of the duration of infections. All 'intensive' sampling studies (i.e., at least monthly sampling) utilising sensitive *Plasmodium* spp.-specific PCR molecular methods observe events of spontaneous clearance regardless of transmission setting (Table 1) [2,3,15,24–29]. The observed duration of submicroscopic infections ranged from days [2,24] to several months [3,25,27–29] before spontaneous clearance was observed, with a large proportion of individuals (17–87%) in most studies clearing infections within 1 month and the majority of individuals (67–100%) spontaneously clearing *P. falciparum* parasitaemia with 12 months (Table 1). Of studies with a follow-up of at least 1 year, ≥98% of submicroscopic infections were spontaneously cleared within the year [25,28,29] (Table 1).

While diagnosis by *Plasmodium* spp.-specific PCR can determine the presence of *P. falciparum* at a given time, additional molecular characterisation of the infecting parasite strain(s) is required to capture the subtleties of multiclonal infection dynamics. Genetic characterisation of parasites in high-transmission settings suggests that, while some individuals appear to be chronically infected when simply reporting their binary infection status, genotyping studies demonstrate that individuals are constantly clearing one infection then being infected with new parasite strains [10,11,13,15,20,24,26,30]. This results in a cycle of concurrent submicroscopic infections that, without genetic characterisation, can appear as a single chronic infection (as demonstrated in Figure 1) [10,11,13,15]. It is therefore possible that longitudinal studies that rely solely on PCR for detection of parasites may overestimate the duration of submicroscopic *P. falciparum* infections and underestimate rates of spontaneous clearance. This overestimation of infection duration may be further compounded by the fact that PCR diagnostics are able to detect parasite debris well after clearance of viable parasites [31–34]. Alternatively, infections may be at densities below the detection limit of PCR diagnostics and may be misclassified as cleared only to subsequently reappear at densities above the detection limit.

Studies which evaluated clonal dynamics of the natural course of submicroscopic infections by genotyping or sequencing various *P. falciparum* genes such as AMA1, MSP1, MSP2, GLURP, and *var* genes demonstrate that spontaneous clearance of clones can occur [2,3,10,15,20,24–30,35–37]. Furthermore, spontaneous clearance of clones occurs more frequently, and faster (days to weeks) than when simply evaluating binary infection status by PCR (months) (Tables 1 and 2) [2,3,10,15,20,24–30,36,37]. Differences in clearance rates have only been quantified in one study which genotyped all microscopy-positive infections and quantified the clearance rate of both microscopy-detectable infections and single clones, demonstrating that clonal clearance is almost three times greater than when evaluating microscopy infection status alone [30].

Generally, the definition of 'clearance' for most studies was the absence of an infection for one timepoint after a positive result; this absence could be a 'negative' result or the absence of a particular clone which was detected earlier. While this single timepoint absence could be a fluctuation of an infection below the limit of detection rather than clearance per se, there is evidence of extended periods of clearance. For example, in two studies undertaken in low-transmission settings, where reinfection rates would be low, participants with a spontaneously cleared infection were negative for 8–11 [29] and 13–23 [28] consecutive monthly samples using the most sensitive PCR diagnostic (high-volume qPCR, known as ultrasensitive 'uPCR')

Glossary

Chronic infection: an untreated infection which persists.
Endogeneity selection bias: a form of selection bias which occurs when one makes assumptions of the risk of a variable from data that were selected based on a particular outcome, in this case, assumptions on the duration of *P. falciparum* infections based on a selection of case studies selected due to long-lasting *P. falciparum* infections.
Naturally acquired immunity: immunity which develops with repeated exposure and generally prevents clinical disease, but not infection.
Sequestration: adherence of *P. falciparum*-infected erythrocytes to microvasculature in tissues and organs, removing themselves from circulation in peripheral blood.
Splenic clearance: where *Plasmodium* spp.-infected erythrocytes, and noninfected erythrocytes which form rosettes around infected erythrocytes, are filtered out of circulation by the spleen. These infected erythrocytes and rosettes are too large to pass through the interendothelial gaps in the splenic structure and are consequently filtered out of circulation.
Spontaneous clearance: the resolution of an infection without antimalarial drug treatment which can occur over varying infection lengths and may be influenced by a combination of parasite factors, splenic clearance, innate and adaptive immunity.
Subclinical infection: an asymptomatic *Plasmodium* spp. infection, often low density and undetectable by conventional diagnostics (rapid diagnostic test and microscopy).
Submicroscopic infection: a low-density *Plasmodium* spp. infection not microscopically detectable and therefore only detectable by molecular methods (such as PCR).

