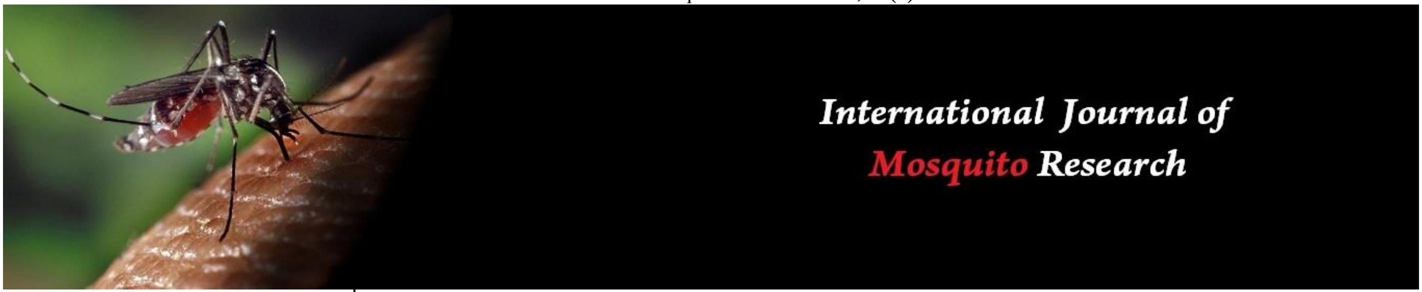

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Evolutionary outlook on the impending endgame of malaria

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Abstract
Malaria is a highly debilitating vector-borne parasitic infection which has been known to be mortal and morbid to human beings for centuries now. As of today, the success rate of effective control strategies is less; however there have been several riveting developments. These include: 1. A global incidence of decrease in malaria cases; 2. The development of several non-malaria transmissible transgenic mosquitoes and 3. The launch of the vaccine has been a game changer as it is now being given to children with the success rate being high. In this review, we discuss if the current control strategies in place and the launch of the vaccine and its speculated success in children has become/been a game changer. Our take on the coevolution of parasites and vector can be the answer to the questions above. We highlight that parasites and vectors have evolved together for millions of years now, and to break this liaison will not be as easy.

Keywords: Mosquirix, RTS, S vaccine, R21/Matrix-M malaria vaccine, the global technical strategy for malaria 2016–2030, transgenic mosquitoes, decrease in malaria cases

1. Introduction
Malaria is a vector-borne disease caused by the parasites *Plasmodium falciparum* (*P. falciparum)* and *Plasmodium malariae* (*P. malariae*). It is transmitted by the vector female *Anopheles* mosquito during blood-feeding [5]. Malaria causes a sizable impact on socioeconomic well-being of the human population in the tropical and subtropics [4]. Malaria infection results in a wide variety of symptoms like absence or very mild fever, sometimes severe that may result in death. As stated by the World Malaria Report (WMR) 2021, the caseload of malaria is said to have increased from 227 million in 2019 to 241 million in 2020 [62]. The malaria-linked mortality has reduced from 148 per 100,000 cases to 56 per 100,000 cases between 2000 and 2019. However, in 2020 it rose to 60 and decreased again to 58 in 2021. These numbers are within the range of yearly fluctuations and coinciding with the global Covid-19 pandemic. This rise might also be because of changes in the norms for calculating cause-of-death based upon the revelations suggesting an updated series of cause-specific mortality for neonates and children in 2000 to 2019 [46]. This is contrary to several reports stating decline in Malaria cases and mortality [61]. In this review we discuss recent developments in malaria control and whether the century-long battle against malaria is finally over.

2. Recent developments in malaria management
Current focus in malaria control involves continual development, upgradation of existing drugs and design of novel agents with improved efficacy. One of the major challenges is to counter the ever-increasing drug and insecticide resistance in pathogens having effect on epidemiological outcomes [67]. As part of the integrated malaria control program enhanced vector control strategies, awareness of sanitation and waste management are being promoted. Experts have pointed out that controlling the menace of malaria requires a multi-pronged strategy rather than fighting it in isolation [30]. However, with advancement in science and technology, tactics to control malaria have changed. It has led to development of new technologies like, 1. A speculated global incidence of decrease in malaria cases; 2.

