UNIVERSITAT DE BARCELONA

Investigating malaria transmission and assessing control activities in preparation for a malaria elimination strategy in the Lihir Islands of Papua New Guinea

Pere Millat Martínez

TESI DOCTORAL

Investigating malaria transmission and assessing control activities in preparation for a malaria elimination strategy in the Lihir Islands of Papua New Guinea

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Investigating malaria transmission and assessing control activities in preparation for a malaria elimination strategy in the Lihir Islands of Papua New Guinea.

Recerca sobre la transmissió de la malària i avaluació d'activitats de control en preparació per a una estratègia d'eliminació a les illes de Lihir de Papua Nova Guinea.

Memòria de tesi doctoral presentada per **Pere Millat Martínez** per optar al grau de doctor per la Universitat de Barcelona.

Dirigida per: **Prof. Quique Bassat** (Institut de Salut Global de Barcelona-ISGlobal, Hospital Clínic - Universitat de Barcelona, Barcelona) i **Dr. Oriol Mitjà Villar** (Fundació Lluita contra les infeccions- Hospital Germans Trias i Pujol, Badalona; Universitat de Barcelona, Barcelona).

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Barcelona, Setembre de 2023.

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Signed on the 20th of September of 2023.

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They declare that they consent that the thesis may be submitted to procedures to verify its originality.

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Dr. Oriol Mitjà Villar

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Pere Millat Martínez

Candidate.

In the Lihir Islands, I found my second home for five years. I would like to dedicate this doctoral dissertation to the land, our house, the wild bush, the volcanoes, the smell, the blue sea, and the red sunset; as well as to all the people living in Lihir whom I met and made me realize of all the beauty and the bloodshed that exist in our world, the one that we can strive to make better.

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Pere Millat Martínez.

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1. ABBREVIATIONS AND ACRONYMS

ACT: artemisinin-based combination therapies

aOR: adjusted Odds Ratio

APLMA: Asia Pacific Leaders Malaria Alliance

CI: confidence interval

DHA: dihydroartemisinin

DHA-PQP: dihydroartemisinin–piperaquine

ECG: electrocardiography

EIR: entomologic inoculation rate

EMA: European Medicine Agency

FDA: Food and Drug Administration, USA

G6PD: glucose-6-phosphate dehydrogenase

IRS: indoor residual spraying

ISGlobal: Barcelona Institute for Global Health

ITN: insecticide-treated bed nets

LLG: Local Level Government

LLIN: Long Lasting Insecticidal Net

LMEP: Lihir Malaria Elimination Programme

malERA: malaria eradication research agenda

MDA: Mass drug administration

MIZ: Mine-impacted zone

ml: millilitre

MMV: Medicines for Malaria Venture

ms: milliseconds

ng: nanograms

PCR: polymerase chain reaction

PD: pharmacodynamic

PK: pharmacokinetic

PNG: Papua New Guinea

PNG-IMR: Papua New Guinea Institute of Medical Research

PQP: piperaquine phosphate

QTcF: QT interval corrected by Fridericia's formula

RDT: Rapid Diagnostic Test

SAE: Serious Adverse Event

SERCaP: Single Encounter Radical Cure and Prophylaxis

WHO: World Health Organization

2. LIST OF ARTICLES IN THE DOCTORAL THESIS

This doctoral thesis is presented as a compendium of publications.

The thesis consists of four objectives and five articles:

1. First article:

   Authors: **Millat-Martínez P***[#], Katusele M*, Baro B*, Kasian B, Jamea E, Lorry L, Casellas A, Ouchi D, Wali C, Raulo S, Elizah A, Omera E, Kaman P, Dau A, Sakur M, Kilepak L, Yabu S, Koata N, Kave J, Toa M, Urakusie C, Kongs C, Kisba F, Laman M, Mitjà O, Pomat W, Karl S, Bassat Q. *Shared first authorship. [#]Corresponding author.

   "Human and entomological determinants of malaria transmission in the Lihir Islands of Papua New Guinea: a cross-sectional study" Circulated to co-authors, to be submitted to PLoS Neglected Tropical Diseases (IF imputed for 2023: 4.78; Q1 under category Medicine-Infectious Diseases).

2. Second article:

   Authors: **Millat-Martínez P*[#],** Gabong R*, Balanza N, Luana S, Sanz S, Raulo S, Elizah E, Wali C, Paivu B, Dalmas J, Tabie S, Karl S, Laman M, Pomat W, Mitjà O, Baro B, Bassat Q. *Shared first authorship. [#]Corresponding author.

   "Coverage, determinants of use and repurposing of long-lasting insecticidal nets two years after a mass distribution in Lihir Islands, Papua New Guinea: a cross-sectional study"**.** Published: Malaria Journal 2021 Aug 4;20(1):336. doi: 10.1186/s12936-021-03867-z. (IF 2021: 3.4; Q1 under category Medicine-Infectious Diseases).

3. Third article:

   Authors: **Millat-Martínez P***[#], Ila R*, Laman M, Robinson L, Karunajeewa H, Abel H, Pulai K, Sanz S, Manning L, Moore B, Bassat Q, Mitjà O. *Shared first authorship. [#]Corresponding author.

   "Electrocardiographic Safety of Repeated Monthly Dihydroartemisinin-Piperaquine as a Candidate for Mass Drug Administration"**.** Published: Antimicrobial Agents and Chemotherapy. 2018 Nov 26;62(12). pii: e01153-18.

doi: 10.1128/AAC.01153-18. (IF 2018: 4.8; Q1 under category Medicine-Infectious Diseases).

4. Fourth article:

   Authors: **Millat-Martínez P*[#],** Salman S*, Moore BR, Baro B, Page-Sharp M, Batty KT, Robinson LJ, Pomat W, Karunajeewa H, Laman M, Manning L, Mitjà O, Bassat Q. *Shared first authorship, [#]Corresponding author.

   "Piperaquine pharmacokinetic and pharmacodynamic profiles in healthy volunteers of Papua New Guinea after administration of three-monthly doses of dihydroartemisinin-piperaquine". Published: Antimicrobial Agents and Chemotherapy 2022 Aug 16;66(8):e0018522. doi: 10.1128/aac.00185-22. (IF 2022: 5.9; Q1 under category Medicine-Infectious Diseases).

5. Fifth article**:**

   Authors: **Millat-Martínez P***, Baro B*[#], Kasian B, Jamea E, Lorry L, Casellas A, Ouchi D, Wali C, Raulo S, Elizah A, Omera E, Kaman P, Dau A, Sakur M, Kilepak L, Yabu S, Koata N, Kave J, Toa M, Urakusie C, Kongs C, Kisba F, Laman M, Mitjà O, Pomat W, Karl S, Bassat Q. *Shared first authorship. [#]Corresponding author.

   "A cross-sectional study to ascertain malaria prevalence among asymptomatic travellers arriving on the Lihir Group of Islands, Papua New Guinea: implications for elimination efforts". Submitted to the Malaria Journal, submission ID: 5b8e0655-764a-41f6-82d8-c3fcc6405601 (IF imputed for 2023: 3.0; Q1 under category Medicine-Infectious Diseases).

3. SUMMARY (ENGLISH)

Investigating malaria transmission and assessing control activities in preparation for a malaria elimination strategy in the Lihir Islands of Papua New Guinea

Introduction:

Papua New Guinea (PNG) has the highest malaria transmission rates in the Pacific region. The Lihir Islands of PNG comprise the islands of Aniolam, where a gold mine is located, Masahet, Mahur and Malie. The primary vector control across Lihir is based on Long-Lasting Insecticidal-treated Nets (LLINs). Additionally, puddles' drainage and larviciding are deployed in the Mine-impacted Zone (MIZ). In 2015, a program to test a malaria elimination strategy in Lihir was proposed.

Elimination programs use human and entomological metrics to identify the parasite reservoir and strategically target interventions for halting transmission and preventing re-establishment. Achieving universal coverage with LLINs is recommended, although vector's behavioural changes and suboptimal use of LLINs undermine its effectiveness. Hence, vector control alone presents significant limitations to achieve malaria elimination. Mass drug administration (MDA) with repeated courses of dihydroartemisinin–piperaquine (DHA-PQP) has been proposed as an additional strategy to effectively interrupt malaria transmission. However, prolongation of the QT segment caused by the piperaquine cumulative effect could lead to serious side effects.

Hypotheses:

1. Malaria transmission is high in Lihir, and distributing LLIN every three years is not sufficient to protect the population. Malaria prevalence in travellers arriving on Lihir is also high, hence there is a moderate-to-high risk of re-establishment if malaria is eliminated.

2. The administration of DHA-PQP for three consecutive months does not pose a cardiac risk to people, making it suitable for MDA.

Objectives:

1. To determine factors driving malaria transmission in Lihir.

2. To determine the effectiveness of LLIN mass distribution campaigns.

3. To determine the cardiac safety of a monthly therapeutic dose of DHA-PQP for three consecutive months.

4. To determine the risk of malaria importation into the Lihir Islands.

Methods:

In the first and forth objective, parasite prevalence was determined by rapid diagnostic test (RDT), microscopy and quantitative polymerase chain reaction (qPCR) through two cross-sectional studies targeting the local and mobile population, respectively. To complete the study of malaria transmission, incidence was determined using passive case detection data. Moreover, *Anopheles* mosquitoes were collected through human landing catches and analysed to characterize species, density and behaviours. For the second objective, coverage, determinants of use and repurposing of LLIN was determined through a cross-sectional survey implemented two years after distribution. For the third objective, we tested a 3-day course of DHA-PQP in healthy individuals for three consecutive months, using regular electrocardiograph readings and PQP determinations. The difference in QTcF prolongation between courses was determined, a pharmacokinetic-pharmacodynamic model using non-linear mixed effects was developed, and 20,000 hypothetical individuals were simulated.

Results:

Malaria prevalence in Lihir was 3.6% by RDT, 4.5% by microscopy, and 15.0% by qPCR. Positive qPCR samples reported 37.1% of *P. vivax*, 34.6% of *P. falciparum*, 24.5% of mixed, 3.0% of *P. malariae*, and 0.2% of *P. ovale* infections. Malaria infection by microscopy and qPCR varied by geographic region ($p<0.001$). The strongest risk factor for infection was living in the non-MIZ of Aniolam [aOR=3.56 (95%CI: 2.72-4.65)]. Other independent risk factors were male sex, younger age, living with a malaria positive individual, and living in a traditional type of house.

Malaria incidence in Lihir was 345 cases per 1,000 inhabitants. Malie, Mahur, and the non-MIZ of Aniolam were the most affected zones. Most malaria cases (53.6%) occurred in children.

An. farauti was the predominant vector in Aniolam-MIZ (94.2%), in Malie (100%) and in Masahet (100%), and showed an early peak-biting time. Biting frequency differed across areas ($p<0.001$), being higher in Malie. In Aniolam non-MIZ, *An. punctulatus* was predominant (75.7%), with a peak-biting time at 6-7pm. Entomological inoculation rates ranged from 0.221 in the MIZ to 2.289 in Malie. Masahet Island had the lowest vector density.

From the 2,694 households surveyed, 27.4% owned at least one LLIN, and only 13.6% of the individuals had slept under a LLIN the preceding night. Head of the household knowing that LLIN prevents malaria was the strongest factor associated with ownership [aOR=30.32 (95%CI: 21.25–3.27)] and with sleeping under LLIN [aOR=16.44 (95%CI: 8.29–32.58)].

There were no differences ($p=0.285$) in the QTcF prolongation after DHA-PQP treatment between the third and the first months. QTcF >60 ms from baseline occurred in 3 (4.3%) participants after the first course and in 2 (2.9%) after the third course. No participants exhibited QTcF >500 ms, and no Serious Adverse Events were reported. In the PK-PD model, gender was found to be a significant co-variate for baseline QTcF, and 0.08% of the simulated males and 0.45% of the females showed QTcF >500 ms after three dosing periods.

Malaria prevalence by qPCR was 5% and 17% in travellers arriving by plane and boat, respectively. Risk factors associated with infection were arriving by boat [OR=4.2 (95%CI: 2.45-7.21)], arriving from provinces with high malaria incidence [OR=5.02 (95%CI: 1.80-14.01)], and having been away from Lihir for ≥91 days [OR=4.15 (95%CI: 2.58-6.66)]. Staying at the mine accommodation was associated with decreased risk.

Conclusions:

1. Malaria transmission is high in the Lihir Islands, and the malaria burden outside of the MIZ is among the highest in PNG. Hence, strengthening malaria control activities is critical before embarking on elimination strategies.

2. The main vector control strategy deployed in Lihir is insufficient to effectively protect the population from malaria infections. Mass distribution of LLINs every three years is not sufficient to maintain adequate coverage and usage, especially if it is not accompanied by strong educational campaigns.

3. Even if LLIN coverage and usage were high and maintained over time, it should be combined with other strategies to halt malaria transmission in Lihir.

4. If MDA is considered as part of a strategy to accelerate progress towards elimination, the administration of three consecutive monthly courses of DHA-PQP does not pose a cardiac risk to the residents of Lihir. However, the implementation of a Pharmacovigilance system is recommended.

5. Importation of malaria infections into Lihir is significant, especially among travellers arriving by boat. It is advisable to implement a surveillance and response system to prevent malaria re-establishment targeting travellers arriving by boat, with a focus on high-risk groups for importation.

4. RESUM (CATALÀ)

Recerca sobre la transmissió de la malària i avaluació d'activitats de control en preparació per a una estratègia d'eliminació a les illes de Lihir de Papua Nova Guinea.

Introducció:

Papua Nova Guinea (PNG) té les taxes de transmissió de malària més altes del Pacífic. Les illes de Lihir, a PNG, inclouen Aniolam -on hi ha una mina-, Masahet, Mahur i Malie. L'única estratègia de control de malària a Lihir es basa en mosquiteres impregnades amb insecticides (LLIN, en anglès). També es drenen basses i s'apliquen larvicides a la Zona d'Impacte de la Mina (MIZ, en anglès). El 2015 s'inicià un programa per testar estratègies d'eliminació a Lihir.

Els programes d'eliminació utilitzen mètriques humanes i entomològiques per identificar el reservori de paràsits i dissenyar estratègies per aturar la transmissió i prevenir el re-establiment de la malària. Es recomana una cobertura universal amb LLIN, però l'ús inadequat i els canvis de comportament del mosquit limiten la seva efectivitat, així que no serveix per assolir l'eliminació per si mateixa. Per fer-ho, s'ha proposat afegir l'administració massiva de fàrmacs (MDA, en anglès) en cicles consecutius. Dihidroartemisinina-piperaquina (DHA-PQP) en MDA podria interrompre la transmissió de malària, però la prolongació del QT per l'acumulació de piperaquina podria causar efectes secundaris greus.

Hipòtesis:

1. La transmissió de malària és alta a Lihir, i distribuir LLIN cada tres anys no és suficient per protegir la població. La prevalença en els viatgers que arriben a Lihir també és alta, amb risc moderat-alt de re-establiment de malària.

2. L'administració de DHA-PQP durant tres mesos consecutius no comporta risc cardíac.

Objectius:

1. Determinar els factors associats a la transmissió de malària a Lihir.

2. Determinar l'efectivitat de LLIN en distribucions massives.

3. Determinar la seguretat cardíaca d'una dosi terapèutica mensual de DHA-PQP durant tres mesos consecutius.

4. Determinar el risc d'importació de malària a Lihir.

Mètodes:

Pel primer i quart objectiu, vam determinar la prevalença d'infeccions mitjançant tests de diagnòstic ràpid (RDT, en anglès), microscòpia, i reacció en cadena de la polimerasa (qPCR), en dos estudis transversals que incloïen població local i mòbil, respectivament. Vam determinar la incidència mitjançant detecció passiva de casos. També vam caracteritzar espècies d'*Anopheles*, la seva densitat i comportament. Pel segon objectiu, vam determinar la cobertura i l'ús de LLINs mitjançant una enquesta transversal dos anys després de la distribució. Pel tercer objectiu, vam administrar DHA-PQP a individus sans durant tres dies per tres mesos consecutius, i vam fer electrocardiogrames i determinacions de PQP. Vam determinar la diferència en la prolongació de QTcF entre mesos, desenvolupàrem un model farmacocinètic-farmacodinàmic utilitzant efectes mixtes no-lineals, i simulàrem 20.000 individus hipotètics.

Resultats:

La prevalença de la malària a Lihir fou del 3,6% (RDT), del 4,5% (microscòpia), i del 15,0% (qPCR). Les qPCR positives van ser-ho per *P. vivax* (37,1%), *P. falciparum* (34,6%), infeccions mixtes (24,5%), *P. malariae* (3,0%) i *P. ovale* (0,2%). La prevalença per microscòpia i qPCR varià segons zones ($p<0,001$). El factor de risc més associat a infecció fou viure fora de la MIZ d'Aniolam [aOR=3,56 (IC95%: 2,72-4,65)]. Altres factors de risc independents van ser el sexe masculí, l'edat més jove, viure amb una altra persona infectada, i viure en una casa tradicional.

La incidència de malària fou de 345 casos per 1,000 habitants. Les zones més afectades eren Malie, Mahur i Aniolam fora de la MIZ. La majoria de casos (53,6%) eren nens.

An. farauti predominà a la MIZ d'Aniolam (94,2%), a Malie (100%) i a Masahet (100%), i mostrà un pic precoç de picada. La freqüència de picades varià per zones (p<0,001), sent més alta a Malie. A la MIZ d'Aniolam, *An. punctulatus* predominà (75,7%), amb un pic de picada a les 18-19 hores. La taxa d'inoculació entomològica varià de 0,221 a la MIZ fins a 2,289 a Malie. L'illa de Masahet mostrà la densitat de vectors més baixa.

De les 2.694 llars enquestades, el 27,4% tenia almenys una LLIN, i només el 13,6% de les persones havien dormit sota LLIN la nit anterior. Que el/la cap de la llar sabés que les LLINs prevenen malària fou el factor més associat amb tenir mosquitera [aOR=30,32 (IC95%: 21,25-3,27)] i amb l'ús d'aquesta [aOR=16,44 (IC95%: 8,29-32,58)].

No hi van haver diferències (p=0,285) en la prolongació del QTcF entre el tercer i el primer mes de DHA-PQP. S'observaren 3 (4,3%) i 2 (2,9%) increments de QTcF >60ms des del basal, en el primer i en el tercer cicle respectivament. Cap participant va presentar QTcF >500ms, ni cap efecte secundari seriós. En el model farmacocinètic-farmacodinàmic, el gènere resultà significatiu per al QTcF basal, i el 0,08% dels homes simulats i el 0,45% de les dones van mostrar QTcF >500ms després de tres cicles.

La prevalença de malària (qPCR) fou del 5% i del 17% en els viatgers que arribaven en avió i en vaixell, respectivament. Els factors de risc associats a infecció van ser arribar en vaixell [(OR=4,2 (IC95%: 2,45-7,21)], arribar de províncies amb alta incidència [OR=5,02 (IC95%: 1,80-14,01)] i haver estat fora de Lihir ≥91 dies [OR=4,15 (IC95%: 2,58-6,66)]. L'allotjament a les instal·lacions de la mina s'associà a menor risc.

Conclusions:

1. La transmissió de malària és alta a Lihir, i la càrrega d'infecció fora de la MIZ és una de les més altes de PNG. És crític reforçar les activitats de control abans d'emprendre estratègies d'eliminació.

2. Les LLIN, principal estratègia de control a Lihir, són insuficients per a protegir la població de la malària. La seva distribució cada tres anys és insuficient per mantenir una cobertura i un ús adequats si no s'acompanya d'intenses campanyes educatives.

3. Inclús si la cobertura i l'ús de les LLINs fossin adequats i mantinguts en el temps, haurien de combinar-se amb altres estratègies per aturar la transmissió.

4. Si es considera la MDA com a part d'una estratègia cap a l'eliminació, l'administració de tres cicles mensuals consecutius de DHA-PQP no suposaria un risc cardíac per als residents de Lihir. Tot i així, es recomana implementar farmaco-vigilància.

5. La importació de malària a Lihir és significativa, especialment per viatgers que arriben en vaixell. És aconsellable implementar un sistema de vigilància i resposta per prevenir el re-establiment de malària, especialment dirigit a aquests viatgers i enfocat a grups d'alt risc d'importació.

5. INTRODUCTION

5.1. General introduction

Malaria is a serious disease caused by the parasite *Plasmodium.* Clinically, it is known to cause high fevers, chills, strong headaches, and tiredness, with children under the age of 5 being the most vulnerable population. Although causing more than 500,000 deaths per year, mortality can be prevented with early diagnosis and treatment. There are five *Plasmodium* species with potential to infect human population. *P. falciparum* accounts for approximately 80% of all malaria infections and it is the deadliest specie causing close to 90% of all malaria deaths (1). On the other side, *P. vivax* is the most geographically widespread specie and the second largest contributor to malaria's clinical burden (2). *P. ovale*, *P. malariae* and *P. knowlesi* are less extended, with a lower clinical burden compared to the other two species.