Table 1. Spontaneous clearance events of circulating submicroscopic *P. falciparum* infection*

Country	Transmission	Study population	Sampling	Detection	Spontaneous clearance events
Tanzania [24]	High	20 children	Daily for 14 days	PCR†	15%[a] of infections cleared‡‡ ≤2 weeks
Burkina Faso [2]	High	45 children	Daily/weekly for 35 days	nPCR†	17%[a] of infections cleared‡ ≤1 month (of these, infection duration median 3.5 [1–35] days)
Ghana [25]	High	143 newborns	Monthly for 24 months	PCR	55%[a] of infections cleared‡ ≤1 month; 98%[a] ≤12 months
Uganda [3]	High	149, all ages	Monthly for 18 months	qPCR	Spontaneous clearance‡‡‡‡ observed (infection duration range 87–536 days[b])
Uganda [26]	High	531, all ages	Monthly for 24 months	qPCR†	Spontaneous clearance‡‡‡ observed
Mozambique [52]	High	32 adult men	Daily/weekly for 28 days	qPCR	19%[b] cleared‡ 28 days
Kenya [27]	Moderate	246 children	Monthly for 12 months	nPCR	15%[b] cleared‡ ≤2 months; 53%[a] ≤6 months; 67%[a] ≤12 months
Gambia [15]	Dry season	37, all ages	Monthly for 6 months	qPCR†	41%[b] of infections cleared‡‡ <1 month; 60%[b] < 6 months
Vietnam [28]	Low	325 adults	Monthly for 24 months	uPCR	30%[b] cleared ≤1 month‡; 100%[b] ≤11 months
Cambodia [29]	Low	19 adults	Monthly for 12 months	uPCR	87%[b] cleared ≤1 month‡; 100%[b] ≤5 months

*Inclusion criteria. Studies evaluating individual-level longitudinal untreated subclinical *P. falciparum* infections using molecular diagnostics and intensive sampling (at least monthly). The sensitivity of PCR is as follows: conventional PCR < nested PCR (nPCR) < quantitative real time PCR (qPCR) < ultrasensitive high-volume qPCR (uPCR). Spontaneous clearance is defined as converting from positive to negative for a minimum of ‡1 sample, ‡‡2 samples, ‡‡‡3 samples, ‡‡‡‡≥4 samples. †Parasites were genotyped (e.g., MSP1/MSP2/GLURP) in the study to describe infection complexity but not to estimate clonal clearance.
[a]Denotes calculations performed from data available.
[b]Denotes estimates reported in original manuscript.

until completion of follow-up. Furthermore, another study demonstrated very little difference in conclusions of spontaneous clearance using more conservative definitions of spontaneous clearance (four consecutive monthly negative results) compared to 'single timepoint' clearance definitions, suggesting that single negative timepoint definitions may be sufficient to support conclusions of spontaneous clearance in some settings [3].

Accurate estimations of the probability of spontaneous clearance across populations are challenging given issues with unstandardised data reporting and heterogeneity in study populations (malaria transmission, participants), study design (sampling frequency, and follow-up times, particularly postspontaneous clearance event) as well as *P. falciparum* diagnosis (microscopy, PCR, genotyping). Additional, long-term, longitudinal studies, with intensive sampling and highly sensitive molecular diagnosis, including genetic characterisation of parasites, are warranted. These need to be undertaken in a range of transmission settings in order to provide the most detailed and robust generalisable evidence of infection duration and spontaneous clearance of *P. falciparum* parasites.

What impacts the likelihood of spontaneous clearance?

