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Applying novel *Plasmodium Vivax* serological exposure markers to quantify residual malaria transmission in the Philippines through repeated health facility surveys: the SMaRT study protocol

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Abstract

Background After decades of progress towards malaria elimination, *Plasmodium vivax* is now the predominant source of infection and the major obstacle towards elimination in the Asia-Pacific region. In the Philippines, the situation is slightly different with *P. falciparum* still accounting for the largest burden. However, there has been a steady increase in the total number of reported *P. vivax* cases in the main transmission hotspot of Palawan, as well as two years of consecutive outbreaks of *P. vivax* in the near-elimination setting of Sultan Kudarat. Here, we describe the protocol for a new study in Sultan Kudarat that aims to identify whether an underlying, hidden, burden of *P. vivax* contributes to the ongoing risk of outbreaks.

Methods A challenge for surveillance of *P. vivax* is the presence of an additional hidden liver-stage, where parasites (hypnozoites) lie dormant for weeks to months before causing a relapse of infection. Hypnozoites cannot be detected with commercial diagnostic tests. We have designed novel serological exposure markers of recent *P. vivax* infection, which indirectly inform on hypnozoite carriage. In this study we will conduct a prospective 18-month survey in health facilities within Kalamansig, Sultan Kudarat, and compare epidemiology and serological data with that in archival samples from Palawan. We will enroll both care-seeking individuals and their companions, and utilise remote geolocation to uncover spatial trends.

Discussion This study will generate important data for the malaria control program in the Philippines whilst also demonstrating utility of *P. vivax* serological exposure markers in near-elimination settings. We will utilise this data

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to build a decision-making framework to support novel, evidence-based elimination strategies relevant for the Philippines and the wider Asia-Pacific region.

Keywords Malaria, Elimination, *Plasmodium vivax*, Serology, Surveillance, Serological exposure markers, Philippines

Background

After decades of progress towards malaria control and elimination, *Plasmodium vivax* is now the predominant source of cases and the major obstacle to malaria elimination in the Asia-Pacific region [1]. Endemic countries have the unified goal of malaria elimination by 2030, but progress has stagnated due to the COVID-19 pandemic [2] and the challenges posed by *P. vivax* [3]. The Philippines have had great success in reducing malaria case numbers from ~50,000 per year in the early 2000s to ~6000 in 2021. Transmission was reported in only three of the country's 81 provinces until 2021, with more than 90% of cases reported in Palawan, and since 2022, only the province of Palawan continued to report indigenous cases [4, 5]. Unstable or recent historical transmission (>5 years ago) has occurred in the provinces of Sultan Kudarat and Occidental Mindoro, respectively. The proportion of cases due to *P. falciparum* versus *P. vivax* (~85:15) has remained relatively stable in the Philippines despite large reductions in overall transmission levels [1], with the transmission and case breakdown in Palawan heavily influencing these proportions. The 2021 World Health Organization (WHO) World Malaria Report [1] highlighted an increase of >40% in case incidence in the Philippines between 2015 and 2020, which included an increase in reported *P. vivax* cases in recent years (i.e., 736 in 2020 compared to 116 in 2018). Together with recent *P. vivax* outbreaks documented in Sultan Kudarat in 2020 and 2021 (Table 1), this suggests there may be a residual reservoir of *P. vivax* transmission that remains undetected.

P. vivax can cause relapsing infections due to the reactivation of liver stage asymptomatic hypnozoites, which contribute up to 80% of all recurrent blood-stage *P. vivax* infections and are a major transmission reservoir [6, 7]. There is likely population heterogeneity in risk of relapse following *P. vivax* infection, due to differing hypnozoite burdens related to variation in exposure to injected sporozoites [8]. In order to control and ultimately eliminate the *P. vivax* reservoir, a combination regimen of antimalarial drugs targeting both the acute blood-stage infection (schizonticidal agents) and the hidden liver-stage hypnozoites (hypnozonticidal agents) is required. The only hypnozonticidal drugs on the market are the 8-aminoquinoline compounds primaquine and, more recently, tafenoquine. Both drugs can cause severe hemolysis in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency, thus requiring G6PD testing prior to administration in most countries [9]. Primaquine requires treatment over 14 days, thus low adherence to the regimen has been linked to poor effectiveness [10]. When hypnozoites are not effectively cleared from patients, the patient will still recover from the acute infection but remain susceptible to relapses and thus contribute to maintaining the transmission reservoir [6].