The approval of the much awaited launch of the anti-malarial parasite vaccine, the Mosquirix (RTS, S). 3. Besides, the development of several non-malaria transmissible transgenic mosquitoes. These groundbreaking developments have certainly raised our hopes, whether the century long quest and battle for the control of malaria is in hindsight.

1.1 Is there really a decrease in malaria cases?

The World Malaria Report (WMR) estimated 247 million cases in 2000 worldwide which more or less remained unchanged in 2020 (226–260 million) [66]. From this, we can clearly say that there is no clear trend in case of endemic, reduction of mortality, decrease in number cases and overall disease burden. While in 2015 ninety-three countries were endemic, by 2020 forty-three countries achieved reduction in mortality [66]. During 2010-2018, the load of cases is said to have dropped by 76% in South-East Asian countries, and the death rates related to malaria fell significantly by 95% [23]. India reported a huge reduction in infections with 2.6 million fewer cases in the year 2018 than in 2017 [43].

However, in the WHO South-East Asia region, India reported about 82% of overall malaria deaths [66, 13]. This reflects a sizable burden of vector-borne diseases in the country. Hence it is unclear if there is a decrease in malaria incidence. Between 2005 and 2015, the mortality rate of children was halved. This was achieved by better implementation of preventive measures like bed nets, improved methods of diagnosis and treatment. However, these cases have since then reached a plateau. A major problem with the WMR is that the method of calculation changes every year [61]. This suggests two things, there is neither consistency nor a clear trend to draw figures and in a short time-span one cannot affirmatively claim anything. While the WHO claims a decrease in malaria mortality on a global scale, it also requests a hefty increase in international funding. There is no clarity about what is happening, where we are going wrong and a justification for the application of the funding [61].

1.2 The development and approval of anti-Malarial vaccines

An important development which can be seen as an important milestone of the century, in 2021 the WHO approved GlaxoSmithKline's Mosquirix (RTS,S/AS01) [26]. It is the only malaria preventive vaccine for application in children at high risk African countries. This development has the potential to create an impact on the disease which has plagued humans for centuries. The Mosquirix is designed to affect the sporozoite phase of the mosquito. It blocks the maturation phase in the liver so that it does not infect the erythrocytes after re-entering the bloodstream [2, 34, 65, 71]. This vaccine has been reported to show up to 56% efficacy in preventing childhood malaria related mortality. The approval of RTS, S was the first-ever vaccine approval for a human parasitic infection, and clearly it is a paradigm shift, a step towards prophylactic control [53, 54]. Recently, WHO has given permission to clinically evaluate (phase-3) R21/Matrix-M malaria vaccine, which is widely claimed to be up to 80% effective [18, 19]. There is an upcoming mRNA-based vaccine for malaria being developed by Bio NTech. Apart from this, a large program of monoclonal antibody-based treatment to opt for clinical trials [71, 38]. It is anticipated that vaccine strategy could dent the impact of disease control. A multipronged strategy like vaccinating right before the rainy season while also using the other tools simultaneously may produce maximum effect. New technology interventions have provided interesting armaments in the fight against malaria.