While the mechanisms behind spontaneous clearance events are yet to be elucidated, both immune-mediated and immune-independent factors may play an important role. **Naturally acquired immunity**, which is acquired after repeated exposure and can reduce parasite densities, may play an important role. The age-dependent acquisition of immunity may explain observations of faster spontaneous clearance of submicroscopic infections in older, compared to younger, children [20,27,38]. Furthermore, the observation that monoclonal infections clear faster than polyclonal infections [3] may also support some role of naturally acquired immunity in spontaneous clearance of parasites. However, direct evidence of the relationship between immunity and parasite clearance in the absence of antimalarial drug treatment has been examined in only two studies. In a historical study in The Gambia, sera from highly immune adults was transferred to children with clinical malaria [39], reducing parasite densities by 99% within 4 days, and

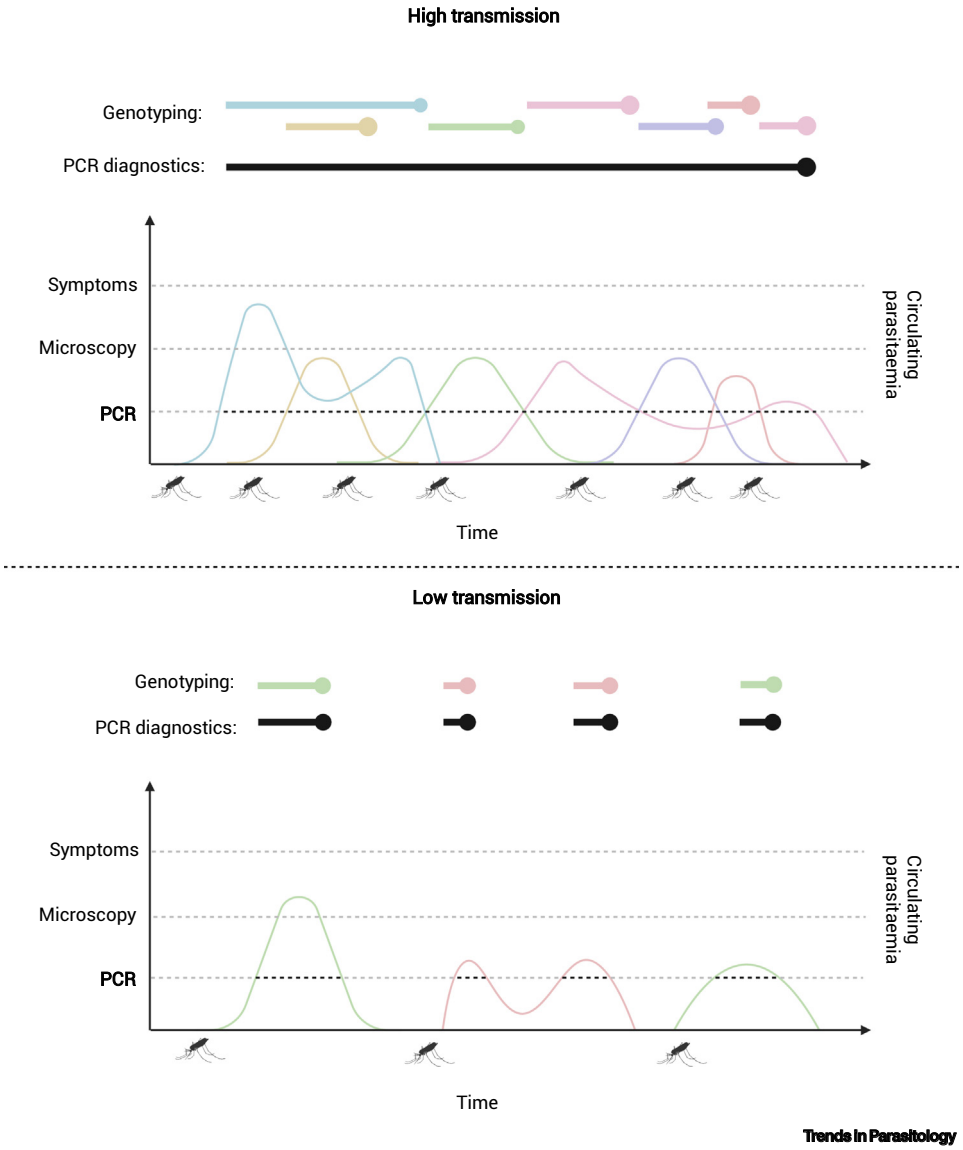


Figure 1. Detection of infections over time in high- and low-transmission settings.