Hypnozoites are not detected by current commercial diagnostic tests. All current diagnostic methods are based upon detecting parasites or parasite antigens in the blood, hence even highly sensitive PCR assays cannot detect hypnozoite carriers [11]. Strategies for elimination in the Asia-Pacific require a novel approach, including actionable surveillance as a core intervention [12]. Recently, researchers have developed serological signatures of recent exposure to *P. vivax* infections that detect individuals at risk of relapse [13]. Exploiting the fact that even low-density, asymptomatic *P. vivax* blood-stage infections induce rapid [14] and persistent antibody responses [15], it was demonstrated that IgG levels to a carefully selected panel of 8 *P. vivax* antigens can identify individuals who have had a *P. vivax* blood-stage infection in the prior 9-months with 80% sensitivity and 80% specificity [13]. This recent exposure signature can identify individuals who go on to have recurrent *P. vivax* infections at far higher rates than those without the signature [13]. Whilst the serological markers do not directly detect hypnozoites, all tropical and sub-tropical *P. vivax* strains cause a primary blood-stage infection followed by a first relapse within 6–9 months [16], thus virtually all individuals who carry hypnozoites will have had a recent blood-stage *P. vivax* infection in the prior 9-months. It is currently not possibly to distinguish whether an individual *P. vivax* blood-stage infection resulted from hypnozoite reactivation or new mosquito bite induced infection, without paired blood samples [17] or outside the context of a clinical trial (arms with/without primaquine treatment [6] or removal from endemic areas [18]). However, identifying individuals that likely have a relapse infection will provide important advances in targeted treatment strategies to support elimination programs.

A variety of options exist for National Malaria Control programs to incorporate surveillance into their ongoing activities. Most commonly surveillance is simply passive case detection via reporting of clinical *P. vivax* case numbers. Options for programmatic active surveillance activities include surveys in easy access groups [19], such as schools, health facilities and churches. These have clear advantages over population-based household surveys given the inefficiencies of this approach in

Table 1 Distribution of confirmed malaria cases in Sultan Kudarat (SK). Data was obtained from two sources: the National epidemiology center (NEC) and the provincial malaria coordinator. There are discrepancies between the numbers, likely due to different reporting templates. *P. vivax* cases started becoming dominant over *P. falciparum* cases in 2019, with all age groups affected (not shown). All cases were located in the municipalities of Palimbang and Kalamansig, dependent on the year (Nd: no data obtained). No cases were reported in SK in 2022. Detection of malaria is by microscopy or RDT, PCR is not currently used by the program

	NEC			Provincial Coordinator		
Year	***P. falciparum***	***P. vivax***	***Total***	***P. falciparum***	***P. vivax***	***Total***
2021	0	71	71	1	71	72
2020	26	116	142	0	142	142
2019	1	14	15	1	15	16
2018	36	6	42	38	6	44
2017	nd	nd		33	16	49
Total	63	207	270	73	250	323

low-transmission settings. A challenge with easy access group surveys can be the inability to spatially map transmission foci if the household location is unknown, however recent advances in the ability for remote geolocation can overcome this issue [20]. A recent health facility-based serological surveillance activity in Indonesia was able to accurately predict an area at risk of future *P. vivax* outbreaks [21], demonstrating the potential power of this approach. Furthermore, repeated cross-sectional health facility based surveys in Palawan in the Philippines [22] were able to show a large burden of low-density submicroscopic infections and higher risk of *P. falciparum* infections in children and indigenous groups. The next logical step in programmatic surveillance, with a focus on surveillance as a core intervention, is a framework to incorporate this data to support public health decision-making for malaria elimination [23].

In this new research project, termed SMaRT (**s**erological **ma**rkers for **r**esidual **m**alaria **t**ransmission), we will assess the potential hidden hypnozoite reservoir in Sultan Kudarat through application of the novel *P. vivax* serological exposure markers. These will be used to delineate the residual *P. vivax* burden, and compared with data from other areas in the Philippines, be used to inform a locally appropriate and evidence-based intervention to accelerate elimination that is applicable not only to the Philippines but to the wider Asia-Pacific region.

Methods/design

Study rationale

Reported *P. vivax* outbreaks occurred in Sultan Kudarat in 2020 and 2021 (Table 1) but there has been no further assessment of the potential underlying *P. vivax* asymptomatic reservoir that may be contributing to these sporadic outbreaks, which would be undetectable via microscopy or rapid diagnostic tests. Cross-sectional surveys in health facilities applying highly-sensitive PCR-based detection of current low-density blood-stage *P. vivax* infections, combined with remote geolocation of households, will enable mapping of the potential *P. vivax* reservoir. *P. vivax* serological exposure markers inform on recent blood-stage *P. vivax* infections in the prior 9-months [13], thus provide a window of data in addition to identifying likely hypnozoite carriers. Applying novel *P. vivax* serological exposure markers will provide more informative data on the residual hidden *P. vivax* burden in Sultan Kudarat. This will be compared to the serological signature in archival samples from other regions in the Philippines [22].

