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Perspective Piece

A Second Chance to Tackle African Malaria Vector Mosquitoes that Avoid Houses and Don't Take Drugs

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Sometimes history gives us a second chance by repeating itself. The timely and lucid modeling analysis presented by Phillip Eckhoff in this issue[1] reminds us all that the challenges and opportunities faced by the malaria control community today remain remarkably similar to those of our predecessors who undertook the Global Malaria Eradication Campaign (GMEP). The anti-malarial drugs we use in 2013 are relatively new to the front lines but they are still overwhelmingly used in the same way for reactive clinical management of symptomatic cases.[2] Long-lasting insecticidal nets (LLINs) now offer a proven alternative to indoor residual spraying (IRS) as the front-line vector control tool of the GMEP era but both approaches target mosquitoes within the same indoor environment and evidence that combining the two yields incremental benefits remains mixed.[3–7] Although efficacious vaccines against malaria now exist and their expected impacts are simulated here,[1] the protection they confer is partial and may wane[8] as naturally acquired immunity fades[9] and/or naturally acquired skin stage infections induce immunotolerance of pre-erythrocytic stages.[10] Therefore, the fundamental properties, applications, and limitations of "off-the-shelf" intervention options available to control program managers today have not dramatically changed since the heyday of GMEP optimism half a century ago.[11] And neither have the most fundamental knowledge gaps we face. The white arrows in Figure 1 crudely illustrate the impacts of intervention strategies we have reasonable experience and understanding of (suppression of high transmission with LLINs or IRS and elimination of sparse residual human parasite reservoirs with drugs), whereas the dark arrows illustrate those we urgently need to develop and learn about through trial and error (long-term resistance management, programmatic-scale vector control outdoors or at source, elimination of mosquito-to-human transmission during the dry season with vaccines, novel vector control tools, or chemoprophylaxis).

The four major issues highlighted by this intricate set of simulations, using a remarkably flexible and extendable modeling architecture, are as follows. 1) We need more intervention layers of vector and parasite control to eliminate malaria from African settings than we presently have available. 2) Options for vector control outdoors or at source remain conspicuous by their absence. 3) Although recently developed vaccines narrow this gap, between the control levels we can achieve today and the elimination target we have set for ourselves, they do not fill it. 4) Parasite populations are buffered against extinction by low levels of persisting transmission during the dry season, which represents a far more manageable target for targeted intervention than peaks of transmission associated with the rainy season. Although none of these insights are entirely unprecedented, they have never before been given such explicit emphasis and integrated consideration as the obstacles we must overcome to eliminate malaria from equatorial Africa. Furthermore, the timing could not possibly be better: These predictions that dramatic reductions of transmission can be achieved in Africa parallels reality on the ground in many countries today, while at the same time, the global financial support base for national control programs is in clear and present danger. Less than 6 years have passed since the dramatic reprioritization of local, national and regional elimination, ultimately leading to worldwide eradication, as the long-term goal of the global malaria control strategy.[12] Since then, high coverage with proven vector control methods and effective drugs has become increasingly commonplace and saves hundreds of thousands of lives annually.[13–15] However, elimination of transmission from African settings remains as elusive as ever, even in settings where these historical cornerstones of malaria control have been supplemented by the most advanced malaria vaccine available.[8] As explained using an unusually explicit and detailed model,[1] the most effective intervention technologies available today have largely delivered on reasonable expectations but their fundamental limitations persist because the biology of malaria parasites and vectors have not changed. Unlike the GMEP generation, we have the privilege of hindsight, embraced malaria elimination and eradication goals fully informed by historical precedents, and should be encouraged that we now find ourselves at this difficult but predictable crossroads again so soon. The recent rapid gains in both coverage and impact of LLINs and IRS are massive and unprecedented but also worryingly fragile.[13] The immediate threat of funding stagnation, or even contraction, belies an even more dangerous loss of confidence by the global public based on media emphasis of the know limitations of established control technologies that still "do what they say on the tin" but no more. So what was written in the historical fine print on the side of the tin that this article illuminates for us? What are the scientific, implementation, and stakeholder engagement issues this article raises? More to the point, how should we address them now that history has given us a second chance?

First, this article outlines in great detail why layering of all available effective interventions (vector control in the form of LLINs or IRS, therapeutic drugs, and vaccines) is required to reduce transmission levels to manageable levels but is unlikely to extinguish it from many parts of sub-Saharan

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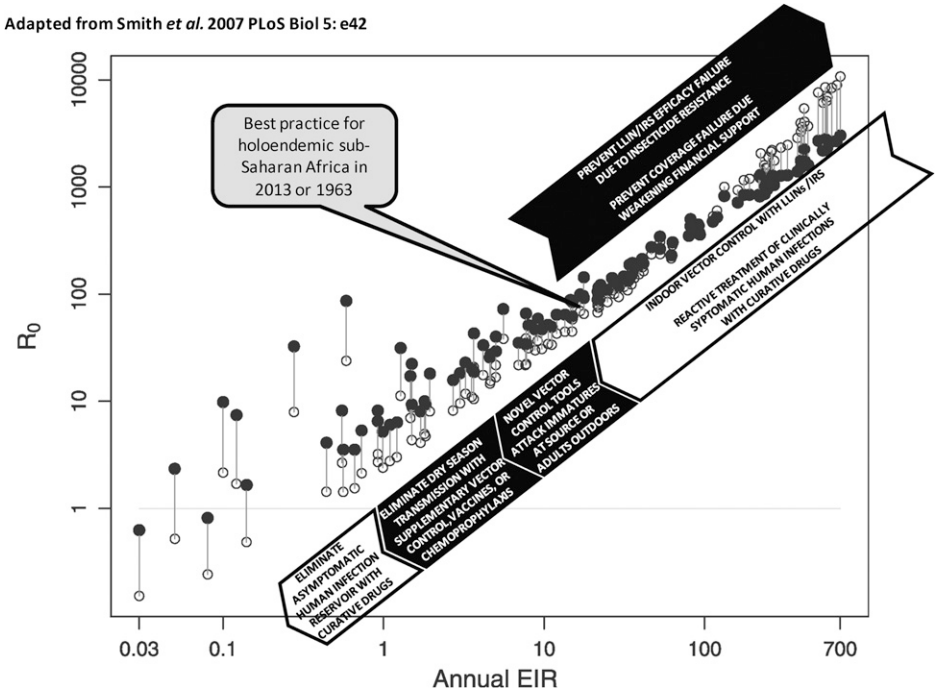