For a Figure360 author presentation of Figure 1, see the figure legend at https://doi.org/10.1016/j.pt.2022.02.005. Each coloured line represents a single parasite strain introduced by a mosquito bite, which can be detected by PCR, microscopy, or symptoms depending on circulating parasite density. When using *Plasmodium* spp.-specific PCR diagnostics individuals can appear to be chronically infected (thick black line); however, genotyping studies demonstrate that individuals are constantly clearing one infection then being infected with new parasite strains. This is more likely to happen in areas of high transmission and high genetic diversity compared to low-transmission areas. Some infections may be recrudescent, that is, low-density or sequestered infections below the lowest detection limits which subsequently reappear at detectable levels. Figure created with BioRender.com.

resulting in 67% of these (microscopically-detected) infections being cleared within 9 days in the absence of antimalarial drug treatment [39]. More recently, in a study in Benin, newborns who were able to spontaneously clear their subclinical microscopically detected infections were found to have higher levels of (likely maternal) cytophilic classes of blood-stage (merozoite) antibodies [18]. Conversely, a recent study of Gambian children and adults found that individuals

Table 2. Spontaneous clearance events of circulating clonal *P. falciparum* infections*

Country	Transmission	Study population	Sampling	Genotyping	Spontaneous clearance events of individual genotypes
Ghana [25]	High	143 newborns	Monthly for 24 months	Microsatellites	50%[a] cleared‡ ≤2 months
Mali [30]	High	50 children	Monthly for 12 months	MSP1	0.38[a] infections cleared‡ per person per month
Sudan [10]	High	74, all ages	Every 14–28 days for 15 months	MSP1&2, GLURP	Spontaneous clearance ‡‡‡‡ observed in 3/3 of patient examples presented
Senegal [36]	High	3, all ages	Every 2–4 days for 2 months	MSP1&2	61%[b] cleared‡‡ in <27 days
	High	5, all ages	Every 1–2 weeks for 4 months		78%[b] cleared‡ in <81 days
Uganda [3]	High	149, all ages	Monthly for 18 months	AMA1	Spontaneous clearance‡‡‡‡ observed (Duration of clonal infections 103-447 days[a])
Uganda [26]	High	531, all ages	Monthly for 24 months	Amplicon deep sequencing AMA1	Spontaneous clearance‡‡‡ observed
PNG [37]	Medium	11 children	Every 2 weeks for 4 months	*var* genes, MSP2**	100%[b] *var* transcripts cleared‡ ≤10 weeks 90%[b] cleared‡ ≥1 MSP2 genotype <14 weeks
PNG [20]	Medium	28 children	Every 3rd day for 61 days	MSP2	90%[a] cleared‡‡ ≤1 month (5–14 years); 33%[a] (4 years)

*Inclusion criteria. Studies evaluating individual-level longitudinal untreated subclinical *P. falciparum* infections using genotyping and intensive sampling (at least monthly).
**Of microscopy-positive samples only. Spontaneous clearance is defined as converting from positive to negative for a minimum of ‡1 sample, ‡‡2 samples, ‡‡‡3 samples, ‡‡‡‡≥4 samples.
[a]Denotes estimates reported in original manuscript.
[b]Denotes calculations performed from data available.

with longer infections (≥3 months) tended to have antibody responses to more antigens than those with shorter infections, although this association was not statistically significant and did not take into account clearance of clonal infections reported in the study [15]. As it is well established that antibodies and other adaptive immune responses are crucial in protection against clinical malaria, it seems likely that similar mechanisms are involved in the regulation of subclinical infections and spontaneous clearance events. These may involve both the innate and adaptive immune response, including antibody-mediated functional responses such as complement deposition and opsonic phagocytosis [40]. Needless to say, further research to elucidate the role of immunity in spontaneous clearance of submicroscopic *P. falciparum* parasites is warranted.

There are a number of parasite factors which have been explored in the context of virulence and severity of disease; these factors are also likely to influence the duration of infection and spontaneous clearance. Many of these mechanisms are part of an intricate relationship between parasite and host innate and acquired immunity. *Plasmodium* spp. parasites produce an array of immunomodulatory factors such as heme, hemozoin, nucleic acids, and other microparticles, which interfere with a variety of host immune responses from inflammation to immune-cell recruitment, likely contributing to duration of infections, and may vary in potency between infecting strains [41]. Antigenic variation by the parasite (both of antigens on the invading merozoite and variant surface antigens expressed on the surface of infected erythrocytes) is an immune evasion strategy by parasites to avoid naturally acquired antibody-mediated immunity. Strain-specific acquired immunity can provide protection against clinical malaria upon re-exposure to genetically and antigenically similar parasites [42] and may also play a role in the chronicity of infection. One may speculate that, in areas of low transmission and low genetic diversity, naturally acquired immunity would develop quicker to circulating parasite strains compared to high-transmission areas, resulting in faster clearance of parasites from the circulation. Alternatively, there may be

