8 | KILLING THE PARASITE

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8.1 | Introduction

The pattern of malaria transmission around the world is highly variable and covers a broad spectrum of epidemiological situations ranging from areas with a high population at risk, high mortality, and high transmission (predominantly *Plasmodium falciparum* malaria) to the other extreme of low population at risk, low mortality, and low mixed-species transmission. As we have seen, a very different approach is needed to achieve elimination of the parasite from low-transmission settings than is required for the attack on disease in high-transmission settings. A conceptual and operational shift must be made, from prevention and treatment of disease in individuals across entire or broad areas of the country, to community-focused strategies aimed at ending transmission and eliminating residual foci of infection. Strategies for elimination must be based on accurate case reporting and precise assessments of the epidemiology and the populations at risk (Chapter 2). It will be necessary for an elimination program to constantly monitor the shifting character of malaria and adapt intervention strategies appropriately to these changes as they occur, as an aggressive intervention program will change the pattern of malaria over time.

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BOX 8.1 | Main Messages

- Strategies developed for malaria elimination should be planned to detect all infections and not just those that are responsible for clinical malaria.
- The progress of a malaria elimination initiative should be monitored regularly, as the epidemiology will change and measures used for parasite killing (and vector control) may need to be modified. It is important to obtain accurate estimates of the numbers of infections persisting in the community.
- Clinical diagnosis is inappropriate for an elimination program and should be replaced by malaria-parasite-specific diagnosis, by either rapid diagnostic tests (RDTs) or microscopy of blood films. Reference facilities, with personnel to provide quality assurance for microscopy and RDTs, are needed.
- Diagnostic measures should assume that all *Plasmodium* species can persist as both subclinical and mixed infections.
- Trials of drug combinations that include a drug capable of killing gametocytes (or stages developing in the mosquito) should be undertaken for both treatment and mass drug administration (MDA). Safety should be a priority, particularly when drugs are likely to be given to a large number of people who are not infected.
- An assessment should be made of the appropriateness of using either MDA or mass screening and treatment (MST) in order to find and kill the last parasites.
- There needs to be greater focus on *P. vivax*, as the number of infections and the severity of the disease are commonly underestimated.
- *P. vivax* and *P. ovale* present particularly challenging problems because they can persist undetected in the liver for 3 to 5 years. A detection and treatment strategy should assume that new blood infections can occur in an individual over several years without exposure to infectious mosquito bites.

8.2 | Non-*falciparum* Malaria: A Challenge to Elimination

PLASMODIUM VIVAX

The focus of malaria control programs has, to date, been largely on *P. falciparum* because this parasite is the major cause of mortality and severe clinical malaria, especially in tropical Africa, although there is recent evidence that the burden of *P. falciparum* infection in Southeast Asia may have been underestimated.[1,2] However, once elimination becomes the target, *P. vivax* needs to