Malaria is transmitted by the mosquito *Anopheles* from human to human; except for the zoonotic malaria resulting from *P. knowlesi* infections, where it is only transmitted from animals (macaques) to humans through the same vector. Therefore, vector control strategies are key to prevent infections and reduce transmission. Actually, universal coverage with insecticide-treated bed nets (ITN) or with indoor residual spraying (IRS) is recommended as the main strategy to achieve community-wide protection in malaria endemic areas (3). A total of 41 species of Anophelines have the capability to transmit malaria parasites to human population, with heterogeneous feeding behaviours and geographic distribution patterns (4). In Africa, the main vectors are *An. arabiensis*, *An. gambiae s.s.*, and *An. funestus*; whereas in the Americas, *An. darlingi*, *An. albotarsis*, and *An. pseudopunctipennis* are more commonly found as primary vectors (4). In contrast, in the Asian-Pacific region the vector distribution exhibits a greater diversity with 19 dominant vector species that are highly co-dominant, being *An. culicifacies* complex, *An. fluviatilis* complex, *An. stephensi*, *An. dirus*, *An. minimus*, *An. punctulatus* complex, *An. farauti* complex, and *An. koliensis* the most extended (5).

Globally, there were an estimated 247 million malaria cases and 619,000 deaths in 84 malaria endemic countries in 2021 (6). In 2020, the World Health Organization (WHO) annual report showed an increase of 12% in deaths and 15 million malaria cases compared to the previous year (1). Such a worrying increase was predominantly

attributed to the restrictions imposed by malaria-endemic governments during the COVID-19 pandemic (1). However, even before the pandemic, the progress in the reductions of malaria morbidity and mortality seemed to have stalled (7). Malaria occurs in tropical and subtropical regions of the world, being the poorer areas the most affected. Sub-Saharan Africa is the region with more infections, due to a combination of factors including weather, scarce resources, and the presence of a very efficient vector, the *An. gambiae* complex. Out of Sub-Saharan Africa, the highest global transmission rates can be found in some parts of Oceania such as Papua New Guinea (PNG) (8). Indeed, the WHO Western Pacific Region has seen an important lack of reduction in malaria incidence and mortality rates for the last years, being attributed to the persistence surge of cases and deaths in PNG (6).

Malaria eradication, which entails achieving a permanent reduction of worldwide incidence to zero and creating a world without malaria, stands as a major ambition of global health. Between 2000 and 2015, the global malaria burden experienced a rapid decline, and the global health community believed that regional malaria elimination and the eventual malaria eradication were plausible in the near future. Aiming for the goal of eradication, the 68th World Health Assembly of 2015 endorsed the objective to reduce in 90% the world malaria incidence and mortality by 2030, and to eliminate malaria from at least 35 more countries (9). At the same time, the Asia Pacific Leaders Malaria Alliance (APLMA) agreed to reach at least 90% reduction in the burden of disease in the Asian Pacific region by 2030 (10). Unfortunately, there has been an increase in the global malaria cases and deaths in the recent years, and significant accomplishments from previous decades may be at risk to be halted or even reversed. Aside of the challenges raised during the COVID-19 pandemic, population growth in vulnerable regions of Sub-Saharan Africa, financial standstill, reduced vector control coverage, and continued poor quality of the health systems, contribute to this lack of progress (11). Therefore, in pursuit of the eradication goal, there is growing consensus within the global health community that a shift in focus is needed. This shift involves transitioning from a primarily elimination-oriented approach to a substantial commitment directed at enhancing malaria control efforts in countries with the highest burdens (12). Simultaneously, elimination strategies could be employed in specific settings characterized by lower transmission rates.

5.2. Malaria in the context of elimination

Malaria elimination is defined as the reduction to zero on the incidence of infection with total interruption of local transmission in a particular geographical area. It is the result of a group of deliberate activities aiming to control cases and stop local transmission, plus the deployment of continued measures to prevent re-establishment of transmission. The WHO certifies malaria elimination when a country has zero locally acquired malaria cases for at least three consecutive years. Various countries and the entire WHO-European region have certified malaria elimination in the last decade. Moreover, between 2000 and 2021, the number of countries that reported less than 100 indigenous cases increased from 6 to 27, and in the last 5 years the number of countries with fewer than 10 indigenous cases increased from 20 to 25 (6).

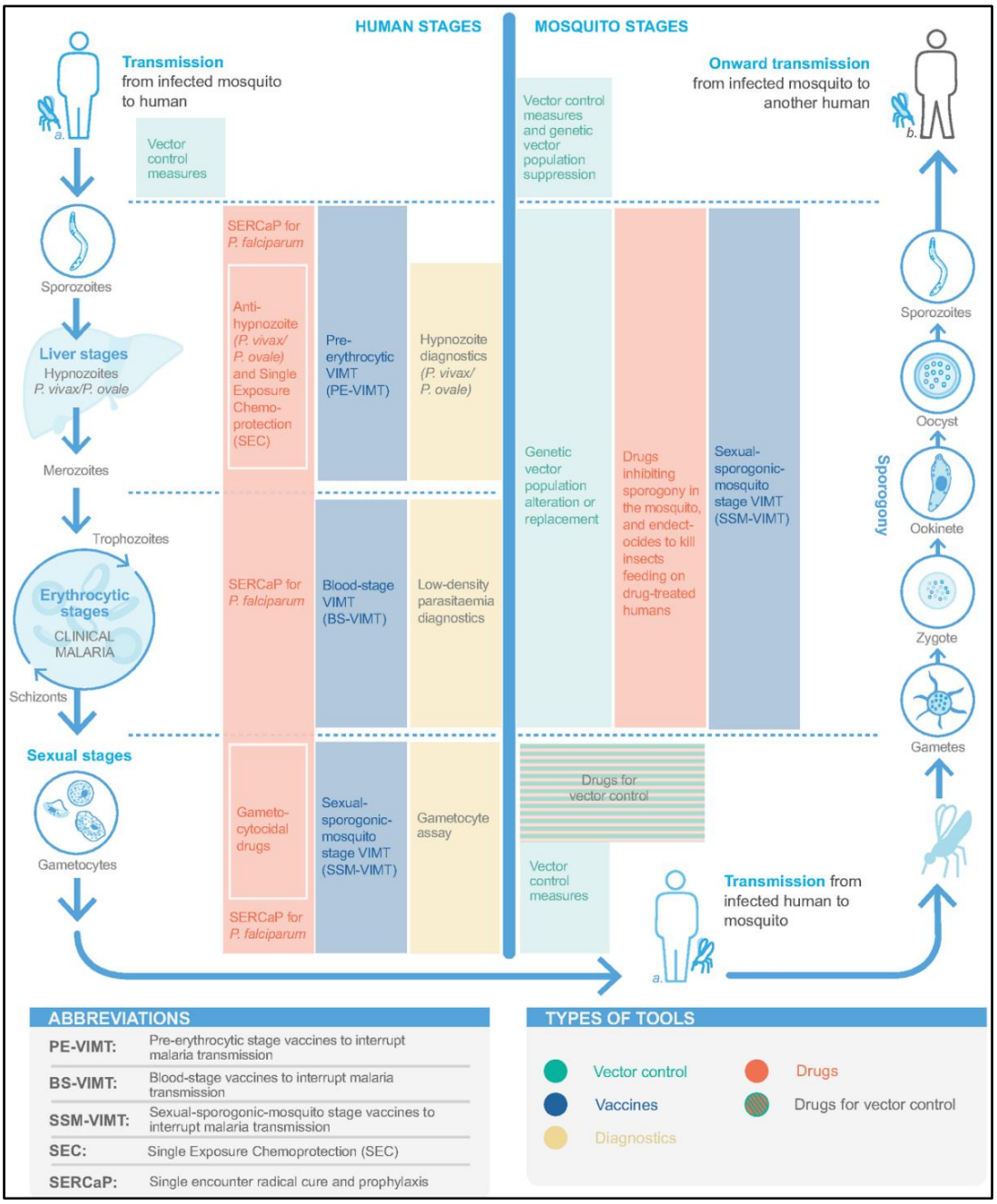
In May 2015, the World Health Assembly endorsed a new Global Technical Strategy for Malaria that includes ambitious goals for malaria control and elimination in the next 15-year period (13). In the same year, the WHO also issued "*A framework for malaria elimination*" providing malaria-endemic countries with a guidance on the tools, activities, and dynamic strategies required to achieve interruption of transmission and to prevent re-establishment of malaria (14). The framework is meant to serve as a basis for national malaria elimination strategic plans and should be adapted to local contexts.

Elimination requires vigorous and proactive multifaceted activities to be put in place, to rapidly decrease the malaria reservoir and eventually interrupt transmission and avoid its re-establishment. The rate of progress depends on the strength of each country's national health system, their level of investment in malaria control and several other factors including biological determinants, the environment, and the social, demographic, political and economic realities of the country.

Although rapid progress can be achieved when increasing access and coverage of the existing malaria control tools, elimination requires a significant amount of innovation and development of new tools. In this respect, the malaria eradication research agenda (malERA), a multi-discipline consultative process (later updated as malERA refresh), has defined and updated the required research agenda that needs to escort any elimination efforts (15, 16). Such an agenda clearly defines knowledge gaps and areas in need of research to support elimination efforts, being key those tools that hinder the

parasite transmission, such as vaccines, drugs and innovative vector control strategies (Figure 1). Unfortunately, most of the proposed tools are still not available nowadays, more than 10 years after the recommended research agenda was launched.

Figure 1. Tools for detecting and interrupting malaria transmission and their action in the malaria transmission cycle. From the malERA Agenda (15).



5.3. Understanding malaria transmission as a tool for planning elimination strategies

Understanding the sources of transmission and the population at risk are of paramount importance to progress towards elimination. Malaria transmission involves the entire process of an infection's life cycle, starting from an infectious mosquito bite on a host, to the subsequent transmission of the resulting infection to a new host. Therefore, both human and entomological transmission metrics are valuable for establishing a baseline measure for elimination programmes, as well as for planning strategies to halt parasite transmission and monitoring their effectiveness.

5.3.1 Detection of malaria infections in human population

Rapid Diagnostic Tests (RDT) and light microscopy techniques are the currently available tests for confirming malaria in febrile patients. The diagnostic sensitivity of microscopy is 50-500 parasites/μL, and RDTs require a minimum diagnostic sensitivity of 200 parasites/μL to be validated (17). The WHO recommends to confirm all the clinically suspected cases with microscopy or with any of the validated RDT tests (18, 19). However, population living in endemic areas can be infected and not showing classical signs of acute infection, what it is known as subclinical infections. Some of these infections can be detected by RDTs or microscopy, but many of them are left out of diagnostic because of their low parasitaemia levels, which are only detectable with highly sensitive diagnostic tools such as molecular amplification methods. Polymerase chain reaction (PCR) can detect twice as many *P. falciparum* infections and between 2 and 5 times as many *P. vivax* infections when compared to microscopy in asymptomatic individuals (20, 21). In addition, gametocytes, the parasites' sexual stage transmissible to the mosquitoes, are more abundant in infected humans of lower age, in those with lower asexual parasite density and in those without fever at presentation (22). Hence, Hence, human subclinical and submicroscopic infections are one of the most relevant factors to address in elimination programs, given they are not targeted by regular control strategies.

In elimination programs, and especially in settings with low transmission rates, it is thus strongly recommended to characterize the spatial and temporal heterogeneity of these infections. This exercise allows the design of elimination programs aimed at defeating

the parasite reservoir (23). The evaluation of malaria prevalence in human populations with molecular tools facilitates the calculation of malaria transmission metrics, such as the proportion of parasitaemic fevers, the parasite or the gametocyte rates (Figure 2). On the other hand, other human metrics indicating malaria transmission and related to the incidence of infection include the number of febrile patients, the number of confirmed cases, the annual blood examination rate, and the RDT positivity rate (Figure 2). These metrics are useful for monitoring symptomatic individuals with a higher risk of transmission, as they typically carry higher parasite loads than afebrile infected individuals

Figure 2. Summary of currently available malaria transmission metrics in humans. From the malERA Refresh Consultative Panel (23).

Metric	Definition [3]	Measure of transmission	Method	Discriminatory power
Annual blood examination rate (ABER)	The number of people receiving a parasitological test for malaria per unit population per year	Level of diagnostic monitoring activity	Microscopy or RDT	• Dependent on health-system provision
Case, confirmed	Malaria case (or infection) in which the parasite has been detected in a diagnostic test	Current transmission or incidence if data collection is repeated or routine	Microscopy or RDT positive	• Insensitive at low transmission; saturates at high transmission • Underestimates due to system inadequacies and poor health-seeking behaviour
Case, fever	The occurrence of fever (current or recent) in a person	Current transmission or incidence if data collection is repeated or routine	Reported or observed fever	• Overestimates malaria infection
Proportion of fevers parasitaemic (PFPf) *	Proportion of fever cases found to be positive for *Plasmodium*	Current transmission or incidence if data collection is repeated or routine	Microscopy; RDT; NAAT	• Depends on diagnostic sensitivity • Insensitive at low transmission
Slide positivity rate (SPR)	Proportion of blood smears found to be positive for *Plasmodium* among all blood smears examined	Current transmission or incidence if data collection is repeated or routine	Microscopy	• Depends on ABER • Insensitive at low transmission
RDT positivity rate (RDT-PR)	Proportion of positive results among all RDTs performed	Current transmission or incidence if data collection is repeated or routine	RDT	• Depends on RDT sensitivity • Insensitive at low transmission
Parasite rate (PR)	Proportion of the population found to carry asexual blood-stage parasites	Current transmission or incidence if data collection is repeated or routine	Microscopy; RDT; NAAT	• Depends on diagnostic sensitivity • Insensitive at low transmission
Gametocyte rate (GR)	Percentage of individuals in a defined population in whom sexual forms of malaria parasites have been detected	Potentially infectious human population	Microscopy; NAAT	• Depends on diagnostic sensitivity • Insensitive at low transmission

*No WHO definition is available for this term.

Abbreviations: ABER, annual blood examination rate; GR, gametocyte rate; NAAT, nucleic acid amplification test; PF*Pf*, proportion of fevers parasitaemic; PR, parasite rate; RDT, rapid diagnostic test; RDT-PR, RDT positivity rate; SPR, slide positivity rate.

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5.3.2 Mosquito density and behaviours driving transmission

Understanding transmission also relies on the fundamental aspects of Anophelines' contribution. It is recommended to characterize the predominant species, their biting behaviours, the mosquito density and the parasite carriage (mosquitoes with infective capacity) (23). Anopheline species show different feeding behaviours, habitats, and ecologies, impeding the generalization of specific vector control strategies, which may

work in some areas and can be less effective in others. For example, *An. gambiae* are very efficient vectors with a usually high biting rate, have preference for humans as a host and a predominantly midnight indoor-feeding behaviour (8). Conversely, *An. farauti* are zoophilic and anthropophilic mosquitoes, have a predominantly outdoor-feeding behaviour and biting peak earlier in the evening (24). In this last case, the effectiveness of ITNs may be reduced, as populations tend to still be working, playing, or socializing during the evening, and with frequency, these activities are conducted outdoors. Specific entomological metrics proposed for malaria control and elimination programs are shown in Figure 3. All of these metrics require mosquitoes' collection through human landing catches or using different kind of traps; and some of the metrics, such as the sporozoite rate, the human biting rate (HBR) and the vectorial capacity, require of examining carriage of sporozoites, usually through molecular tools.

Figure 3. Summary of currently available entomological malaria transmission metrics. From the malERA Refresh Consultative Panel (23).

Metric	Definition [3]	Measure of transmission	Sampling method and resolution	Discriminatory power
Entomological inoculation rate (EIR)	Number of infective bites received per person in a given unit of time, in a human population	Transmission intensity	• Human landing collection; light traps • Resolution: Household or community level	• Insensitive at low transmission • Lack of standardised sampling design • Collected by malaria control programmes
Sporozoite rate (SR)	Percentage of female *Anopheles* mosquitoes with sporozoites in the salivary glands	Risk of infection	• Human landing catch; baited traps; gravid traps • Resolution: Community level	• Insensitive at low transmission
Human biting rate (HBR)	Average number of mosquito bites received by a host in a unit of time, specified according to host and mosquito species	Risk of exposure	• Human landing collection • Resolution: Person or community level	• Allows determination of the primary vector
Vectorial capacity	Rate at which given vector population generates new infections caused by a currently infectious human case	Efficiency of transmission	• Derived from human biting rate, parasite inoculation period, mosquito to human density and mosquito survival • Resolution: Community level	• Measures potential, not actual, rate of transmission—includes no parasitological information • Sensitive to changes in mosquito survival and biting behaviour but may not translate to significant change in human incidence • Can be useful when infection rates are low and mosquito sampling difficult

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5.4. Vector control strategies for elimination programs

Universal coverage with ITNs or IRS constitute the recommended core interventions for all malaria control programs. In the last 3 years, more than 500 million ITNs have been distributed worldwide and undoubtedly contributed in reducing malaria burden. Progress in their distribution have been ongoing, with 233 million ITNs distributed in

2020 and 171 million in 2021 in malaria endemic countries (6). Long-lasting insecticidal nets (LLINs) are nets impregnated with insecticide that can be used for 3-5 years, and have shown a higher cost-effectiveness. Currently, LLINs are the single most important malaria control intervention, responsible for averting approximately 68% of *P. falciparum* cases in Africa (25). Although IRS is a less extended strategy due to its programmatic challenges, it has been successfully used in malaria endemic countries for long time and it has contributed in averting 13% of all malaria cases (3).

Increasing coverage for these two core interventions and maintaining their effectiveness are basic in all elimination programs (26). However, the last decades have seen an important decrease in ITN and LLIN bioefficacy due to *Anopheles* insecticide resistance (25). All currently recommended nets are treated with pyrethroids and the malERA agenda urges to find new insecticides with different modes of action aiming to hinder Anophelines' resistance level (26). For instance, the number of reports on pyrethroid resistance (less than 90% of mosquito mortality after exposure to a discriminating dose) in *An. gambiae* and *An. funestus* have increased over time and it is rare to find sites in Africa where these vectors do not show some resistance (27, 28). *Anopheles* resistance to at least one insecticide class is now widespread and reported in more than two thirds of countries with malaria transmission, and 29 countries have already detected resistance to pyrethroids, organochlorines, carbamates and organophosphates across different sites (6). For these areas, if the population is already covered with LLINs, the WHO recommends to add IRS with an insecticide different than pyrethroid as a second core strategy (3). A recent systematic review showed that the addition of IRS with non-pyrethroid-like insecticide reduces malaria parasite prevalence and may also reduce incidence of infection and prevalence of anaemia (29).

However, despite the efforts made to increase LLIN coverage and overcome insecticide resistance, the low use of LLINs by the human population is one of the most significant threats for the overall effectiveness of this strategy. Some programs have reported coverage decrease over time with heterogeneous maintenance and use of nets, which are sometimes misused or repurposed (30, 31). The main reasons for lack of maintenance and use of LLINs include dislike and discomfort due to heat, along with perceived low mosquito density (32-34). Studies conducted in different regions showed that

repurposing of nets are common, and old nets (and sometimes new nets) are used for fishing, gardening, or fencing (30-32, 35, 36). Therefore, distributing LLINs without ensuring their correct use and maintenance may not be sufficient if malaria elimination is to be achieved. Furthermore, another factor that can diminish the effectiveness of vector control strategies is the mosquito's behavioural adaptation to the core vector control measures, such as changes in biting times, resting locations, and zoophagic rates. For example, in PNG, after the first nationwide mass distribution of LLINs, *An. punctulatus* and *An. farauti* exhibited an earlier shift in the median biting time towards early evening (37). Hence, it is advisable to monitor mosquitoes' behaviours over time and develop vector control strategies aligned with these behavioural changes (26).