other parasite virulence factors which confer a fitness advantage by promoting longer-lasting infections to aid onward transmission. It has recently been hypothesised that, in low-transmission areas where there is low competition with more monoclonal infections, less-virulent parasites have less competition from more-virulent strains (which can cause high-density clinical infections which are subsequently eliminated earlier with effective diagnosis and treatment) and therefore persist for longer at low parasite densities compared to highly virulent strains [43,44]. The complex interplay between host immunity and parasite virulence, competition, and diversity, and the impact on the probability and speed of spontaneous clearance in both high- and low-transmission settings, remains to be elucidated.

Immune-independent host and parasite factors such as **splenic clearance**, splenic accumulation, and undetected tissue reservoirs of infection through **sequestration** may also play a role. Sequestration is the process whereby *P. falciparum*-infected erythrocytes adhere to endothelial receptors, via parasite-expressed erythrocyte membrane protein 1 (PfEMP1, the most abundant variant surface antigen), in the microvasculature and organs. This process is a normal part of the final maturation stage of the 48 h *P. falciparum* asexual life cycle. Sequestration enables the parasite to avoid the spleen which removes infected erythrocytes from circulation through splenic clearance, a process in which infected erythrocytes are unable to pass through interendothelial gaps and are filtered out of circulation [45]. Sequestration may play a role in maintaining low circulating parasite densities, below the diagnostic detection limit, which can later re-emerge causing a recrudescent blood-stage infection. This is supported by several case studies of recrudescent infections post-splenectomy in individuals testing negative for *P. falciparum*, by either microscopy or PCR, prior to surgery [46–48]. Furthermore, the ability of parasites to sequester, and be cleared by the spleen, may also help to maintain parasites at low, undetectable levels through the dry season. A recent study demonstrated that parasites maintained in circulation throughout the dry season are transcriptionally distinct, are maintained in circulation for longer within each replicative cycle, have decreased cytoadhering capacity and increased rates of splenic clearance [6]. The authors hypothesised that these traits kept parasitaemia below the 'immunological radar' [6].

The spleen may also be a reservoir for infections in its own right. Recently, case studies of asymptomatic and untreated splenectomised individuals from Papua, Indonesia, demonstrated significant viable blood-stage parasite densities in the spleens of infected individuals [4,5]. In three of the nine patients with splenic *P. falciparum* infections there were no PCR-detectable infections in peripheral blood, indicating that a self-replicating and viable splenic parasite reservoir may give the appearance of clearance from circulation when parasites are, in fact, persisting in the spleen [4,5]. Furthermore, splenic infection, together with sequestered parasites, may cause recrudescence of circulating parasitaemia. This may explain what is often stated as strong evidence for long-lasting subclinical *P. falciparum* infections in the absence of treatment – numerous clinical case studies of infections acquired from transfusions or transplants, demonstrating that donor products can be infectious for as long as 13 years (median 1.9 years) [47,49] after the donor had last been in a malaria-endemic area as well as clinical case studies in immigrants from malaria-endemic areas being diagnosed with clinical *P. falciparum* malaria up to 15 years (median 3 years) after leaving malaria-endemic regions [47,50,51].

These case studies of splenic infection and long-lasting *P. falciparum* infections demonstrate that circulating parasites can be maintained subclinically for extended periods of time and below the limit of detection of conventional diagnostics. However, these studies are not designed to investigate the natural course of *Plasmodium* spp. infection. Importantly, case studies are also inherently subject to **endogeneity selection bias**, whereby all cases were selected based on their outcome

– a recrudescent or splenic *P. falciparum* infection. Therefore, a collection of case studies is not evidence that all infections are chronic and/or persist in the spleen and may instead represent outliers. The contribution of the spleen, or other sites of *P. falciparum* sequestration, in the evaluation of spontaneous parasite clearance is important to elucidate and quantify, especially when measures of parasite clearance rely solely on measures of circulating parasites.