Aim

The overall aim is to evaluate novel serological surveillance strategies to delineate the residual *P. vivax* malaria burden in different endemic areas in the Philippines and inform targeted elimination strategies. Specifically, the study aims to: [1] determine the population level of exposure to, or infection with, *Plasmodium* through repeated cross-sectional surveys in health facilities in Sultan Kudarat, Philippines; [2] estimate the hidden hypnozoite reservoir through application of *P. vivax* serological exposure markers; and [3] describe the epidemiology of residual *P. vivax* transmission, including spatial hotspots, and identify variables associated with infections.

Study design

This is an observational cross-sectional study that will implement repeated ("rolling") health facility-based surveys. From prior work in the Philippines, a large proportion of the burden of infection is expected to be in rural areas [22], thus surveys will be conducted both at the village (*barangay* or *sitio*) and rural health centers/units (RHUs). In Kalamansig in Sultan Kudarat province, surveys will be implemented for two weeks every two months over an 18-month period, sampling from 10 to 15 facilities/centers. These will be selected from a list of villages with a comparatively higher number of reported malaria cases in the past 3–5 years, including the ones with recent reported outbreaks, guided by local health officials. Similar surveys were implemented in previous studies in Occidental Mindoro and Palawan [22] from

which stored samples will be tested with the novel *P. vivax* serological markers, and archived datasets will be included and re-analyzed in this study.

Study area/setting

Transmission was reported in only three of the 81 provinces in the Philippines, namely Sultan Kudarat, Occidental Mindoro and Palawan until 2022, when Palawan became the only province reporting indigenous cases. The study will have 4 municipalities from these provinces as study sites: Kalamansig in Sultan Kudarat, Abra de Ilog in Occidental Mindoro, Rizal, Rizal and/or Puerto Princesa in Palawan and Morong in Bataan (Fig. 1). Morong, Bataan, declared as "malaria-free" in 2018, will serve as a "negative control" area. Kalamansig was selected based on the distribution of confirmed cases in Sultan Kudarat (Table 1) and access as guided by the local officials.

Study population and sample size estimation

All individuals consulting at the selected health facilities in Sultan Kudarat, and their companion/s, will be included in the study. The final sample size (maximum $n = 6000$) was estimated based on a past study in Rizal, Palawan (ENSURE) which had an almost similar population size [22]. Given a number of variables are unknown, including the seroprevalence of *P. vivax* in Sultan Kudarat, sample size calculations based on low seropositivity/seroconversion would suggest 105–248 individuals per facility per time point should be sampled to achieve seroprevalence estimates with a precision of between 2.5 and 5%. This was based on a low seroconversion rate of 0.0036–0.0108 on sample size calculations for serology [24]. The sample size for this study was estimated based on an expected prevalence of 3%.

The archival samples are: $n = 4105$ from Palawan, $n = 1501$ from Occidental Mindoro and $n = 559$ from Bataan [22], dependent on availability of dried blood spots (DBS).

Inclusion and exclusion criteria

Inclusion criteria: (a) individuals one year old and above of either sex, (b) with signed written informed consent (for 15 years old and above), parental consent (for 1–17 years old), verbal assent (for 7–11 years old), or written assent (for 12 to 14 years old), and (c) seeking care or accompanying someone seeking care at included facilities.

Exclusion criteria: (a) individuals less than one year of age, (b) with serious and acute illness/condition needing immediate attention or transport to a higher-level health care facility, and/or (c) no signed informed consent/assent. Serious and acute illnesses include: bleeding, difficulty breathing/shortness of breath, chest pain, choking, severe or persistent coughing or vomiting, fainting or loss of consciousness, head or spine injury, change in mental status (such as unusual behavior, confusion, difficulty arousing), and sudden dizziness.

Infants less than one year of age were excluded due to logistical issues for the study team and due to possible confounding from maternally transferred antibodies [25].