FIGURE 1. A schematic representation of the sequential layers of intervention required to eliminate malaria from the most staunchly endemic regions of Africa in the long term and where we stand in terms of best practice today or 50 years ago during the Global Malaria Eradication Program.

Africa where it can exceed minimum stability thresholds by up to four orders of magnitude (Figure 1). Although the exceptional positive externality delivered by the community level impact, or the *mass effect*, of LLINs or IRS can indeed reduce transmission by two orders of magnitude, a very small fraction of a very, very, very big number is still a very big number. Most countries in sub-Saharan Africa have historically experienced the full range of baseline transmission intensities described in Figure 1 and it is the exceptional, most extreme values in the top right corner that bolster parasite populations against our best efforts to eliminate them.[16,17] Settings exist somewhere in most nations of equatorial Africa where optimal conditions for propagation of malaria parasites (R_0 approaching 10,000, entomologic inoculation (EIR) approaching 1,000) necessitate 99.99% control to push reproductive numbers below the threshold at which local extinction occurs ($R_0 < 1$). To do so will require that we not only sustain 99% suppression of indoor transmission with the vector control tools we already have: we must also develop and apply complementary tools that layer on a further 99% impact by controlling vectors outdoors and at source. The wide range across which African malaria transmission intensity has historically varied (Figure 1) therefore creates the counterintuitive situation in which successful application of the best tools available can be accurately and simultaneously described, as a glass that is either almost full or almost empty. Thus, a given level of impact on malaria transmission can be accurately described as either highly successful control or abject failure to eliminate it:

The combination of mass administration of sulfalene-pyrimethamine with residual spraying of propoxur failed to interrupt transmission for any length of time. This was true even when drugs were given every 2 weeks in the wet season and every 10 weeks in the dry season with coverage of 85%. A high level of control was, however, achieved at that frequency of mass drug administration (MDA): the prevalence of parasitemia decreased very rapidly and varied in the l–5% range, according to season.[18]

The correct interpretation of contemporary progress along the logarithmic scales in Figure 1 is that we are approximately half way along the road to elimination from areas of such intense transmission. As elegantly outlined by Eckhoff, the glass is half full and this situation is neither surprising nor unprecedented. The primary immediate threat to sustained malaria control is neither the results of such simulations, nor parallel empirical observations from the field, but rather the way in which we formulate and communicate our interpretation of them. We should all therefore carefully consider past errors of interpretation that ultimately caused collapse of public and political support for the GMEP, and for malaria control generally, for decades afterward. Would any of us agree with the following statements today?

It may be concluded that in the rural areas of the Sudan savanna of Africa residual spraying is not to be recommended as a malaria control method.[18]

It may not be feasible at an acceptable cost at the present time to control malaria in the rural areas of the Sudan savanna by an attack on transmission. It should, however, be possible to reduce the morbidity and mortality as a result of malaria by the treatment of clinical cases.[18]

Despite valid concerns regarding physiological resistance to insecticides,[19] and clear evidence of vector population rebound in two cases,[20–22] IRS and LLINs continue to deliver the valuable levels of transmission control predicted by Eckhoff in most contexts.[13–15] Encouragingly, pyrethroid resistance was successfully managed in the South African example by reverting to DDT as the active ingredient for IRS, so that vector control and even elimination has been

sustained on a national scale for half a century with only one major interruption.[22,23] Recent evidence of rebounding vector populations and malaria transmission in Senegal soon after LLIN introduction,[20] is undoubtedly worrying. Furthermore, resistance management strategies for nets are far more challenging because direct contact with the user currently limits choice of active ingredients to a single insecticides class—the pyrethroids upon which we have become so dangerously dependent. Nevertheless, in most parts of Africa the glass remains half full for now.[13–15] Weakening global commitment to support delivery of these proven life-saving measures, arising from the perception of a half-empty glass, represents an equally important and immediate threat to successful malaria control programs in Africa today. Sharing a balanced view of our long-term struggle with the global public, and sustaining political will to save lives with the tools we have now, is as important, challenging, and achievable as managing insecticide resistance among mosquitoes. Central to that communication strategy must be open acceptance that we will need to enhance and maintain delivery of current interventions for many decades, generations, or even indefinitely. The real beauty of an eradication strategy is that even if you never ultimately achieve it, vast numbers of lives and dollars are saved in the attempt so long as indefinite timelines for sustaining control and elimination programs are formally incorporated into policy, practice, and funding mechanisms. It is therefore vital that we simply keep trying, never give up, and convince the global public to formalize this philosophy in global funding mechanisms. If we really wish to go further than the boom-and-bust experience of the GMEP, we have to accept the uncomfortable truth that setbacks must be expected and stubbornly tackled as part of our long-term strategy. We cannot allow the political history of GMEP to repeat itself.