1.3 The development of transgenic non-malaria transmitting mosquitoes

A major development in malaria control is the transgenic non-malaria-transmitting mosquitoes. This technique has the potential to break-down the vector-pathogen liaison responsible for disease spread. Various techniques reported are: 1) Release of gene-modified male mosquitoes comprising a lethal gene with ability to kill their offspring after mating with wild species [48]; 2) In the *Wolbachia* method, endosymbiont bacterium developed in the laboratory is introduced in *Ae. aegypti* mosquitoes which are then released into the environment [25, 39, 42]. The *Wolbachia*-infected mosquitoes breed with their wild counterparts and ultimately increase the number of desired transgenic mosquitoes. Although this approach is reported for *Ae. aegypti* but it could also be applicable to *An. gambiae* [15]. 3) Another method is Gene drives – in which certain hereditary genetic elements tend to spread through the population at a fast rate. This is a rapidly emerging approach to vector and pathogen control [9, 22, 24]. The gene drive method could be applied to fieldwork in about two ways: a) suppression of insect populations by targeting genes associated with parameters such as insect fecundity, lifespan, or mortality; or b) reducing the vector competency through targeting pathogen associated genes. 4) Oxitec Mosquito Technology has put to use two simple genes which create mosquitoes that are safe, non-biting, self-limiting as well as non-persistent male mosquitoes [45].

The effectiveness of these mosquitoes has been tested in parts of Brazil, India, Singapore, the Cayman Islands, and Panama to control the *Ae. Aegypti* mosquito population [10]. These types of experiments are being carried out in significant numbers. A drawback of this method is that it works to reduce only the target mosquito species but most communities have various types of mosquitoes [48]. The future of modern biotechnology and enhanced genetic modifications relies on regulations that govern their release into the environment and our ability to monitor their impact [28]. However, it would be interesting to cross-examine whether comparative genomics provides suggestions for evolutionary mechanisms between vectors and pathogens [52].

2 Other latest developments in malaria control

Using artemisinin in combination with other drugs for treatment of malaria at an early stage, preventive treatment for pregnant women, and residual spraying or mosquito nets have proven to be effective tools in malaria control [8, 49]. New tools developed are production of new drugs with better efficiency, vaccines for treatment and prevention, new diagnostic tests, innovative materials that can be treated with insecticide, better systems for delivering and evaluating malaria control [11]. Another task is understanding the efficacy, effectiveness and safety of the new interventions and advising endemic countries about when and where to introduce them [12].

In remote health facilities of endemic communities, it is difficult to diagnose malaria due to absence of pathology laboratories and unavailability of functioning microscopes. Rapid malaria diagnostic tests expand laboratory diagnosis to health facilities in remote health facilities of endemic countries. Experts in the field always emphasized that in order to fight/control malaria only a multi-pronged strategy might work. Nevertheless, despite all the promising developments in malaria cases and decrease in related deaths, there is significant prevalence of the disease based on recent data [43, 44].

3. Is the centuries-long battle of malaria control nearing end?

So a bigger question arises - whether the century long battle against malaria is finally coming to an end? Have we finally defeated this vicious malaria, which was believed to be impossible once upon a time? Before we celebrate, we should look into history and its evolution. Biggest threat to these early conclusions might come from the rising challenge of development of resistance in malaria parasites. The emergence of drug-resistant parasites represents one of the greatest threats to malaria control and results in increased morbidity and mortality [64]. This is because malaria pathogens are rendering the drugs in-effective and insisting on upgradation of existing drugs from time to time [6, 59]. This uprising represents formidable challenges to existing treatment regimens. To deal with problems like drug-resistance requires coordinated action, greater innovation, investments in operational research along with development of new drugs, vaccines, diagnostic tools as well as innovative ways to vector control [63].

Even though many early signs look promising, assuming anything as big as control over much complicated infection like malaria on a short-term lead would be a mis-calculation. Sometimes, the pathogen's infectivity may bend down temporarily, take a pause and re-emerge powerfully in resistant forms. In recent history, there have been several incidents like this. Familiar problems with challenges to the efficacy vaccines might arise from variation in pathogen genotype and antigenic characteristics or rendering vaccines ineffective. There have been numerous reports with regards to genetic and phenotypic mutations in *P. falciparum* [14, 17, 41, 57, 58, 68]. A few infamous examples are frequently-emerging Influenza virus and one of the most prevalent *Mycobacterium tuberculosis*, which are known for their remarkable ability to change its genetic and antigenic structures. It is possible for the pathogens to cause a pandemic because the human immune system is not prepared to recognize and defend against the new variants and existing medications are ineffective. For a huge shift in these genetic changes to occur and pass into humans, we have coexisted in close proximity with farm animals such as chickens, ducks and pigs.