Health facility (HF)-based surveys (*aim 1*)

Enrollment and obtaining informed consent and assent from participants After being attended and treated for their primary health concern by the HF physician/nurse/midwife, all care-seeking individuals, regardless of reason for consultation, and their companion/s, will be approached by a trained Barangay Health Worker (BHW) or midwife. She/he will be assessed if the individual/s satisfy the inclusion and exclusion criteria, before recruiting them to participate in the study. The BHW/midwife will then obtain written informed consent, and assent where applicable, from potential participant/s. A separate consent will be obtained each time an individual is invited to participate in the study within the 18-month survey period. All consenting individuals will be enrolled in the study by entering their information in a designated log book and assigning unique identification code (barcode) to each individual to protect privacy. Logbooks and informed consent forms (ICFs) will be stored in locked cabinets in the RHU before transfer to the Research Institute of Tropical Medicine (RITM), Manila, at the end of the study.

Blood sample collection rapid diagnostic tests (RDTs) and preparation of DBS After obtaining written consent (and assent, if applicable) from each participant, blood samples from finger prick will be collected by a trained BHW/midwife, followed by on-site immediate testing for malaria by RDTs (the Abbott Bioline Ag Pf/Pan kit or the FalciVax from Tulip Diagnostics, depending on availability). All RDT-positive individuals at the time of the survey will be treated by trained local health workers following the Philippines' national treatment guidelines for malaria. From the same finger prick, 3 blood spots (~20 µl each) will be prepared on filter paper. When the spots are completely air-dried, they will be kept individually in a primary container (resealable bag with desiccant) and placed inside a secondary container (conical tube/canister with screw cap) before storage in the refrigerator (2 to 8°C) in the study site. They will be transported at ambient temperature every two months to RITM, and will be stored in the freezer (-20°C to -70°C or lower for long-term storage) for later detection of low-density *P. vivax* infections by PCR and for serological assessment. For children 1–2 years old, blood sampling may be by finger or heel puncture.

In 1 or 2 identified barangay that have access to required facilities (a refrigerator and centrifuge e.g. RHU

