Title of project: Use of serological surveillance to unravel malaria trends and impact of antimalarial interventions among pregnant women at antenatal care clinics in Mozambique (Serosurv).

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Background

Mozambique is among the ten countries with the highest burden of malaria worldwide, with an estimated 9.8 million cases in 2021(1). In order to reduce this high burden of malaria, the national malaria control program (NMPC) from Mozambique is implementing intermittent preventive treatment of malaria during pregnancy with sulfadoxine-pyrimethamine (IPTp-SP). In addition to IPTp-SP seasonal malaria chemoprevention (SMC), perennial malaria chemoprevention (PMC), mass drug administration (MDA), are going to be introduced in the country from 2024.

The primary source to estimate malaria burden in Africa has been the prevalence of infection in children from nationally representative household surveys(2). Due to high cost and operational challenges such surveys are typically undertaken at every 2-5 years, that do not allow monitoring yearly transmission trends (2). The other source for estimating malaria burden is the open source platform(3–5) District Health Information System (DHIS2), which is not accurate(4). As an alternative, surveillance strategies targeting easy-access groups have been suggested as a cost-efficient approach to obtain more reliable information on malaria burden at a more operational spatial and temporal resolution(6).

Together with schoolchildren and children in immunization programs(7–9), pregnant women at first antenatal care (ANC) visit represent a promising convenience group for surveillance of infectious diseases (10–16). In sub-Saharan Africa, 79% of pregnant women have at least one ANC visit (17), providing an easy-access population representative of the overall population.

Previous data from our group show that malaria in pregnant women at their first ANC visit reflect malaria transmission trends observed in the community *(Matambisso et al., 2023; Pujol et al.,* 2023) and during antimalarial interventions *(Brokhattingen et al., 2023, submitted)*. Concretely, drops in malaria transmission from high to low in Southern Mozambique were accompanied by reductions in parasite densities among anti-VAR2CSA-naive primigravidae women in spite of low levels of immunity(18). However, among secundigravidae in Ilha Josina, parasite densities and the relative abundance of

detectable infections increased with declining malaria, as expected from les malaria exposure in the previous pregnancies(18). Thus, malaria parasites may develop mechanisms to adjust their transmissibility and densities to changes in transmission(19). This parasite adaptability observed in primigravidae women at ANC clinics suggests that factors other than acquired immunity emerge as potentially important for producing less detectable infections over short but marked declines in malaria transmission(20). These acquired immune and non-immune factors contribute to the heterogeneity in the detectability of *P. falciparum* infections in pregnancy and the detectability is dependent on gravidity and changes in transmission intensity, affecting the sensitivity of current diagnostic tools(18). Thus, in areas with low-to-moderate malaria transmission intensity in Southern Mozambique, pregnant women reflect malaria transmission trends in the community and during antimalarial interventions(18,21), but this was observed in areas with low-to-moderate malaria transmission intensities(18,21).

Therefore, the importance of our future work is underlined by significant findings and their broader implications for malaria surveillance and intervention strategies, particularly in the context of Mozambique:

First, the finding that molecular and serological malaria outcomes in pregnant women reflect malaria transmission trends in the community and during interventions(18,21) in Mozambique, was observed in areas with low-to-moderate malaria transmission intensities. Evidence for all malaria transmission intensities and high transmission areas is lacking, and this work will allow us to obtain more informative data that can guide programmatic decisions in establishing ANC-based malaria surveillance in the country.

Second, decreasing transmission in three different epidemiological settings in Maputo province, were followed by changes in parasite densities, which was gravidity-dependent. Falling parasite rates were associated with declining parasite densities among anti-VAR2CSA-naive primigravidae women, over short but marked declines in malaria burden(18). However, it is important to explore the dynamics of parasitic densities and acquired immunity at different intensities of malaria transmission throughout the country, for a better discussion of the dogma that all malaria infections evolve into symptomatic infections as immunity decreases with a declining transmission. This analysis will also be important to inform the national malaria control program about potential sub-RDT infections as a result of declining transmission.

Third, in addition to IPTp-SP seasonal malaria chemoprevention (SMC), perennial malaria chemoprevention (PMC), mass drug administration (MDA) are going to be introduced in the country from 2024. Timely monitoring of malaria transmission (22,23), and acquired immunity against malaria is needed to track the impact of these interventions in the dynamics of malaria transmission.

