Chapter

Malaria Elimination: The Role and Value of Sero-Surveillance

Kingsley Badu, Amma Aboagyewa Larbi and Kwadwo Boampong

Abstract

As countries move from intense malaria transmission to low transmission there will be a demand for more sensitive tools and approaches in tracking malaria transmission dynamics. Surveillance tools that are sensitive in tracking real time infectious bites as well as infectious reservoir will be preferred to counting number of cases in the hospital or parasite prevalence. The acquisition and maintenance of anti-malarial antibodies is a direct function of parasite exposure, seroprevalence rates has been used as an efficient tool in assessing malaria endemicity and confirming malaria elimination. Plasmodium antibodies are explicit biomarkers that can be utilised to track parasite exposure over more extensive time spans than microscopy, rapid diagnostic testing or molecular testing and the conventional entomological inoculation rate. Seroprevalence studies can therefore help monitor the impact of malaria control interventions, especially when the parasite occurrence is low. As a result, antibody responses to Anopheles salivary proteins or Plasmodium species may potentially offer reliable information of recent or past exposure; recognise short-term or gradual changes in exposure to Plasmodium infection or to estimate individual-level exposure to infection. This book chapter will present about four studies we have conducted across eastern and western Africa on the efficiency of salivary gland proteins and antimalarial antibodies in tracking malaria transmission intensity. We hope that these could be used as surveillance tools in malaria elimination efforts.

Keywords: malaria elimination, sero-surveilance, antibody response, MSP1 malaria surveilance, seroepidemiology

1. Introduction

1.1 Malaria elimination

Since the inception of initiatives such as the Roll Back Malaria and the creation of the United Nations Millennium Development Goals (MDGs) on combating HIV/AIDS, malaria, and other diseases in September 2000, unprecedented progress has been made in the fight against malaria. Malaria vector control interventions such as indoor residual spraying (IRS), the provision of insecticide-treated bed nets (ITNs) and the use of artemisinin-based combination therapy for the prompt treatment of clinical malaria cases has led to a decrease in the number of malaria morbidity and mortality rates in many endemic areas in the world [1]. Globally, the rate of new malaria infections and deaths decreased by 41% and 62% respectively between 2000 and 2015

[2]. Despite the many interventions, malaria remains a major public health concern in many African countries. According to the World health Organisation (WHO), an estimated 229 million malaria cases were recorded in 2019 with 409,000 deaths [3]. Increasing insecticide resistance and behavioural changes by vectors that transmit malaria parasites as well as drug-resistant parasites threaten the progress made so far.

The advances made in malaria control in many countries have in effect led to the disease becoming more varied, leading to a new challenge of understanding the disposition of lingering transmissions in certain populations [4]. The remedy to this conundrum is a rigorous surveillance system with the ability to measure spatial and temporal variations in malaria transmission accurately and efficiently. The Global technical strategy for malaria 2016–2030 provides a comprehensive framework to guide countries in their efforts to accelerate progress towards malaria elimination [5, 6]. The strategy sets the target of reducing global malaria incidence and mortality rates by at least 90% by 2030 [6]. This technical strategy recognises the critical role that surveillance plays in malaria elimination.

Malaria surveillance metrics used over the years to measure transmission include both entomological and parasitological metrics such as parasite prevalence, clinical cases and serological measurements which rely on host antibody responses to both the vector and parasite antigens [4, 7].

2. The significance of malaria surveillance

Malaria surveillance deals with continually and systematically collecting malaria specific data, analysing and interpreting such data [6]. This could be essential in the planning, implementation and evaluation of public health practice. As part of the Global Technical Strategy for Malaria 2016–2030, WHO has urged endemic countries and those that have achieved elimination to strengthen their disease surveillance in order to identify any reinfection, reemergence or resistance. Malaria surveillance also makes available data to help all transmission settings make appropriate decisions regarding malaria control strategies, priorities and resource allocation. In order to achieve malaria elimination globally, surveillance is crucial because it will provide accurate data at any point in time regarding the state of a particular country in connection with malaria.

3. Challenges to malaria surveillance

The continuous and systematic collection, analysis and interpretation of malaria-specific data and the use of such data in the planning, implementation and evaluation of public health practice is paramount in the quest to eradicate malaria. Surveillance programmes can enable resource-poor malaria-endemic countries to prioritise populations that are most in need, re-strategize in cases where interventions have not resulted in decrease in cases and appropriately respond to pockets of outbreak cases without wasting resources. Malaria surveillance programmes should therefore seek to identify the most affected populations, trends in incidence and mortalities as well as the overall impact of control measures [5].