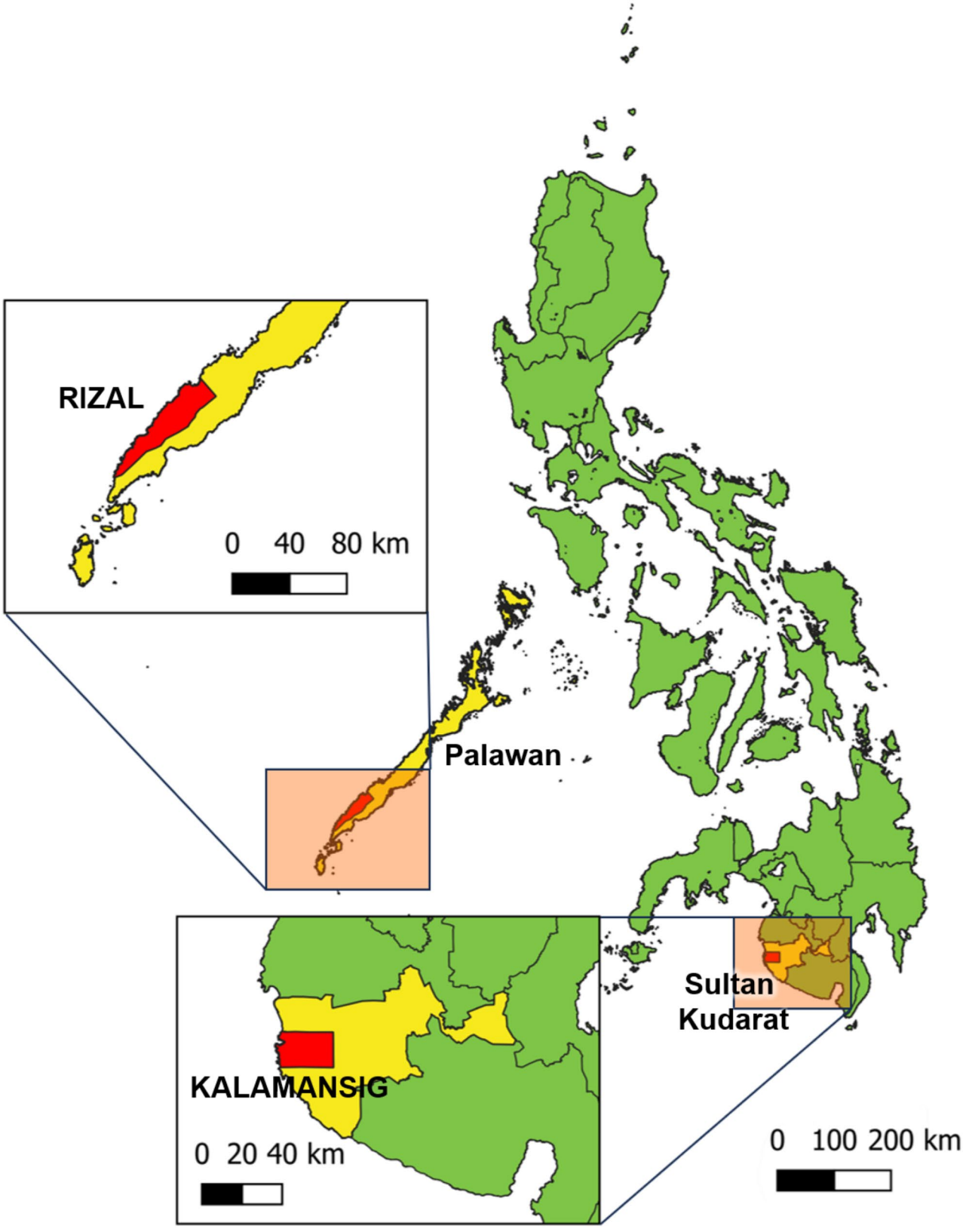

Fig. 1 Location of the main two *P. vivax* study sites in Palawan (endemic) and Sultan Kudarat (unstable transmission). Rizal in Palawan has been chosen due to ongoing endemic transmission and availability of archival samples. Kalamansig in Sultan Kudarat was selected for the prospective health-facility based surveys due to the recent *P. vivax* outbreaks described in Table 1. Other study sites (Occidental Mindoro and Bataan) not shown will provide archival samples as negative controls

or nearby district hospital), additional ~ 500μL of whole blood will be collected from 50 individuals at one time point only, to allow matched analysis with DBS samples for the quantification of antibodies to validate the protocol using different sample types. The additional blood will be collected from the same finger prick into a small (microtainer) EDTA tube, which will be immediately refrigerated (maximum 8 h) until it is separated into packed red cells and plasma, and subsequently stored frozen (-20ºC to -70ºC or lower) prior to testing. This data will be combined with other samples collected outside this protocol, from clinical *P. vivax* cases, to ensure a range of antibody levels.

Interview After enrolment to the study, all participants will be interviewed by trained staff (e.g., local HCWs) to complete a standard tablet computer-based questionnaire using the Open Data Kit (ODK) electronic data collection software. In case of minors aged 1–11 years old, the parent or guardian will serve as the respondent. The data to be collected will include any past malaria infections, and history of illness and travel. Information on recent reported travel will facilitate analysing the potential impact of imported malaria on the study results. Both the tablet questionnaire and the ICF are available in English and Tagalog, and the trained technical staff will interview the participants to enter data into the questionnaire. They will ask the questions in Tagalog (or will be assisted by a local HCW if participants speak a different language). The participant's axillary temperature will also be recorded.

Geolocating households Health facility-based surveys will be combined with remote geolocation of households, to enable mapping of the potential hidden *P. vivax* reservoir. This will be performed using satellite imagery with known landmarks and spatial coordinates that allows zooming in/out and locating the participant's residence, as viewed on tablets by the participants, as previously described [20] and successfully undertaken in the Philippines [22]. In case of poor internet connectivity in the area during the survey, a generated offline map can be imported in the ODK application.

Data processing and management Each participant will be assigned a unique identification number in the form of a barcode sticker. Only one participant will be assisted at a time by the trained BHW or midwife to ensure accuracy. The trained BHW and midwife will carry out these procedures at the HF. In the RHU, where patient consultations are more frequent, the project coordinator may assist. The project coordinator will also conduct daily quality control checks and inventory assessments of forms and samples during each collection period. During each collection period, at least one member of the research team from RITM will visit the study site to oversee the HF survey and address any challenges encountered, under the guidance of the PI. This proactive approach aims to swiftly resolve any issues that may arise. Subsequently, the data collected will be reviewed for completeness and inconsistencies by the designated data manager at RITM. A summary report will be generated, and if any data verification or correction is necessary, the project coordinator will be tasked with addressing these matters. This meticulous process ensures the accuracy and integrity of the data collected throughout the project.

PCR To detect and confirm infection, PCR will be performed at the RITM on all samples collected from the HF surveys in Sultan Kudarat, as previously described [22]. Briefly, a nested PCR assay targeting the *Plasmodium* small subunit ribosomal RNA genes will first identify positive samples, followed by species-specific PCR (for the 5 species found in humans) on any genus-positive samples [26–28]. High throughput pooling of samples will be considered as a modification to the protocol. In the DNA extraction procedure, pooling will be done by groups of 10 blood samples (from 10 individuals). Each pool that gives a positive result in the first-round genus assay will be re-analyzed by extracting and testing all samples individually for a second-round species-specific assay.