4. Do evolutionary factors hold the key?

Until recent times, there has been little evidence to show the evolutionary forces underlying the vectorial capacity of anopheline mosquitoes for malaria parasites [37, 47, 51]. In order to find the cure, the main focus has to be *Plasmodium* and the evolution of vectors, unfortunately this crucial aspect has received less importance [21]. Various factors have contributed to opportunities for parasite adaptation for local vector species and populations such as 1. Genetic composition of the mosquito species, 2. The genetic inter-relatedness and compatibility between malaria pathogens and their target vectors. Which exists at the interspecies (mosquitoes, birds, gorillas and humans) and at the intra-species (*P. falciparum, P. malariae* and *An. gambiae*) level.

Uninfected mosquitoes are attracted to humans that contain gametocytes of malaria [20, 31]. By biting those humans, the transmission rate of *P. falciparum* increases in vectors. Infected mosquitoes containing sporozoites are unable to feed properly due to the reduction of apyrase activity in their saliva [7, 32]. They are unable to consume enough blood in a meal, which leads to more meals and subsequently increases the number of hosts infected [7, 32]. The selection of uninfected mosquitoes in the race of survival of the fittest seems favorable since evolution of mosquito biting and transmission rates affect the reproductive success of the vector. However, experimental evidence demonstrates that most mosquitoes are prone to malaria [16, 40]. By altering the sex and reproduction mechanism of their hosts, parasites could improve their survival which results in evolution of sex [27, 60].

The resistance of mosquitoes to malaria has always focused on the genetic angle. However, it also depends on various immune responses, which are initiated by pattern recognition receptors that undergo signal modulation and transduction that helps in regulating downstream effector mechanisms [1, 71]. However, the relationship between malaria and the human immune system is a highly complex mechanism to comprehend. Resistance has not developed due to a single gene modification or mutation; there have been multiple gene mutations in the parasite and the vector. It results in resistant variants and permutation-combination in the vector population. Thus, when we search for genes immune to malaria infection, it becomes difficult to find the same genes in different malaria hosts. The huge number of variants in the environment make it difficult to find concrete evidence between the host, vector and parasite [33, 50]. This is why ecological approaches can bring answers to the questions about malaria-mosquito interactions [35, 55, 56].

The vector population intervention strategy, a genetic modification method for producing transgenic mosquitoes, could reflect several defects in the evolutionary aspect. The parasite could develop a sense of refractoriness to malaria infection which could impact the overall control program on malaria prevalence in the human population. To avoid this co-evolutionary balance, we could transform the natural mechanisms of mosquitos and increase their resistance to refractoriness. It is less likely that such mechanisms are known to occur in the parasites. The co-evolutionary response could remain un-unnoticed until necessary changes have taken place [7, 29].

Another important factor to be considered is the evolutionary response of the wild vector population. There might be a significant risk to the suppression strategies in the population due to the release of mosquitoes comprising a predominant, subdued, fatal genetic construct. Several models which make the use of mathematics and statistics, have illustrated that resistant alleles are incapable of spreading the fixation. However, alternative susceptible alleles can become widespread and could have an adverse impact on the field applications [3, 36]. Several factors must be considered while designing experiments for future research such as; they must emulate more closely with the natural conditions for parasite transmission, the influence of abiotic factors, and the mechanistic link between environmental heterogeneity and vectorial capacity. These are some of the critical points that have to be considered if we wish to decipher the mosquito-parasite dynamics and co-evolution.

