Asymptomatic and submicroscopic malaria: the danger in the cases we don't see

William R Brieger discusses the complexities that the end-game in the defeat of malaria is going to have to face. How to interrupt transmission from the unknowingly affected hosts

As countries move closer to eliminating malaria a debate arises about the importance of asymptomatic plasmodial infections (API) in the overall transmission of the disease. The new Malaria Elimination Framework[1] from the Global Malaria Programme of the World Health Organization (WHO) stresses that malaria surveillance and case detection are crucial aspects in a national malaria strategy that must be built on a thorough stratification of a country according to malaria transmission characteristics. In practical terms people ask whether in fact an asymptomatic infection actually contributes to transmission.

The WHO explains that, 'A suspected malaria case cannot be considered a malaria case until parasitological confirmation', whether this case is symptomatic or asymptomatic. A variety of tests enable the parasitological confirmation, including the basic microscopy where parasites are seen, to rapid diagnostic tests (RDTs), polymerase chain reaction (PCR) or other newly developing tools. In most settings at present it is microscopy and RTDs that are most practical for real time study. In short WHO says that, 'In settings where malaria is actively being eliminated or has been eliminated, a 'case' is the occurrence of any confirmed malaria infection with or without symptoms'.

Furthermore, according to WHO, 'A malaria case can be classified as indigenous, induced, introduced, imported, relapsing or recrudescent (depending on the origin of infection).' Information on the origins and locations of malaria cases must guide the choice and implementation of strategies to bring about the end of transmission in a particular locality. Several recent studies describe the importance of paying attention to asymptomatic infections.

As the WHO outlines, passive case detection tests and treats persons who present at clinics with suspected malaria.[1] On the other hand, active case detection 'requires extra effort to find malaria cases among people who do not present to health facilities, for various reasons, including living in a remote area, populations such as migrants and refugees who may not use or have access to routine healthcare and asymptomatic infections'. This extra effort benefits elimination programmes, 'by detecting infected people who may risk transmitting malaria but are not detected by passive case detection'.

William R Brieger is a Professor for the Department of International Health, the Johns Hopkins Bloomberg School of Public Health, and Senior Malaria Specialist, Jhpiego, an affiliate of Johns Hopkins University.

Threat to health and transmission

In the Bagamoyo District of Tanzania, Sumari and colleagues collected blood samples and examined them for *Plasmodium falciparum* prevalence using RDT, light microscopy (LM) and reverse transcription quantitative PCR.[2] While overall prevalence was higher in symptomatic children using all three methods, asymptomatic children had a higher prevalence of gametocytes using light microscopy and PCR. They concluded that, 'The higher gametocytemia observed in asymptomatic children indicates the reservoir infections and points to the need for detection and treatment of both asymptomatic and symptomatic malaria'. Therefore, 'asymptomatic infections become increasingly important for interrupting transmission'.

The health effects of active pharmaceutical ingredients (API) on children were documented in Rwanda. These included 'Plasmodium infection was associated with anaemia, fever, underweight, clinically assessed malnutrition, and histories of fever, tiredness, weakness, poor appetite, abdominal pain, and vomiting', and were generally more common with submicroscopic infection.[3]

Besides children, other groups are at risk from API. Malaria during pregnancy is a health threat to both the pregnant woman and the unborn child. Thirty-seven percent (37%) of asymptomatic pregnant women who had just delivered in Colombia were found to have parasitemia.[4] Using microscopy only 8% were identified, such that without PCR the true extent of the problem would not have been identified. Thus, there is also concern for submicroscopic malaria, as well as API generally. Asympto-matic and submicroscopic infections in areas co-endemic for *P. falciparum* and *P. vivax* are major contributors to anaemia, not only in children but also in adults.[5]