Fourth, evidence shows that serological surveillance can be used for monitoring and evaluation of malaria trends and the impact of antimalarial interventions. Seroprevalence against the pregnancy-specific

antigen VAR2CSA reflected declining malaria trends in Southern Mozambique(21). The efficacy of the serological markers, especially Etramp5.Ag1 (short-lived antibody), for assessing anti-malarial interventions (such as Reactive focal mass drug administration [rfMDA] or indoor residual spraying [IRS]) was proven(24). Thus, the impact of anti-malarial interventions could be assessed by using serological data from pregnant women attending ANC.

Overall, ANC malaria surveillance has a high potential to be a source of much higher spatial and temporal resolution prevalence data (molecular and serological) to better monitor progress towards malaria control targets and to tailor interventions according to local levels of transmission(12). In another hand, Schistosomiasis is an important neglected tropical disease in Mozambique(25) and *Salmonella spp* is the main cause of diarrhea in individuals infected and not infected with HIV in Mozambique(26). The potential use of serological data from pregnant women to conduct surveillance to multi-pathogens (*P. falciparum*, Schistosoma, Salmonella) is a cost-efficient approach.

Research Question

1- Is serological data from ANC useful to stratify different malaria transmission levels and reflect changes of transmission in these different regions?
2- Are the dynamics of parasite densities and acquired immunity (pregnancy-specific and non-pregnancy-specific) gravidity-dependent, in areas with different transmission levels and after declines in transmission resulting from drug-based anti-malarial interventions?
3- Is there a serological marker that allows us to infer the impact of drug-based anti-malarial interventions?
4- Can we use pregnant women at ANC to conduct surveillance to multiple pathogens (*Plasmodium falciparum*, *Schistosoma*, *Salmonella*)?

Primary Objectives

1- To analyse Pregnancy-specific and non-pregnancy-specific serology in pregnant women at ANC and correlate ANC-seroprevalences with *Pf*PR_{qPCR} in children.
2- To compare parasite densities and VAR2CSA and non-VRA2CSA immunity levels and seroprevalences between pregnant women at ANC, from areas with different transmission levels and compare the metrics after declines in transmission, resulting from programmatic drug-based anti-malarial interventions.

3- To correlate VAR2CSA and non-VRA2CSA immunity levels and seroprevalences in pre-intervention and post intervention ANC samples with *Pf*PR_{RDT} and *Pf*PR_{qPCR} in children.

Secondary Objective

4- Carry out multi-pathogen (*Plasmodium falciparum*, *Salmonella*, *Schistosoma*) surveillance based on ANC serological data and compare the seroprevalences with qPCR multi-pathogen positivity rates.

Inclusion Criteria

ANC surveiilance

- ✓ Pregnant women seen during their first prenatal consultation
- ✓ Age ≥ 12 years
- ✓ Resident in the Study area (District).

Health facility survey

- ✓ Children between 2 - 10 years of age
- ✓ Resident in the study area (district)
- ✓ Axillary temperature >= 37.5 C (using a thermometer according to the current version of SOP_HO_003_PT) or history of fever in the last 24 hours.

District Health Information System (DHIS2)

- ✓ Children aged 0-5 years

Household surveys conducted as part of the malaria indicator survey (MIS).

- ✓ Children between 2 - 10 years of age

Exclusion Criteria

ANC surveillance

- ✓ Lack of willingness to participate in the study or take a sample
- ✓ Age <12 years
- ✓ Not being from the study area
- ✓ Pregnant woman with more than one prenatal visit made during the current pregnancy.
- ✓ Any sign or symptom of severe malaria in accordance with national guidelines[24].

Health facility survey

- ✓ Any sign or symptom of severe malaria in accordance with national guidelines[24].
- ✓ Age < 2 years or >10 years
- ✓ Not being from the study area
- ✓ History of antimalarial treatment in the last 14 days: for children it is verified through their yellow health card

DHIS2

- ✓ Children over 5 years of age

Household surveys conducted as part of MIS

- ✓ Age < 2 years or >10 years

Study Population

The present work will be carried out within the framework of the GenMoz project (*Plasmodium falciparum* genomic intelligence in Mozambique; https://www.isglobal.org/en/-/genmoz). The main objective of GenMoz Project is to establish a malaria genomic surveillance approach in Mozambique for informing programmatic activities.