In many countries, malaria surveillance systems remain inadequate to support the goal of elimination [8]. The intensity of malaria transmission is closely linked to the epidemiology of the disease. Transmission intensity is often measured using the parasite prevalence metric, which measures the proportion of the population with detectable parasites in the blood in a given locale. The entomological inoculation rate, malaria-positive fraction, incidence rate and deaths are other metrics used to

measure malaria transmission intensity [9]. The 2020 world Malaria Report rightly recognised the weak surveillance systems in moderate to high malaria transmission countries that rely solely on parasite prevalence or clinical case reporting for surveillance [3]. This greatly affects the quality of data for epidemiological studies. Improving the scope and quality of surveillance is critical to malaria elimination.

Many malaria endemic countries use clinical case reports for surveillance due to its ease and affordability. However, clinical case reporting has some limitations such as the inability to measure asymptomatic infections as well as infections among transient populations who contribute to transmission [10, 11]. Parasite prevalence surveys are also useful in malaria surveillance, however, in cases of low transmission, large sample sizes are required before useful prevalence data can be obtained. Other seldomly used metrics such as entomological inoculation rate is very labor intensive and costly to implement in malaria endemic populations on routine basis, making it less useful [12]. It is therefore critical that countries adopt surveillance metrics such as serum anti-malarial antibody based seroprevalence surveys that can effectively measure both high and low transmission as well as the temporal and spatial variations in malaria transmission. Such a metric can complement the already existing clinical case reporting and parasite prevalence surveys.

Seroprevalence is a surveillance metric used to measure the antibody markers that are elicited by host cells in response to vectors and parasites. Measuring the proportion of the population who are seropositive for malaria could inform the rate of malaria transmission over time and space [13, 14].

4. Markers of malaria exposure

Measurement of serum anti-malarial antibodies is a useful marker of malaria exposure that indicates long-term transmission potential especially if such measurements are done over a period of time. This is mainly because anti-malarial antibodies develop after repeated exposures and can persist for months to years after infection [7, 14]. In very low transmission settings, where parasite prevalence and entomological inoculation rate (EIR) are insensitive, serological measures offer a way of accurately assessing endemicity and identifying focal areas of transmission supporting the potential for elimination [7]. Evaluating serological evidence of malaria exposure in the human population provides insight into malaria endemicity. Seroconversion rates are related to the force of infection of malaria as reflected through the immune responses of exposed individuals. Thus, the seroconversion rates provide measures of malaria exposure that compare with the malaria transmission intensity [14]. Different antibodies can be used in malaria intensity studies depending on the life span of that protein and how well it is produced in low or high endemic regions as a direct response to exposure.

IgG response to whole saliva extracts of *Anopheles gambiae* has been observed as a marker of exposure to *Anopheles gambiae* bite, and consequently, high anti-saliva IgG levels is a predictive indicator of malaria morbidity [15]. One protein that is being explored is the salivary gland protein and its peptide P1. The *Anopheles gambiae* salivary gland gSG6 protein and derived P1 peptide are specific to *An. gambiae* and elicit specific antibody response in the human host. It is said to have the potential to represent a general epidemiological marker of exposure of the main Afro-tropical malaria vectorial system, *Anopheles arabiensis* and *Anopheles funestus* up to 99% and 80% respectively [15].

Other malaria antibodies that are used in most malaria antibody studies include circumsporozoite protein (CSP), apical merozoite antigen-1 (AMA1), merozoite surface proteins 1 and 3 (MSP -1, MSP-3) [16, 17].

5. Seroprevalence as malaria transmission metric for surveillance

Changes in vector exposure, parasite infections in humans and human immunity can alter the metrics of malaria transmission. Thus, the malaria transmission metric used to determine transmission intensity should depend on the intrinsic variability of the metric across space and time [18]. These variations that might go undetected by parasite prevalence can be identified with the use of seroprevalence data, antibody density and seroconversion rates. In the era where transmission intensity of malaria is decreasing it becomes particularly difficult to use popular methods like the entomological inoculation rate (EIR). Thus, the use of other serological tools based on antibody responses to parasite and vector antigens are potentially valuable for robust transmission measurement.