The question of appropriate methods for identifying API was raised in a survey of health individuals living in the China-Myanmar

border area.[6] Zhao et al explained that, 'Sensitive methods for detecting asymptomatic malaria infections are essential for identifying potential transmission reservoirs and obtaining an accurate assessment of malaria epidemiology in low-endemicity areas aiming to eliminate malaria'. PCR, while common, was seen as 'low throughout and cannot be used for large-scale molecular epidemiological studies'. This led them to compare 'three molecular detection methods side-by-side, namely nested PCR targeting the rRNA genes, nested RT-PCR to detect parasite rRNA, and CLIP-PCR to detect parasite rRNA'. Light microscopy, CLIP-PCR, nested PCR to detect parasite DNA, identified only 1–2% of Plasmodium infections (vivax, falciparum and mixed) compared to 18% using qRT-PCR. The latter test also highlighted that 'a large proportion of asymptomatic individuals were gametocyte carriers', and thus contributors to continuing transmission.

Interestingly the presence of fever is no guarantee that malaria parasites will be found. A study in Gabon demonstrated that among febrile patients only 1% had parasites found through microscopy compared to 32% through molecular testing.[7] Similarly in Ethiopia where both *P. falciparum* and *P. vivax* are present a study found 'evidence on the wide-scale presence of submicroscopic parasitaemia by quantifying submicroscopic parasite densities and concurrent gametocyte densities'

in low-endemicity areas and helped identify appropriate interventions for such areas.[8]

Practical considerations

The value in these studies was pointed out by Abdul-Ghani et al when they observed that, 'An evidence-based estimation of the 'true' reservoir of resistant parasites can help target the existing and emerging foci of resistant parasites before they spread'. This knowledge will contribute toward preventing emergence and spread of artemisinin-resistant *P. falciparum* malaria. In particular, Pava et al stressed that 'Novel public health strategies are needed to detect and eliminate these parasite reservoirs, for the benefit both of the patient and the community'.[5]

Tripura and colleagues addressed the heterogeneity of API and submicroscopic malaria epidemiology across villages and districts. This stratification can lead to better targeting of 'radical' treatment that ensures gametocytes are targeted and transmission interrupted.[9] The same need for better stratification of transmission zones and hence better targeting of interventions was also seen in Uganda by Rek et al when they reported that 'Across a range of transmission intensities in Uganda, microscopy vastly underestimated parasite prevalence, especially among adults'.[10]

Lessons from other parts of the world apply to malaria eliminating countries in Africa. Lennon et al discovered that a considerable number of asymptomatic *P. vivax* infections were mostly submicroscopic, 'Robust surveillance systems, molecular diagnostic tests and tailored malaria detection activities for each endemic site may prove to be imperative in accelerating malaria elimination in Guatemala and possibly across all of Mesoamerica'.[11]

In Ghana, high submicroscopic gametocyte carriage among children led researchers to call for 'more effective elimination approaches like the development of transmission-blocking vaccines and safer gametocytocidal drugs'.[12] As noted previously, febrile children may have submicroscopic malaria infections. In the process of studying paediatric admissions a Brazzaville hospital, researchers discovered that 21% of febrile children had submicroscopic malaria.[13] Not only did these findings imply the 'necessity to investigate carefully other causes of fever', but also to emphasise the importance of developing and applying better guidance for health staff in malaria and overall case management.

Although people worry more about transmission of HIV and Hepatitis C through blood transfusion, submicroscopic malaria transmission can also occur. This led Lima and colleagues to report that, 'Reservoirs of Plasmodium represent a challenge for blood banks, since studies have shown that high levels of submicroscopic infections can occur in low-transmission areas. The risk of transfusion-transmitted malaria points to the need to conduct molecular investigations of candidate donors with any positive malarial antibody test'.[14]

Findings that school-age children represent an under-recognised reservoir of malaria infection and 'frequently carry gametocytes in communities of southern Malawi', many of whom were submicroscopic carriers.[15] This has practical implications for control programmes. The researchers recommend better housing construction to protect these children and families, but in the short run, strong attention to universal coverage of insecticide treated bednets is needed.

In conclusion, these studies have demonstrated the need for a better understanding of malaria transmission across different zones and strata in a country in the light of asymptomatic and submicroscopic malaria, especially gametocytemia. This should lead to better targeting of case detection, improved treatment, and better compliance with preventive measures.

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