Another effective approach in settings undertaking elimination efforts is to target the vector natural environment with the objective of reducing mosquito density. Environmental management or the use of larvicides in water bodies, particularly for aquatic habitats smaller than 1 km², have proven to be successful; although they are extremely resource-intensive (38). Larviciding is a common strategy; however, few malaria programs in endemic settings have used environmental management. The main evidence comes from a century ago in Zambia, where after combining this strategy with other vector control tools and mass population treatment with quinine, malaria incidence and mortality were reduced by 70-95% over a 3-5 years period (39). Although the program was cost-effective at long term, the initial expenditures were exceptionally high. Strategies such as improvement of housing and water and waste management could be of help; however, they are not well studied and further investigations are needed to understand their epidemiological impact (26). Finally, novel vector control measures could supplement the core strategies, such as genetically modified mosquitoes through population suppression (modified mosquitoes which progeny results sterile or dysfunctional once have procreated with the wild type) or through population alteration (modified mosquitoes which their progeny results refractory to malaria parasite infection) (26). These technologies are still under development and the quality and consistency of the procedures to create genetically modified mosquitoes need to be fostered and improved. Moreover, there are still some uncertainties related to their possible ecosystem interactions that should be solved before expanding their use (40).

5.5. Use of drugs for malaria elimination

In the absence of a highly effective malaria vaccine, new strategies that are actively considered for interrupting malaria transmission include the use of antimalarial drugs in large populations. The malERA group of experts recommends the search for a Single Encounter Radical Cure and Prophylaxis (SERCaP) remedy (26, 41), a drug therapy that would effectively eliminate all parasites in the human body and prevent relapse and reinfection for at least 1 month in a single encounter with the human population (see Figure 1). However, the SERCaP concept describes an ideal and theoretical antimalarial drug that is still inexistent: it cures malaria in a single dose, has reliable absorption, and has little variability in pharmacokinetic properties with slow body elimination (prophylaxis effect).

While research is ongoing to find a suitable drug for SERCaP, antimalarial drugs for malaria treatment and prophylaxis have been implemented since the 1900's, even in some elimination programs of the mid-twentieth century (41, 42). Mass drug administration (MDA) consist in the administration of therapeutic and prophylactic antimalarial drugs to a specific population in a designated region within a brief timeframe, regardless of their infectious status. Usually, multiple rounds of MDA are implemented to maximizing its effectiveness in preventing re-infection. The rationale behind MDA is to render the entire human population both, non-susceptible to new infections and incapable of transmitting to the vector, for a total period that exceeds the lifespan of the local Anopheline population. Hence, MDA campaigns would lower malaria prevalence in three ways: by reducing the parasite reservoir in a population, by preventing infection of uninfected people, and by preventing transmission from infected people (41).

Past MDA campaigns with antimalarials have met with mixed success. The largest experience with MDA comes from China, where it was used in more than 30 million subjects treated with chloroquine and sulfadoxine plus piperaquine or primaquine between 1962 and 2000, achieving a reduction from 6.9 million annual cases to 25,000 (43, 44). These campaigns were accompanied with high coverage of vector control strategies and followed by an intensive surveillance and response system with passive and active case detection, strong political commitment, and collaboration with

bordering countries (45). Experience from the South Pacific Island of Aneytum (Vanuatu), demonstrated the efficacy of MDA to completely interrupt parasite transmission with weekly chloroquine plus primaquine, and monthly sulfadoxine-pyrimethamine given over a 9-week period (46). This strategy was also accompanied by the massive distribution and use of ITNs and larviciding. Part of the success of the Aneytum program is explained by the fact that it is an island with less than 1,000 inhabitants, so malaria risk was circumscribed and re-establishment risk was low (41). In contrast, other programs failed to maintain success, like in northern Nigeria, where MDA with chloroquine and pyrimethamine was given to 52,000 individuals between 1967 and 1968, together with intensive IRS. The program achieved a reduction of parasite prevalence from 19% to 1%, followed by a rebound to a prevalence of 24% one year after the MDA (47).

Hence, after MDA, if malaria transmission is not interrupted and importation of malaria is not prevented, malaria prevalence can eventually return to its original level, especially in regions of high endemicity. Furthermore, if parasites are not completely eliminated from the target population, MDA could lead to emergence of resistance due to the selective pressure generated on the parasite. In turn, loss of acquired immunity would result in increasing morbidity and mortality (19). For this reason, MDA is not recommended unless there is a good chance that local elimination will be achieved.

The factors contributing to the success of MDA include a meticulous and intensive planned preparatory phase, effective social and community mobilization (ensuring program ownership within the target communities), robust healthcare infrastructure through strengthening the existent health system, and the concomitant use of effective vector control strategies (41, 48). Additional factors linked to favourable outcomes of this strategy encompass achieving at least 80-90% of MDA coverage, using direct observed treatment during the MDA, employing short-term interventions, using 8-aminoquinolines as one of the drugs, and targeting population living in areas with low risk of re-establishment, such as islands (49).

Since MDA involves treating entire populations regardless of their infectious status, several considerations must be taken into account when selecting the optimal drug. Firstly, MDA should be implemented in consecutive treatment rounds, ideally

administered at intervals determined by the expected duration of the drug's post-treatment prophylaxis. The post-treatment prophylaxis refers to the period during which new malarial infections are suppressed following the administration of a dose. This duration is determined by maintaining the drug concentration above the *in vivo* parasite's minimal inhibitory concentration (50). Longer post-treatment prophylaxis periods render the intervention more feasible and cost-effective. Secondly, ensuring drug safety is of paramount importance when treating large populations, requiring a high threshold for drug toxicity. Drugs with a wide therapeutic range and minimal pharmacokinetic variability offer the greatest potential for safely optimization of dosage (51). Any unforeseeable side effects that can occur, even with the safest drugs, is one of the possible caveats of mass population treatments. Hence, when massively treating healthy individuals for the communal good, it is fundamental to establish adequate pharmaco-surveillance systems to detect even the uncommonest but potentially life-threatening side effects.

5.5.1. Dihydroartemisinin-piperaquine as a candidate for mass drug administration

Artemisinin-based combination therapies (ACTs) have been considered suitable candidates for rapidly eliminating malaria in specific regions where resistance is present (19, 26). Furthermore, Dihydroartemisinin-piperaquine (DHA-PQP) has been postulated as one of the best candidates for MDA in this group of drugs. The short half-life artemisinin-derivative component (DHA) rapidly reduces the parasite load; while the PQP component has a large distribution volume and reduced excretion rate with an estimated prolonged post-treatment prophylaxis effect of 28 days, which could potentially prevent new infections for a period of at least 90 to 120 days if administered during three consecutive monthly courses (52, 53). This period of time would be enough to surpass the lifespan of infective mosquitoes, hence both human and vector population would be theoretically clean of parasites after this period.

The effectiveness of different schedules of MDA with DHA-PQP is well proven: in a meta-analysis of 11 studies using DHA-PQP versus placebo or other antimalarial, monthly DHA-PQP for high-risk populations was associated with an 84% reduction in the incidence of malaria parasitaemia (54); in Zambia, *P. falciparum* prevalence decreased from 31.3% in 2014 to 4.0% in 2016 after 4 rounds of MDA with a largest

short-term effect size in areas of lower transmission (55); a before-after study in Mozambique after two rounds of yearly MDAs over two years showed an 84.7% overall *P. falciparum* reduction in all-age prevalence (56); and in Myanmar, after three monthly consecutive rounds of MDA with DHA-PQP plus single dose of primaquine, *P. falciparum* prevalence decreased in 10.6% at month 3 and in 4.5% at month 10 compared to the control population (57).

DHA-PQP has been shown to be well tolerated and effective in mass treatments and in intermittent preventative therapy for high-risk populations (58). However, one of the most feared undesirable risks of the PQP component, given its cumulative effect, is the potential to prolong the QT interval (59, 60). Prolongation of QT reflects delayed cardiac repolarization which, if severe, may put patients at risk of polymorphic ventricular tachycardia, Torsade de Pointes, and sudden cardiac death. Therefore, the drug manufacturer and the European Medicines Agency (EMA) recommend that repeated treatment courses of DHA-PQP should not be administered within 2 months of initial treatment (61). Thus, it seems that to date there is not enough evidence to recommend the extended use of DHA-PQP in repeated monthly doses, particularly among healthy populations targeted for MDA programs. Previous studies have demonstrated that this QT interval prolongation is mild in most cases and not associated with severe cardiac adverse events. When used repeatedly for treatment of malaria episodes, only 6 patients of 797 receiving DHA-PQP in a multi-site clinical trial had a post-dose QT interval corrected by Fridericia's formula (QTcF) > 500 ms; moreover, there was no evidence of QTcF prolongation being more frequent in retreatment than after a single course (62). In a meta-analysis including close to 4000 patients under repeated courses of DHA-PQP as chemoprevention, this drug was well tolerated and associated with a lower odd of Serious Adverse Events (SAEs) compared to placebo or other antimalarials, and there was not association with the risk of QT prolongation or an increased risk of cardiac events; however, only 56 of the included participants had electrocardiographic (ECG) measurements during the repeated courses (54). In a clinical trial including Ugandan children, DHA-PQP was given monthly since the 6 months until the 24 months of age in one of the trial arms, and the monthly ECG conducted in 19 of the participants (total of 145 occasions) showed all QTc intervals within normal limits (63). On the other hand, very few studies assessing ECG

determinations in individuals receiving consecutive monthly courses of DHA-PQP have been conducted. A systematic review and a Bayesian meta-analysis including close to 200,000 people receiving DHA-PQP in different regimens, showed only one unexplained death that could potentially be linked to the intake of this drug. In this review, risk estimates for people taking the medication were not higher than the baseline risk of sudden cardiac death (64). An opinion piece in The Lancet Infectious Diseases, co-authored by this PhD student, commented the findings of this meta-analysis and its implication for the use in MDA campaigns. This opinion piece can be found in the supplementary material section of this Introduction (see section 5.10.1). As a summary, we consider that just a single unexplained fatality case over the 200,000 people studied, represents an infrequent SAE potentially linked to DHA-PQP. Hence, this drug should be shed of its cardiotoxic reputation, allowing malaria-endemic areas to fully utilize its capabilities.

On top of that, bioavailability of PQP is influenced by food intake, leading to higher blood levels; consequently, it is advisable to administer PQP under fasting conditions of at least 3 hours before and 3 hours after food intake (61), adding complexity to a possible MDA campaign. However, few information is known about the pharmacokinetic (PK) - pharmacodynamic (PD) profile of this drug when given repeatedly. A PK-PD model including blood levels of 256 participants receiving repeating doses of DHA-PQP, showed maximum plasma concentrations at 4 hours post-dosing and a long terminal half-life (21.3 days), prolonging the QT segment by 5 ms per 100 ng/ml plasmatic levels of PQP (65). In this case, the time since the previous meal did not have any additive effect on QT prolongation. However, the cohort was predominantly composed of male participants, who usually have a shorter QT segment at baseline (66). A PK study conducted in pregnant women undertaking monthly courses for intermittent preventive therapy in pregnancy, reported suitable PQP blood concentrations for malaria protection after three rounds of DHA-PQP, even though 9% of the participants had lower concentrations than the targeted (10.3 ng/ml) (67). In this cohort, ECG was performed only for a subset of 33 participants not showing any increase in absolute QTc prolongation with repeated courses, and this data was not included in the PK-PD model. Finally, a linear PK-PD model mimicking the effect of monthly courses of DHA-PQP in 16 healthy volunteers, showed a mean QT interval

prolongation of 4 ms per 100 ng/ml of PQP plasmatic levels (68). This model, with data from 20,000 simulated subjects, showed that monthly MDA in healthy subjects would result in median maximum QT interval prolongations of 18.9 ms. In summary, effectiveness of DHA-PQP is proven when given in repeated monthly courses, and cardiac safety should not be a big concern in MDA campaigns. While keeping this into consideration, a pharmacovigilance system should still be in place during MDA with DHA-PQP, and further evidence of cardiac safety and PK-PD data is therefore warranted.

5.5.2. Mass drug administration campaigns in settings where *Plasmodium vivax* is endemic

In areas where *P. vivax* is endemic, the hypnozoites of the parasite could be the major driver of ongoing transmission (69). Indeed, it has been estimated that 4 out of every 5 *P. vivax* infections in PNG are caused by relapses from the dormant liver hypnozoites, rather than from new infections (70). Moreover, *P. vivax* relapses in tropical areas are common to occur 3-6 weeks apart from the primary infection (71). Theoretically, if the human population is treated with blood-stage drugs during MDA campaigns for at least 3-months, it could also eliminate the circulating *P. vivax* parasites in blood caused by the relapses occurring during this period. Therefore, the same MDA schedule could be equally effective in preventing transmission of *P. vivax* gametocytes to mosquitoes, eliminating the need for liver-stage treatment. However, a study performed in PNG showed high level of *P. vivax* and *P. ovale* relapses in children treated only with blood-stage drugs, predicting that MDA including liver-stage treatment would be a more effective strategy (70). A study conducted in Myanmar, Cambodia, and Lao, showed a major decline of *P. falciparum* prevalence after three consecutive monthly rounds of DHA-PQP and a single low dose of primaquine (with gametocidal action). However, prevalence of *P. vivax* infection rebounded to 5.81% at month 6 post-treatment after having decreased from 9.31% to 0.89% at month 3 (72). Thus, MDA with an ACT alone (or in combination with a single dose of primaquine) is not sufficient to achieve and maintain reduction in *P. vivax* infections. Consequently, other MDA schemes should be studied in *P. vivax* endemic settings.

Aside of its effects in reducing transmission due to its gametocidal action, primaquine is a well-known 8-aminoquinoline that it is used as radical cure for *P. vivax* and *P. ovale* infections. It targets the hypnozoite stages, being efficacious on preventing relapses. The effectiveness of primaquine in preventing relapses has also been demonstrated in MDA programs (48, 49). However, a full course of primaquine requires between 7 and 14 days of treatment, a fact that limits its effectiveness due to the challenges in ensuring an adequate compliance of such a long treatment course. Recently, a new 8-aminoquinoline called tafenoquine, which can be taken as a single-dose treatment, was developed. Although it has not demonstrated to be non-inferior compared to primaquine, tafenoquine showed efficacy for the radical cure of *P. vivax* malaria (73, 74) and it has been recently approved by the USA Food and Drug Administration (FDA) for its use as treatment for dormant forms (75). Tafenoquine could be a much-improved operational alternative to primaquine in an MDA schedule for *P. vivax* endemic settings.

Nevertheless, the main concern in using 8-aminoquinolines in healthy population such as in MDA campaigns is their toxicity potential. Their main side effects are mild gastrointestinal symptoms and moderate to life-threatening haemolysis reactions in those patients with severe glucose-6-phosphate dehydrogenase (G6PD) deficiency (19). The treatment of these haemolysis reactions, if not severe, consists of withdrawal of treatment. This could be easily done with primaquine because its half-life is less than 8 hours; however, it would be highly problematic with tafenoquine, with a half-life of 14 days. Therefore, and although the risk of severe haemolysis in mass administrations has been estimated at 1.8 cases per million (76), a MDA program including an 8-aminoquinoline should not be run without understanding the prevalence of G6PD deficiency in the target population, and performing G6PD deficiency screening for all MDA participants if the prevalence of this anomaly is high in the target population (19, 77).

Furthermore, the correct primaquine dosage for radical cure is still controversial. While the dose of 0.5 mg/kg/day for 14 days seems more suitable to clear the liver-stage parasites (78); the WHO still recommends courses of 0.25 to 0.5 mg/kg/day for 14 days in their most recent guidelines (79). This is likely due to the limited availability of data

and the concern about potential haemolysis in individuals with G6PD deficiency. A recent patient meta-analysis assessed the risk of the first *P. vivax* recurrence between days 7 and 180 after infection, comparing different dosages (80). This analysis highlights the superior efficacy of the higher dose (~7 mg/kg/total), with a recurrence risk as low as 8%. This was significantly lower than the recurrence with the low dose primaquine (~3·5 mg/kg/total) and within the non-treated patients, being 19% and 51% respectively. Of note, the high dose of 7 mg/kg/total would be equivalent to 0.50 mg/kg/day for 14 days. In addition, another systematic review, has shown that using standard doses (either 0.25 mg/kg/day or 0.50 mg/kg/day for 14 days) when G6PD activity was ≥ 30% did not entail an increased risk of haemolysis; which only appeared when doses of 1mg/kg/day were used (81). A recent opinion piece co-authored by this PhD candidate, commented the findings of these two articles and their implication for *P. vivax* radical cure. This opinion piece can be found in the supplementary material section of this Introduction (see section 5.10.2). As a summary, we consider that the findings of these two systematic reviews should reassure the use of primaquine for the radical cure of liver-stage parasites, and the higher dose of 0.5 mg/kg/day for 14 consecutive days can be taken as safe and effective. Even in MDA programs, this higher dose remains effective and safe, provided that G6PD status is assessed in the participants.

5.6. Understanding the risk of malaria re-establishment

Re-establishment of malaria transmission is the detection of at least three indigenous malaria cases of the same species in the same focus for 3 consecutive years, in an area in which transmission had been interrupted (82). Between 2000 and 2021, no country with malaria-free certification suffered re-establishment (6). Following elimination, imported cases of malaria are expected; however, any introduced or indigenous case means local transmission, indicating deficiencies in strategies to prevent re-establishment. Hence, all countries should remain vigilant until global eradication is achieved. According to WHO, it is considered mandatory for all countries to have sufficient vigilance activities in place to uphold their malaria-free status (83, 84). Since malaria elimination often begins in specific regions within a country, this prevention of re-establishment should commence

at subnational level, at the same time that the country achieves the complete interruption of transmission. These activities should be tailored to the particularities of each country and should be planned considering the malariogenic potential of the territory. This malariogenic potential depends of three factors: receptivity, infectivity, and vulnerability. Receptivity is determined by the presence and abundance of *Anopheles spp.*; and infectivity is the possibility that the *Plasmodium* sporogonic cycle may be completed within the existent vector and the local climatologic and geographical conditions present in that area. On the other hand, vulnerability is determined by the number of gametocyte carriers (human population) during the period in which malaria transmission is possible (82, 85). After malaria elimination is achieved, vulnerability is considered by the frequency of influx of infected individuals and/or infective Anopheline mosquitoes into the malaria-free territory. Currently, there is no agreement on the methods to be used in measuring the malariogenic potential (86). Nevertheless, mathematical models considering receptivity, infectivity and different scenarios of vulnerability can predict the highest risk areas and guide towards preventing future outbreaks during and after the elimination phase (87). An example can be seen in the Maremma region of Italy, where malariogenic potential was assessed using a multifactorial model with receptivity data (bionomics, distribution, and abundance of local *Anopheles*, weather-based statistical models for the vectorial seasonal dynamics, and models of the length of the transmission seasons for *P. vivax* and *P. falciparum),* infectivity data (vector competence assessment for *Plasmodium* infection), and vulnerability data (retrospective registers on imported cases) (88).

Imported cases into a territory that is pursuing elimination can reverse the major achievements of the pre-elimination phase. As an example, malaria in Cabo Verde has resurged after interruption of local transmission in two occasions within the last 50 years, due to the number of travellers arriving from the main African continent and carrying malaria parasites transmitting to local mosquito population (89). Hence, after malaria elimination is achieved, vulnerability is considered to be the most determinant factor for threatening the elimination status of a territory (90). Examples of outbreaks of locally transmitted malaria due to initially imported cases can be seen in Trinidad and Tobago (91), Jamaica (92) or Greece (93).

However, migrants and travelers are a common source of imported cases in all settings, not only in malaria-free countries. For example, on Bioko Island, Equatorial Guinea, a prevalence survey revealed a strong correlation between malaria infection and a history of travel to mainland Equatorial Guinea, with an odds ratio (OR) ranging from 3.28 to 4.78 for the risk of infection among individuals who had traveled in the previous month (94). These imported cases may well contribute to the local transmission, even before elimination is achieved. A stochastic model of malaria transmission including prevalence and mobility data of Zanzibar, Tanzania, showed that imported cases were up to 18% and introduced cases (locally transmitted from an imported source) were up to 25% (95). In consequence, targeting these infections are crucial to decrease the local malaria burden. Therefore, it is critical to determine the parasite reservoirs and the risk groups in the surrounding populations and, in especial, in those people arriving to the malaria-free or low endemic setting from a higher endemic area.

In a territory that has eliminated malaria but is surrounded by endemic territories, the influx of travellers exhibiting malaria symptoms can be seen as just the tip of the iceberg in comparison to those arriving with subclinical infections. Hence, understanding the influx of clinical and subclinical infections are crucial, as well as knowing the possibility of arriving travellers with submicroscopic parasitaemia that could transmit to the local Anophelines. Also, in areas where local mosquito is able to transmit *P. vivax*, special attention should be paid to the risk of re-establishment of this specie, as relapses over time (from local human population or from travellers) might develop important outbreaks (96-98).