P. vivax serology assay (*aim 2*)

The study will apply validated *P. vivax* serological exposure markers (at least 8 *P. vivax* proteins) that detect polyclonal antibodies in human blood samples using a multiplexed bead-based assay as previously described [13]. Briefly, this assay uses the Luminex xMAP technology with the scaffold being unique internally colored magnetic microspheres to which we couple the *P. vivax* proteins. This enables measurements of total IgG antibody responses against all *P. vivax* proteins included in the multiplex, i.e., in one well using 1 μl plasma, reducing requirements for large volumes of sample. The 96-well assay plates are run on a MAGPIX or Luminex-200 instrument (both generate essentially the same readings when the same beads are used [29, 30]). The raw assay data, mean fluorescent intensity (MFI), will be converted to relative antibody units (RAU) based on the standard curve generated with a hyperimmune plasma pool. The RAU data will then be used to classify each individual sample as recently exposed to *P. vivax* in the prior 9-months or not using our validated random forest algorithm [13], trained on prior data from low-transmission settings [13, 31, 32].

The blood samples that will be analyzed for the serological assay are:

a. DBS that will be collected in HF-based surveys in Sultan Kudarat (maximum $n = 6000$), regardless of the parasite status as determined by blood-stage diagnostic methods (serological markers are predicted to uncover hidden *P. vivax* infections).
b. Archived DBS ($n \sim 4000$) from previous surveys in Rizal, Palawan, collected in 2016–2018 (RITM-IRB 2016-04) [22], a municipality and province with stable *P. vivax* transmission, to allow comparison between areas of varying endemicity. About 841 of these samples had *Plasmodium* parasites detected by PCR, with 12% of those samples positive for *P. vivax*. In case the DBS samples from this old study are insufficient for the serological assays and analysis, DBS collected from 2021 to 2023 in Rizal (RITM-IRB 2019-03) and Puerto Princesa (RITM-IRB 2022-01) for other studies may be used.
c. Archived DBS from previous surveys in Occidental Mindoro ($n \sim 1501$) and Bataan ($n \sim 559$) [22] collected in 2017–2018, (RITM IRB 2016-04), areas with no reported *P. vivax* transmission in recent years, as negative control populations. The known epidemiology of *P. vivax* in Bataan and Occidental Mindoro was based on microscopy (as reported by the Department of Health) and confirmed by PCR [22].

DBS samples from Palawan, Occidental Mindoro and Bataan are currently stored at -20ºC in the RITM. Storage of DBS at -20ºC is considered optimal for antibody measurements [33, 34]. For Palawan, the additional province with ongoing *P. vivax* transmission, the sample sizes were pre-determined given they are archival, however; a sample size of 4000 will ensure we have a 90% probability of estimating seroprevalence with a precision of 3.1% at the 2-sided 95% confidence level.

Epidemiological analysis of the *P. vivax* reservoir (*aim 3*)

As no *P. vivax* serological data will be available in the first year, preliminary development of the epidemiological framework to support decision making will commence using existing *P. falciparum* serology data [30] generated in the archival samples from Palawan (stable transmission), Occidental Mindoro (historical transmission) and Bataan (no transmission) [22]. This data was also be generated using the Luminex platform thus is appropriate for use in methods development. Once *P. vivax* serology data is available from the archival samples, the first phase of data analysis will aim to check whether observed trends in the serological data are consistent with what is expected based on the known epidemiology in the region. Areas or households at risk for residual transmission and/or future *P. vivax* outbreaks will be assessed, and any associated risk factors will be defined for both exposure and infection (using mixed effects logistic regression models). Zero-inflated model forms will be fit if warranted. Covariables will consist of the survey round to account for seasonality, sex, age, use of vector control, malaria history and others being collected by questionnaire. Other key focus will include assessments for any potential cross-reactivity with other *Plasmodium* species present in the population, antibody levels according to age and expected degree of past exposure (differentiation for present and past infection), and any spatial trends. For potential cross-reactivity, we will assess for any statistical associations between *P. vivax* serology and detected *P. falciparum* infections, and if needed, we will adjust algorithms to use only *P. vivax* antigens with low levels of sequence similarity with their *P. falciparum* ortholog, as we have done previously for *P. knowlesi* [35]. Geostatistical analysis will be applied to the final dataset (archival and new) to map the residual *P. vivax* reservoir and assess how it evolves over time using the geolocated households from study participants. The spatial analysis will employ methods appropriate for low transmission settings. The participant household location, obtained during the questionnaire at the health facility, and validated by Fornace et al. [20], for use in the Philippines will be the primary unit of analysis. Potential covariates will be derived from satellite-imagery at a 250 m resolution and include topographic measures, distance to landcover types, forest cover, population density, and climatic variables. The spatial effects will be modeled as a Matérn covariance function using the stochastic partial differential equation (SPDE) approach in Integrated Nested Laplace Approximation (R-INLA), as described by Byrne et al. [36]. Predictive modelling will be applied to determine how well the serological markers predict areas at risk for outbreaks by combining the generated data with any outbreaks according to the passive surveillance system during the study period.