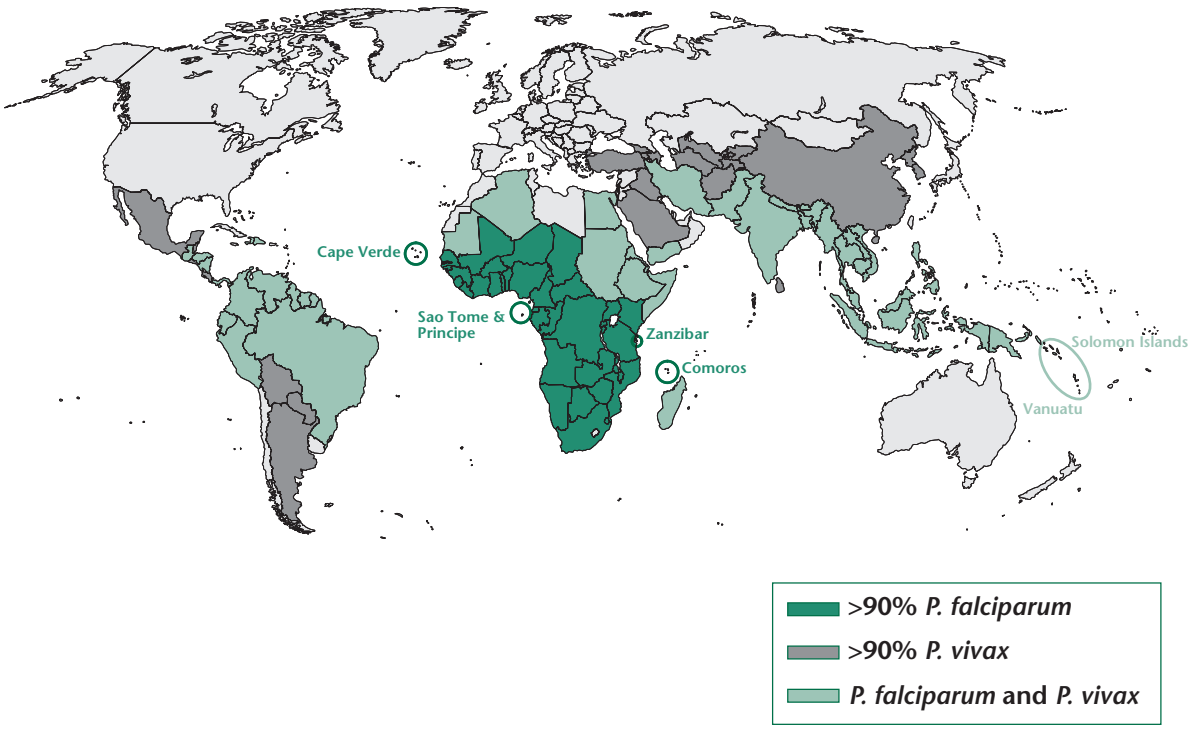

FIGURE 8.1 | The distribution of *P. falciparum* and *P. vivax* by country

be given much more attention. As discussed in Chapter 5, the proportion of the 3.6 billion people who were living at risk of malaria in 2005 was higher for *P. vivax* than for *P. falciparum*.[3] As many as 250 million infections may be due to *P. vivax* each year.[4] In many places outside Africa, such as in some countries of Central and South America, *P. vivax* is the dominant malaria problem.[5] As shown in Figure 8.1, *P. vivax* and *P. falciparum* coexist in many countries around the world.

Issues such as underdiagnosis, relapse from dormant liver stages, a poor understanding of mechanisms of acquisition of immunity, and interspecies interaction complicate any malaria control intervention in areas where *P. vivax* infection predominates and will block achievement of the goal of eradication unless taken into account.[6]

The low priority given to *P. vivax* infections by policy makers, funders, and researchers stems in part from the historical under-recognition of the scale of the problem, an issue which is now being acknowledged. Even more of an obstacle has been the definition of *P. vivax* malaria as "benign" malaria, implying that it does not present as serious an infection and can be ignored until the *P. falciparum* malaria problem is controlled. This perception is being seriously

challenged by a growing number of case studies that indicate that *P. vivax* can cause severe malaria.[7] Two recent studies on the island of New Guinea, from both the Papua, Indonesian, side and the Papua New Guinean (PNG) side, have shown that *P. vivax* can cause severe disease.[8, 9] In the PNG study of almost 10,000 children, mainly under 5 years old, the proportion of cases with a WHO definition of severe malaria caused by *P. falciparum* was 11.7%, while *P. vivax* followed closely behind at a substantial 8.8%.