The second major facet of malaria transmission and control elucidated by Eckhoff is the robust buffering of malaria transmission against IRS and LLIN impact by vectors that feed or rest outdoors. In other words, the most obvious missing layer from the intervention suite described in Figure 1 is vector control of adult mosquitoes outside of houses or immature aquatic stages at source. Even for the most potently anthropophagic but conveniently endophagic African vectors, a minority transmission has always naturally occurred outdoors (Figure 2). This small gap in protective coverage[28] typically represents at least half of the residual transmission experienced by LLIN users in Africa, even without assuming any induced change in vector population composition or behavior: As depicted in Figure 2, an average of 55% of LLIN user exposure to members of the *Anopheles gambiae* complex is estimated to occur outdoors and 66% for *Anopheles funestus*.

However, it is often the less anthropophagic, and correspondingly less potent, primary vectors that usually present the greatest obstacle to malaria elimination. Weaker, but nevertheless important, vectors like *Anopheles arabiensis* transmit less malaria but are correspondingly less vulnerable to LLINs and IRS because they are far less dependent upon human blood.[28,29] Taking *An. arabiensis* as an excellent African example, such zoophagic primary vectors often also exhibit significant exophagy and exophily so that they are resilient to attack within houses using LLINS or IRS. As beautifully outlined with the best empirical data[30–32] and models[33] available at the end of the GMEP, the simulations presented here by Eckhoff confirm that heterogeneity of vector population behaviors present a diverse set of targets that no single vector control measure can effectively tackle.[1] Recent studies of residual transmission systems where highly endophagic mosquitoes have been effectively tackled by IRS or LLINs remind us that behavioral avoidance of indoor insecticide exposure, by such a naturally evasive species as *An. arabiensis*,[6,7,34,35] does not necessarily represent failure, or even waning impact, of these measures but rather their inherent limitations from the outset.[18,31–33,36–38] Recent reports of surprising[39] and previously undescribed[40] primary vectors most probably does not reflect the emergence of new vector systems but rather that their role in transmission is now more obvious because they are no longer outnumbered by *An. gambiae* and *An. funestus*.

Crucially, none of the simulations of Eckhoff assume any selection-induced change in such behaviors[1] and are therefore consistent with the long-established view[18,31–33,36] that pre-existing behavioral preferences or plasticity define the limits of what is possible with IRS or LLINs before a single house is sprayed or a single net distributed. Although the term *behavioral resistance* has been applied to the zoophagic, exophagic and exophilic habits of such robust vector populations, this may be misleading because physiological resistance to insecticides is defined in terms of altered frequencies of heritable traits:

Insecticide resistance (WHO): The ability of an insect to withstand the effects of an insecticide by becoming resistant to its toxic effects by means of natural selection and mutations. Operational field resistance (Insecticide Resistance Action Committee): A heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species.[19]

The term *behavioral resilience*, as applied to the stability of ecological systems,[41] may therefore be more appropriate to describe the pre-existing evasive traits that stabilize the tripartite relationship between vectors, parasites, and humans against perturbation with indoor vector control measures, in such robust transmission ecosystems.

The analysis of vaccine impact upon transmission stability clearly illustrates how the success of each additional intervention layer depends on the success and limitations of those which it supplements. For example, whereas curative drugs can suppress human-to-mosquito transmission in some settings where mosquito-to-human transmission intensity is quite low,[42–45] negligible impact upon transmission has been observed in rural African settings[46–49] where rapid re-infection rates stabilize saturated human reservoirs of parasites.[2,50] Here, this principle is extended to vaccines with similar conclusions that parallel those of similar analyses by others[9]: at current levels of efficacy, vaccines will enhance malaria control impact and narrow the gap between the two white arrows in Figure 1 but will not close it. As elegantly outlined by Eckhoff, the modest attenuation of LLIN and IRS impact by zoophagic, exophagic, and exophilic primary vectors simply leaves far too high a level of residual transmission for conventional drug or vaccine-based strategies to completely mop up the reservoir of parasite infection.[1]

Perhaps the most important issue highlighted by these simulations is the critical importance of dry season transmission in stabilizing populations of falciparum malaria. Most transmission of *Plasmodium falciparum* malaria occurs in predictable annual peaks during, or soon after, rainy periods. These