Study participants are being recruited at first antenatal visits (ANC) and health facility surveys (HFS), in the different *Plasmodium* transmission zones across the country: a) high transmission (Cabo-Delgado, Zambézia, Manica, Nampula and Inhambane provinces), b) moderate (Tete, Niassa, Sofala and Gaza) and c) low transmission (Maputo province), which can represent the epidemiology and quality of service provision in the country, for each zone, from 2022 to 2025.

The recruitment of participants is taking place in a sample of 64 public health units in the country that offer outpatient malaria case management services. To ensure that the sample is representative, a stratified random sampling method was used so that the health units selected could effectively represent the provinces under study, based on WHO standardized methodology[25]. Rapid diagnostic tests results are being recorded and dried blood samples (DBS) on filter paper are being collected.

The interventions, whose impact will be evaluated, are the seasonal malaria chemoprevention (SMC) with sulphadoxin-pirimetamine + amodiaquine (SPAQ); perennial malaria chemoprevention (PMC) with sulphadoxin-pirimetamine (SP) and mass drug administration (MDA) with dihydroartemisinin-piperaquine (DHAp), between 2024 and 2025. The SMC will take place in the provinces of Nampula, Niassa, Cabo-delgado and Manica. PMC intervention will include Gaza, Inhambane, Cabo-delgado, Zambézia, Tete and Sofala provinces. The MDA will take place in cabo Delgado.

The household survey as part of malaria indicator survey (MIS) conducted by the National Malaria control Program, took place from November to December 2023 in the provinces of Maputo and Gaza (areas of low intensity of *Plamsodium* transmission).

This work will be focused on the malaria epidemiology of pregnant women at their first ANC visit and representative children of the community (Children will be from HFS, DHIS2 and household survey as part of MIS), recruited from 2022 to 2025 all over the country, in the context of GenMoz project. The present study will represent the analysis of blood samples on filter paper and clinic-demographic data collected across the country from 2022 to 2025.

Study Design and Methods

Study design:

For primary objective 1, serological outcomes from pregnant women at ANC visit in areas with different intensities of *P. falciparum* infection transmission will be correlated with *Pf*PR_{qPCR} in children from HFS aged 2 -10 years and data from MIS, in each province. Serological outcomes will include Pregnancy-

specific (VAR2CSA) antibodies (Duffy binding-like recombinant domains DBL34 as well as peptides targeting the NTS region [P1] and ID1 [P8 and PD]) and non-pregnancy-specific antigens (full-length *P. falciparum* reticulocyte-binding homologue protein 2 and 5 [PfRH2 and PfRH5], 19-kDa fragment of the merozoite surface protein-1 [MSP1], region II/F2 of erythrocyte-binding antigen-175 [EBA175], gametocyte exported protein 18 [GEXP18], acyl CoA synthetase 5 [ACS5] ag3, early transcribed membrane protein 5 [ETRAMP5] ag1, heat shock protein 40 [HSP40] ag1 and thrombospondin-related apical merozoite protein [PfTRAMP]**).**

For primary objective 2, parasite densities and Pregnancy-specific (VAR2CSA) and non-pregnancy-specific antibodies in pregnant women at ANC visit, will be compared between areas with different intensities of *P. falciparum* transmission in Mozambique from 2022 to 2025. Parasite densities and Immunoglobulin G levels against *P. falciparum* antigens will be compared before and after the deployment of programmatic drug-based antimalarial intervention. Drug-based antimalarial interventions will include seasonal malaria chemoprevention SMC), perennial malaria chemoprevention (PMC), mass drug administration (MDA).

For primary objective 3, VAR2CSA and non-VAR2CSA antibodies levels and seroprevalences in pregnant women from ANC before and after interventions, will be correlated with *Pf*PR_{RDT} and $PfPR_{qPCR}$ in children. For *Pf*PR_{RDT} correlation analysis, children will be from DHIS2 (aged 0-5 years) and HFS (aged 2-10 years).

For secondary objective 1, the prevalence of Salmonella and *Schistosoma* by qPCR in pregnant women across different provinces will be compared with the immunity profiles against Salmonella and *Schistosoma* across different provinces.