Although there is increasing recognition that *P. vivax* contributes significantly to the global malaria burden, the number of infections persisting in the community is probably still being massively underestimated. This has significant implications for those countries where *P. vivax* malaria is endemic and that are already setting an elimination goal, for example, Vanuatu and the Solomon Islands. The extent of this underestimation has been revealed by the use of sensitive methods based on polymerase chain reaction (PCR) for diagnosis of blood-stage infections in large community studies in areas of PNG where the four human malaria species are co-transmitted.[10, 11] Increases in the estimated prevalence of *P. vivax* by 2- to 3.5-fold were observed, and even greater increases in the prevalence of *P. malariae* and *P. ovale* were seen.[12] The number of estimated mixed infections increased by orders of magnitude when these sensitive detection methods were used. We should note that the same problem of underestimation of prevalence can occur with *P. falciparum* in apparently low-endemicity areas, unless the sensitive diagnostic methods are employed.[13]

One of the big obstacles to stopping transmission of *P. vivax,* and one of its major distinctions from *P. falciparum*, is the ability of *P. vivax* to relapse after cure of the original bloodstream infection. A proportion of sporozoites remain dormant as hypnozoites for periods as short as a few weeks or as long as 5 years before emerging to cause a clinical, blood-stage infection (Chapter 5). The dormant stages are not detectable, and the ability to relapse will hinder elimination of this parasite. In order to interrupt transmission completely, it will be necessary to kill the hypnozoites.

MIXED INFECTIONS

PCR-based studies such as those discussed above have shown that there is a much larger pool of mixed infections than suspected, which raises another difficulty for elimination. In areas where transmission of more than one malaria species is common, a malaria-infected person is very likely to be co-infected with more than one species of *Plasmodium*. In such circumstances, there may be interspecies interactions that are modified by interventions that alter the bal-

ance between species, as has been seen in the highlands of Papua New Guinea.[14, 15] The question remains open as to whether the simultaneous presence of non-*falciparum* malaria can reduce the clinical impact of infection with *P. falciparum*. Good examples can be found in the literature arguing either way, although a recent meta-analysis of all available studies fell on the side of a significant negative association between mixed infection and clinical disease.[16] Most of these earlier studies are, however, colored by the underestimation inherent in the use of non-PCR-based techniques for diagnosis, and more research is needed to determine how the pattern of malaria might be altered in areas where infection with multiple species is common as a program moves toward elimination.

8.3 Malaria Immunity and Elimination

People who live in malaria-endemic areas show an age-structured burden of clinical disease, with older children and adults having resistance to severe morbidity and death due to the acquisition of natural immunity, although the nature of the immunological changes that are involved is still not fully understood.[17] Once control programs have reached the stage at which elimination in a particular community is a possibility, it is likely that there will have been a reduction in the level of naturally acquired immunity in that community, though it may be a number of years before there is a substantial loss in the community as a whole. This progressive change may have a significant impact on the final attempts to achieve elimination. Some examples of the changes that may be encountered are considered below.

Reduction in naturally acquired immunity in a community may result in a change in the age pattern of the few clinical infections that continue to occur, with more cases being seen, first in older children and then in adults, than had been the case previously. This necessitates a change in treatment programs with, for example, an increased focus on older schoolchildren.

There is strong evidence that in malaria-endemic areas where some level of drug resistance is present, treatment success is often enhanced by naturally acquired immunity. As control improves and elimination becomes a feasible target, highly effective drug combinations will be needed that can achieve cure without any help from naturally acquired immunity.

Reduction in the community level of acquired immunity as a result of successful control programs over a period of years will also increase the risk of an epidemic resurgence of the infection, as seen in the highlands of Madagascar[18] and on the island of Mauritius (Chapter 10) when control programs failed after a lengthy period of success. Much still needs to be understood about the impor-

tant and dynamic interplay between immunity and exposure before we can be confident in predicting the effect of interventions and can formulate strategies to minimize adverse impact.