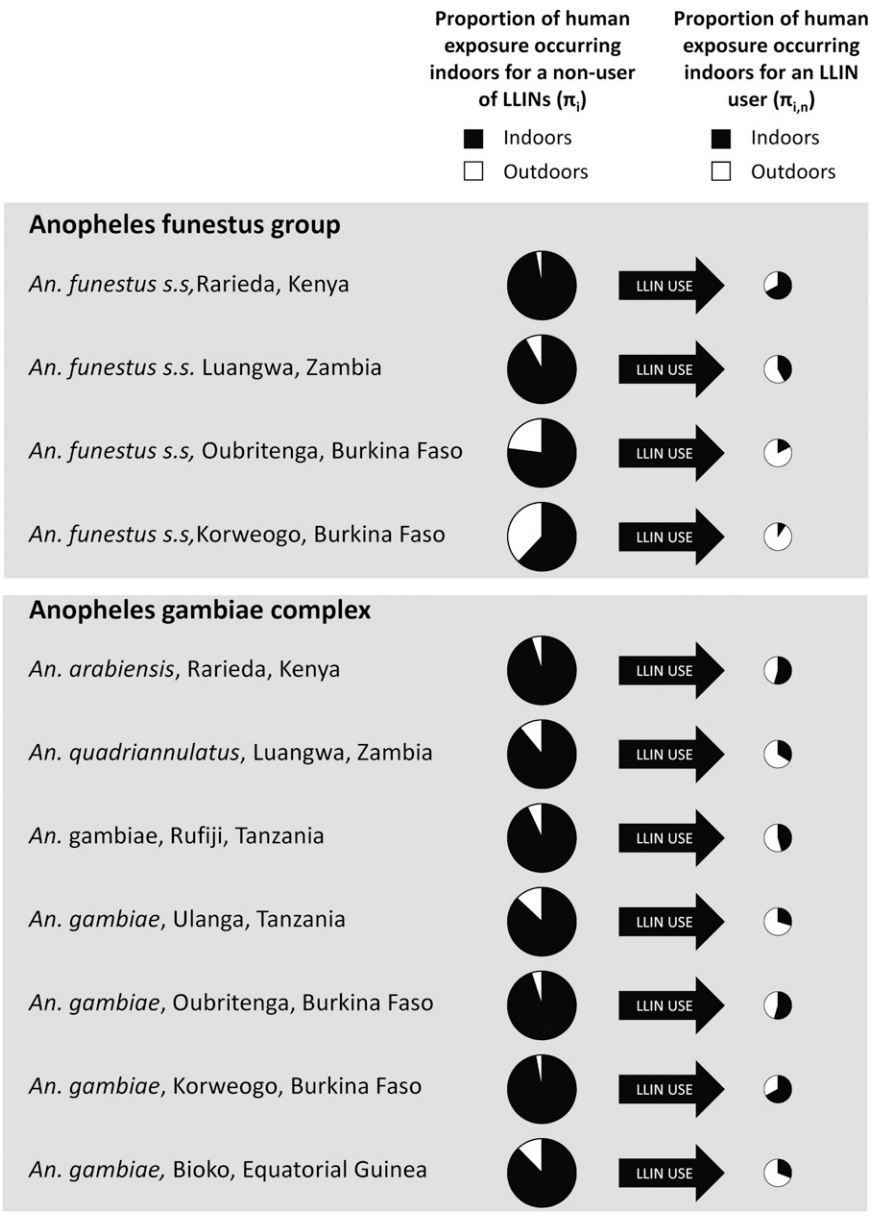

FIGURE 2. Estimates of the proportion of human exposure to African vector populations for users ($\pi_{i,n}$) and non-users (π_i) of long-lasting insecticidal nets (LLINs). All estimates of π_i for vector populations in Kenya, Tanzania, Zambia, and Burkina Faso were obtained directly from a recently published analysis.[24] For *Anopheles gambiae* in Equatorial Guinea, an approximate value was derived from published estimates[25] of the proportion of mosquitoes caught indoors (P_i) and the proportion caught during the first and last hours that the majority of residents were considered to be asleep indoors (P_{fl}): $\pi_i = P_{fl}\, P_i/[P_{fl}\, P_i + (1 - P_{fl})(1 - P_i]$. Values for $\pi_{i,n}$ were calculated without explicit calculation of hourly behavior-weighted biting rates, discounted for indoor personal protection (ρ), as originally described for *Anopheles funestus* and *Anopheles quadriannulatus* in Zambia.[26] Instead $\pi_{i,n}$ was calculated based on these estimates of π_i and a mean of published estimates[27] for the personal protection provided by an LLIN ($\rho = 0.937$): $\pi_{i,n} = \pi_i\, (1\text{-}\rho)/[\pi_i\, (1 - \rho) + (1 - \pi_i)]$. Note that Figure 3 and the abstract of the original report from Zambia[26] mistakenly report a cruder binomial estimate for the proportion of non-user exposure occurring indoors (described by Equation 4 of Seyoum and others and distinguished as π_i^B in Huho and others) rather than the more refined estimate based on mosquito biting rates weighted according population mean human behavior (described as Equation 1 in Seyoum and others and annotated as π_i in both works), which are described in Figure 3 of Huho and others and used to derive the $\pi_{i,n}$ estimates presented here.

peaks account for the vast majority of cumulative malaria transmission and correspondingly attract most attention from malariologists. However, the minority fraction of transmission that occurs during annual minima, typically during the dry season, may actually be a more important determinant of success or failure. As originally highlighted in Figure 77 of the historic Garki monograph,[18] suppression and elimination of *P. falciparum* populations within humans is, by definition, always going to be easiest to achieve during annual dry seasons and periodic droughts when rates of human re-infection are lowest, particularly in localities where vector-borne transmission actually reaches zero rather than merely approaching it. The persistence of low but non-zero transmission in foci of perennial vector breeding bolsters *P. falciparum* populations against the effects of vector control and vaccine products that may remain efficacious for months or even years. However, the implications for how best we use anti-malarial drugs with half lives of hours, days, or weeks merit special consideration.

Unlike *Plasmodium vivax*, which can survive for years hidden cryptically as hypnozoites within the liver of its human hosts,[51] *P. falciparum* must primarily survive seasonal and periodic minima of transmission as blood stages that are vulnerable to treatment with curative drugs. However, asexual blood stages, and the infectious sexual-stage gametocytes they give rise to, enable temporary escape from the human body as sporogonic stages living in the bodies of mosquitoes that may survive for up to 14 feeding cycles, equivalent to at approximately one and a half months.[52] The fact that sporogonic stage infections can persist longer than single complete doses of therapeutic drugs enables *P. falciparum* populations to partially evade such exclusively human-directed chemical attack for the simple reason that mosquitoes do not take drugs. Although the benefits of controlling reservoirs of human parasite infection with therapeutic drugs are intuitive, appealing, and well established, only limited impact can be expected where high transmission levels persist[18,46,50,53] and the dangers of applying strong selection pressure for drug resistance upon robust parasite populations are also obvious.[50] These observations have a number of very important and direct implications for the final stages of *P. falciparum* elimination.