Sample analysis:

Parasitological determinations: DNA extracted from dried blood spots (DBS) will be used for detection and quantification of *Salmonella* using real-time quantitative polymerase chain reaction (qPCR) assay. qPCR for the diagnosis *Salmonella* will be adapted from existing methods. DBS will be screened for detection of Circulating Anodic Antigen (CAA) from *Schistosoma* using an *Enzyme-linked immunosorbent assay* (ELISA).

Serology: The Luminex xMAP technology, a quantitative Suspension Array technology (qSAT) that allows multiplexing biological assays by using colored beads or microspheres, will be used for detection of a total of 17 immunoglobulins G of *Plasmodium falciparum*, *Schistossoma spa* and *Salmonella ssp*. By coding different beads with spectral sets, each bead set will be coupled with a particular capture

molecule, allowing the multiplexing of several beads and the detection of the corresponding analytes from DBS samples.

Statistical analysis: For seroprevalences and parasite densities, categorical and continuous data will be compared between provinces, gravidity groups, and periods using Pearson's chi-square test or Fisher's exact test and Student t-test, respectively. Seroprevalences between pregnant women and *Pf*PR_{qPCR} in children will be correlated by Pearson correlation coefficient. To assess the impact of antimalarial interventions the seroprevalence reported in children and pregnant women before and after the interventions (MDA, SMC and PMC) will be compared, in districts with interventions and in neighboring districts (as controls). Multivariate analysis including clinic, study period, season, gravidity, gestational stage at ANC and at delivery visit, reported use of IRS and *ITNs*, will be performed.
P-values <0.05 will be considered statistically significant in all tests. Univariate and multivariate analysis will be performed with R studio software (version R version 4.3.2). The correlation analyzes between pregnant women and children will be performed using Python software.

Estimated study start date:

Month	Year
November	2025

Estimated study completion date:

Month	Year
Maio	2027

Budget

Materials and consumables	64,597
Equipment	13,550
Field Work	0
Travel and subsistence	2,675
Research Assistance	0
Training	4,229
Publication and dissemination	1,840
General and administrative services:	2,560
Other indirect costs	6,504
Total	**95,955**

References

1. World Health Organization. World Malaria Report. Vol. WHO/HTM/GM, World Health. 2022. 238 p.
2. Hay SI, Smith DL, Snow RW. Measuring malaria endemicity from intense to interrupted transmission. Lancet Infect Dis. 2008;8(6):369–78.
3. WHO. Word Malaria Report 2021. Word Malaria report Geneva: World Health Organization. (2021). Licence: CC. 2021. 2013–2015 p.
4. Sahay S, Rashidian A, Doctor H V. Challenges and opportunities of using DHIS2 to strengthen health information systems in the Eastern Mediterranean Region: A regional approach. Electron J Inf Syst Dev Ctries. 2020;86(1):1–5.
5. DHIS2. About DHIS2 [Internet]. 2022. Available from: https://dhis2.org/about/
6. Mayor A, Menéndez C, Walker PGT. Targeting Pregnant Women for Malaria Surveillance. Trends Parasitol. 2019;35(9):677–86.
7. Ataíde R, Mayor A, Rogerson SJ. Malaria, primigravidae, and antibodies: Knowledge gained and future perspectives. Trends Parasitol. 2014;30(2):85–94.
8. Brooker S, Kolaczinski JH, Gitonga CW, Noor AM, Snow RW. The use of schools for malaria surveillance and programme evaluation in Africa. Malar J. 2009;8(1):1–9.
9. malERA - The malERA Consultative Group on Diagnoses. A research agenda for malaria eradication: Diagnoses and diagnostics. PLoS Med [Internet]. 2011 [cited 2020 May 2];8(1). Available from: https://journals.plos.org/plosmedicine/article/file?id=10.1371/journal.pmed.1000396&type=printable
10. Kwesigabo G, Killewo JZ, Urassa W, Mbena E, Mhalu F, Lugalla JL, Godoy C, Biberfeld G, Emmelin M, Wall S SA. Monitoring of HIV-1 infection prevalence and trends in the general population using pregnant women as a sentinel population: 9 years experience from the Kagera region of Tanzania. J Acquir Immune Defic Syndr [Internet]. 2000;(23(5):410-417). Available from: doi: 10.1097/00126334-200004150-00008
11. Korenromp EL, Mahiané SG, Nagelkerke N, Taylor MM, Williams R, Chico RM, et al. Syphilis prevalence trends in adult women in 132 countries – estimations using the Spectrum Sexually Transmitted Infections model. Sci Rep. 2018;8(1):1–10.
12. Mayor A, Menéndez C, Walker PGT. Targeting Pregnant Women for Malaria Surveillance.