Concluding remarks

In this opinion article we reorientated the narrative on infection chronicity and demonstrated that, while some *P. falciparum* infections do persist for extended periods, there is evidence that *P. falciparum* parasites can spontaneously clear in the absence of antimalarial drug treatment in different malaria-endemic settings. However, further intensive longitudinal studies, with highly sensitive molecular diagnosis, including genetic characterisation of parasites across a range of transmission settings, are warranted. The epidemiological data infer a role for both host and parasite factors in spontaneous clearance of *P. falciparum*; however, there is a paucity of studies that have directly investigated either factor in conjunction with the natural course of infection. It is most likely that immune-mediated and immune-independent mechanisms form part of a complex interaction between parasite virulence, competition, and diversity with host factors such as immunity and splenic clearance. Further investigation is particularly warranted in areas of varying transmission intensity where the epidemiology of malaria, genetic diversity of parasites, and acquisition of naturally acquired immunity will vary. Consequently, there are several outstanding questions that need to be addressed to advance our understanding of spontaneous clearance of *P. falciparum* infections as well as those caused by other *Plasmodium* spp. (see Outstanding questions).

Understanding spontaneous clearance of *Plasmodium* spp. infections will advance our knowledge of malaria epidemiology and transmission potential of malaria parasites in populations. This is particularly pertinent for subclinical transmission reservoirs, which are likely to go undetected and untreated by conventional test-and-treat approaches. Therefore, quantifying the duration of subclinical infections, and underlying risk factors, is paramount to advance our knowledge of malaria epidemiology and the transmission potential of malaria parasites, as well as inform interventions for malaria control and elimination.

Outstanding questions

How frequently does spontaneous clearance in the absence of antimalarial drug treatment occur in malaria-endemic populations?

How does spontaneous clearance vary according to *Plasmodium* spp., clonality, and malaria transmission setting?

How does spontaneous clearance influence the duration of infections and the transmissibility of malaria within populations?

Does naturally acquired immunity affect the probability of, and time to, spontaneous clearance of subclinical infections? Is immune-mediated clearance strain-specific, and what are the functional mechanisms?

What parasite factors play a role in spontaneous clearance?

What is the contribution of undetected sequestered parasites, splenic infections, and ultra-low parasitaemia to infection chronicity? And what is the relative frequency of these phenomena in malaria-endemic populations?

Acknowledgments

The authors would like to acknowledge the financial support from the National Health and Medical Research Council of Australia (Career Development Fellowship 1166753 to F.J.I.F); the Australian Centre for Research Excellence in Malaria Elimination (Centres for Research Excellence grant 1134989 to F.J.I.F); Independent Research Institutes Infrastructure Support Scheme to Burnet Institute and a Victorian State Government Infrastructure Support grant to Burnet Institute, and the Australian Commonwealth Government (Australian Government Research Training Program scholarship to M.S.R.).

Declaration of interests

The authors declare no competing interests.

References

1. Nyarko, P.B. and Claessens, A. (2020) Understanding host–pathogen–vector interactions with chronic asymptomatic malaria infections. *Trends Parasitol.* 37, 195–204
2. Barry, A. *et al.* (2021) Higher gametocyte production and mosquito infectivity in chronic compared to incident *Plasmodium falciparum* infections. *Nat. Commun.* 12, 1–12
3. Briggs, J. *et al.* (2020) Sex-based differences in clearance of chronic *Plasmodium falciparum* infection. *eLife* 9, e59872. https://doi.org/10.7554/eLife.59872
4. Kho, S. *et al.* (2021) Hidden biomass of intact malaria parasites in the human spleen. *N. Engl. J. Med.* 384, 2067–2069
5. Kho, S. *et al.* (2021) Evaluation of splenic accumulation and colocalization of immature reticulocytes and *Plasmodium vivax* in asymptomatic malaria: a prospective human splenectomy study. *PLoS Med.* 18, e1003632 Posted May 26, 2021. https://doi.org/10.1371/journal.pmed.1003632
6. Andrade, C.M. *et al.* (2020) Increased circulation time of *Plasmodium falciparum* underlies persistent asymptomatic infection in the dry season. *Nat. Med.* 26, 1929–1940
7. Portugal, S. *et al.* (2017) Treatment of chronic asymptomatic *Plasmodium falciparum* infection does not increase the risk of clinical malaria upon reinfection. *Clin. Infect. Dis.* 64, 645–653