First of all, the ability of parasite populations to "leap frog" well implemented drug distribution campaigns (Figure 3) inherently depends on opportunities to infect mosquitoes; therefore, the best time to implement these is when the size of the sporogonic-stage parasite population and rate of human re-infection from this flying reservoir is minimized.[2] The final stage of malaria elimination programs in many areas of currently stable endemic transmission will most probably rely upon drug therapy campaigns to remove persisting human infections.[2,54,55] Some notable successes have been reported from areas of modest endemicity[42–45] where theory suggests that anti-parasitic drugs may have their greatest impact.[50,53,54] Consistent with the post-GMEP view, all theoretical, observational and experimental studies since then confirm that population-wide drug administration campaigns have limited impact upon the high levels of transmission commonly observed in sub-Saharan Africa, even when layered upon an effective vector control intervention[48–50]:

Previous trials using the combination of MDA and residual spraying in the Sudan savanna of Africa had achieved variable degrees of control, but all had failed to interrupt transmission.[18]

MDA strategies entail treating entire populations without any diagnostic surveys to determine their infection status,[48]

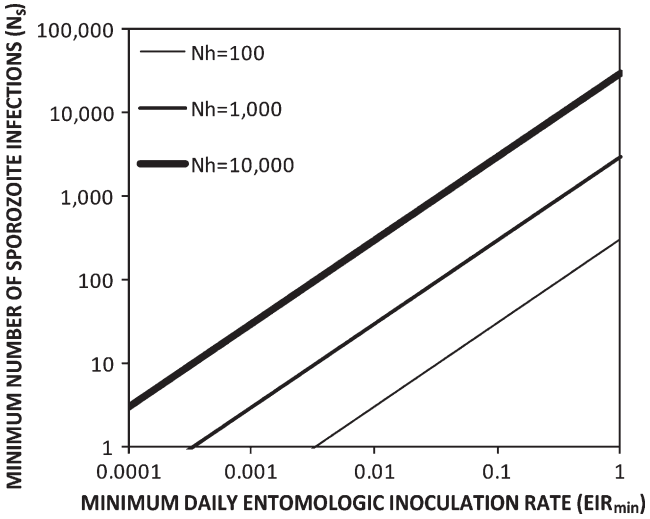

FIGURE 4. Relationship between the total number of sporozoite-stage infections of mosquitoes (N_s) present in human communities of varying size (N_h) with varying minimum levels of human-to-mosquito transmission, expressed as the daily entomologic inoculation rate (EIR_{min}). A mean feeding cycle length of 3 days (f = 3) was assumed so that the size of the sporozoite-stage parasite population could be calculated as $N_s = EIR_{min}\, f\, N_h$.

whereas mass screening and treatment (MSAT), historically referred to as mass blood examination,[56] entails a diagnostic census with treatment provided to all population members confirmed to be infected with parasites. Both of these approaches rely upon achieving comprehensive coverage of at risk populations, as well as high levels of drug efficacy, compliance, and adherence. The Achilles' heel of both these strategies is the inherent assumption that the entire parasite population is accessible to drug treatment within the bodies of human secondary hosts. The mosquitoes that act as primary hosts for *Plasmodia* are obviously non-adherent to any drug regimen so the sporogonic stages that occur in the airborne vector population remain beyond the reach of MDA and MSAT. Figure 4 illustrates just how many sporogonic-stage infections may be present in even quite modestly-sized human communities with very low seasonal transmission minima. To get these figures into perspective, a daily EIR inoculation rate of 0.001 infectious bites per person per day is equivalent to an annual rate of only 0.365 infectious bites per person per year

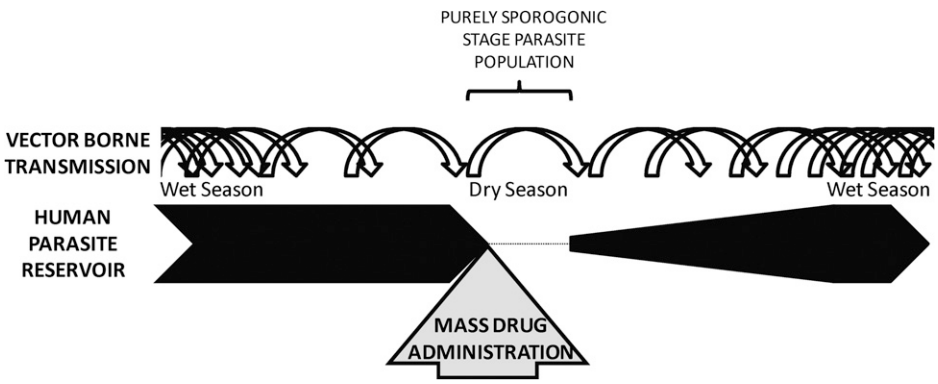

FIGURE 3. A schematic representation of how parasite populations can "leap-frog" mass drug administration (or mass screen and treat) interventions by surviving as purely sporogonic stages unless vector-to-human transmission is terminated for at least 2 months.

and could readily escape detection with standard entomological survey methods.[57] It is also equivalent to a monthly rate of ~0.03 infectious bites per person per month, a level matched or comfortably exceeded by the reported EIR minima for 5 of the 6 African settings examined in another excellent recent theoretical analysis of prospects for malaria elimination using existing vector control and chemotherapy tools.[38] The levels of malaria transmission that often persist throughout the dry season in African communities can therefore keep dozens, hundreds or even thousands of individual parasite infections safe from the effects of anti-parasitic drugs inside the bodies of mosquitoes. To prevent reinitiation of endemic transmission by mosquito-borne parasite stages, it will be essential to completely suppress mosquito-to-human transmission for at least 2 months after a mass drug administration program. It has therefore been suggested that dry season MDA interventions should be supplemented with a correspondingly timed supplementary round of IRS to minimize human re-infection rates[38] and that radical cure should be followed by a period of prophylaxis at least as long as the maximum life span of the mosquito.[2]