Serosurveillance offers an approximation of the antibody levels elicited against an infectious disease. Many developed countries have well established national serosurveillance programs for different infectious diseases [19]. Serosurveillance of malaria offers the advantage of making known active transmission in cases that would otherwise be deemed as interrupted transmission [20]. A case example was observed in Ghana where the parasite prevalence was well below 5% throughout the year, whereas an equivalent seroprevalence of mosquito salivary protein gSG6-P1 and *Plasmodium falciparum* merozoite surface protein $MSP1_{19}$ were above 40% (**Figure 1**) [21]. A similar observation was made in a study in Somalia where the prevalences of $MSP1_{19}$ were 17.9% and 19.3% in the wet and dry seasons respectively when no parasites had previously been observed [20]. A prevalence of 10% and 50% were observed for parasite and $MSP1_{19}$ respectively in the uphill dwellers of the Western Kenyan highlands, emphasising robustness of serological markers in tracking temporal changes in vector exposure, especially in younger populations [17]. Human immune response to *Anopheles* salivary gSG6-P1 varies in relation to exposure to mosquitoes. Measuring human-mosquito contact using gSG6-P1 has therefore been shown to be very reliable.

Seroconversion rates (SCR) which is a function of age and exposure have been estimated using $MSP1_{19}$ age-specific seroprevalence. This has been used to measure the transmission intensity of malaria and has been shown to correlate with EIR measurements [7, 22]. Age-specific seroprevalence of $MSP1_{19}$ can distinguish between transmission intensities in low versus high malaria transmission areas or periods (**Figure 2**). When transmission intensity is below 5%, parasite prevalence is an inefficient metric for establishing changes in transmission or evaluating the impact of interventions [23].

Seroprevalence helps to overcome the challenge of subpatent malaria since microscopy and rapid diagnostic test (RDT) used to measure parasite prevalence

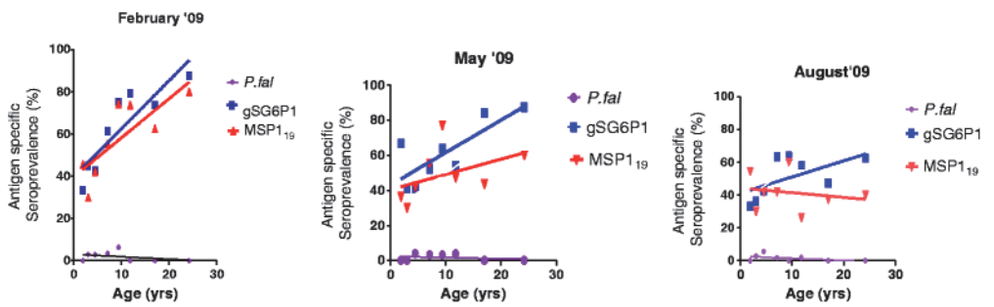

Figure 1.
Relationship between malaria parasite prevalence and antigen specific seroprevalence (adapted from Badu et al. 2015) [21].

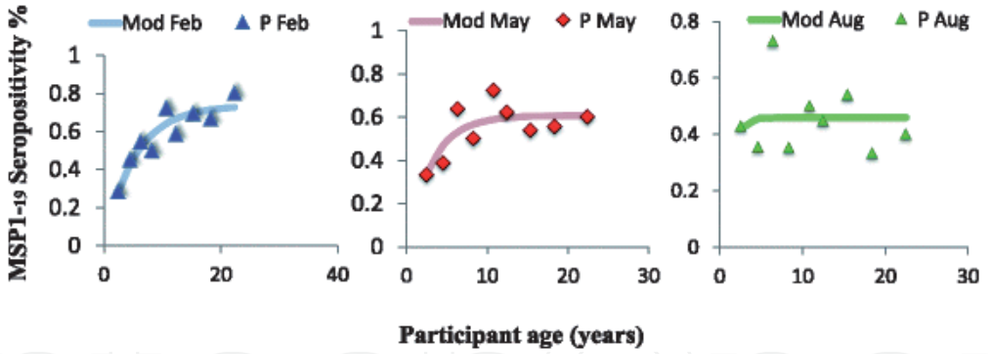

Figure 2.
Seroconversion rate of MSP1$_{19}$ (adapted from Badu et al. 2015 [21].

are less accurate where parasite load is less than 250 parasites per μl [24]. The profound effect of antimalarial drug intake on parasite density is also not accounted for in parasite prevalence measurements. With changing parasite load in the blood in the course of an infection, the accuracy of parasite prevalence is often unreliable [24].