5. Tables and Figures

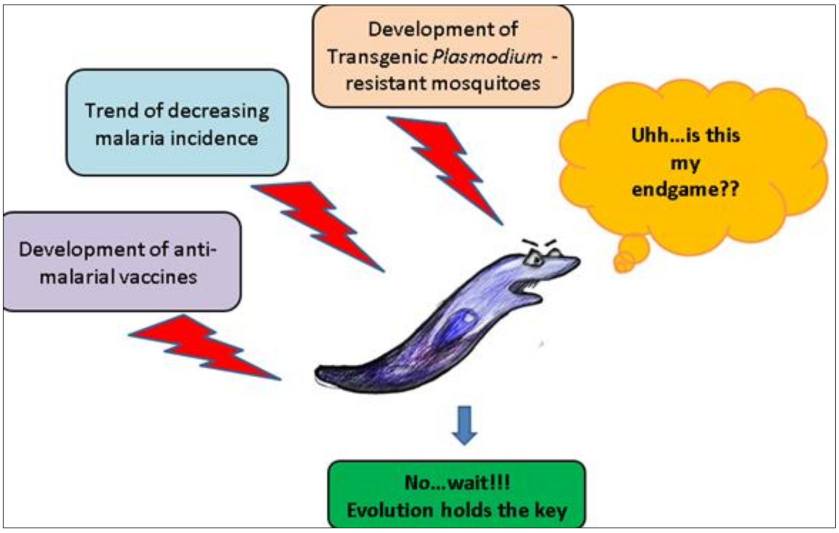

Fig 1: Schematic representation of recent developments in malaria control

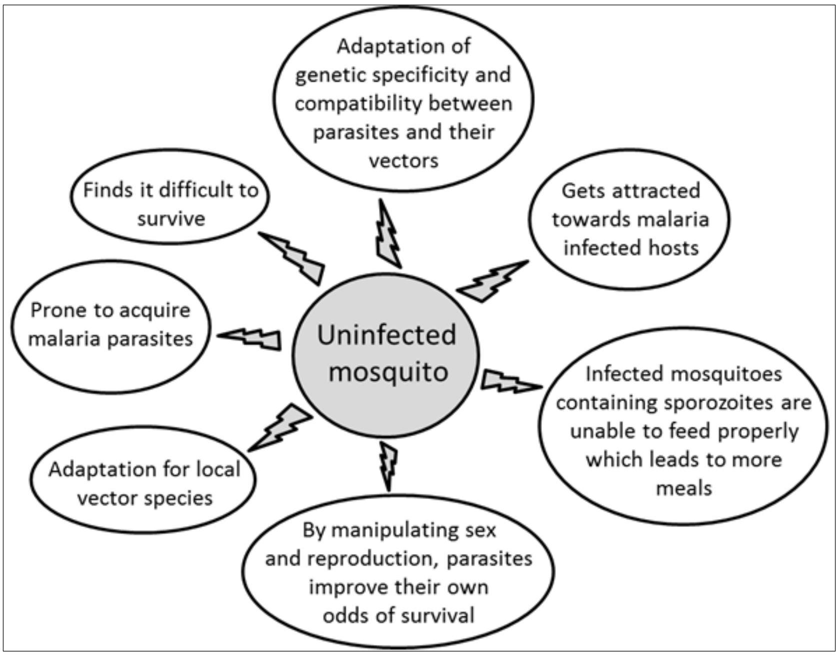

Fig 2: Illustration of physiological and evolutionary relationship between pathogen and mosquito

6. Conclusion

Mosquito-borne infectious diseases like malaria, dengue, and chikungunya have been a threat to global health for hundreds of years. Recent developments in science and technology are promising and equip the integrated malaria control program. However, recent examples suggest some of these pathogens are showing signs of resurgence; which were conquered through large-scale and coordinated public health efforts. In the past whenever new initiatives were launched; authorities nodded their heads affirmatively in accordance with the mandate, only to realize later that the problem persisted. Majority of the treatment and prevention methods for malaria pivot on vectors and their transmission. However, focusing on the evolution of the vectors and pathogens might provide answers for their resistance capacity. Unfortunately, this aspect remains largely ignored. Diseases that were believed to be eradicated are making come-back such as Polio, Measles, Mumps, and Grover's disease. The reasons could be vaccine refusal, waning or insufficient immunity, drug resistance, and climate change. Hence it is difficult to identify if the decline in cases suggests eradication of the disease or the calm before a storm.

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