Integrating this logic into the picture outlined by the simulations presented in this issue[1] prompts a number of further strategic recommendations. 1) It is essential to judiciously plan the sequence in which malaria control intervention are layered to optimize levels of impact and maximize chances of sustained control or successful elimination. 2) Novel vaccine, vector control, and chemoprophylaxis strategies that prevent mosquito-to-human transmission for at least 2 months during the dry season should precede any MDA or MSAT campaign in the layering sequence. 3) These complementary measures need not necessarily be implemented or deliver valuable impact throughout the year so long as they do so during the dry season, specifically in the limited geographic foci where transmission persists all year round. 4) Vector control measures that exploit the specific ecology of mosquitoes during the dry season and the practical logistic advantages of this period could readily fill the intervention gap in the middle of Figure 1. 5) Primary vectors such as *An. funestus* that are known to predominantly depend on perennial aquatic habitats, and therefore mediate a disproportionate amount of dry season transmission, merit correspondingly weighted attention. 6) MDA or MSAT should only be considered as end-game strategies in malaria elimination for situations where confidence of success, established by impacts of preceding intervention layers, is high and should use alternative therapeutic drugs to those essential for treating clinical cases to minimize risk of compromising their efficacy by selecting for resistance.

The GMEP was defeated by dry season transmission, and by mosquitoes which avoid houses, because it simply ignored them, never took them on, and did not acknowledge their importance until it was too late. These are challenges that can be feasibly addressed, however. Ample opportunity now exists to finally tackle these robust minor fractions of transmission[58–60] that have always existed but only become obvious as obstacles to elimination once the bulk of transmission occurring indoors during the rainy season has been suppressed. This, however, will take time and investment. In the meantime, we need to defend the efficacy, financial support base, and delivery capacity for the interventions we already have and remain dependent on for at least another decade. History has given us a second chance on both counts. And history will judge us.

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REFERENCES

1. Eckhoff PA, 2013. Mathematical models of within-host and transmission dynamics to determine effects of malaria interventions in a variety of transmission settings. *Am J Trop Med Hyg 88:* 817–827.
2. The malERA Consultative Group on Drugs, 2011. A research agenda for malaria eradication: drugs. *PLoS Med 8:* e1000402.
3. Hamel MJ, Otieno P, Bayoh N, Kariuki S, Were V, Marwanga D, Laserson KF, Williamson J, Slutsker L, Gimnig J, 2011. The combination of indoor residual spraying and insecticide-treated nets provides added protection against malaria compared with insecticide-treated nets alone. *Am J Trop Med Hyg 85:* 1080–1086.
4. Kleinschmidt I, Schwabe C, Shiva M, Segura JL, Sima V, Mabunda SJA, Coleman M, 2009. Combining indoor residual spraying and insecticide-treated net interventions. *Am J Trop Med Hyg 81:* 519–524.
5. Corbel V, Akogbeto M, Damien GB, Djenontin A, Chandre F, Rogier C, Moiroux N, Chabi J, Banganna B, Padonou GG, Henry MC, 2012. Combination of malaria vector control interventions in pyrethroid resistance area in Benin: a cluster randomized controlled trial. *Lancet Infect Dis 12:* 617–626.
6. Okumu FO, Mbeyela E, Ligamba G, Moore J, Ntamatungiro AJ, Kavishe DR, Kenward MG, Turner E, Lorenz LM, Moore SJ, 2013. Comparative evaluation of combinations of long lasting insecticidal nets and indoor residual spraying, relative to either method alone, for malaria vector control in an area dominated by *Anopheles arabiensis*. *Parasit Vectors 6:* 46.
7. Okumu FO, Kiware SS, Moore SJ, Killeen GF, 2013. Mathematical evaluation of community level impact of combining bed nets and indoor residual spraying upon malaria transmission in areas where the main vectors are *Anopheles arabiensis* mosquitoes. *Parasit Vectors 6:* 17.
8. The RTS,S Clinical Trials Partnership, 2012. A phase 3 trial of RTS,S/AS01 malaria vaccine in African infants. *N Engl J Med 367:* 2284–2295.
9. Smith T, Ross A, Maire N, Chitnis N, Studer A, Hardy D, Brooks A, Penny M, Tanner M, 2012. Ensemble modeling of the likely public health impact of a pre-erythrocytic malaria vaccine. *PLoS Med 9:* e1001157.
10. Guilbride DL, Guilbride PDL, Gawlinski P, 2012. Malaria's deadly secret: a skin stage. *Trends Parasitol 28:* 142–150.
11. Garrett-Jones C, 1964. Prognosis for interruption of malaria transmission through assessment of the mosquito's vectorial capacity. *Nature 204:* 1173–1175.
12. Roberts L, Enserink M, 2007. Did they really say. . .eradication? *Science 318:* 1544–1545.
13. WHO, 2012. *World Malaria Report 2012*. Geneva: World Health Organization.
14. Eisele TP, Larsen DA, Walker N, Cibulskis RE, Yukich JO, Zikusooka CM, Steketee RW, 2012. Estimates of child deaths prevented from malaria prevention scale-up in Africa 2001–2010. *Malar J 11:* 93.
15. Murray CJL, Rosenfeld LC, Lim SS, Andrews KG, Foreman KJ, Haring D, Fullman N, Naghavi M, Lozano R, Lopez AD, 2012. Global malaria mortality between 1980 and 2010: a systematic analysis. *Lancet 379:* 413–431.