A study has also indicated that to assess malaria endemicity at varying altitudes, the most suitable immunological marker to use is MSP -119 [7]. It has been demonstrated in our studies in Kenya that the prevalence of MSP-1 19 antibody in residents at the valley bottom was almost two-fold higher than that of the uphill residents with median total IgG titers indicating a 13-fold difference between the uphill and valley bottom residents suggesting a higher intensity of malaria

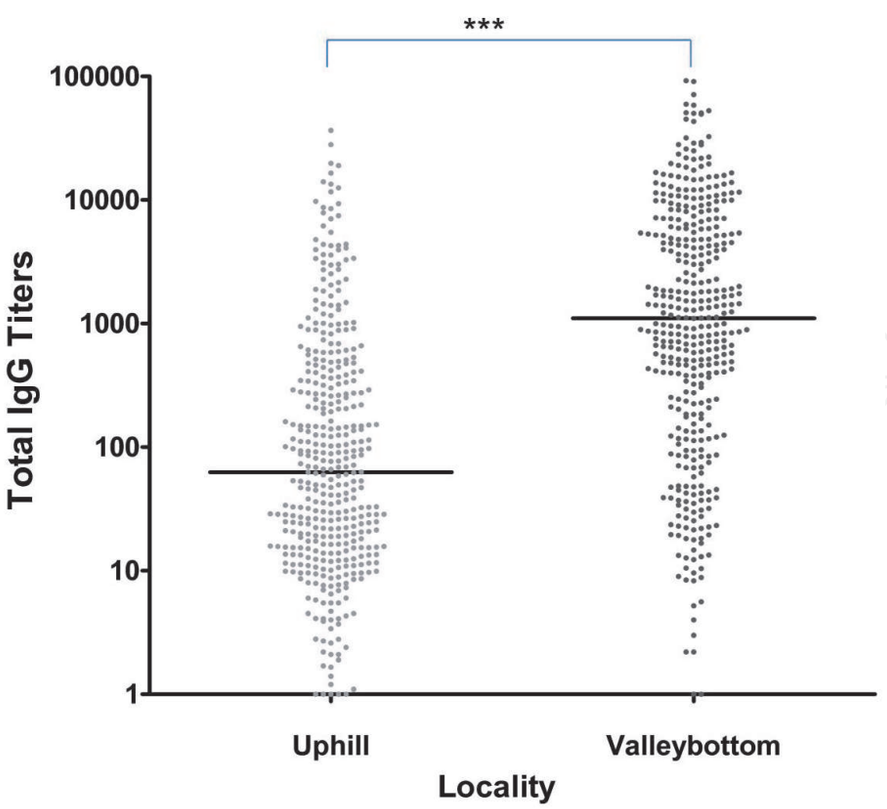

Figure 3.
*Differences in IgG titers among different localities. ***indicates the significance test by Mann-Whitney test, P value < 0.001 (Uphill n = 401, Valley n = 394). Source: Badu et al. [16].*

transmission in the valley area than the uphill area [17]. This was corroborated by a related study which revealed that in 82% of all malaria mosquitoes in the highland aggregated in <300 m area the valley bottom (Guof et al. 2004), [18] This clearly demonstrates that seroprevalence reflects cumulative exposure and thus, is less affected by seasonality or unstable transmission due to the longer duration of the specific antibody response. Age-specific seroprevalence was also used to estimate seroconversion rates (SCR) as a measure of malaria transmission intensity. Age seroprevalence curves of the study showed that in low transmission settings, development of antibodies is slow and is mainly exhibited by the adult population, whereas in a high transmission area, much of the population will be seropositive even at a younger age. **Figures 3** and **4** and **Table 1** below show the findings of this experiment.

Another study showed differences in gSG6-P1 specific seroprevalence and antibody levels depending on your location. The study observed differences in parasite prevalence and gSG6-P1 levels across different transmission settings. Thus, salivary gland gSG6-P1 could be effective in differentiating between vector densities. Again, the study showed that the risk of exposure to malaria parasite is higher in individuals presenting with anti -gSG6 P1 antibody and that gSG6-P1 seroprevalence is not cumulative [17].