Data generated from Aims 1 and 2, routinely reported malaria data, and key informant interviews of health facility staff will be used to adapt existing Freedom from Infection (FFI) models [23] to the context of *P. vivax*. These models have been developed for the context of *P. falciparum* and briefly, involve estimating the sensitivity of the surveillance system for detecting malaria if it exists in the community and the corresponding probability of having achieved malaria elimination with known uncertainty [23]. The model [23, 37, 38] uses a Bayesian joint-inferential framework that simultaneously estimates the observed malaria transmission based on the data available and the potential for unobserved transmission. Integrating the data from novel *P. vivax* serological markers that identify potential hypnozoite carriers into the FFI framework will ensure that the risk of residual transmission can be accounted for when confirming the absence

of *P. vivax* transmission. Overall, the results of the FFI analysis will inform areas for programmatic action to support malaria surveillance for confirming elimination based on factors driving the sensitivity of the malaria surveillance system and the corresponding facilities according to their probability of having achieved elimination.

We intend to have two classes of primary endpoints, depending on the results obtained. Firstly, the primary endpoint of interest to assess presence of infection/hypnozoites, if detected, is *P. vivax* seroprevalence derived according to the classification from the random forest algorithm [13]. Secondary endpoints to detect infection will be PCR and RDT prevalence as described above. However, if results suggest absence of transmission, the FFI outputs (described above) will be considered as the primary endpoints, specifically, the sensitivity of the surveillance system to detect infections if they exist at a threshold of 1/10,000 people and the corresponding probability of freedom from infection [37].

Discussion

P. vivax remains a challenge for low-transmission and pre-elimination settings due to the potentially hidden hypnozoite reservoir. *P. vivax* hypnozoites, that cause relapse of blood-stage infection, can contribute towards residual transmission within communities and potentially result in outbreaks as observed in Sultan Kudarat in 2020 and 2021. The protocol presented here aims to determine whether there is an underlying undetected burden of *P. vivax* in Sultan Kudarat that may have contributed to these outbreaks, through application of novel *P. vivax* serological exposure markers [39]. The protocol utilises innovative approaches to collect data in an elimination setting in a robust and cost-effective manner through health facility-based surveys and remote geolocation of households. Importantly, we will recruit not only patients seeking treatment but also their non-care seeking companions– adding important information that would not be detected if the survey focused on febrile patients only. The use of rolling health-facility surveys every 2 months will also enable adjustment for any seasonality in malaria infection/exposure [22] and variation in abundance of *Anopheles* mosquito vectors [40]. Taken together, this project will enable a much-needed assessment of how and why *P. vivax* outbreaks continue to occur. The project will provide results directly to the Department of Health on the extent of the hidden *P. vivax* reservoir, which will inform the most appropriate surveillance and intervention strategies that would be recommended to target *P. vivax* in the Philippines and the wider Asia-Pacific region.

Novel aspects of this study protocol are inclusion of the *P. vivax* serological exposure markers as surveillance tools and translating the data to a programmatic decision-making framework [41, 42]. *P. vivax* serological exposure markers are the first tool that can be used to indirectly detect hypnozoite carriers, and match the WHO preferred product characteristics (PPCs) for a test that can detect risk of relapse [42]. Data from the study protocol described here will provide the first evidence of the utility of a lab-based *P. vivax* serological exposure marker assay for the use-case 2 described in the PPCs, population-based screening to identify recent *P. vivax* infections/hypnozoite carriage for programmatic applications, in a near elimination setting. Importantly, the protocol described here also progresses to the next step - using this data to inform and adapt local elimination strategies through development of a novel decision-making framework. Such a framework could inform programs of specific factors that could be targeted to strengthen malaria surveillance and translate the available surveillance data into an actionable approach for achieving and sustaining malaria elimination.