5.7. Epidemiology of malaria in Papua New Guinea

PNG is the country with the highest malaria prevalence and incidence of the WHO Western Pacific Region. It accounted for 87% of the malaria cases and over 94% of all malaria deaths in this region, and contributed with 1% of all global malaria deaths in 2021 (6). Hence, reducing malaria burden in PNG should be a priority if achieving malaria control and progressing towards elimination is aimed in the Pacific region. There were a total of 651,963 confirmed cases in 2021, and the WHO estimated between 856,000 and 1,652,000 malaria infections for the same year (99). This is equivalent to an average of 86 -166 annual malaria cases per 1,000 population.

Between the years 2000 and 2015, PNG made major strides against malaria, mainly through the massive implementation of core vector control strategies. LLINs have been distributed in PNG since 2004 and mass distribution campaigns targeting almost 100% of the population were initiated in 2009 and repeated every three years. According to a nationwide survey conducted in 2011 during the second mass distribution, 81.8% of households retained at least one LLIN from the previous campaign (100). Overall prevalence decreased from 15.7% in 2009 to 4.8% in 2011 (101). Moreover, a study conducted in selected sites, showed a steep decrease in malaria annual incidence: from 20-115 cases per 1,000 population in 2010, to 1-79 cases per 1,000 in 2014. Although other strategies were implemented during this period (widespread availability of RDTs and ACTs), this effect was mainly attributed to LLIN mass coverage and usage (102).

Despite this success, PNG has experienced a large increase of infections since 2015, between 5% and 25% in number of cases (6). Such worsening in the burden of infections is reflected in the Sector Performance Annual Review reports from the PNG National Department of Health published in 2018 and 2020: there was a total increase of 40.2% malaria cases between 2015 and 2017, and a continuous increase in infections since then (103, 104). Table 1 shows data from WHO estimates in malaria incidence in PNG, with a significant decrease in cases for the period 2001-2015 and a clearly rise in cases after 2015 (105). Interestingly, incidence for 2021 decreased compared to 2020; however, it was higher than in 2019, before the COVID-19 pandemic.

Table 1. World Health Organization estimation on malaria incidence in Papua New Guinea for the period 2001-2021, from the Global Health Observatory (WHO) (99).

Year	Incidence estimates
2021	124.3 [86.0 – 166.1]
2020	150.8 [103.7 – 202.5]
2019	117.9 [82.1 – 156.1]
2018	139.5 [90.0 – 197.7]
2017	135.0 [88.2 – 191.0]
2016	136.0 [92.0 – 186.3]
2015	101.9 [66.7 – 143.8]
2014	190.7 [111.4 – 297.7]
2013	172.2 [94.6 – 271.6]
2012	155.7 [50.8 – 319.3]

2011	115.2 [41.5 – 206.1]
2010	142.3 [50.2 – 252.2]
2009	212.4 [80.7 – 375.0]
2008	165.4 [53.4 – 299.4]
2007	180.8 [56.4 – 328.7]
2006	220.9 [79.1 – 397.7]
2005	217.5 [71.5 – 397.0]
2004	271.4 [95.9 – 487.8]
2003	218.9 [71.5 – 395.0]
2002	209.7 [65.7 – 380.7]
2001	253.3 [86.4 – 452.4]

Values are shown in number of cases per 1,000 inhabitants. Between square brackets are shown the low-level and the high-level cases estimates.

Nevertheless, malaria transmission is heterogeneous in PNG. It is estimated that 35.7% of the population (approximately 3.33 million inhabitants) live in areas with high to moderate malaria risk (incidence of >100 cases per 1,000 inhabitants), with New Ireland, East and West New Britain, Sandaun and Milne Bay being the most affected provinces (>200 yearly cases per 1,000 inhabitants) (106). Moreover, in some areas of the country this infection reaches transmission levels rarely found outside of Sub-Saharan Africa (8). Is in these areas where the ideal environment for a rapid and uninterrupted malaria transmission can be found: a tropical rainforest climate, a limited health infrastructure, a near-inaccessible geography, and low international and national funding targeting malaria control activities. On the other hand, the Highlands region of PNG, being at > 1,500 m of altitude, and the National Capital District (Port Moresby) register the lowest incidence in the country (104). In terms of prevalence, the last malaria indicator survey conducted between 2019 and 2020 showed similar heterogeneous results (107). Overall, when measured by light microscopy, malaria infection was detected in 2.1% of the population living in altitudes below of 1600 m. On contrary, this was the case for only 0.03% in the population living in highland areas. The PNG provinces with the highest prevalence in 2019-2020 were Sandaun (10.6%), East Sepik (8.6%), Oro (3.7%), East New Britain (2.6%), Madang (2.5%), and Milne Bay (2.2%) (107).

Regarding *Plasmodium* species, PNG is a unique setting. The two highly endemic species are *P. falciparum* and *P. vivax*, which are widely distributed across the country; but *P. malariae* and *P. ovale* are also present. A longitudinal study conducted with infection data collected at sentinel health facilities between 2010-2014 showed *P. falciparum* accounting for more than 60% of the cases diagnosed by RDT in most of the sites (102). However, after the LLIN distribution campaigns in 2008-2009, there has been an upsurge in the proportion of infections and cases attributed to *P. vivax* in certain geographical areas (101). This difference showing less protective effect of LLINs against *P. vivax* infections can be attributed to this species' relapses. For instance, in PNG, up to 80% of *P. vivax* infections in children are known to be relapses (70). On the other hand, malaria diagnosed cases of *P. malariae* and *P. ovale* species account only for 0.2% and 0.02% respectively (102). Nevertheless, these two "minor" species are not correctly identified with the diagnostic tools available at the health facilities. As an example, prevalence of these infections in a cross-sectional study in the Sepik Province increased from 0% to 4.8% for *P. ovale*, and from 3.9% to 13.4% for *P. malariae* when using qPCR instead of microscopy (108).

5.8. Malaria vectors in Papua New Guinea

The main mosquito species in PNG are *An. farauti*, *An. punctulatus* and *An. koliensis* (109). The major costal malaria vectors thorough PNG are mosquitoes of the *An. farauti* complex, which have anthropophilic and zoophilic behaviours; while *An. punctulatus* and *An. koliensis* are considered major vectors due to their capacity on completing the parasite life-cycle, their wide-spread distribution and their mainly anthropophilic behaviour (110).

In 2009, a nationwide study showed an earlier shift in the median biting time of *An. punctulatus* and *An. farauti* after the LLIN mass distributions; which did not translate in an increase of the proportion of infective bites occurring before 22:00 hours (37). However, a posterior study conducted in Madang Province demonstrated that this earlier feeding behaviour of *An. farauti* was epidemiologically significant (111). This shift in feeding behaviours was confirmed in a new survey conducted in 2016, which also showed an increase in the proportion of Anophelines feeding outdoor (24). The outdoor-feeding behaviour and the early evening biting peak of the PNG Anophelines

are crucial differences with the malaria vectors frequent in other geographical areas (112). In addition to vector behaviour, there is significant variation in vector abundance and infective biting rates. For example, in the coastal area of Madang Province, the entomological inoculation rates (EIR) ranges from 0.03 to 0.5 per person-night depending on the surveyed village (24).

In view of the ramp up of malaria cases in PNG these recent years, several studies have been conducted to understand if there is a loss of effectiveness in the country's vector control strategies. Although *An. punctulatus* has recently shown reduced mortality to pyrethroids in some areas of PNG, overall *Anopheles* studied have demonstrated susceptibility to deltamethrin, the sole insecticide used in the distributed LLINs in PNG (113). Plausible explanations may lie in the aforementioned changes in mosquito behaviours (24, 111), a reduced usage or improper maintenance of LLINs (114), and the decreased bioefficacy of distributed nets in PNG (115). Regarding this last issue, only 17% of tested nets distributed between 2013 and 2019 exhibited ≥80% 24-hour mortality or ≥95% 60-minute knockdown (115).

5.9. The Lihir Group of Islands

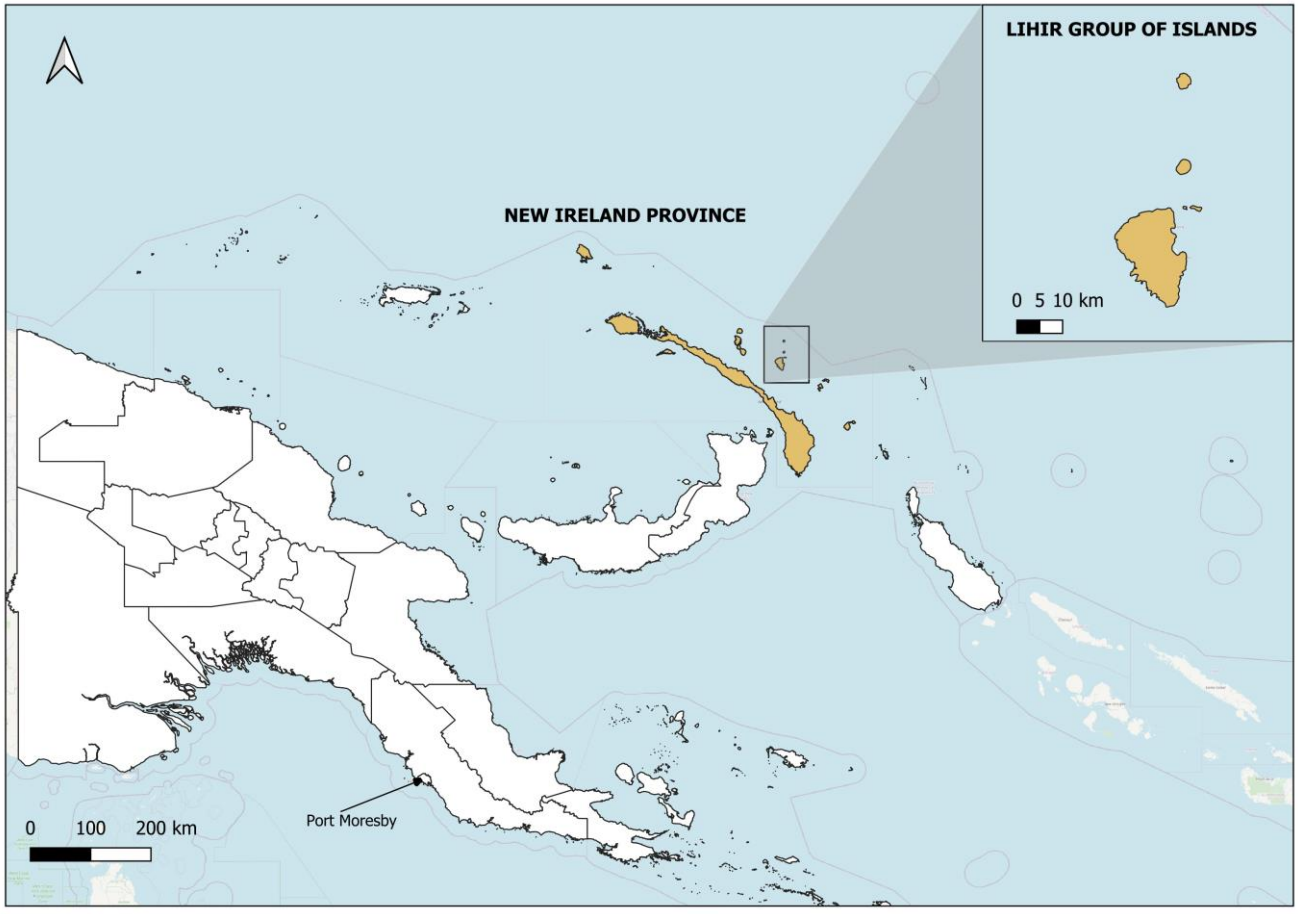
All the studies included in this doctoral dissertation have been conducted in the Lihir Islands, a rural setting in PNG.

Lihir is a group of six islands located 900 km northeast of Port Moresby off the coast of mainland New Ireland Province. The main volcanic island is Aniolam and it is surrounded by two low coralline islets of Sinambiet and Mando, and three larger raised coral platform islands of Malie, Masahet and Mahur (116). See Figure 4 for the Lihir Islands' location in PNG.

A gold mine located in Aniolam is the main source of employment in Lihir. The mine began its gold production in 1995 with an expected mine and processing life past 2040. Currently, it is operated by Newcrest Mining Limited (NML) which employs about 5,000 people of whom 3,000 are direct employees; the rest of the employees are contracted by other organisations that provide services to NML. Approximately, 90% of employees are PNG nationals and about 35% of them are Lihirians; with close to 3,000 mobile employees who arrive by plane or boat from different provinces of the country and reside in the mine accommodation (117). The Mine-Impacted Zone (MIZ) in

Aniolam includes the communities surrounding the open-pit, the main town Londolovit, the mining accommodation (a well-conditioned housing camp within a 2 km^2 area), and the airport. The mine company provides services such as health services, electric power and waste management to the villages in the MIZ, and it has a potable water plant that supplies water to the mine accommodation facilities, the mine process plant, the airport, and to the households at Londolovit Town, Londolovit village and the relocated Putput village.

Figure 4. Location of the Lihir Islands within Papua New Guinea (original figure).



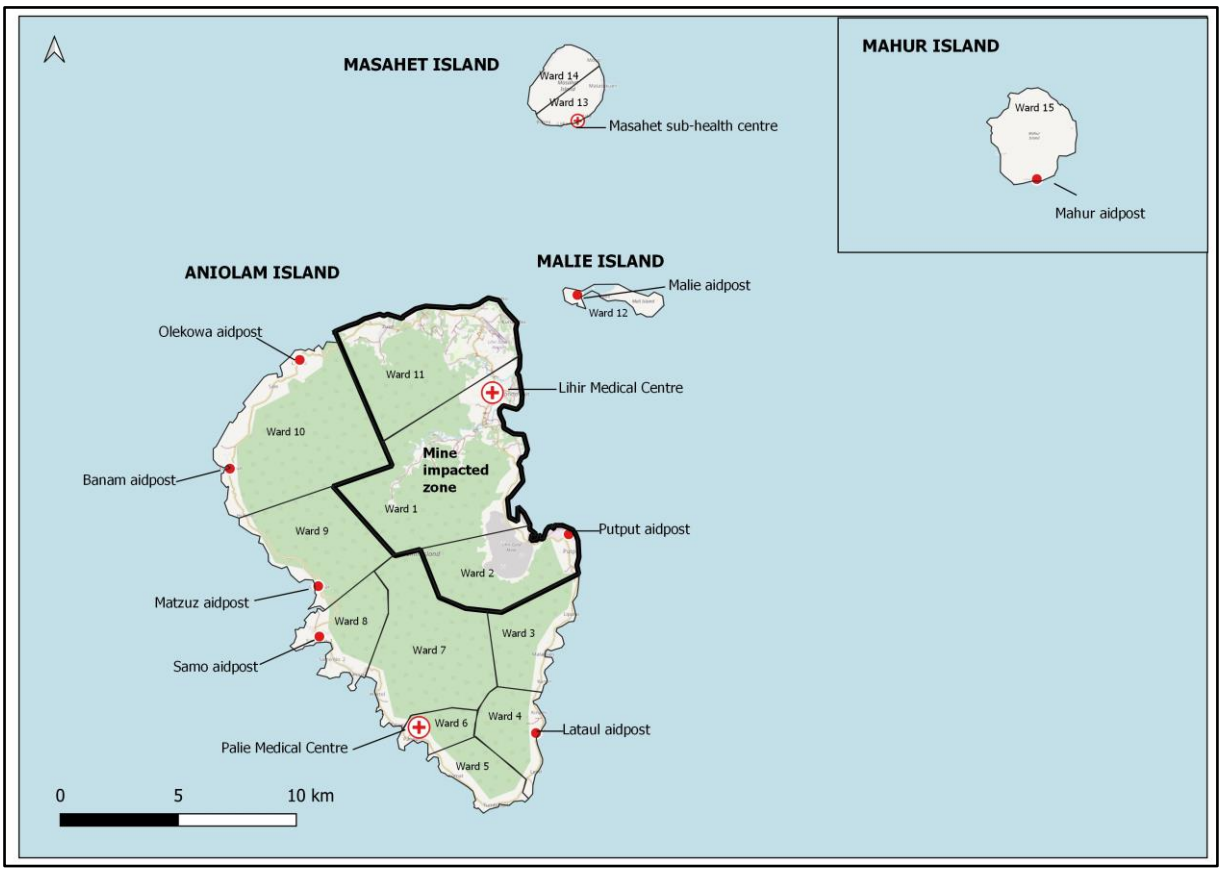
Nowadays, the Lihir islands are administratively divided into 15 wards (see Figure 5 for wards distribution). Aniolam contains 11 of the wards, with the MIZ comprising wards 1, 2 and 11; Sinambiet and Malie are under ward 12; Masahet island comprises wards 13 and 14; and Mahur is under ward 15. There are a total of 40 villages, and according to the official census in 2011, the estimated population was 25,608; however, a more

recent official census conducted in 2021 pointed an estimated population of 30,688, indicating a significant and rapid population growth in the last decade (118).

The health profile in Lihir is inaccurate due to a fragmented health system and poor reliable data. A health report conducted in 2010 underlined an under-5 mortality rate of 8.1 per 1,000 live births with an infant mortality rate of 13.9 per 1,000 live births (119). The total fertility rate for Lihirians in 2010 was 4.0, below the PNG average of 4.7. Communicable diseases are the main cause of morbidity with malaria leading the list, followed by skin diseases, respiratory infections, diarrhoeal and gastrointestinal infections, and obstetrics and gynaecology-related diseases.

There are 8 aid posts, 1 sub-health centre and 2 health centres in Lihir (see Figure 5 for locating the health facilities). Currently, there are four major health providers in Lihir: The New Ireland Provincial Government, the Nimamar (Lihirian) Local Level Government, the Catholic Church, and NML. The Lihir Medical Centre (LMC) located in the MIZ is managed by NML, and aside of providing services to the mine workers and the villagers of the MIZ, it serves as referral centre for the other health facilities, which it usually causes service saturation. The LMC has under its workforce more than 40 staff (community health workers, nursing officers, health extension officers and medical doctors), and it has its own drug purchase chain. On the other hand, the Catholic Church manages the Palie Health Centre (in ward 6), which it is the largest public health facility in Lihir. And the local and provincial governments manage all the Lihirian aid posts and the Masahet sub-Health Centre. The Palie Health Centre and the rest of the public facilities are understaffed and their workers have different salaries and conditions compared to the private facility. Also, these facilities lack of basic medical equipment, cold chain, as well as stable electricity and water supply.

Figure 5. Administrative map of Lihir islands (original figure).



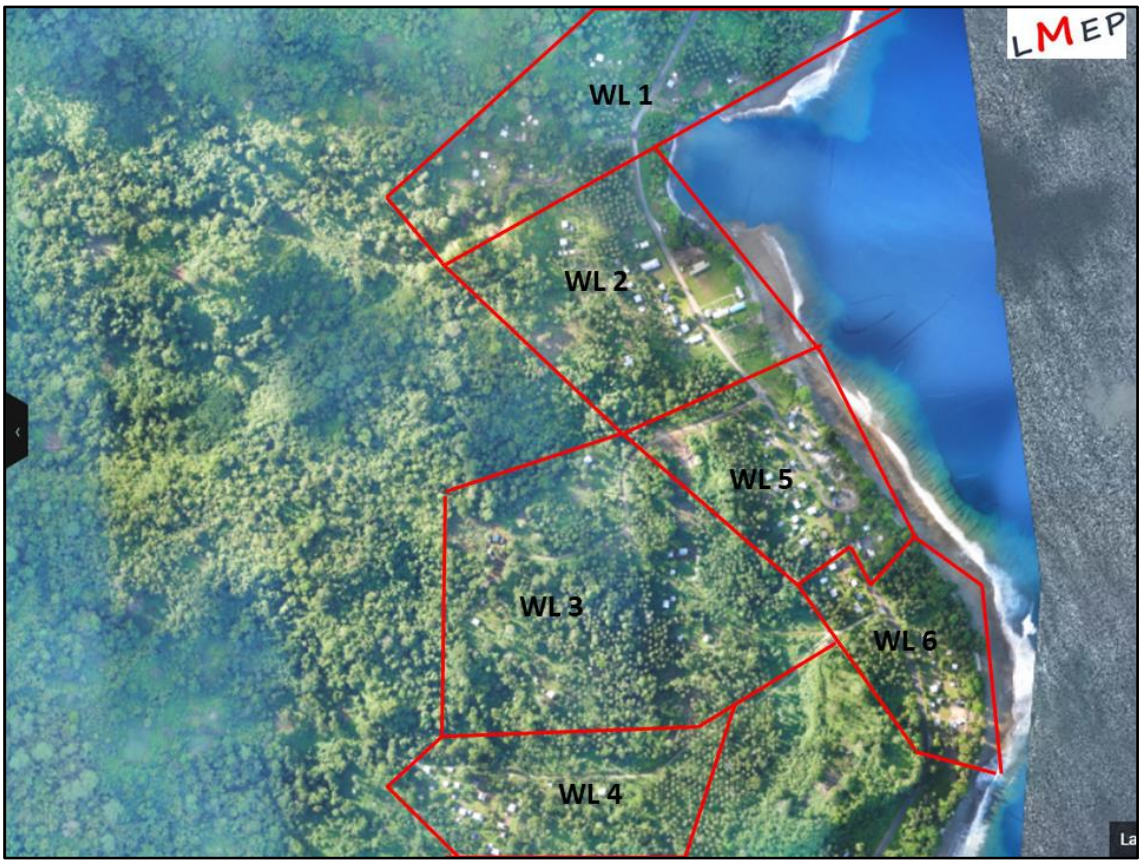
Legend: The Lihir Islands with the 15 administrative wards and the health facilities location. The area limited by the thick black line indicates the Mine Impacted Zone (MIZ).