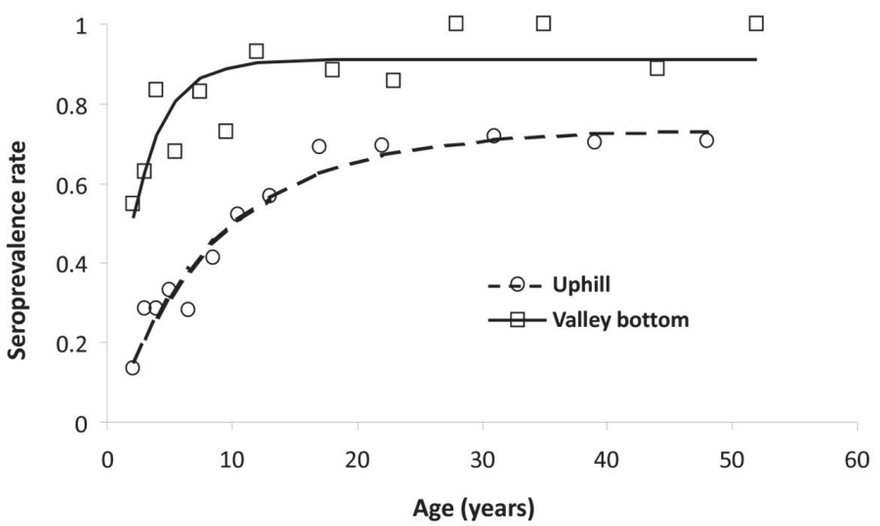

Figure 4.
Kinetics of the age-dependent antibody prevalence at different altitudes. Phill model: Pt = 0.73(1-e-0.11t), R2 = 0.95, P<0.001; valley bottom model: Pt = 0.91(1-e-0.39t), R2 = 067, P<0.001. Source: Badu et al. [16].

Parasite prevalence			
MSP-1_{19} seroprevalence			
Locality (n)	***Odds Ratio***	***95% CI***	***P value***
Uphill (401)	2.798	[1.018, 7.693]	0.046
Valley (394)	3.167	[1.196, 8.386]	0.020
Total Uphill and Valley (795)	4.282	[2.200, 8.330]	< 0.001

Table 1.
Showing the association between MSP-119 seroprevalence with plasmodium parasite prevalence at the different localities. Source: Badu et al. *(2012).*

We utilise a very simple reversible catalytic conversion model which estimates the rate of seroconversion from seronegative to seropositive and vice versa with respect to specific study sites. This we fit with standard maximum likelihood and which assumes a binomial error distribution [7, 16].

Thus seropositive are the percentage individuals whose antibody titers of specific malaria antigens are above the threshold of unexposed or naive individuals (Defined as three standard deviations above the value determine for the negative controls).

Reversible catalytic conversion models uses the average yearly rate of conversion to seropositive, λ, and the average yearly rate of reversion from seropositive to seronegative, ρ [7], The equation is thus fitted for each study site (uphill dwellers and that of valley bottom dwellers). Thus Pt is the proportion of individuals aged t that are seropositive

$$P_t = \frac{\lambda}{\lambda + \rho}(1 - \exp(-(\lambda + \rho)t))$$

Where λ is the respective site rate of seroconversion, and ρ is the site specific rate of reversion to seronegative [7, 16].

Box 1.
Reversible catalytic conversion model.

Age trends of gSG6-P1 is influenced by the differences in the transmission intensity and thus, children had higher responses to whole salivary gSG6 proteins while adults had diminished Antibody responses, suggesting desensitisation of the immune response to the salivary proteins. From all the above, gSG6-P1 measurement shows transient exposure (or seasonal) in a hypoendemic population and would be more useful even under low malaria transmission period as envisaged in the pre-elimination and elimination phase of malaria [17].