Trends Parasitol [Internet]. 2019 [cited 2020 May 1];35(9):677–86. Available from: https://doi.org/10.1016/j.pt.2019.07.005
13. Hellewell J, Walker P, Ghani A, Rao B, Churcher TS. Using ante-natal clinic prevalence data to monitor temporal changes in malaria incidence in a humanitarian setting in the Democratic Republic of Congo. Malar J [Internet]. 2018;17(1):1–11. Available from: https://doi.org/10.1186/s12936-018-2460-9
14. Mayor A, Bardají A, Macete E, Nhampossa T, Fonseca AM, González R, et al. Changing trends in P. Falciparum burden, immunity, and disease in pregnancy. N Engl J Med. 2015;373(17):1607–17.
15. Willilo RA, Molteni F, Mandike R, Mugalura FE, Mutafungwa A, Thadeo A, et al. Pregnant women and infants as sentinel populations to monitor prevalence of malaria: Results of pilot study in Lake Zone of Tanzania. Malar J. 2016;15(1):1–10.
16. van Eijk AM, Hill J, Noor AM, Snow RW, ter Kuile FO. Prevalence of malaria infection in pregnant women compared with children for tracking malaria transmission in sub-Saharan Africa: A systematic review and meta-analysis. Lancet Glob Heal. 2015;3(10):e617–28.
17. Walker PGT, Floyd J, ter Kuile F, Cairns M. Estimated impact on birth weight of scaling up intermittent preventive treatment of malaria in pregnancy given sulphadoxine-pyrimethamine resistance in Africa: A mathematical model. PLoS Med. 2017;14(2):1–19.
18. Matambisso G, Brokhattingen N, Maculuve S, Cisteró P, Mbeve H, Escoda A, et al. Gravidity and malaria trends interact to modify P. falciparum densities and detectability in pregnancy: a 3-year prospective multi-site observational study. BMC Med [Internet]. 2022;20(1):1–12. Available from: https://doi.org/10.1186/s12916-022-02597-6
19. Andrade CM, Fleckenstein H, Thomson-Luque R, Doumbo S, Lima NF, Anderson C, et al. Increased circulation time of Plasmodium falciparum underlies persistent asymptomatic infection in the dry season. Nat Med. 2020;26(12):1929–40.
20. Rono MK, Nyonda MA, Simam JJ, Ngoi JM, Mok S, Kortok MM, et al. Adaptation of Plasmodium falciparum to its transmission environment. Nat Ecol Evol. 2018;2(2):377–87.
21. Pujol A, Brokhattingen N, Matambisso G, Mbeve H, Cisteró P, Escoda A, et al. Detecting temporal and spatial malaria patterns from first antenatal care visits. Nat Commun. 2023;14(1):1–12.
22. Ohrt C, Roberts KW, Sturrock HJW, Wegbreit J, Lee BY, Gosling RD. Information Systems to Support Surveillance for Malaria Elimination. 2015;93(1):145–52.
23. WHO WHO. Global Technical Strategy for Malaria 2016–2030 [Internet]. 2015 [cited 2020 May 31]. Available from: https://www.who.int/malaria/publications/atoz/9789241564991/en/
24. Wu L, Mwesigwa J, Affara M, Bah M, Correa S, Hall T, et al. Antibody responses to a suite of novel serological markers for malaria surveillance demonstrate strong correlation with clinical and parasitological infection across seasons and transmission settings in The Gambia. BMC Med. 2020;18(1):304.
25. Grau-Pujol B, Massangaie M, Cano J, Maroto C, Ndeve A, Saute F, et al. Frequency and distribution of neglected tropical diseases in Mozambique: A systematic review. Infect Dis Poverty. 2019;8(1):1–11.
26. Hlashwayo DF, Noormahomed EV, Bahule L, Benson C, Schooley RT, Sigaúque B, et al. Microbiological assessment reveals that Salmonella, Shigella and Campylobacter infections are widespread in HIV infected and uninfected patients with diarrhea in Mozambique. PLOS Glob Public Heal. 2023;3(5):e0001877.