5.9.1. Population census in the Lihir Islands

Given that PNG conducts its official national census every ten years, the most recent census available for reference during this doctoral work was conducted in 2011. Consequently, this PhD student designed, implemented, and supervised a population census in the Lihir Islands between 2018 and 2020. The goal of this census was to obtain the most accurate population numbers and demographic information possible, which would later facilitate the implementation of better-planned health programs and support the research studies included in this thesis. In 2020, census activities were halted due to the restrictions imposed in response to the Covid-19 pandemic. At that point, we had completed the census for wards 1-8 and 11. The census was conducted using a household survey after having obtained aerial images of all the villages using a drone (see Figure 6 as an example). Every building seen in the aerial images was

numbered and annotated in the map, and GPS points were collected for all buildings, independently of their given use (church, household, kitchen, toilet, etc). Then, all the households were targeted in a house-by-house survey. Demographic information, employment and education data were collected for every single person living in the household. Additionally, information on sanitation, water supply, waste management and amenities/utilities owned were collected for households in wards 3 to 8 as a sample of the population.

Figure 6. Aerial image of Kinami village in ward 3 (original figure).



Kinami village as an example of the aerial images obtained, and the division in different workloads (WL) for the household survey completion during the population census.

A total of 20,693 inhabitants were living in the nine of the fifteen Lihirian wards where the census was completed. The MIZ concentrated most of the population censed (13,474 people living in Wards 1, 2 and 11). The gender distribution was 55.2% (11,429) men and 44.8% (9,264) female. The age distribution showed an expansive population pyramid, with 30.6% of the population being ≤15 years-old, 45.6% being

between 16 to 45 years-old, and 14.8% being 46 years-old or older. See Table 2 for the detailed population data in each of the wards censed. In regards of the education received, most of the adult population (97.8%) had undertaken the full Elementary grades, and 47.6% of them had completed Primary education. On the other hand, the 62.2% of the children aged 4 to 15 were attending school (Elementary or Primary grades).

The MIZ of Aniolam showed some unique characteristics. The most populated villages of Lihir (Upper and Lower Londolovit, Kunaiye 1, Putput 1 and Zuen) are contained in this geographic area. Also, it was the only place where the proportion of population originated in other places of PNG (non-Lihirian population) was higher than the Lihirian population: 56.8% non-Lihirian vs. 43.2% Lihirian. Interestingly, Landolam, a settlement area located in the MIZ and one of the most populated places, had 93.5% of migrant population. The employment rate of adult population was also higher in the MIZ, with 40.1% of them being employed compared to the 19.5% of those living outside the MIZ.

Table 2. Population distribution obtained from the 2018-2020 census (wards 1-8 and 11).

Ward	Village	Households surveyed	Total population	Gender		Age							
				Male	Female	0 to 3	4 to 9	10 to 15	16 to 25	26 to 35	36 to 45	46 to 55	56 or older
11	Zuen	392	1830	1022	808	184	303	202			1141		
	Kunaiye 1	537	2284	1288	996	197	390	178			1519		
	Kunaiye 2	141	772	417	355	81	111	67			513		
	Khul	139	699	398	301	64	113	40			482		
	Total Ward 11	**1209**	**5585**	**3125**	**2460**	**526**	**917**	**487**			**3655**		
1	Londo Town	91	413	289	124	18	36	9			350		
	Lower Londo	414	1997	1141	856	171	311	151			1364		
	Upper Londo	299	1261	729	532	110	227	98			826		
	Potzlaka	20	160	88	72	11	31	19			99		
	Landolam	400	1779	984	795	164	275	90			1250		
	Total Ward 1	**1224**	**5610**	**3231**	**2379**	**474**	**880**	**367**			**3889**		
2	Putput 1	313	1253	758	495	95	192	109			857		

	Putput 2	168	1026	572	454	101	193	109			623			
	Total Ward 2	**481**	**2279**	**1330**	**949**	**196**	**385**	**218**			**1480**			
3	Lipukuo	177	797	428	369	76	135	107	128	142	112	43	54	
	Matakues	153	632	325	307	63	115	91	110	85	72	54	42	
	Kanaan	67	254	132	122	22	47	37	49	31	28	19	21	
	Total Ward 3	**397**	**1683**	**885**	**798**	**161**	**297**	**235**	**287**	**258**	**212**	**116**	**117**	
4	Kinami	75	332	178	154	21	62	43	59	43	44	39	21	
	Lissel	138	626	344	282	70	102	89	128	83	66	49	39	
	Lataul	56	274	146	128	37	55	38	41	41	28	14	20	
	Total Ward 4	**269**	**1232**	**668**	**564**	**128**	**219**	**170**	**228**	**167**	**138**	**102**	**80**	
5	Tumbuapil	118	548	289	259	63	102	78	91	92	58	26	38	
	Komat 1	54	239	111	128	23	31	26	60	32	22	20	25	
	Komat 2	112	479	232	247	50	98	55	95	75	36	27	43	
	Total Ward 5	**284**	**1266**	**632**	**634**	**136**	**231**	**159**	**246**	**199**	**116**	**73**	**106**	
6	Pangoh	127	601	310	291	57	99	84	111	82	78	43	47	
	Palie	27	138	71	67	11	23	14	31	32	10	6	11	
	Total Ward 6	**154**	**739**	**381**	**358**	**68**	**122**	**98**	**142**	**114**	**88**	**49**	**58**	
7	Talies	65	252	128	124	16	41	33	54	31	28	26	23	
	Hurtol	149	714	371	343	57	124	93	150	116	69	55	50	
	Total Ward 7	**214**	**966**	**499**	**467**	**73**	**165**	**126**	**204**	**147**	**97**	**81**	**73**	
8	Sianus	97	405	193	212	27	63	53	83	73	40	40	26	
	Samo 1	161	629	333	296	46	90	83	142	130	53	42	43	
	Samo 2	73	299	152	147	29	41	37	50	51	26	30	35	
	Total Ward 8	**331**	**1333**	**678**	**655**	**102**	**194**	**173**	**275**	**254**	**119**	**112**	**104**	
TOTAL		**4563**	**20693**	**11429**	**9264**	**1864**	**3410**	**2033**	**13386**					
				55.2%	**44.8%**	**9.0%**	**16.5%**	**9.8%**	**64.7%**					

For obtaining a total number of people living in the Lihir Islands, the population number had to be estimated for the areas that were not censed. The 2011 National Census was considered as a baseline for calculating a population growth for the surveyed wards (118). Population growth figures and total estimated population for each ward can be

seen in Table 3. In wards 1-2 and 11 (MIZ) the population growth was of + 19% in 8 years; and in the non-MIZ the growth was of – 9%. These differences in population growth between one area and the other could be explained by two factors: 1) the increase of migrant people established in the MIZ due to the economic activities driven by the mine, and 2) the house relocation/movement of people living in the non-MIZ towards the MIZ. We took the assumption that the population growth in the areas where the census was not conducted was the same than in the wards from the non-MIZ where the census was completed (- 9%). This approach obtained an estimated + 4% general population growth since 2011. Hence, the total estimated population for the Lihir Group of Islands was 26,528 (close to 1,000 more individuals than those estimated in the 2011 census).

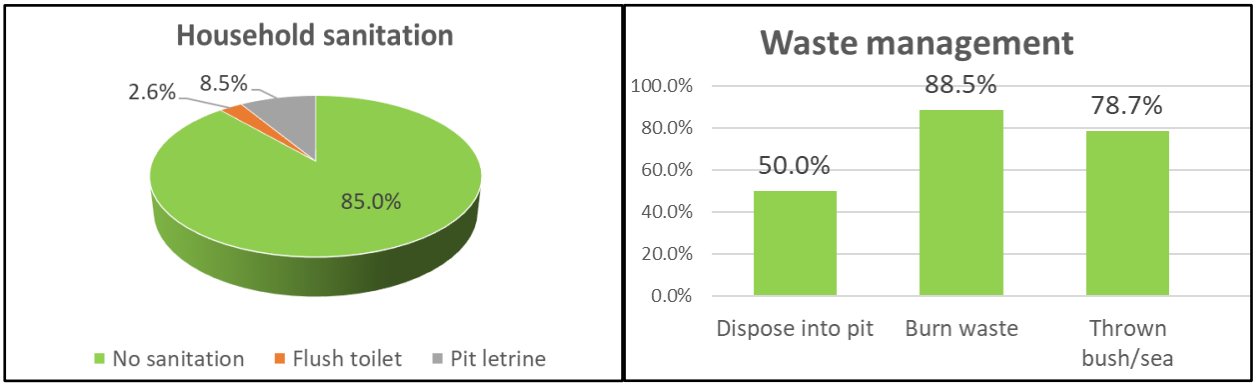
Table 3. Population growth and total estimated population in Lihir Islands by wards.

Area	**Ward**	**National Census 2011**	**Local census 2018-2020**	**Population growth (ward level)**	**Population growth (area)**	**Estimated population**
MIZ	11	4,984	5,585	+ 12%	+ 19%	5,585
	1	4,252	5,610	+ 32%		5,610
	2	2,067	2,279	+ 10%		2,279
Non-MIZ census conducted	3	1,838	1,683	- 8%	- 9%	1,683
	4	1,611	1,232	- 23%		1,232
	5	1,308	1,266	- 3%		1,266
	6	774	739	- 5%		739
	7	901	966	+ 7%		966
	8	1,464	1,333	- 9%		1,333
Non-MIZ census not conducted	9	1,558			-9 % (estimated)	1,418
	10	1,004				914
	12	874				796
	13	974				887
	14	943				859
	15	1,056				961
TOTAL		**25,608**	**20,693**	**+ 4% (estimated)**		**26,528**

Abbreviations: MIZ = mine impacted zone.

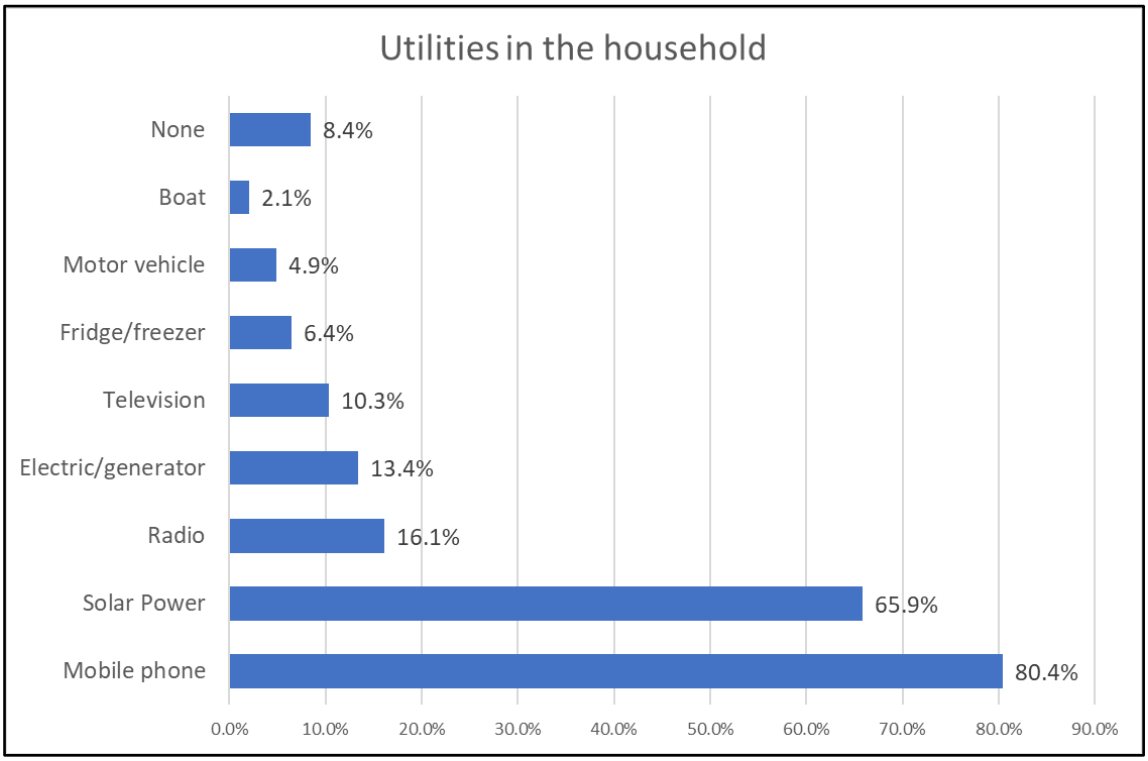
Regarding water supply, most of the households surveyed in wards 3-8 (non-MIZ of Aniolam) obtained their drinking water directly from rivers or creeks (58.7%), and/or had a water tank for rainwater collection (52.2%). There was no piped treated water available for any of the houses in this area. Sanitation measures were poor, with the majority of households (85.5%) lacking latrines and resorting to open defecation in the forest, rivers, or sea. Waste management was also inadequate, as 78.7% of the population disposed of waste into the sea or forest (see Figure 7 for information on household sanitation and waste management).

Figure 7. Household sanitation and waste management at wards 3-8 (original figure).



In terms of household utilities, the majority of surveyed households owned at least a mobile phone (80.4%) and a source of power, with solar panels being the choice for 65.9% of households and fuel generators for 16.1%. For more details on utilities' ownership, refer to Figure 8. Of note, 8.4% of the households did not earn any of the listed utilities.

Figure 8. Utilities owned in the households surveyed at wards 3-8 (original figure).



5.9.2. Epidemiology of malaria and malaria control programs in the Lihir Islands

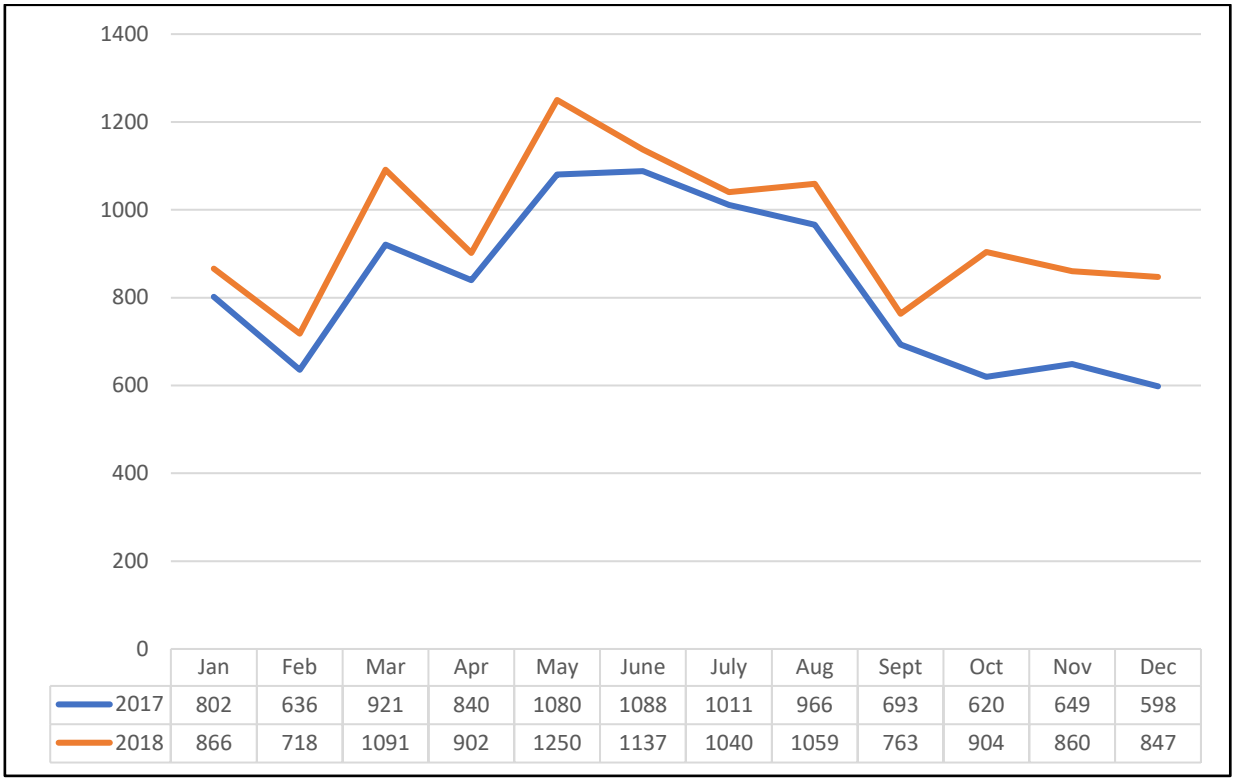
New Ireland Province, where the Lihir Islands are located, is one of the PNG provinces with higher malaria incidence (> 250 annual cases per 1,000 inhabitants) (120). Malaria in Lihir is an important health issue, being one of the main causes of mortality and morbidity in the islands. The last estimates of malaria transmission intensity in Lihir were reported in a study comparing data from the 2006-2012 period (121). It showed a decrease in incidence in the MIZ, from 601 new cases per 1,000 population in 2006 to 437 cases per 1,000 inhabitants in 2011. In this study, significant seasonality could be observed for *P. falciparum* cases coinciding with the months of higher precipitation average (January to May), while the peak for *P. vivax* infections was less marked. The same publication reported the results of a cross-sectional survey assessing malaria prevalence in children using microscopy technique. It showed a prevalence of infection of 5.8% in the MIZ of and 26.9% in the non-MIZ of Aniolam in 2010 (121). This resulted in a decrease of prevalence compared to 2006, when it was of 31.5% and 34.9% in the MIZ and non-MIZ of Aniolam, respectively. In terms of species distribution, of

the total new infections diagnosed with microscopy for this period, 70.8% were positive for *P. falciparum*, 27.0% for *P. vivax*, and 2.3% for *P. malariae* (121).

Concerning the malaria control programs implemented in the Lihir Islands, NML introduced a vector control program in 2006, mostly based on draining puddles or using *Bacillus thuringiensis israelensis* for larviciding big water bodies within the MIZ, and specially around the mine accommodation. Distribution of mosquito nets was also deployed in 2004 and again in 2010 in the MIZ. The rest of the Lihir Islands have followed the same vector control strategies than the PNG Government implemented across the country, relying only on universal coverage with LLINs for vector control. All the villages in Lihir received LLINs distributed in mass campaigns in 2009, 2013, 2016 and 2019. Coverage of this intervention was 97% and 98% in the 2016 and 2019 campaigns, respectively (122, 123).

Although the implemented strategies and the progress in decreasing malaria burden for the 2000-2014 period, malaria incidence has arisen in Lihir since 2015, like in the rest of the country. No other studies on malaria transmission have been conducted since 2010-2012; however, the number of febrile patients and malaria cases are mandatorily registered in the public facilities and in the LMC. Since 2017, data of both of these reporting systems are electronically digitalized and summarised. In 2017 and 2018, the number of cases diagnosed were 9,904 and 11,437 respectively, with an annual incidence rate of 478 cases per 1,000 inhabitants in 2018, one of the highest in the country (104). Figure 9 shows the monthly variation in number of cases for these two years of registers.

Figure 9. Number of confirmed malaria cases in the Lihir Islands, period 2017-2018 (original figure).



	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
2017	802	636	921	840	1080	1088	1011	966	693	620	649	598
2018	866	718	1091	902	1250	1137	1040	1059	763	904	860	847

5.9.3 The Lihir Malaria Elimination Programme

The Lihir Malaria Elimination Programme (LMEP) is a non-for-profit organization operating in the Lihir islands between 2015 and 2020. The organization was established after an agreement between NML and Medicines for Malaria Venture (MMV). The overall goal of the LMEP was to improve the health of all individuals residing in the Lihir Islands, with a specific focus on malaria control and elimination. Aiming to be successful and operational, the LMEP established a consortium of partners where the leadership for strategy implementation and operational activities was coordinated by the LMEP based in Lihir, and the scientific leadership of the program was coordinated and shared between the Papua New Guinea Institute of Medical Research (PNG-IMR) and the Barcelona Institute for Global Health (ISGlobal).