8.4 Finding and Killing the Last Parasites

In an elimination program, treatment of a sufficient number of infected subjects in a community, whether they are symptomatic or asymptomatic, to interrupt transmission becomes the primary goal. Two possible approaches to this objective can be adopted—detection and treatment of infected individuals capable of transmitting the infection, or MDA given to as large a proportion of the population as possible on the grounds that this will cover a high proportion of those infected. As naturally acquired immunity wanes, the proportion of symptomatic individuals increases, making it easier to detect them as they are more likely to seek treatment. However, as we have seen, even in areas of relatively low transmission, asymptomatic individuals are still detected, and they need to be treated in order to interrupt transmission. The availability of a sensitive method for diagnosing malaria is essential for this strategy of malaria elimination.

DIAGNOSIS OF MALARIA INFECTION

When killing the last remaining parasites becomes the goal, an ability to identify all parasites becomes increasingly important. Good-quality microscopy conducted by skilled technicians with capacity to manage appropriate quality control, and currently available RDTs, whose effective use requires less training than microscopy, are generally adequate for diagnosis in persons who are acutely ill with malaria. However, there are particular issues to be addressed with both procedures. Ensuring the quality of microscopy used for routine diagnosis has often proved difficult, as the sensitivity and specificity of routine microscopy is significantly lower when compared with that of qualified microscopists based in central reference laboratories. This underlines the need for good training in microscopy for staff in primary health centers, coupled with the provision of reliable, well-maintained equipment and regular monitoring and quality control (Chapter 2).

There is a wide range of commercially available RDTs. Each one incorporates a monoclonal antibody that detects one of three well-characterized proteins of the malaria parasites. Though cost is a problem, they are becoming widely used. Among the many tests being manufactured, there is considerable vari-

ability in quality, however, so it is important to establish quality assurance programs for quality of manufacture, plus measures of their stability and performance over time.[19, 20] Some RDTs detect only *P. falciparum*, but others can distinguish between *P. falciparum* and non-*falciparum* malarias, although RDTs are generally less sensitive at detecting non-*falciparum* infections.[21]

When compared against each other, microscopy and RDTs detect a similar minimum threshold density of parasites (about 50 parasites per microliter of blood). Thus, the choice for routine use is this: use microscopy, which is technically more difficult but is better for species identification (especially non-*falciparum* species) and for estimating parasite densities, or diagnose with the user-friendly RDT, which gives a positive or negative result (but not a measure of the density of parasites) and is not as good for detecting *P. vivax* and the other non-*falciparum* parasites.

Since most elimination efforts will need to deal with both low-density parasitemias and non-*falciparum* species, diagnosis becomes a major challenge for elimination programs. More-sensitive methods of diagnosis than microscopy and RDTs are likely to be needed, including those that can detect small numbers of gametocytes. Although the propensity of a gametocyte carrier to transmit infection is related to the density of gametocytemia, individuals with very low gametocyte numbers can still transmit infection and can be an important part of the reservoir of infection. Thus, if an elimination program is to be based on detection and treatment of all potential transmitters of infection, much more sensitive detection tests will be needed.

PCR assays provide the sensitivity needed to detect low parasitemias, including low-level gametocyte infections. Studies in Kenya and Tanzania using the QT-NASBA real time PCR assay have shown that this increases the number of gametocyte carriers detected in the population 40-fold over the number detected by microscopy. LAMP assays may prove to be equally sensitive.[22] Developing tests with the sensitivity of these assays that can be employed in field situations is a key priority for the operational research agenda (Chapter 10) in elimination.

Serology, which employs relatively crude assays such as the measurement of antibodies against the whole parasite by fluorescence, was occasionally used during previous eradication programs to monitor their impact, but serology has, until recently, been a largely neglected aspect of malaria research. In China, immunofluorescence assays are being used in schools at the end of malaria transmission seasons to measure how much *P. vivax* transmission has occurred, and it is used as a guide to whether any control interventions are needed. New studies using antibody assays to defined malaria antigens, particularly MSP-1,

have shown that serology can play an important role in assessing malaria endemicity, and it could therefore make an important contribution to elimination programs. It is unlikely to be used to detect infection in individual subjects, but it may prove to be very useful in monitoring the progress of elimination efforts and for detecting foci where transmission is still continuing, and where extra control efforts are needed.