One limitation or risk associated with the study protocol is the necessity for primary data collection in an area that rarely experiences isolated and sporadic local political and social conflicts. To mitigate this challenge, the research team will work with, and train local staff in Kalamansig on the data and sample collection procedures to limit any possible interruptions due to travel restrictions or potential security risks to the study team, and to further capacity building and maximize integration into routine surveillance. Study investigators in the Philippines will travel routinely to Kalamansig to continue relationship building and study oversight, whilst study investigators from Australia and the USA will have annual meetings with the Philippines investigators in another location that has no travel advisory warnings such as Davao City, Palawan or Manila. An additional risk related to the primary data collection is travel restrictions related to any surge in local COVID-19 cases. To potentially enable the HF-based surveys to continue even amidst a surge, COVID-19 antigens [43] could be included into the multiplex serology panel if requested by the Department of Health.

An additional limitation of our study is that whilst sensitivity and specificity of the *P. vivax* serological markers is expected to remain constant despite changes in actual prevalence, the positive predictive value will decrease with decreasing transmission [44]. For example, with 80% sensitivity and 80% specificity, and a true disease prevalence of 3%, the positive predictive value (PPV) is 11%, meaning there would be a high rate of false positives. Therefore, any positive serological results will be interpreted with this low PPV in mind, and in context of the PCR results, and will be used to look at potential risk of ongoing transmission at a small-spatial scale in local areas (i.e. any clustering of seropositivity). Despite

the lower PPV, serological tests are particularly useful in confirming absence of transmission or infections in a population. Whereas the PPV is low, the negative predictive value (NPV) of this serological tool is 99.2%, assuming a 3% seroprevalence, or 99.7% at 1% seroprevalence. Additionally, the absence of serological signatures have a distinct advantage as they provide temporal insight regarding exposure over a larger period of time (e.g. previous 9 months), as compared to infection metrics such as PCR which reflect experience over the past 4–6 weeks. This historical information providing a time-series of absence in a single sample is particularly salient to improve confidence in absence of transmission as has been recently described [37].

Overall, successful completion of the study described here in this protocol will enable new insights into a novel surveillance tool for *P. vivax* malaria, along with generation of a new decision-making framework to support the Philippines malaria elimination strategy. This framework will be data driven and thus informed in part by our study results, however will build upon the FFI framework developed for *P. falciparum. P. vivax* is more complex, given the inability to detect individuals with the hidden hypnozoite burden, and thus incorporate this measure (by serology) into these models will provide information to the health system that they cannot currently access through detection of incident cases of clinical malaria. This project therefore has the capacity to show proof-of-concept for this integrated public health approach to support utilisation in further *P. vivax*-endemic countries throughout the Asia-Pacific, and in other global hot-spots such as the Americas, the horn of Africa and Madagascar.

Abbreviations

BHW/HCW	Barangay health worker / health care work
CRF	Case report form
DBS	Dried blood spots
G6PD	Glucose-6-phosphate dehydrogenase
HF	Health facility
ICFs	Informed consent Forms
ODK	Open data kit
PI	Principal investigator
RAU	Relative antibody units
RDTs	Rapid diagnostic tests
RHU	Rural health unit
RITM	Research institute for tropical medicine

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Author contributions

JB MM JL GS and RJL led writing of the manuscript. JB and MM prepared figure 1. FE, JL, GS and RJL prepared Table 1. JB MM KC BB IM FE JL GS and RJL contributed to the writing of the study protocol and reviewed the manuscript.

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Data availability

Data is provided within the manuscript.

Declarations

Ethics approval and consent to participate

All study documents (research protocol, information sheets and written informed consent forms) have been submitted to RITM (Philippines, approved: #2023-17), USF (USA, approved: #STUDY005669) and WEHI (Australia, approved: #HREC23/24) IRBs for ethical approval following local guidelines. Investigators will use the latest Ethics Committee approved version of these documents at all times. The stored blood samples (DBS, EDTA-preserved whole blood, plasma) and datasets that will be collected from this study may be used in the future for new and related research such as surveillance and diagnosis of other diseases of public health importance in the Philippines, and for training of RITM staff on molecular and/or serological assays. A new protocol will be written and IRB approval will be obtained for the use of the samples and/or datasets for the new and related research. Participants will be allowed to withdraw their consent/assent for the storage of their blood samples at any time.

Consent for publication

Not applicable.

Competing interests

IM and RJL are named inventors on a patent describing P. vivax serological exposure markers (PCT/US17/67926). All other authors declare that they have no competing interests.

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