In a comprehensive study conducted in two malaria endemic western Kenyan highland areas, 107 proteins of *P. falciparum* that elicited antibody responses were identified. Many of these immunogenic proteins had been previously identified in other studies. Hsp70, ETRAMP10.2, MSP1, and conserved *Plasmodium* protein PF3D7. These were the most frequently recognised proteins among the sera groups studied [25]. Expectedly, more proteins were identified in sera from valley bottom than sera from hilltop residence. When comparing the breadth of antibody response to these proteins, the study observed that there was no difference between

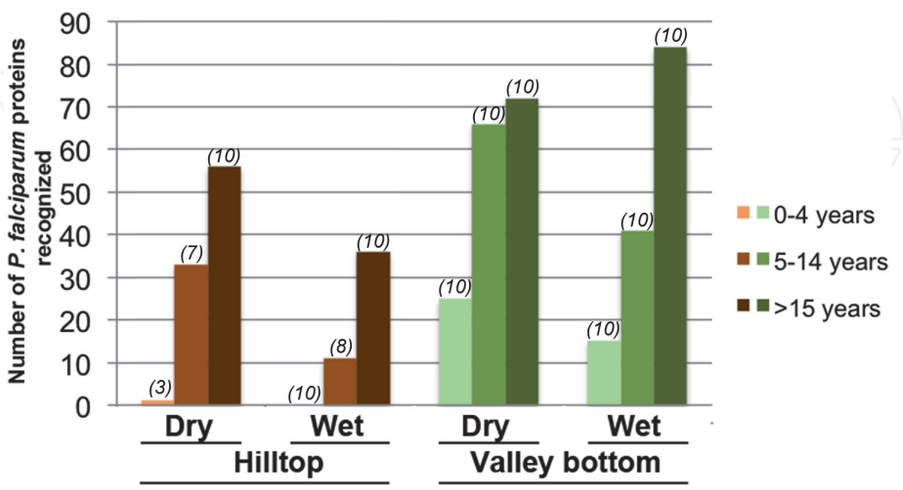

Figure 5.
Breadth of antibody binding to immunogenic P. falciparum *proteins by study sera groups. The number of proteins considered immunogenic by the study sera (Y axis) is plotted against sera cohorts stratified by age (0–4, 5–14 and > 15 years old), season (wet and dry) and site (hilltop and valley bottom) of sample collection (X axis). The number of serum samples tested in each sera group is provided in parenthesis above the corresponding bar (adapted from Baum et al. 2013) [25].*

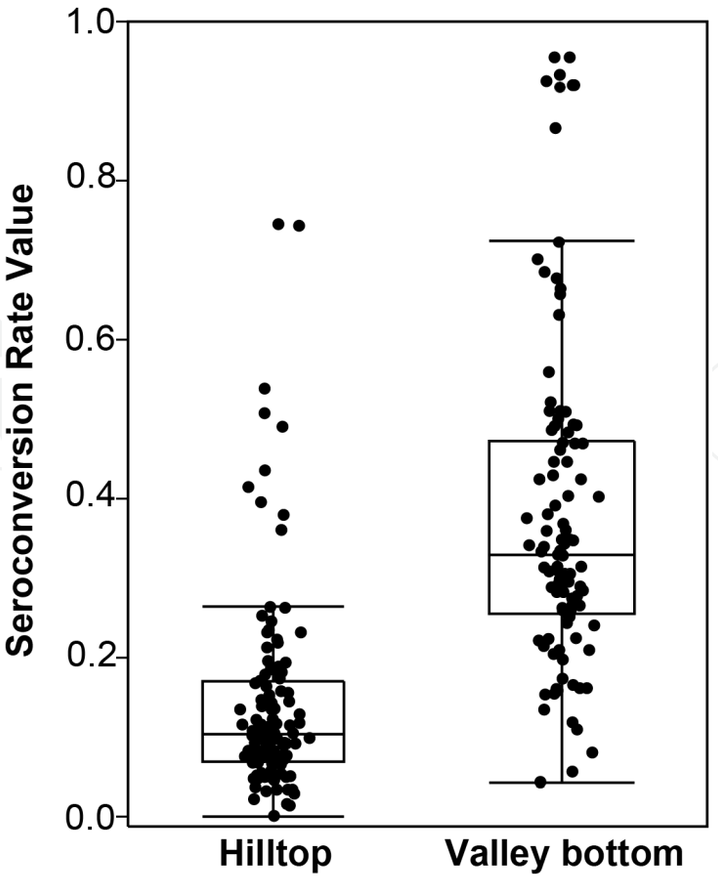

Figure 6.
Whisker box plot of seroconversion rate (SCR) values of immunogenic polypeptides of P. falciparum *for the hilltop and valley bottom sites. The SCR of 98 immunogenic polypeptides for which paired values were calculated (Y axis) is plotted against the site of serum sample collection (X axis). The lower and upper edges of the box indicate the first and the third quartiles, the line in the middle represents the median. The 1.5X interquartile range is indicated by the vertical line outside the boxes. Outlying values are indicated by dots outside boxes (adapted from Baum et al. 2013) [25].*

immunogenic proteins identified during the wet or dry season among valley bottom residents. However, seasonal variation in protein was observed in hilltop sera. Between donors below 5 years and those above 5 years, difference between antibody response was observed (**Figure 5**). This aligns with surveillance studies that used clinical cases or parasite prevalence metrics [16, 26].