The Lihir program was expected to serve as a testbed for enhancing malaria control and test strategies towards elimination in a unique setting with *Plasmodium* species co-endemicity and high transmission levels. The program would generate evidence of useful strategies aimed to eliminate malaria, and provide experience and learnings to inform the public health authorities and policy leaders in the country. However, the

LMEP ceased its activities at the end of 2020 due to the impact of the COVID-19 pandemics, the increase in malaria cases (in PNG and globally), and the loss of interest by the funding partners of the program.

The LMEP project envisioned a program based on progressive steps: 1) deployment of public health strategies including health system strengthening, enhancement of vector control strategies, and increase of community involvement in health-related issues, as part of a preparation phase for elimination; 2) implementation of evidence-based strategies to achieve a fast and maintained reduction in malaria cases (elimination phase), with a possible strategy based on MDA rounds as the centre of these strategies; and 3) set up a surveillance and response system to monitor the effectiveness of these strategies and suffocate new malaria hotspots in the future (after elimination).

As part of the preparation phase for elimination, the LMEP implemented a health system strengthening plan and a community engagement program, following the recommendations from the PNG National Health Plan 2011-2020. This national plan emphasized the importance of strategies involving primary health care through universal health coverage and equity in health access (124). At the same time, the malERA consultative panel pointed up that health systems are basic to support disease elimination, and highlighted that effectiveness of interventions within the health systems rely on patient adherence, provider compliance, availability of diagnostic tools and access to health services (125). However, the lack of human workforce in the rural areas of PNG leads into work overload; the problems with securing essential drugs causes frequently stock outs; the poor quality of services in rural areas induces to an important deterioration of health facilities; and the lack of policies for holding accountability endangers any health program implemented (126).

The PhD student of this doctoral dissertation was the supervisor in the field for implementing the mentioned programs since 2015 until 2020. These strategies are elaborated upon in this section, so a better understanding of the situation in the Lihir Islands during the preparation phase can be provided for context within this thesis.

The health system strengthening program in Lihir consisted in supporting the public health facilities in performing correct malaria diagnosis and treatment, reporting of

cases, ensuring the stocks of antimalarial drugs and RDTs, responding to monthly infections' increases, and supporting a better diagnose and management of febrile diseases. For achieving these objectives, we delivered a training and coaching program consisting of monthly visits to all health facilities where a LMEP physician or a nursing officer conducted patients' visits hand-by-hand with the health workers. During these visits, the LMEP health staff provided clinical guidance for the patients visited, paying special attention to all possible malaria cases. The facility health staff were also guided in how to collect data for correctly reporting malaria cases and in how to keep a good stock management. We delivered biannual training sessions for all health staff, comprising different topics such as updates on malaria diagnose and treatment, malaria prophylaxis for pregnant women, maternal and child health, causes of fever and their clinical diagnose and management, among others. The monthly stocks were monitored in all the facilities and stock outs were reported to the health manageress of the Local Level Government. The LMEP periodically provided RDTs and antimalarial treatment to the health facilities when the stocks were low. All malaria cases diagnosed in each facility were collected on monthly bases and registered in a database; in case of increases, there were extra visits to those facilities for understanding the possible causes and targeting them.

With this program, an accurate monitoring of malaria registers in all health facilities of Lihir was achieved, and the health staff could correctly diagnose and manage the malaria cases. While at the beginning of 2017, the reporting of cases was scarce and through different systems, at the end of the program, in 2020, all health facilities used the official National Health Information System. Mistakes in recording cases, diagnoses and treatments were very low in 2020. Also, malaria diagnoses relying only on clinical signs and symptoms (without a confirmatory test) decreased from 6.0% in 2017, to only 0.6% in 2019. Finally, the stock outs of antimalarial drugs and RDTs were punctual in some facilities and for very few days (2-3 days) since the beginning of the program.

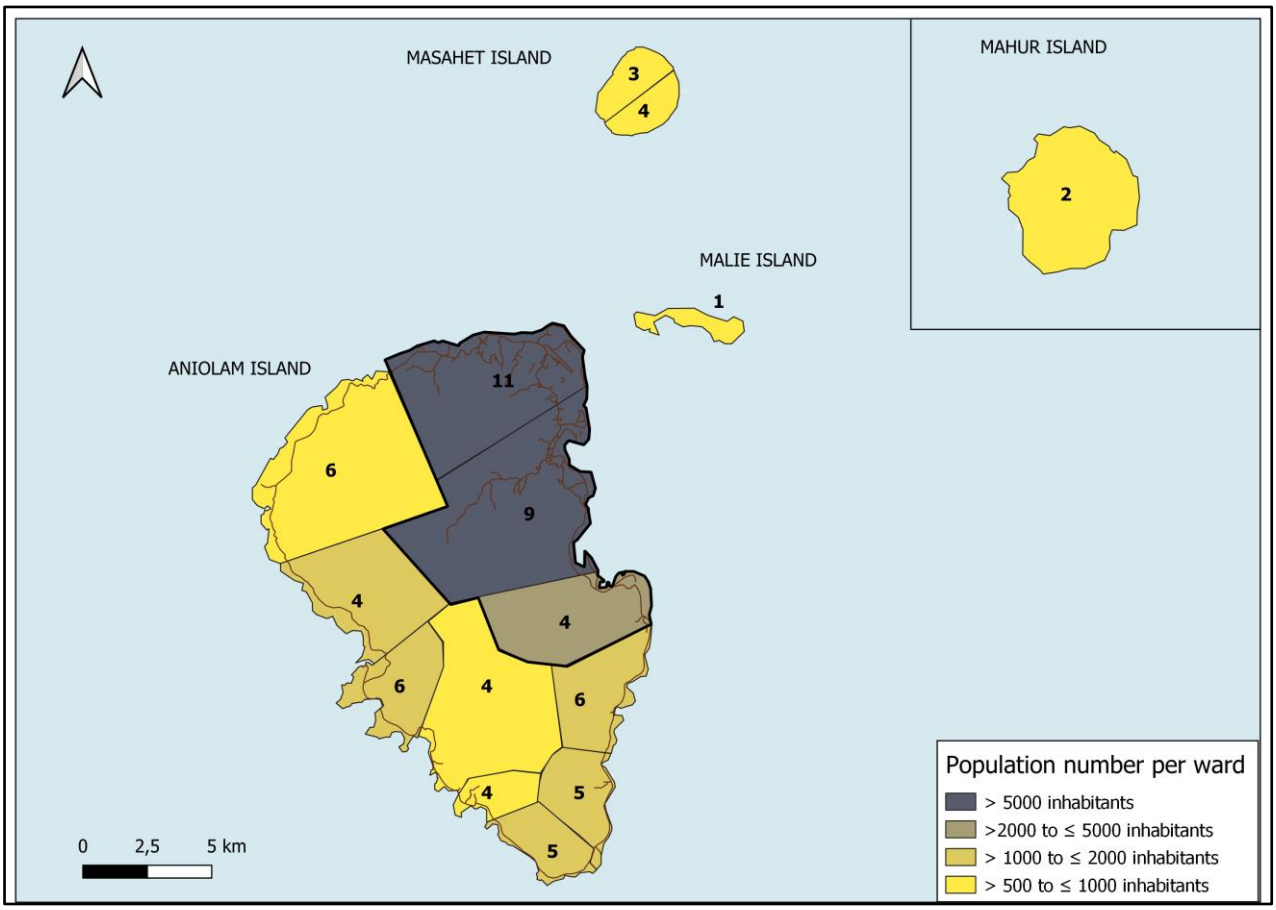
On the other hand, the objectives of the community engagement program were to create and maintain a robust community network where health-related messages could arrive to all hamlets of Lihir. The program pursued to link the community with the health facilities and increase community health seeking behaviours, increase the coverage and

usage of malaria preventative measures such as LLINs, increase the compliance of malaria treatments, and increase the knowledge in malaria transmission and prevention.

A network of Village Malaria Assistants (VMAs) was created to achieve most of these objectives. After introducing the program to the communities, we set up links with the Village Planning Committees and village leaders for recruiting community volunteers following agreed selection criteria. The village leaders proposed their candidates and we selected them considering the number of VMAs needed in each village (1 VMA for every 200-500 inhabitants, or more if the population was scattered). Women candidates had preference to be chosen as VMA candidates. The VMAs training was undertaken in two phases; first, a 1-week theorical training was conducted by the LMEP based on malaria, its prevention, diagnose and treatment. Secondly, a two-three months period of direct supervision and coaching directly at the community was established. After this period, the VMAs were contracted by the LMEP, and frequent supervision and coaching was undertaken. Substitution for VMAs not willing to continue in the program was facilitated and new VMA candidates undertook the same training process.

In this way, we obtained a robust network of VMAs that were able to deliver awareness and other activities. In January of 2020, a total of 74 VMAs were actively working in the Islands and there was at least one VMA in each community (see Figure 10 for the exact number of VMAs in each area); most of the VMAs were able to effectively deploy awareness information on malaria signs and symptoms, prevention, diagnosis and treatment. Moreover, 44 of the active VMAs were trained in data collection for a research survey and effectively collected information about maintenance and use of LLINs (this study is included as the second article of this doctoral thesis). Also, during and after the national LLIN distribution campaign in October 2019, all the VMAs deployed an intensive awareness campaign (hamlet-by-hamlet) on maintenance and use of LLINs.

Figure 10. Number of active VMAs and their location in the Lihir Islands, January 2020 (original figure).



Other community engagement strategies, such as direct awareness sessions in all communities of Lihir, were also implemented. In these sessions, the villagers were gathered around the LMEP team, and we delivered information on incidence of malaria in that village, awareness on the importance of using the available prevention measures, and on treatment compliance. The community members asked questions during the sessions and raised new topics for the next meetings. The VMAs were also present and coached to conduct part of the awareness session. Additionally, education campaigns in schools were conducted 2 times per year since mid of 2018, using games and educational material. Evaluations of knowledge acquired by scholar children were positive and children and their teachers displayed the work done at schools in front of the communities.

Furthermore, and together with the community and health system activities, the LMEP created the ideal environment for research during the pre-elimination phase. A strategic

document was developed by the two research entities involved in the program. In this strategic plan, repeated MDA campaigns and a surveillance-response system were at the core of the interventions, recommending a previous characterization of the malaria transmission in the Lihir Islands, an increasing of vector control coverage, and the assessment of the safety and effectiveness of the strategies to be used during the elimination phase. The PhD student of this doctoral dissertation could collaborate in the design and implementation of these research activities. See in Figure 11 an example of the implementation of a research activity.

Figure 11. The PhD student collecting samples for a cross-sectional study on malaria prevalence in Lihir (original photo).

5.10. Supplementary material for the Introduction

5.10.1 Opinion piece: commentary article on cardiac safety of dihydroartemisinin-piperaquine

Reappraising the cardiosafety of dihydroartemisinin-piperaquine.

Millat-Martinez P, Bassat Q.

Lancet Infect Dis. 2018 Aug;18(8):824-826. doi: 10.1016/S1473-3099(18)30360-8. Epub 2018 Jun 7.

Reappraising the cardiosafety of dihydroartemisinin-piperaquine

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The arsenal of efficacious drugs for the treatment of malaria remains small and is clearly insufficient to tackle the global burden of malaria, with more than 216 million clinical episodes and nearly half a million deaths annually.[1] Among the new antimalarials that have been developed in the past decade, the artemisinin-based combination dihydroartemisinin–piperaquine is one of the most promising, on account of its good efficacy and tolerability, simplified dose schedule (ie, once daily for 3 days), and long post-treatment prophylactic effect.[2] The only brand of dihydroartemisinin–piperaquine that has been registered under stringent regulatory authority is Eurartesim (licensed by the European Medicines Agency [EMA] in 2011), although at least three other brands exist: Duo-cotecxin (also prequalified by WHO), D-ARTEPP, and Arterakine. Dihydroartemisinin–piperaquine is not only used as a treatment of uncomplicated malaria but also has been proposed as an alternative to sulphadoxine–pyrimethamine for intermittent preventive treatment of malaria during pregnancy,[3] or as a suitable drug for the mass treatment of entire populations as part of malaria-elimination endeavours.[4]

However, because piperaquine can cause a dose-dependent effect on cardiac repolarisation, which can manifest as a prolonged QT interval in surface electrocardiograms (ECG), concerns have been raised regarding the cardiosafety of dihydroartemisinin–piperaquine, which have limited its much wider deployment and use. Indeed, such potentially pro-arrhythmogenic characteristics, which theoretically could be linked to an increased risk of ventricular fibrillation and sudden death, led the EMA to recommend that an ECG should be done before the drug's administration, that the drug should be administered under strict fasting conditions (because food increases piperaquine's plasma concentration), and that repeated doses should be limited in number and frequency.[5] These recommendations reduced the potential use of the drug in most areas of the world where malaria remains highly endemic. Dihydroartemisinin–piperaquine has since become one of the most studied drugs in relation to its associated repolarisation-related cardiotoxicity, conditions that were not applied to other antimalarial drugs with similar potential (quinine, amodiaquine, or chloroquine) when they were licensed, more than half a century ago.

Although the occurrence of clinically relevant adverse cardiovascular effects associated with dihydroartemisinin–piperaquine or piperaquine on its own after several years of extensive use seems rare, the drug's association with life-threatening cardiac events remains unchallenged. In this issue of *Lancet Infectious Diseases*, Xin Hui S Chan and colleagues[6] present the results of a systematic review and meta-analysis of 94 studies including nearly 200 000 pooled individuals given dihydroartemisinin–piperaquine for several indications, including treatment of uncomplicated malaria, intermittent preventive treatment, and mass drug administration. The investigators compared the risk of sudden unexplained death after dihydro-artemisinin–piperaquine with the baseline rate of sudden cardiac death in a reference population aged younger than 35 years, chosen to reflect the typical age of antimalarial users in malaria-endemic areas.

The median pooled risk estimate of sudden unexplained death after dihydroartemisinin–piperaquine was 1 in 757 950 (95% CI 1 in 2 854 490 to 1 in 209 114), a finding deemed to be similar (and not higher) than the baseline rate of sudden cardiac death in the reference population after standardisation to 30-day risks (0·7–11·9 per 100 000 person-years or 1 in 1 714 280 to 1 in 100 835). The investigators also examined the 61 deaths of individuals in the studies who received the drug, 31 of which occurred during the 3-day treatment period, and a further 30 that occurred during one terminal elimination half-life of piperaquine (around 30 days). Members of a WHO Expert Review Group, specifically convened to review the cardiosafety of antimalarial drugs,[7] concluded that only one of these deaths (of a healthy woman aged 16 in Mozambique who developed heart palpitations several hours after the second dose of dihydroartemisinin–piperaquine and collapsed and died on the way to hospital) was consistent with sudden cardiac death and possibly causally related to drug exposure. Although under-reporting is likely, that only one death among nearly 200 000 studied individuals could be linked to the drug strongly supports the idea that sudden deaths potentially attributable to repolarisation-related tachyarrhythmia after treatment with dihydroartemisinin–piperaquine are infrequent.

These reassuring data further corroborate the conclusions of the aforementioned WHO expert panel,[7] of findings from a previous smaller systematic review and meta-analysis on this subject,[3] and of various surveillance efforts undertaken by many independent research groups to study the safety of dihydroartemisinin–piperaquine.[2,8–10] Additionally, a multicentre clinical trial in five sub-Saharan African countries of a new dispersible paediatric formulation of dihydroartemisinin–piperaquine in infants aged 6–12 months did not note any clinical cardiosafety concerns.[11] Further studies (NCT02605720) assessing the safety in healthy individuals (including detailed ECG assessment) of cumulative repeated monthly treatment with dihydroartemisinin–piperaquine, mimicking regimen used in mass-drug administration are underway.

Reappraising the value of the few available effective antimalarials is more important now than ever. The global effort against malaria is at a crossroads;[12] malaria incidence seems to be rising again after many years of decreases. Dihydroartemisinin–piperaquine should be shed of its cardiotoxic reputation, so that malaria-endemic areas can benefit from its full potential and to decrease the toll that malaria still imposes globally.

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We declare no competing interests. QB is a member of the WHO Malaria Treatment Guidelines Group, which produces global guidance on the treatment of malaria including decisions about dihydroartemisinin–piperaquine. The views expressed by the authors are personal opinions and do not represent the recommendations of WHO.

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5.10.2 Opinion piece: commentary article on safety and effectiveness of primaquine

Primaquine dose and the risk of haemolysis and P. vivax recurrence: Pooling the available data to reassure the unconvinced.

Millat-Martinez P, Bassat Q.

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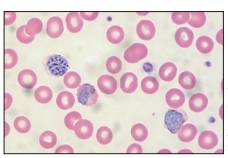
Primaquine dose and the risk of haemolysis and *Plasmodium vivax* recurrence: pooling the available data to reassure the unconvinced



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71 years after its US Food and Drug Administration approval for the treatment of *Plasmodium vivax* and *Plasmodium ovale*, and despite the hundreds of millions of doses cumulatively used in the world, the use of primaquine remains hampered by the many unknowns and allegations that have cast a shadow over its reputation. As an 8-aminoquinoline, primaquine is part of the only family of drugs with the potential to eliminate hypnozoites and thus prevent delayed relapses arising from the dormant stages of these two malaria species, a major cause of morbidity and a threat to global public health. Primaquine can also clear all-stage gametocytes of any malaria species, preventing their uptake by the next mosquito vector and is therefore an efficacious tool to interrupt ulterior malaria transmission.[1] Given its unique characteristics, what has hampered its trustworthiness and more widespread deployment?

Primaquine implementation has always been eclipsed by the many uncertainties regarding its mechanism of action and dosage,[2] its long duration of treatment (generally 14 days) and associated poor compliance, and the sensible safety concerns regarding its haemolytic potential.[3] Among individuals with varying degrees of G6PD deficiency, primaquine and the other 8-aminoquinolines can all cause mild to life-threatening hemolysis.[3,4] In spite of very clear policy recommendations in vivax-endemic areas, this fear has primarily driven a rather conservative uptake, often leading to the prioritisation of the avoidance of individual risks rather than the potential benefits of preventing relapsing clinical episodes.[5] In this context, the two systematic reviews and individual patient data meta-analyses published by the WorldWide Antimalarial Resistance Network in *The Lancet Infectious Diseases*[6,7] are instrumental at collating some of the most relevant information on the safety and efficacy against relapses of primaquine generated globally in the past two decades. Such analyses should complement the existing expert opinion and less recent literature by providing a more robust backbone on our current understanding of some of the key aspects related to the use of primaquine in malaria.

Megha Rajasekhar and colleagues[6] explore the association between primaquine dose and the risk of haemolysis in patients with uncomplicated *Plasmodium vivax* malaria. From the 18 studies selected including 5462 patients from 15 countries, the findings suggests that, in patients with a glucose-6-phosphate dehydrogenase (G6PD) activity of 30% or higher, the use of primaquine at the standard doses (ie, 0·25–0·5 mg/kg per day) does not entail an enhanced haemolytic risk compared with not using it. Severe haemolytic events were rare, and their frequency was higher only when the primaquine dose used was increased to 1 mg/kg per day, supporting the safe use of standard doses of primaquine for radical cure of hypnozoites (ie, by targeting both the blood and liver-stage parasites). In the complementary analysis, Robert J Commons and colleagues[7] explore the effect of primaquine dose on the risk of *P vivax* recurrence in a dataset of 6879 patients from 23 studies and 16 countries, representative of regions with high and low relapse periodicity (ie, the time from initial infection to vivax malaria relapse). The findings indicate that, compared with not using primaquine, low (approximately 3·5 mg/kg) or high (approximately 7 mg/kg) total doses of primaquine substantially lower the rate of vivax recurrence, and the high total dose regimen can potentially halve recurrences in most endemic regions, with an acceptable safety and tolerability profile. Altogether, these results should reassure clinicians and policy makers on the importance and impact of using primaquine for the radical cure of hypnozoites, particularly because, in highly endemic *P vivax* areas, relapses might account for up to four of five new clinical cases.[8] In individuals with a G6PD activity of 30% or higher, the use of primaquine is not more dangerous than its non-inclusion, and it will clearly avert the cumulative effects of repeated clinical episodes. Moreover, in areas endemic for *Plasmodium*

falciparum and *P vivax*, the high risk of *P vivax* recurrences after a *P falciparum* case is well established,[9] further widening the potential benefit of using primaquine more routinely for any malaria episode in these settings.[10]

For many years, widespread expectation has been placed into tafenoquine, a new long-lasting single-dose 8-aminoquinoline, which achieves a similar anti-relapse activity to multidose primaquine regimens.[11] Although the single dose approach can bypass the many challenges related to primaquine's poor associated compliance, it also entails a much less controllable risk related to its inherent haemolytic potential in patients with G6PD deficiency (<30%). For primaquine, this risk is more controllable because the drug can be interrupted if any noticeable side-effect appears. However, for tafenoquine, such risk has resulted in the obligatory co-deployment of G6PD point-of-care testing before administering the drug. Although, as a field, we strive to address the many implementation challenges that tafenoquine entails, we can take solace in the fact that primaquine will remain an indispensable part of our current global arsenal against relapsing malaria and will continue to safely address its high public health burden.