DRUGS TO KILL THE LAST PARASITES

Treatment of malaria in the context of elimination necessitates achieving a complete parasitological cure, including killing of the parasites in their sexual stages, either in the blood of the infected subject or in the midgut of any vector mosquito that ingests them. Artemisinin-based combination therapies (ACTs), now the first-line treatment for *P. falciparum* malaria in nearly all countries, have an advantage over many other antimalarials used for treatment—they have some effect on gametocytes, thus reducing the potential for transmission. The introduction of ACTs may have contributed to the marked reduction in the incidence of *P. falciparum* malaria seen on the Thailand-Myanmar border[23] and, more recently, in some countries in Africa, such as South Africa[24] and Zanzibar. However, the effect of artemisinins on gametocytes of *P. falciparum* is not complete, and patients treated with artemisinins can still transmit malaria infection.[25] In fact, the mature gametocytes of *P. falciparum* are resistant to most of the antimalarial drugs that affect the asexual stages, and they develop much more slowly than gametocytes of the other three species. Currently, the only licensed drug that can ensure complete killing of *P. falciparum* gametocytes is the 8-aminoquinoline drug primaquine, which is very effective at preventing transmission when given as a single treatment. Thus, in the context of elimination, any patient treated for *P. falciparum* malaria should also receive primaquine in addition to the primary treatment unless he or she is glucose-6-phosphate dehydrogenase (G6PD) deficient and thus at risk from hemolysis.[26] Within the context of an active case detection program, the inclusion of screening for G6PD deficiency is recommended, but the tests available are not readily applicable, and testing becomes increasingly difficult for mass treatment programs. Development of simple, cheap, field-friendly tests for G6PD deficiency (Chapter 10) would greatly facilitate the elimination agenda, particularly because there are different forms of G6PD deficiency, some of them relatively mild and therefore perhaps not presenting such a serious risk to the treated patient.

There are many factors that can lead to an increase in the number of game-

tocytes of *P. falciparum* circulating in the blood and hence capable of increasing transmission to vector mosquitoes. Most of these are not well defined, but the numbers can increase during the course of a long infection (being higher at the end of a season of transmission than at the beginning), when the patient is anemic, and as a consequence of the development of drug resistance. This last effect is particularly important as the increased transmissibility contributes to the spread of resistance. Increase in gametocyte numbers has been identified as the first indication that a drug is beginning to fail and emphasizes the need for treatment to include drugs that will kill the sexual stages—what has been called "prevention by treatment."[19, 27]

Gametocytes of *P. vivax, P. ovale*, and *P. malariae* appear in the circulation at the same time as the asexual stages and, unlike the gametocytes of *P. falciparum*, are killed by the antimalarial drugs that are effective against the asexual blood stages. *P. vivax* transmits well at very low parasite densities, so transmission can already have occurred before a patient has become symptomatic and sought treatment.[19]

Obtaining a complete cure of *P. vivax* or *P. ovale* malaria is a more complex procedure than is the case for *P. falciparum* infections, as it involves not only killing sexual and asexual blood-stage parasites but also eliminating residual inactive parasites in the liver (hypnozoites). Currently, primaquine is the only licensed drug that can do this.[28] As mentioned above, primaquine can cause hemolysis when given to subjects who are G6PD deficient, and this complication is more likely to occur when the drug is used to eliminate hypnozoites, as opposed to killing gametocytes, as a much more prolonged course of treatment is needed—for example, a 14-day course.[26] Tafenoquine is a new 8-aminoquinoline under development that has the advantage over primaquine that a much shorter course of treatment is needed.[29] However, it still has a propensity to cause hemolysis in G6PD-deficient subjects, and development of a safer treatment for killing *P. vivax* hypnozoites is a high research priority that is now being addressed by organizations such as the Medicines for Malaria Venture (MMV).