16. Smith DL, McKenzie FE, Snow RW, Hay SI, 2007. Revisiting the basic reproductive number for malaria and its implications for malaria control. *PLoS Biol 5:* e42.
17. Smith DL, Dushoff J, Snow RW, Hay SI, 2005. The entomological inoculation rate and *Plasmodium falciparum* infection in African children. *Nature 438:* 492–495.
18. Molineaux L, Gramiccia G, 1980. *The Garki Project*. Geneva: World Health Organization.
19. Ranson H, N'Guessan R, Lines J, Moiroux N, Nkuni Z, Corbel V, 2011. Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? *Trends Parasitol 27:* 91–98.
20. Trape JF, Tall A, Diagne N, Ndiath O, Ly AB, Faye J, Dieye-Ba F, Roucher C, Bouganali C, Badiane A, Sarr FD, Mazenot C, Toure-Balde A, Raoult D, Druilhe P, Mercereau-Puijalon O, Rogier C, Sokhna C, 2011. Malaria morbidity and pyrethroid resistance after the introduction of insecticide-treated bednets and artemisinin-based combination therapies: a longitudinal study. *Lancet Infect Dis 11:* 925–932.
21. Hargreaves K, Koekemoer L, Brooke BD, Hunt RH, Mthembu J, Coetzee M, 2000. *Anopheles funestus* resistant to pyrethroids in South Africa. *Med Vet Entomol 14:* 181–189.
22. Sharp BL, Kleinschmidt I, Streat E, Maharaj R, Barnes KI, Durrheim DN, Ridl FC, Morris N, Seocharan I, Kunene S, LA Grange JJ, Mthembu JD, Maartens F, Martin CL, Barreto A, 2007. Seven years of regional malaria control collaboration-Mozambique, South Africa, and Swaziland. *Am J Trop Med Hyg 76:* 42–47.
23. Sharp B, Le Sueur D, 1996. Malaria in South Africa-the past, present and selected implications for the future. *South African Journal of Medical Research 86:* 83–89.
24. Huho BJ, Briët O, Seyoum A, Sikaala CH, Bayoh N, Gimnig JE, Okumu FO, Diallo D, Abdulla S, Smith TA, Killeen GF, 2013. Consistently high estimates for the proportion of human exposure to malaria vector populations occurring indoors in rural Africa. *Int J Epidemiol 42:* 235–247.
25. Reddy M, Overgaard HJ, Abaga S, Reddy VP, Caccone A, Kiszewski AE, Slotman MA, 2011. Outdoor host seeking behavior of *Anopheles gambiae* mosquitoes following initiation of malaria vector control on Bioko Island, Equatorial Guinea. *Malar J 10:* 184.
26. Seyoum A, Sikaala CH, Chanda J, Chinula D, Ntamatungiro AJ, Hawela M, Miller JM, Russell TL, Briët OJ, Killeen GF, 2012. Most exposure to *Anopheles funestus* and *Anopheles quadriannulatus* in Luangwa valley, south-east Zambia occurs indoors, even for users of insecticidal nets. *Parasit Vectors 5:* 101.
27. Okumu FO, Moore SJ, 2011. Combining indoor residual spraying and insecticide-treated nets for malaria control in Africa: a review of possible outcomes and an outline of suggestions for the future. *Malar J 10:* 208.
28. Kiware SS, Chitnis N, Devine GJ, Moore SJ, Majambere S, Killeen GF, 2012. Biologically meaningful coverage indicators for eliminating malaria transmission. *Biol Lett 8:* 874–877.
29. Kiware SS, Chitnis N, Moore SJ, Devine GJ, Majambere S, Killeen GF, 2012. Simplified models of vector control impact upon malaria transmission by zoophagic mosquitoes. *PLoS ONE 7:* e37661.
30. Molineaux L, Shidrawi GR, Clarke JL, Boulzagwet R, Ashkar T, Dietz K, 1976. Impact of propoxur on *Anopheles gambiae* s.l. and some other anopheline populations, and its relationship with some pre-spraying variables. *Bull World Health Organ 54:* 379–389.
31. Coluzzi M, Sabatini A, Petrarca V, Di Deco MA, 1977. Behavioral divergences between mosquitoes with different inversion karyotypes in polymorphic populations of the *Anopheles gambiae* complex. *Nature 266:* 832–833.
32. Coluzzi M, Sabatini A, Petrarca V, Dideco MA, 1979. Chromosomal differentiation and adaptation to human environments in the *Anopheles gambiae* complex. *Trans R Soc Trop Med Hyg 73:* 483–497.
33. Molineaux L, Shidrawi GR, Clarke JL, Boulzaguet JR, Ashkar TS, 1979. Assessment of insecticidal impact on the malaria mosquitoe's vectorial capacity, from data on the man-biting rate and age composition. *Bull World Health Organ 57:* 265–274.
34. Kitau J, Oxborough RM, Tungu PK, Matowo J, Malima RC, Magesa SM, Bruce J, Mosha F, Rowland MW, 2012. Species shifts in the *Anopheles gambiae* complex: do LLINs successfully control *Anopheles arabiensis? PLoS ONE 7:* e31481.
35. Russell TL, Govella NJ, Azizi S, Drakeley CJ, Kachur SP, Killeen GF, 2011. Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania. *Malar J 10:* 80.
36. Elliott R, 1972. The influence of vector behavior upon malaria transmission. *Am J Trop Med Hyg 21:* 755–763.
37. Govella NJ, Okumu FO, Killeen GF, 2010. Insecticide-treated nets can reduce malaria transmission by mosquitoes which feed outdoors. *Am J Trop Med Hyg 82:* 415–419.
38. Griffin JT, Hollingsworth TD, Okell LC, Churcher TS, White M, Hinsley W, Bousema T, Drakeley CJ, Ferguson NM, Basáñez MG, Ghani AC, 2010. Strategies towards *Plasmodium falciparum* malaria elimination in Africa using currently available tools. *PLoS Med 7:* e1000324.
39. Kawada H, Dida GO, Sonye G, Njenga SM, Mwandawiro C, Minakawa N, 2012. Reconsideration of *Anopheles rivulorum* as a vector of *Plasmodium falciparum* in western Kenya: some evidence from biting time, blood preference, sporozoite positive rate, and pyrethroid resistance. *Parasit Vectors 5:* 230.
40. Stevenson J, St Laurent B, Lobo NF, Cooke MK, Kahindi SC, Oriango RM, Harbach RE, Cox J, Drakeley C, 2012. Novel vectors of malaria parasites in the western highlands of Kenya. *Emerg Infect Dis 18:* 1547–1549.
41. Hollings CS, 1973. Resilience and stability of ecological systems. *Annu Rev Ecol Syst 4:* 1–23.
42. Kaneko A, Taleo G, Kalkoa M, Yamar S, Kobayakawa T, Bjorkman A, 2000. Malaria eradication on islands. *Lancet 356:* 1560–1564.
43. Zulueta J, Kafuko GW, McCrae AW, Cullen JR, Pedrsen CK, Wasswa DF, 1964. A malaria eradication experiment in the highlands of Kigezi (Uganda). *East Afr Med J 41:* 102–120.
44. Song J, Socheat D, Tan B, Dara P, Deng C, Sokunthea S, Seila S, Ou F, Jian H, Lee G, 2010. Rapid and effective malaria control in Cambodia through mass administration of artemisinin-piperaquine. *Malar J 9:* 57.
45. Shah NK, Tyagi P, Sharma SK, 2013. The impact of artemisinin combination therapy and long-lasting insecticidal nets on forest malaria incidence in tribal villages of India, 2006–2011. *PLoS ONE 8:* e56740.
46. Huho BJ, Killeen GF, Ferguson HM, Tami A, Lengeler C, Charlwood JD, Kihonda A, Kihonda J, Kachur SP, Smith TA, Abdulla SM, 2012. Artemisinin-based combination therapy does not measurably reduce human infectiousness to vectors in a setting of intense malaria transmission. *Malar J 11:* 118.
47. Tiono AB, Ouédraogo A, Ogutu B, Diarra A, Coulibaly S, Gansané A, Sirima SB, O'Neil G, Mukhopadhyay A, Hamed K, 2013. A controlled, parallel, cluster-randomized trial of community-wide screening and treatment of asymptomatic carriers of *Plasmodium falciparum* in Burkina Faso. *Malar J 12:* 79.
48. von Seidlein L, Greenwood BM, 2003. Mass administrations of antimalarial drugs. *Trends Parasitol 19:* 452–460.
49. Shekalaghe SA, Drakeley CJ, van den Bosch S, ter Braak R, van den Bijllaardt W, Mwanziva C, Semvua S, Masokoto A, Mosha F, Teelen K, Hermsen R, Okell LC, Gosling R, Sauerwein R, Bousema T, 2011. A cluster-randomized trial of mass drug administration with a gametocytocidal drug combination to interrupt malaria transmission in a low endemic area in Tanzania. *Malar J 10:* 247.
50. Okell LC, Griffin JT, Kleinschmidt I, Hollingsworth TD, Churcher TS, White MT, Bousema T, Drakeley CJ, Ghani AC, 2011. The potential contribution of mass treatment to the control of *Plasmodium falciparum* malaria. *PLoS ONE 6:* e20179.
51. Verhave JP, 2013. Experimental, therapeutic and natural transmission of *Plasmodium vivax* tertian malaria: scientific and anecdotal data on the history of Dutch malaria studies. *Parasit Vectors 6:* 19.
52. Gillies MT, Wilkes TJ, 1965. A study of the age-composition of populations of *Anopheles gambiae* Giles and *A. funestus* Giles in north-eastern Tanzania. *Bull Entomol Res 56:* 237–262.
53. Crowell V, Briët OJT, Hardy D, Chitnis N, Maire N, Di Pasquale A, Smith TA, 2013. Modelling the cost-effectiveness of mass