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6. HYPOTHESIS

In 2015, the Lihir Islands of Papua New Guinea met some of the ideal characteristics for a successful malaria elimination program: a favourable geographic location and condition (islands), a consistent and gradual decrease in malaria incidence, a population covered with core vector control strategies, a technical plan for elimination designed by international and national research institutions, and a funding agreement to implement a program with the ultimate goal of eliminating malaria, the Lihir Malaria Elimination Programme (LMEP). A pre-elimination phase was planned and initiated in 2016, with research activities aiming to generate evidence on the safety and effectiveness of the strategies that would be used in the future. It is within this context that the research studies included in this dissertation were conducted.

The investigations conducted in this doctoral dissertation try to answer the following hypotheses:

Hypothesis 1: Malaria transmission is high in the Lihir Islands, and distributing long-lasting insecticidal-treated nets every three years is not sufficient to protect the population living on Lihir. Malaria prevalence in travellers arriving on Lihir is also high, hence there is a moderate-to-high risk of re-establishment of malaria if it is eliminated.

Hypothesis 2: The administration of dihydroartemisinin-piperaquine for three consecutive months does not pose a cardiac risk to people, making it suitable for mass drug administration in the Lihir Islands.

7. OBJECTIVES

OBJECTIVE 1: To determine factors driving malaria transmission in the Lihir Islands (First article).

Specific objectives:

1. To determine the blood carriage of *Plasmodium* species in the population living in Lihir.
2. To identify which human populations are at highest risk for carriage of microscopic and submicroscopic parasitaemia.
3. To determine the malaria incidence in the Lihir Islands and the population groups at highest risk.
4. To characterize the *Anopheles* species in the Lihir Islands and their biting behaviour.
5. To determine the blood carriage of *Plasmodium* species in the Anopheline population.

OBJECTIVE 2: To determine the effectiveness of the long-lasting insecticidal-treated nets mass distribution campaigns in the Lihir Islands (Second article).

Specific objectives:

1. To evaluate the coverage and determinants of use of long-lasting insecticidal-treated nets in the population of Lihir Islands.
2. To determine how the population uses the long-lasting insecticidal-treated nets distributed to them.

OBJECTIVE 3: To determine the cardiac safety of a monthly therapeutic dose of dihydroartemisinin-piperaquine for three consecutive months (Third and Fourth articles).

Specific objectives:

1. To assess the cardiac and overall safety of three-monthly doses of dihydroartemisinin-piperaquine based on electrocardiographic and clinical parameters.
2. To model the safety of three-monthly doses of dihydroartemisinin-piperaquine according to the pharmacokinetic/pharmacodynamic profile of piperaquine.

OBJECTIVE 4: To determine the risk of malaria importation into the Lihir Islands (Fifth article).

Specific objectives:

1. To determine the overall prevalence of malaria among travellers arriving to Lihir by boat and by plane.
2. To identify the groups of travellers at highest risk of having *Plasmodium* parasitaemia.

8. MATERIAL, METHODS, AND RESULTS

8.1. First article: Human and entomological determinants of malaria transmission in the Lihir Islands of Papua New Guinea: a cross-sectional study.

Human and entomological determinants of malaria transmission in the Lihir Islands of Papua New Guinea: a cross-sectional study.

Short tittle: Malaria transmission in the Lihir Islands, Papua New Guinea.

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ABSTRACT

Background: The Lihir Islands of Papua New Guinea, located in a high transmission setting and hosting a mining operation, provide the opportunity to describe malaria burden and transmission intensity, and test control strategies. We characterized human and vector determinants for malaria transmission in the mine-impacted zone (MIZ) and the other areas of Lihir. **Methods:** in 2019 we conducted a cross-sectional study assessing prevalence through microscopy, rapid diagnostic test (RDT), and quantitative PCR (qPCR), and infection-associated factors were identified through logistic regression. Same year's passive case detection data were collected and analysed. Finally, we assessed *Anopheles* species and abundance, their biting behaviours and sporozoite carriage after human landing catches and larvae surveys. **Results:** 2,914 individuals participated in the cross-sectional survey, revealing a malaria

infection prevalence (any species) of 3.6% by RDT, 4.5% by microscopy, and 15.0% by qPCR. 37.1% of infections were *P. vivax*, 34.6% *P. falciparum*, 3.0% *P. malariae*, 0.2% *P. ovale*, and 24.5% mixed infections by qPCR. 6.8% of the individuals had haemoglobin <8.0 g/dL, and 4.8% had splenomegaly. Malaria incidence ranged from 116/1,000 inhabitants in Masahet Island to 828/1,000 in Malie Island (p<0.001). Factors associated with malaria prevalence included living in the non-MIZ of Aniolam (aOR=3.56, 95%CI: 2.72, 4.65) or in Malie (aOR=1.83, 95%CI: 1.04, 3.21), sharing household with an infected individual (aOR=1.94, 95%CI: 1.56, 2.42), and residing in a traditional housing (aOR=1.65, 95%CI: 1.21, 2.25). Children had twice the infection risk than adults, and the referred use of long-lasting insecticide-treated nets did not increase protection. *An. punctulatus* was the major vector (75.7%) in the non-MIZ of Aniolam, with an early hour biting behaviour; while *An. farauti* was the predominant (94.2-100%) in the rest of the areas, differing in biting behaviours. Entomological inoculation rates ranged from 0.221 (95%CI: 0.120, 0.322) in the MIZ to 2.289 (95%CI: 1.560, 3.018) in Malie. **Conclusions:** Malaria transmission patterns differed across areas, emphasizing the need for targeted measures. Interventions focusing on at-risk groups, including vector-control and transmission interruption methods, are warranted to decrease the island's malaria burden.

AUTHOR SUMMARY

The Lihir Islands are located in a high malaria burden province of Papua New Guinea. They host a mining operation in Aniolam, that conducts specific vector control strategies in the mine-impacted zone (MIZ); while the rest of the Islands rely only on long-lasting insecticidal-treated nets (LLIN). We assessed human and vector factors for malaria transmission in Lihir. The most frequent *Plasmodium* species infecting humans was *P. vivax,* followed by *P. falciparum*, with a high rate of mixed infections. The MIZ of Aniolam and Masahet Island had the lowest malaria burden, as well as mosquito densities and biting intensities. In contrast, living in the non-MIZ of Aniolam and in Malie Island was associated with higher risk of infection. Other risk factors

for infection included younger age, cohabiting with infected individuals, and living in traditional housing; while use of LLINs did not appear to increase protection. Our study reveals specific malaria transmission patterns in each area of Lihir Islands, with an important *P. vivax* burden, calling for tailored strategies towards interrupting malaria transmission. Strengthening current malaria control methods may not be enough and new approaches are needed. Our findings can guide ongoing initiatives and suggest solutions for malaria control and elimination in the Lihir Islands or in similar settings.

INTRODUCTION

Progress in reducing the global malaria burden has stagnated since 2015, and the milestones proposed by the World Health Organization (WHO) have not been met (1). Although the increase in malaria cases seen in 2020 was predominantly attributed to the restrictions imposed by malaria-endemic governments during the COVID-19 pandemic (2), the progress in malaria burden reduction had already plateaued before the pandemic (3). The WHO Western Pacific region is an example of this lack of progress, since malaria incidence increased by 10% and the mortality rate by 4% between 2015 and 2021 (4). This is largely due to a rise in the malaria burden in Papua New Guinea (PNG), as it accounts for 87% of all malaria cases and 94% of all malaria deaths in the region (5). It is estimated that 35.7% of the population (approximately 3.33 million inhabitants) live in areas with high to moderate malaria risk (6). Malaria transmission in PNG exhibits geographical heterogeneity, with the northern coast and the islands' region being affected with high levels rarely found outside of Sub-Saharan Africa (7). *Plasmodium falciparum* and *P. vivax* are highly endemic in PNG, although the other two human parasite species, *P. malariae* and *P. ovale*, are also present (8). Hence, focusing efforts in PNG, with this unique transmission patterns and high transmission intensities, is warranted for reducing malaria transmission in the region (5).

While malaria symptomatic cases can be confirmed by microscopy or rapid diagnostic test (RDT), molecular tools such as polymerase-chain reaction (PCR) are required to confidently detect sub-clinical infections, given many present with sub-microscopic parasite densities (9). These sub-microscopic infections could constitute a source of ongoing transmission, and are a common feature in malaria endemic areas, involving all *Plasmodium* species and individuals of all ages (10). In PNG, prevalence of microscopic parasitaemia in household surveys varied among different geographic areas, ranging from 0.1% in the Highlands to 10.6% in the coastal areas of the country (11). When PCR is used to include sub-microscopic infections, prevalence of malaria parasites increases, especially for *P. vivax* infection. As an example, in a cross-sectional study performed in Madang Province during 2014, *P. falciparum* prevalence increased from 2.8% to 9.0% when comparing microscopy to PCR results, while *P. vivax* prevalence increased from 2.7% to 19.0% (12).

On the other hand, the main mosquito species in PNG are *Anopheles farauti*, *An. punctulatus* and *An. koliensis* (13, 14). Significant variation in vector abundance and infective biting rates are observed across villages, even in the same region (15). In addition, after the first nationwide distribution of long-lasting insecticidal treated-nets (LLINs) between 2005 and 2009, shifts towards an earlier peak of biting activity was observed in *An. punctulatus* and *An. farauti* (16). A new survey conducted in 2016, confirmed that 25.5–50.8% of the vectors in the studied villages encountered human hosts in the evening, with an increased proportion of Anophelines feeding outdoors (15).

Lihir Islands, located in New Ireland province of PNG, present unique characteristics to study malaria transmission (17). Aside of being located in one of the provinces with highest malaria transmission (>200 yearly cases per 1,000 inhabitants) (7), they host a gold mining operation on their largest island, Aniolam. Newcrest Mining Ltd, the mine company, provides essential services to those communities in the Mine Impacted Zone (MIZ) surrounding the open-pit,

whereby a specific vector control program has been deployed. Since 2006, the company conducts drainage of puddles and larviciding of big water bodies using *Bacillus thuringiensis israelensis* within this area. A prevalence study conducted in 2010, showed a marked reduction in malaria positive children found by microscopy in the MIZ (from 31.5% in 2006, to 5.8% in 2010) (18). In contrast, the rest of the Lihir Islands relies only on universal coverage with LLINs. Nevertheless, despite achieving 97%-98% coverage during the distribution campaigns, only 8.7% of households maintained the nets two years after distribution (19).

Nearly a decade later, in 2019, we conducted a study to characterize transmission, obtaining incidence data and performing a prevalence survey and an entomological survey in the MIZ and in the other geographic areas of the Lihir Islands. Factors associated with high malaria transmission, both in the human and the vector population, were assessed.

METHODS

Study setting

This study was conducted in the Lihir Islands, which are located 900 km northeast of Port Moresby. In addition to Aniolam, the Lihir group is formed by Malie (a raised coral platform island and 2 low coralline islets) and two more larger raised coral platform islands (Masahet and Mahur). A population census conducted between 2018 and 2020, estimated a population of 26,528 inhabitants in the Lihir Islands. The villages of the MIZ accommodate half of the Islands' population, and there are 2,000-3,000 extra mobile workers staying at the mine housing facilities in Londolovit, at the MIZ. Most of the people staying in the MIZ are migrants from other places of PNG (57% of all inhabitants), living in permanent or makeshift houses and few of them in traditional houses compared to the other areas of Lihir.

Ethical statement and informed consent

This study was approved by the PNG Medical Research Advisory Committee (PNG-MRAC) with MRAC No.18.07. The informed consent process followed community and cultural values of PNG. Following consultation with and approval by community leaders, awareness meetings or notes (*tok saves*) were delivered in each village to explain the study aims and announce the visit of a study team. Permission for collecting malaria incidence data registered at health facilities was obtained from the health department of the local level government and from the district level government (Namatanai, New Ireland Province, PNG).

For the cross-sectional study, individual written informed consent was obtained from all participants, or the parent or legal guardian of children below 18 years old, after explanation of the risks and potential benefits of the study. Children under the age of 18 were verbally assented. Those participants unable to read and/or write were verbally consented with a witness countersigning the consent form. For the human landing catches, individual, written informed consent was obtained from all participants after explanation of the risks and potential benefits of the study, and chemoprophylaxis was offered to prevent infection from exposure to infectious mosquito bites.

Collection of health system data

The health system in Lihir is highly fragmented. The MIZ contains the Lihir Medical Centre, run by an external health provider contracted by the mine company. In contrast, in the non-MIZ there are a public health centre, a health sub-centre and eight aidposts, which are under-staffed with community health workers and some nursing officers. Passive case detection data registered between January and December of 2019 in all the health facilities (public and private) were extracted and digitalized for analysis of malaria incidence. The collection and analysis of passive case detection data was a regular activity conducted since January 2017. It consisted in monthly collection of data using photocopies of the national health information

system of all the health facilities, data digitalization in a central database classifying them in age groups, village of origin, diagnostic tool used and treatment given to each patient.

Study population, data and sample collection

We conducted a cross-sectional study during October and November of 2019. To obtain a representative population sample of Lihir Islands, we used a stratified random sampling strategy. In this case, the strata were each of the four Islands of Lihir and separated random samples were selected from each of the villages of the strata. The sampling sizes of each stratum and village were calculated considering the data from the population census. Inside each strata all households were enumerated, geo-positioned and randomly selected. All individuals living in the selected households, defined as non-visitor individuals staying in that household for at least the last 2 weeks, and present at the moment of the survey, were eligible for recruitment to the study until the achievement of the sample size. A total of 2,914 individuals of all ages above 6 months and residing in 696 households across the 43 villages of Lihir Islands were included.

Following informed consent, a questionnaire including demographic and clinical data, relation with head of the household, LLIN usage and mobility information was administered to all participants. Data were collected using Open Data Kit directly at the field and then uploaded to the online server at the end of the day. Pregnancy was assessed through asking the pregnancy status to all female participants aged 16 years or older, no confirmatory test was conducted. A finger prick was performed by a health practitioner, and blood drops were collected for a malaria RDT (Malaria Pf/PAN Ag Combo RDT, Carestart™, USA), a blood slide with thin and thick smears for microscopy examination, 2 dry blood spots in filter paper, and a drop for haemoglobin analysis with Hemocue® HB 301 analyser. A short clinical assessment, including axillary temperature, spleen size assessment and history of last malaria episode was conducted by a clinician, who did also interpret the result of the RDT. In case of a positive RDT result,

antimalarial treatment with artemether/lumefantrine (± primaquine if needed) was delivered following PNG guidelines (20). In case of detecting anaemia with the haemoglobin analysis, the participants were referred to the nearest health facility for assessment.

Light microscopy detection of *Plasmodium spp.* parasites

Blood films were fixed and stained with Giemsa 10 % at the local laboratory upon arrival from the field, and they were later sent to the Institute of Medical Research (IMR) Vector-borne Diseases Unit at Madang (PNG) for examination. The slides were examined under x1000 power by two independent level 1-2 microscopists having completed WHO quality assurance courses. A sample was considered negative after examining one hundred fields of view. When a parasite was observed, counts of white cells and parasites were conducted until 300 white cells had been counted. The parasite count was calculated assuming a white cell count of 8,000 cells/ µL. Gametocyte stages were recorded separately. Any discrepancies were addressed with the involvement of a different WHO-certified level 1 microscopist

DNA extraction and molecular detection of *Plasmodium spp* parasites

Filter papers with dry blood spots were dried in the field, placed in separated zip-lock bags, and stored at -20 ºC. Subsequently, they were sent to the IMR Vector-borne Diseases Unit at Madang (PNG) for further processing. DNA was extracted using FavorPrepTM 96-well Genomic DNA kit (FAVORGEN®) and performed according to the manufacturer protocol for extraction of genomic DNA from blood. Following DNA extraction, a generic quantitative PCR (QMAL) that amplifies a conserved region of the 18S rRNA gene was run on all samples (21); and for all positive samples, a species-specific quantitative PCRs (qPCR) detecting all *Plasmodium* species were performed as previously described (22). Finally, in PCR QMAL positive samples that yielded negative by the species-specific qPCR, ultra-sensitive qPCRs targeting Pf-varATS for *P. falciparum* and Pv-mtCOX1 for *P. vivax* were conducted (23, 24).

Mosquito and larval sampling

In parallel to the cross-sectional study, we conducted an entomological survey including human landing catches (HLC) and larval collection at eight sentinel sites distributed throughout Lihir: 3 sites in Aniolam MIZ, 3 sites in Aniolam non-MIZ, 1 site in Malie and 1 site in Masahet. These sites were selected after studying the environmental characteristics of Lihir Islands to represent the ecological diversity found within Aniolam, as well as in the outer Islands.

For the HLC, 20-25 healthy consented volunteers were selected in each site. The inclusion criteria were those individuals, male or female, from 18 to 75 years old living in the selected site, and expressing a willingness to participate in human landing catches. Exclusion criteria were those participants who were not willing to give informed consent, and individuals with any apparent acute or chronic illness. Participants were seated comfortably with the lower part of their legs (from the knee to the feet) exposed to host-seeking mosquitoes. Mosquitoes that rested and attempted to bite on the exposed part of the legs were captured using a mouth aspirator aided by a flashlight/torch to see the mosquitoes (25). The HLC were done from 6:00 pm to 6:00 am each night during a collection round, and next to sleeping spaces inside the houses (indoor collections) or outside but near the houses (outdoor collections). Captured mosquitoes were placed into screened paper cups and classified according to the date and hour of the night, and location (geolocation, household, indoor/outdoor). Mosquitoes were separated into their respective genera with the aid of a light microscope. Each of the female *Anopheles* were morphologically identified to species (26), and placed in a 2-ml microcentrifuge tube with a unique identification number. Data on mosquito biting frequency, biting time (mainly the peak biting time), whether they were exo- or endophagic, their biting density, and their transmission intensity were also annotated.

All potential larval habitats at the eight collection sites were surveyed and categorized as confirmed or potential larval habitat depending on the presence of *Anopheles spp*. larvae. Two more sites for potential larval habitats were included (one in Aniolam-MIZ and one in Aniolam

non-MIZ). Density was estimated using a larval dipper, and GPS coordinates and environmental variables were recorded for each habitat to map and characterize anopheline breeding habitats. All larvae were reared to adults in a temporary field insectary and in the PNGIMR entomology insectary in Madang, and they were identified to species following the same methods as above.

Molecular identification of *Anopheles* species and sporozoites

At the entomology unit at IMR Vector-borne diseases unit (Madang, PNG), the abdomen of each *Anopheles* mosquito was separated from the rest of the body and DNA was extracted from the abdomen-detached body part (i.e., head and prothorax) using DNeasy Blood and Tissue Kit (Product number: 69582; Qiagen, Valencia, CA, USA). Anophelines were analysed using a standard PCR method by ITS2-RFLP for species determination (27). Sporozoite positive mosquitoes were Identified using a multiplex quantitative PCR with two fluorescent-labelled TaqMan probes targeting the 18S rRNA gene of *P. falciparum* and *P. vivax* as previously described (28, 29). As the mosquito DNA was isolated from part of the body anterior to the abdomen, it was considered devoid of oocysts and other human stages of the malaria parasites that might have been present in the midgut. Thus, the PCR-positive mosquitoes were assumed to carry the infective sporozoite stage which inhabits the salivary glands in the head and thorax (30).

Statistical analysis

The sample size for the cross-sectional survey was calculated for a precision of 1.5% and a confidence interval (CI) of 95%, with an estimated malaria prevalence of approximately 20% in the general population based on the 2010 survey (18), yielding a minimum required population to screen of 2734 participants, calculated after stratifying by age and village based on a previous population census.