8.5 | Mass Drug Administration and Elimination

MDA has a mixed reputation and is not recommended by WHO. Part of the antagonism comes from a form of MDA that involved use of salt fortified with chloroquine or pyrimethamine (the Pinotti method) that, predictably, led to the rapid development of resistance. However, other forms of targeted MDA have been much more successful, for example, intermittent preventive treatment

(IPT) in infants and children[30] (though IPT is not appropriate in low-endemic settings). Many large community-based studies of MDA, such as those undertaken in Nicaragua and Garki, Nigeria, have shown that community-based MDA can be highly effective in reducing parasite prevalence to a very low level but that parasitemia soon rebounds to its previous level once MDA is stopped.[31] Thus, this form of MDA has no role in disease control programs, except during epidemics. However, MDA could play a key role in the final stages of an elimination program as an alternative to an active case detection program, once the level of infection has been reduced to a low level.[27] Although a difficult and labor-intensive process, MDA may be easier and more effective than mass screening and treatment, and previous studies have shown that a high level of coverage can be achieved for a limited number of treatment rounds, provided there is full involvement of the community. MDA probably played an important role in the elimination of *P. falciparum* and *P. vivax* malaria from Aneityum, Vanuatu.[32]

Drugs used for MDA should ideally be active against sexual-stage parasites (and hypnozoites, if used in an area where *P. vivax* or *P. ovale* infections are present), and they must be very safe, as a high proportion of the subjects treated are likely to be uninfected. Any serious adverse event that could clearly be linked to the medication would end a community's participation, no matter what the long-term risk-benefit equation indicated. Whether it would be safe to use primaquine for MDA in large populations where G6PD deficiency prevalence is high without screening is uncertain; a safer drug, or drug combination, for MDA is urgently needed.[28]

8.6 Vaccines

This *Prospectus* focuses on the tools available to eliminate malaria today and/or in the near future, and it therefore pays little attention to malaria vaccines. This is because it is unlikely that a malaria vaccine that is effective enough to play a significant role in malaria elimination will become available in the next few years. However, in the longer term, malaria vaccines may have a very important role to play in malaria elimination programs, especially in areas where the infection is otherwise difficult to control.

Any malaria vaccine that is highly effective at preventing infection, regardless of whether it acts at the pre-erythrocytic or erythrocytic stage of parasite development, will have an impact on transmission. However, in areas of mod-

erate or high transmission, modeling indicates that for a significant effect to be achieved, efficacy will need to be very high, probably as high as 95%.

Thus, as elimination becomes an increasingly realistic prospect, there has been renewed interest in the development of vaccines which are targeted specifically at preventing transmission either by inducing an immune response that destroys gametocytes or interferes with the development of the parasite in the mosquito. A move to elimination has raised the development of transmission blocking vaccines higher up the malaria research agenda than in the past and a number of candidates are now reaching the stage of early clinical trials.[33] For transmission blocking vaccines to be most effective they will need to be given to as large a proportion of the population as possible, and probably delivered through mass campaigns in a manner analogous to that used to deliver drugs in MDA programs.

8.7 | Conclusion

Elimination of malaria involves a paradigm shift away from treating patients with malaria toward killing the last few malaria parasites. Relapsing malaria such as *P. vivax* will become increasingly important as current measures limit transmission of *P. falciparum* malaria. Improved means to detect asymptomatic persons with low parasitemia will be crucial to malaria elimination. Effective chemotherapy is and will remain a primary means of achieving malaria control and eventually elimination. Mass screening (active case detection) and MDA are alternative approaches toward this goal, but both are hindered by the lack of a safe and effective drug that is highly effective at killing both the sexual stages of all the main human malaria parasites and the resting stages of the relapsing malaria infections.

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