The study also noted that seroconversion rates differed between hilltop and valley bottom donors, with lower SCR values recorded for hilltop than in the valley bottom (**Figure 6**). Overall, the antigens identified in the study can be used as protein candidates to sero-survey the intensity of malaria transmission across stable and unstable transmission areas and compared different age stratifications to give an overview of the effect of temporal and spatial variations on malaria transmission.

6. Sero-surveilance as for the confirmation of elimination

The development and utilisation of serological tools, especially, the antibody response to specific malaria antigens has a great potential to support malaria elimination agenda. A recent study [14] has identified about five priority areas in which this approached could be utilised to support the malaria elimination agenda. Among these, the confirmation of malaria elimination can be a practical benefit. "Certification of malaria elimination is the official recognition by WHO of a country's

malaria-free status". WHO may grant this recognition to a country only when it can prove beyond reasonable doubt, that the indigenous malaria transmission of all human parasites has been interrupted across the whole country for at least three consecutive years. Not only that but the country also has "a fully functional surveillance and response system that can prevent re-establishment of local transmission" [27]. Practically, antibodies to malaria antigens are sensitive biomarkers of population-level malaria exposure and can be used among other things to confirm malaria elimination, and monitor re-emergence of malaria [28]. Specific anti malaria antibodies such as $MSP1_{19}$ and AMA that tend to accumulate in the population can be used to confirm malaria elimination in children born in the last three years. When antibody response in children born in the last three years are undetectable across all subnational sentinel sites, this will be a good indication of the confirmation of the malaria elimination.

7. Sero-surveilance for monitoring recent exposure and the impact of interventions

Reliable serological markers of recent malaria exposure could dramatically improve current surveillance methods by allowing for accurate estimates of infection incidence from limited data. According to Greenhouse et al. [14], Sero-surveilance could be implemented in two scenarios settings: 1) when new tools are being deployed for the first time; and 2) regularly to evaluate the success of such tools during implementation.

For the purpose of regular evaluation of intervention tools antibodies with relatively short half life will be more useful. Yman et al. 2016, studied We IgG antibody responses to over one hundred 111 *malaria antigens* in a longitudinal cohort of travellers who did not have a second express following heir initial exposure. They identified five serological markers (GAMA, MSP1, MSPDBL1 C- and N-terminal, and PfSEA1) which could detect exposure within the previous 3-months with >80% sensitivity and specificity. Others (Kerkhof et al. 2016) have studied through several cross-sectional studies among 8439 participants have demonstrated that *Plasmodium falciparum*, antibodies against LSA3.RE, GLURP and Pf.GLURP.R2 are capable of detecting exposure range of 6–8 months. The quest for standard antigenes or peptides as well as standard technologies that will ensure smooth implementation and comparable results across the world is a subject of intense research currently ongoing.

8. Conclusion

Currently, the utility of sero-surveilance in the support of malaria elimination (measuring transmission intensity, assessing the impact evaluation, confirmation of elimination and prevention of re-introduction) have moved beyond the prove of principle. However, full implementation is hampered by the current requirement for expensive high-throughput technologies for specimen storage and functioning assays, which are largely not available in many malaria-endemic communities. Therefore, governments and research programs should critically analyse the value being added by antibody tests before considering using it to complement other metrics [14, 28].

In their current state, sero-surveillance tools can only be performed by technicians who are well trained on the use of antibody detecting equipment. However, surveillance studies are often carried out by field workers who might lack the

adequate knowledge and training needed to perform antibody tests. As such, it is very important that antibody detecting tools are easy to use, with test results being easy to interpret [14, 29]. This will ensure the generation of data that can easily be analysed to translate to action implementation.

The integration of seroprevalence as a malaria surveillance metric in endemic communities will require the understanding of stakeholders in malaria control and eradication at the local, national, and global level. This involves engaging and sensitising local communities, governmental and non-governmental organisations, research bodies and donor agencies on the need for sero-surveillance as a function of malaria exposure.

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