8. Eyles, D.E. and Young, M.D. (1951) The duration of untreated or inadequately treated *Plasmodium falciparum* infections in the human host. *J. Natl. Malar. Soc.* 10, 327–336
9. Bruce-Chwatt, L.J. (1962) A longitudinal survey of natural malaria infection in a group of West African adults. *West Afr. Med. J.* 12, 199–217
10. Babiker, H.A. *et al.* (1998) Characteristics of *Plasmodium falciparum* parasites that survive the lengthy dry season in eastern Sudan where malaria transmission is markedly seasonal. *Am. J. Trop. Med. Hyg.* 59, 582–590
11. Roper, C. *et al.* (1998) Seasonal changes in the *Plasmodium falciparum* population in individuals and their relationship to clinical malaria: a longitudinal study in a Sudanese village. *Parasitology* 116, 501–510
12. Delley, V. *et al.* (2000) What does a single determination of malaria parasite density mean? A longitudinal survey in Mali. *Tropical Med. Int. Health* 5, 404–412
13. Sondén, K. *et al.* (2015) Asymptomatic multiclonal *Plasmodium falciparum* infections carried through the dry season predict protection against subsequent clinical malaria. *J. Infect. Dis.* 212, 608–616
14. Cucunubá, Z.M. *et al.* (2008) Asymptomatic *Plasmodium* spp. infection in Tierralta, Colombia. *Mem. Inst. Oswaldo Cruz* 103, 668–673
15. Collins, K.A. *et al.* (2021) A cohort study on the duration of *Plasmodium falciparum* infections during the dry season in The Gambia. *medRxiv* Posted November 14, 2021. https://doi.org/10.1101/2021.11.12.21266275
16. Earle, W.C. and World Health Organization (1962) The course of naturally acquired malaria. Institutional Repository for Information Sharing. https://apps.who.int/iris/handle/10665/64925
17. Pinkevych, M. *et al.* (2012) The dynamics of naturally acquired immunity to *Plasmodium falciparum* infection. *PLoS Comput. Biol.* 8, e1002729. https://doi.org/10.1371/journal.pcbi.1002729
18. Adamou, R. *et al.* (2019) *Plasmodium falciparum* merozoite surface antigen-specific cytophilic IgG and control of malaria infection in a Beninese birth cohort. *Malar. J.* 18, 194
19. Botwe, A.K. *et al.* (2020) Profiles of *Plasmodium falciparum* infections detected by microscopy through the first year of life in Kintampo a high transmission area of Ghana. *PLoS One* 15, e0240814. https://doi.org/10.1371/journal.pone.0240814
20. Bruce, M. *et al.* (2000) Genetic diversity and dynamics of *Plasmodium falciparum* and *P. vivax* populations in multiply infected children with asymptomatic malaria infections in Papua New Guinea. *Parasitology* 121, 257–272
21. Branch, O. *et al.* (2005) Clustered local transmission and asymptomatic *Plasmodium falciparum* and *Plasmodium vivax* malaria infections in a recently emerged, hypoendemic Peruvian Amazon community. *Malar. J.* 4, 27
22. Tangpukdee, N. *et al.* (2009) Malaria diagnosis: a brief review. *Korean J. Parasitol.* 47, 93–102
23. Hofmann, N. *et al.* (2015) Ultra-sensitive detection of *Plasmodium falciparum* by amplification of multi-copy subtelomeric targets. *PLoS Med.* 12, e1001788. https://doi.org/10.1371/journal.pmed.1001788
24. Farnert, A. *et al.* (1997) Daily dynamics of *Plasmodium falciparum* subpopulations in asymptomatic children in a holoendemic area. *Am. J. Trop. Med. Hyg.* 56, 538–547
25. Franks, S. *et al.* (2001) Frequent and persistent, asymptomatic *Plasmodium falciparum* infections in African infants, characterized by multilocus genotyping. *J. Infect. Dis.* 183, 796–804
26. Andolina, C. *et al.* (2021) Sources of persistent malaria transmission in a setting with effective malaria control in eastern Uganda: a longitudinal, observational cohort study. *Lancet Infect. Dis.* 21, 1568–1578
27. Baliraine, F.N. *et al.* (2009) High prevalence of asymptomatic *Plasmodium falciparum* infections in a highland area of western Kenya: a cohort study. *J. Infect. Dis.* 200, 66–74