screening and treatment for reducing *Plasmodium falciparum* malaria burden. *Malar J 12:* 4.
54. Moonen B, Cohen JM, Snow RW, Slutsker L, Drakeley C, Smith DL, Abeyasinghe RR, Rodriguez MH, Maharaj R, Tanner M, Targett G, 2010. Operational strategies to achieve and maintain malaria elimination. *Lancet 376:* 1592–1603.
55. White NJ, 2008. The role of antimalarial drugs in eliminating malaria. *Malar J 7* (Suppl 1)*:* S8.
56. Gabaldon A, Garnham PC, MacDonald CM, Pampana EJ, Alvarado CA, Bruce-Chwatt LJ, 1963. *Terminology of Malaria and Malaria Eradication: Report of a Drafting Committee*. Monograph Series. Geneva: World Health Organization, 126.
57. Beier JC, Killeen GF, Githure J, 1999. Short report: entomologic inoculation rates and *Plasmodium falciparum* malaria prevalence in Africa. *Am J Trop Med Hyg 61:* 109–113.
58. Ferguson HM, Dornhaus A, Beeche A, Borgemeister C, Gottlieb M, Mulla MS, Gimnig JE, Fish D, Killeen GF, 2010. Ecology: a prerequisite for malaria elimination and eradication. *PLoS Med 7:* e1000303.
59. The malERA Consultative Group on Vector Control, 2011. A research agenda for malaria eradication: vector control. *PLoS Med 8:* e1000401.
60. Govella NJ, Ferguson HM, 2012. Why use of interventions targeting outdoor biting mosquitoes will be necessary to achieve malaria elimination. *Front Physiol 3:* 199.