28. Nguyen, T.-N. *et al.* (2018) The persistence and oscillations of submicroscopic *Plasmodium falciparum* and *Plasmodium vivax* infections over time in Vietnam: an open cohort study. *Lancet Infect. Dis.* 18, 565–572
29. Tripura, R. *et al.* (2016) Persistent *Plasmodium falciparum* and *Plasmodium vivax* infections in a western Cambodian population: implications for prevention, treatment and elimination strategies. *Malar. J.* 15, 181
30. Krogstad, D.J. *et al.* (2015) Molecular incidence and clearance of *Plasmodium falciparum* infection. *Malar. J.* 14, 415
31. Homann, M.V. *et al.* (2017) Detection of malaria parasites after treatment in travelers: a 12-months longitudinal study and statistical modelling analysis. *EBioMed.* 25, 66–72
32. Dakić, Z. *et al.* (2014) Clinical significance of molecular methods in the diagnosis of imported malaria in returning travelers in Serbia. *Int. J. Infect. Dis.* 29, 24–30
33. Phuong, M. *et al.* (2015) Survival analysis of diagnostic assays in *Plasmodium falciparum* malaria. *Malar. J.* 14, 350
34. Aydin-Schmidt, B. *et al.* (2013) Usefulness of *Plasmodium falciparum*-specific rapid diagnostic tests for assessment of parasite clearance and detection of recurrent infections after artemisinin-based combination therapy. *Malar. J.* 12, 349
35. Bretscher, M.T. *et al.* (2010) Detectability of *Plasmodium falciparum* clones. *Malar. J.* 9, 234
36. Daubersies, P. *et al.* (1996) Rapid turnover of *Plasmodium falciparum* populations in asymptomatic individuals living in a high transmission area. *Am. J. Trop. Med. Hyg.* 54, 18–26
37. Kaestli, M. *et al.* (2004) Longitudinal assessment of *Plasmodium falciparum* var gene transcription in naturally infected asymptomatic children in Papua New Guinea. *J. Infect. Dis.* 189, 1942–1951
38. Felger, I. *et al.* (2012) The dynamics of natural *Plasmodium falciparum* infections. *PLoS One* 7, e45542. https://doi.org/10.1371/journal.pone.0045542
39. Cohen, S. *et al.* (1961) Gamma-globulin and acquired immunity to human malaria. *Nature* 192, 733–737
40. O'Flaherty, K. *et al.* (2022) The role of naturally acquired antimalarial antibodies in subclinical *Plasmodium* spp. infection. *J. Leukoc. Biol.*, 1–9 Posted January 20, 2022. https://doi.org/10.1002/JLB.5MR1021-537R
41. Deroost, K. *et al.* (2016) The immunological balance between host and parasite in malaria. *FEMS Microbiol. Rev.* 40, 208–257
42. Doolan, D.L. *et al.* (2009) Acquired immunity to malaria. *Clin. Microbiol. Rev.* 22, 13–36
43. Björkman, A. and Morris, U. (2020) Why asymptomatic *Plasmodium falciparum* infections are common in low-transmission settings. *Trends Parasitol.* 36, 898–905
44. Björkman, A.B. (2018) Asymptomatic low-density malaria infections: a parasite survival strategy? *Lancet Infect. Dis.* 18, 485–486
45. Chotivanich, K. *et al.* (2002) Central role of the spleen in malaria parasite clearance. *J. Infect. Dis.* 185, 1538–1541
46. Bachmann, A. *et al.* (2009) Absence of erythrocyte sequestration and lack of multicopy gene family expression in *Plasmodium falciparum* from a splenectomized malaria patient. *PLoS One* 4, e7459. https://doi.org/10.1371/journal.pone.0007459
47. Ashley, E.A. and White, N.J. (2014) The duration of *Plasmodium falciparum* infections. *Malar. J.* 13, 500
48. Hviid, L. (2020) Precipitated malaria: it never rains but it pours. *Trends Parasitol.* 36, 721–723
49. Anand, A. *et al.* (2018) Investigation of a case of suspected transfusion-transmitted malaria. *Transfusion* 58, 2115–2121
50. Zammarchi, L. *et al.* (2018) Cryptic severe *Plasmodium falciparum* malaria in a Moroccan man living in Tuscany, Italy, August 2018. *Euro Surveill.* 23, 1800527
51. Dauby, N. *et al.* (2018) Case report: delayed or recurrent *Plasmodium falciparum* malaria in migrants: a report of three cases with a literature review. *Am. J. Trop. Med. Hyg.* 98, 1102–1106
52. Galatas, B. *et al.* (2018) Dynamics of afebrile *Plasmodium falciparum* infections in Mozambican men. *Clin. Infect. Dis.* 67, 1045–1052