For the analysis of prevalence and incidence, data were analysed using the computer package STATA (31). Data were described as frequencies and mean (standard deviation, SD) for qualitative and quantitative variables, respectively. For typically skewed quantitative variables, median (interquartile range, IQR) was also considered. Chi-squared test (or Fisher's exact test) and t-test were performed to assess differences between groups for qualitative and quantitative variables, respectively. Spearman correlations were calculated to estimate the relationship between quantitative variables. Univariable and multivariable logistic regression models were used to determine the factors that were associated with PCR positivity. For the incidence, we fitted a binomial regression model to estimate the incidence risk ratio (IRR) and the Wald 95% CI; and we employed the Mann-Kendall statistical test for trend analysis to consider for seasonal patterns present in monthly incidence time series. The significance level was set at 0.05.

For the entomological survey, data were analysed using R software version 3.4.2 (32). The proportion of each vector species in a sample of *Anopheles* mosquitoes from each site was calculated. Collections conducted over the course of one night were equivalent to one person-night. It was assumed that the number of mosquitoes landing on a collector is equivalent to the number of mosquito bites. Biting times were shown as mean frequency of mosquitoes sampled hourly from 6-7 pm (hour 1) to 5-6 am (hour 12). Sporozoite rate was quantified as the proportion of PCR-tested mosquitoes that were positive for malaria parasites. Malaria transmission intensity was expressed in terms of the nightly entomological inoculation rate (EIR). Human biting rate (HBR) was quantified determining the total number of mosquitoes collected divided by the number of nights of collection and the number of collectors. For each HLC collector, the EIR was estimated by taking the product of two quantities: the HBR and the sporozoite rate. Variation in vector composition among sampling location was tested using Chi-squared test, the non-parametric Kruskal–Wallis rank sum analysis was used to test for variation in biting rates among collection sites. The proportion of mosquitoes in indoor and

294 outdoor collections were calculated using Chi-squared analysis. As all the collectors in a village
295 had equal number of nights during which mosquitoes were collected (balanced sampling
296 effort), the frequency distribution of mosquitoes was analysed to characterize patterns of
297 spatial distribution of vectors. Significance level of all statistical tests was based on type I error
298 rate of 0.05.

299 # RESULTS

300 **Study population**

301 A total of 2,914 participants living in 696 households were included in the cross-sectional
302 survey to determine parasite prevalence. Demographic, geographic and clinical characteristics
303 are summarized in Table 1. Sex and age of the participants were representative of the Lihir
304 Islands population, with 54.5% females and a predominance of younger individuals: <5 years,
305 12.4%; 5-14 years, 20.8%; 25-34 years, 20.4%; 35-44 years, 11.4%; ≥45 years, 13.9%. Half of
306 the participants (49.2%) were living in the MIZ of Aniolam, 33.5% in the rest of Aniolam (non-
307 MIZ), 8.3% in Masahet Island, 4.9% in Mahur Island, and 4.0% in Malie Island. The type of
308 house differed across Aniolam, with less traditional houses in the MIZ compared to the non-
309 MIZ (9.3% vs 17.7%, p<0.001). Regarding population's mobility, most participants travelled to
310 other villages within Lihir Islands at least once a month (60.9%) or at least once a week
311 (44.5%). In contrast, most of them never travelled outside Lihir (60.5%), or they only did it
312 once or twice a year (34.5%). Most visited places were other nearby islands of New Ireland
313 (84.0%) and East New Britain (31.1%).

314 Table 1. Characteristics of the participants included in the prevalence survey.

Variable		N (%)
Demographic characteristics		
Sex	Male	1327 (45.5)

	Female	1587 (54.5)
Age (years)	< 5	361 (12.4)
	5 to 14	606 (20.8)
	15 to 24	615 (21.1)
	25 to 34	595 (20.4)
	35 to 44	332 (11.4)
	≥ 45	405 (13.9)
Origin[a]	Born in Lihir Islands	1850 (63.6)
	Born in other PNG regions	1061 (36.4)
Pregnant[b]	Yes	63 (5.6)
Attending school[c]	Yes	492 (61.1)
Geographic characteristics		
Geographic area in Lihir	Aniolam MIZ	1433 (49.2)
	Aniolam non-MIZ	976 (33.5)
	Malie	118 (4.0)
	Masahet	243 (8.3)
	Mahur	144 (4.9)
Type of house[d]	Permanent	1580 (54.3)
	Traditional	333 (11.4)
	Makeshift	999 (34.3)
Slept indoors previous night	Yes	2776 (95.4)
Slept under LLIN previous night	Yes	1082 (37.2)
Clinical characteristics		
Presence of fever preceding 2 weeks[a]	Yes	649 (22.3)
Temperature (ºC) mean (SD)[e]		36.4 (0.5)
Temperature (ºC) [e]	≥ 37.8	23 (0.8)
Received antimalarial the previous month	Yes	325 (11.2)
Receiving antimalarial at time of the survey	Yes	46 (1.6)

Splenomegaly[f]	Yes (Hackett grades 1-5)	71 (4.8)
Haemoglobin[e] (g/dL)	< 8.0	127 (6.8)
	≥ 8.0 to < 10.0	431 (23.0)
	≥ 10.0 to < 12.0	764 (40.7)
	≥ 12.0	555 (29.6)

Abbreviations: MIZ = mine-impacted zone, SD = Standard deviation. [a]n = 2911 (0.1 % missing), [b]n = 1116 (0 % missing, only for females with ≥ 16 years-old), [c]n = 805 (0 % missing, only for participants between 5-18 years-old), [d]n = 2912 (0.1 % missing), [e]n = 2884 (1.0 % missing), [f]n = 1486 (49.0 % missing), [e]n = 1877 (35.6 % missing).

Of all participants, 649 (22.3%) had a history of fever in the preceding month, but only 23 (0.8%) had a documented axillary body temperature ≥37.8 ºC, and 46 (1.6%) were taking an antimalarial at recruitment. Haemoglobin levels were below 12 g/dL in 70.5% of the participants and increased with age, with a mean of 9.4 g/dL in under 5 years-old, 10.5 g/dL in 5-14 years-old, and >11.0 g/dL in all groups older than 15 years-old (p<0.0001). 6.8% of the individuals assessed were considered to have severe anaemia (haemoglobin <8.0 g/dL). The mean (±SD) haemoglobin levels were lower in the people living in the non-MIZ of Aniolam (10.8 ±2.0 g/dL), Mahur (10.8 ±1.9 g/dL) and the MIZ of Aniolam (10.9 ±2.1 g/dL), compared to those living in Masahet (11.4 ±1.9 g/dL) and Malie (11.1 ±2.2 g/dL); p=0.0194. In addition, pregnant women had lower haemoglobin levels than their non-pregnant child bearing age counterparts (9.6 g/dL vs 10.6 g/dL, p=0.0005). On the other hand, 71 (4.8%) participants had an enlarged spleen, which was not associated to sex (p=0.33) or pregnancy status (p=0.31). However, prevalence of splenomegaly decreased with age, being 7.9% in participants under 5 years-old, 7.5% in 5-14 years-old, and <4.0% in all age groups older than 15 years-old (p=0.009). Splenomegaly was also more frequent in individuals living in the non-MIZ of Aniolam (7.3%) compared to the other geographic areas, where prevalence of splenomegaly was below 4% (p=0.02).

Finally, 37.2% of the participants reported having slept under an LLIN the previous night. LLIN use was more common among females compared to males (41.3% vs 32.3 %, p<0.0001), while sleeping outdoors was more frequent in males compared to females (6.7% vs 2.9%, p<0.0001). LLIN use also differed across age (p<0.0001), with children under 5 years-old being the ones using LLIN the most (49.3%). When asked on prevention measures to avoid being sick of malaria, less than half of the population (39.6%) responded that sleeping under LLIN prevents malaria.

Malaria prevalence in the Lihir Islands

Overall, 105 participants (3.6%) had blood-stages *Plasmodium* parasites detectable by RDT, 132 (4.5%) by light microscopy and 437 (15.0%) by qPCR.

RDT showed 91 (86.7%) cases of *P. falciparum* infection, 14 (13.3%) of non-*P. falciparum* species, and no cases of mixed infection. In contrast, microscopy showed 67 (50.8%) cases of *P. falciparum* infection, 51 (38.6%) of *P. vivax*, 7 (5.3%) of *P. malariae*, and 7 (5.3%) mixed infections (*P. falciparum* and *P. vivax)*. No *P. ovale* infection was detected by microscopy. Regarding the presence of gametocytes, they were detected in 23 (31.1%) of all positive *P. falciparum* samples, with a mean (IQR) concentration of 164 (45-1854) parasites/µL; and in 12 (20.7%) of all positive *P. vivax* samples, at 70 (28- 229) parasites/µL. Gametocyte prevalence considering both species was 1.2 % of the population. On the other hand, median asexual parasitaemia was 442 (147- 5313) parasites/µL in *P. falciparum* positive samples, and 164 (78-853) parasites/µL in *P. vivax* cases. Aiming to compare with a previous prevalence study performed in Aniolam, we quantified the positive cases found by microscopy in children under 5 years-old, which were 5.0% in the MIZ of Aniolam and 19.9% in the non-MIZ (p <0.0001).

On the other hand, from the 2908 samples with DNA material for qPCR analysis, species differentiation showed 162 (37.1%) cases of *P. vivax* infection, 151 (34.6%) of *P. falciparum*, 13 (3.0%) of *P. malariae,* 1 (0.2 %) of *P. ovale,* and 107 (24.5%) of mixed infection. Mixed

infections included mostly two species, being 83 cases positive for *P. falciparum* and *P. vivax*, 15 for *P. falciparum* and *P. malariae*, 2 for *P. falciparum* and *P. ovale*, and 2 for *P. vivax* and *P. malariae*. In addition, there were 2 cases of a triple combination of species, which included *P. falciparum, P. vivax* and *P. malariae*; and 1 infection that included the four species. Positive cases for *P. falciparum*, *P. vivax* or mixed infections detected by qPCR compared with microscopy and age distribution are shown in Figure 1.

FIGURE CAPTION:

Figure 1. *Plasmodium* species detected in the cross-sectional survey.

Legend: (A) Number of *Plasmodium* infections detected by qPCR stratified by age group and species; (B) Prevalence of *P. falciparum* infections by age groups (light microscopy and qPCR); (C) Prevalence of *P. vivax* infections by age groups (light microscopy and qPCR); (D) Prevalence of mixed infections by age groups (light microscopy and qPCR). Prevalence data are shown in percentage and 95% confidence intervals.

Factors associated with malaria infection

Association of the population characteristics with presence of *Plasmodium* parasites by qPCR is shown in Table 2. Prevalence by qPCR was higher in males compared to females (17.6% vs 12.9%, p=0.0004), and also varied across age groups, with higher prevalence in children younger than 15 years-old (p<0.0001). On the other hand, there were more positive cases in those participants with lower haemoglobin levels (p=0.0197), with 22% of positive cases among participants with haemoglobin <8.0 g/dL, 16% in those with 8-10 g/dL, 14% in those with 10-12 g/dL, and 11.9 % in those with Hb ≥12.0 g/dL. Finally, there were more positive cases by qPCR in those participants with splenomegaly compared to those without (23.9% vs 13.1%, p=0.0095).

384 Table 2. Variables associations with *Plasmodium* infection by qPCR and logistic regression

385 models for risk factors.

Variable		qPCR positive N (%)	p-value	Univariate OR (95 % CI)	p-value	Multivariate aOR (95 % CI)[#]	
Sex[a]	Male	233 (17.6)	0.0004	reference group	0.0004	reference group	
	Female	204 (12.9)		0.69 (0.56, 0.85)		0.75 (0.60, 0.93)	
Age (years)[a]	< 5	63 (17.5)	< 0.0001	reference group	<0.0001	reference group	<
	≥ 5 to < 15	128 (21.1)		1.26 (0.90, 1.76)		1.33 (0.93, 1.89)	
	≥ 15 to < 25	102 (16.6)		0.93 (0.66, 1.32)		1.00 (0.69, 1.44)	
	≥ 25 to < 35	71 (12.0)		0.64 (0.44, 0.92)		0.67 (0.46, 1.00)	
	≥ 35 to < 45	31 (9.4)		0.49 (0.31, 0.77)		0.50 (0.31, 0.81)	
	≥ 45	42 (10.4)		0.55 (0.36, 0.83)		0.52 (0.34, 0.81)	
Origin[b]	Born in Lihir Islands	314 (17.0)	0.0001	1.55 (1.24, 1.94)	0.0001	0.96 (0.73, 1.24)	
	Born outside Lihir	123 (11.6)		reference group		reference group	
Pregnant[c]	Yes	12 (19.0)	0.0493	not included		not included	
	No	115 (10.9)					
Attending school[d]	Yes	100 (20.3)	0.7124	not included		not included	
	No	67 (21.4)					
Geographic area[a]	Aniolam MIZ	121 (8.5)	< 0.0001	reference group	< 0.0001	reference group	<
	Aniolam non-MIZ	263 (27.0)		3.99 (3.16, 5.04)		3.56 (2.72, 4.65)	
	Malie Island	17 (14.4)		1.82 (1.05, 3.14)		1.83 (1.04, 3.21)	
	Masahet Island	21 (8.6)		1.02 (0.63, 1.66)		1.13 (0.68, 1.89)	
	Mahur Island	15 (10.4)		1.26 (0.71, 2.21)		1.36 (0.75, 2.49)	
Type of house[e]	Permanent	226 (14.4)	< 0.0001	reference group	< 0.0001	reference group	
	Traditional	84 (25.4)		2.04 (1.54, 2.71)		1.65 (1.21, 2.25)	
	Makeshift	127 (12.7)		0.87 (0.69, 1.10)		0.93 (0.72, 1.19)	
	Yes	241 (23.8)	< 0.0001	2.70 (2.20, 3.32)	< 0.0001	1.94 (1.56, 2.42)	<

Living with a malaria positive[a]	No	196 (10.3)		reference group		reference group
Slept indoors previous night[b]	Yes	413 (14.9)	0.3627	0.81 (0.51, 1.28)	0.3635	0.82 (0.50, 1.35)
	No	24 (17.8)		reference group		reference group
Slept under LLIN previous night[b]	Yes	169 (15.7)	0.4528	1.08 (0.88, 1.34)	0.4529	0.88 (0.70, 1.12)
	No	268 (14.7)		reference group		reference group
Travelling outside Lihir[b]	Never	189 (16.6)	0.7876	not included		not included
	Once/twice year	157 (15.7)				
	3 to 5 times/year	13 (14.4)				
	Once per month	7 (16.7)				
	Once per week	1 (20.0)				
	> Once per week	2 (25.0)				
Temperature (ºC)[f]	< 37.8	434 (15.2)	0.5623	not included		not included
	≥ 37.8	2 (8.7)				
Haemoglobin (g/dL)[g]	< 8.0	28 (22.0)	0.0197	not included		not included
	≥ 8.0 to < 10.0	69 (16.0)				
	≥ 10.0 to < 12.0	107 (14.0)				
	≥ 12.0	66 (11.9)				
Splenomegaly[h]	Yes (Hackett 1-5)	17 (23.9)	0.0095	not included		not included
	No	185 (13.1)				

386 Abbreviations: aOR=adjusted odds ratio, MIZ=mine-impacted zone, OR=odds ratio, PNG=Papua New
387 Guinea, qPCR=quantitative polymerase chain reaction. [#]Multivariable analysis conducted for 2906
388 observations. [a]N=2908 (0% missing), [b]n=2905 (0.1% missing), [c]n=1114 (0.1% missing, only for females
389 with ≥16 years-old), [d]n=805 (0% missing, only for participants between ≥5 and ≤18 years-old),[e]n=2906
390 (0.1% missing), [f]n=2878 (1.0% missing), [g]n=1877 (35.5% missing), [h]n=1481 (49.1% missing).

391

392 Regarding the geographic distribution, prevalence of both microscopic and sub-microscopic
393 infections varied across areas (p<0.0001). The lowest prevalence was found in the MIZ of

Aniolam (8.5%) and in Masahet Island (8.6%), followed by Mahur Island (10.4%), Malie Island (14.4%) and the non-MIZ of Aniolam (27.0%). Of note, we found a highly heterogeneous malaria prevalence across villages and administrative divisions, especially in the non-MIZ, ranging from 12.4% in the south-western coast to 38.9% in the north-western coast of Aniolam. Distribution of positive cases by qPCR across all administrative divisions is shown in Figure 2A.

FIGURE CAPTION:

Figure 2. Malaria prevalence and incidence in each geographic area of the Lihir Islands.
Legend: (A) Malaria prevalence by qPCR presented in percentages, with the denominator being the number of participants included in each area; (B) Incidence rates presented in annual cases per 1,000 inhabitants, with the denominator being the total population of each area (population census conducted in 2018-2020). In both maps, areas are divided by wards (administrative divisions), and the mine impacted zone comprises wards 1, 2 and 11 (area indicated by the thick black line).

We performed uni- and multivariate logistic regression analyses to identify the risk associated with being positive for *Plasmodium* parasites by qPCR (Table 2). Haemoglobin levels, splenomegaly and other clinical variables were excluded of the analysis. Females had a decreased risk of infection, with an OR of 0.69 (95%CI: 0.56, 0.85). Adults above 25 years-old also had a decreased risk of infection [OR = 0.64 (95%CI: 0.44, 0.92) for those between 25 and 34 years-old; OR = 0.49 (95% CI: 0.31, 0.77) for those between 35 and 44 years-old; and OR = 0.55 (95% CI: 0.36, 0.83) for ≥ 45 years-old individuals]. In addition, living with at least one other member infected by malaria increased risk of infection, with an OR of 2.7 (95% CI: 2.20, 3.32). On the other hand, we found an increased risk of infection for individuals living in the non-MIZ of Aniolam [OR=3.99 (95% CI: 3.16, 5.04)] and for those living in Malie [OR=1.82 (95% CI: 1.05, 3.14)]. Participants born in Lihir Islands were also more frequently infected [OR = 1.55 (95% CI: 1.24, 1.94)], as well as those living in traditional houses [OR = 2.04 (95% CI: 1.54,

2.71)]. Surprisingly, individuals that had slept indoors or had slept under LLIN the preceding night did not appear to show a decreased risk of malaria infection. In the multivariate analysis, the strongest independent risk factor for carrying *Plasmodium* parasites was the geographic area, with the highest risk in the non-MIZ of Aniolam, presenting an adjusted OR (aOR) of 3.56 (95%CI: 2.72, 4.65). Sex was also independently associated with parasite prevalence, with an aOR of 0.75 (95% CI: 0.60, 0.93) in females. Risk of malaria infection also decreased with age older than 35 years-old (p<0.0001). Finally, other independent risk factors associated with parasite prevalence by qPCR were living with a malaria positive individual [aOR = 1.94 (95 % CI: 1.56, 2.42; p < 0.0001], and living in a traditional type of house [aOR = 1.65 (95 % CI: 1.21, 2.25; p = 0.0017].

Passive case detection and malaria incidence in the Lihir Islands

In 2019, a total of 18,419 patients were attended in the Lihirian health facilities with suspected malaria, of whom 9,207 were considered positive. Of the positive, 7,774 (84.4%) were diagnosed by RDT, 1,377 (15.0%) by microscopy, and 56 (0.6%) based solely on clinical grounds, without using diagnostic tools. Considering only the confirmed cases, malaria incidence in Lihir was 345 cases per 1,000 inhabitants in 2019, with an annual blood examination rate of 69.2%.

Incidence was similar across months (p=0.054 for the Mann-Kendall test), with a mean (±SD) of 767.3 (±169.7) cases per month. Similar to parasite prevalence, incidence varied substantially by geographic areas (p<0.001), with higher incidence in Malie Island (828/1000), followed by Mahur Island (712/1000), the non-MIZ of Aniolam (596/1000), and being substantially lower in the MIZ of Aniolam (142/1000) and Masahet Island (116/1000). Malaria incidence across all administrative divisions is shown in Figure 2B. In addition, incidence varied across age (p<0.001), with 53.6% of malaria cases occurring in children below 15 years-old (Supporting

material S1). Incidence rates and incidence risk ratios between the different geographic areas and age groups is shown in Supplementary Table 1 (Supporting material S2).

Incidence of each *Plasmodium spp.* varied depending on the diagnostic tool used. Microscopy is exclusively available in the MIZ, at the Lihir Medical Center, where slide positivity rate was 36.7% (total of 3,752 blood slides examined). From all positive blood slides, 51.3% were positive for *P. falciparum*, 26.8% for *P. vivax*, 3.1 % for *P. malariae* or *P. ovale,* and 18.8% were mixed infections. On the other hand, the rest of health facilities relay on RDT for malaria diagnosis. RDT positivity rate was of 55.5% (total of 13,851 tests performed). From all positive RDT, 24.9% were positive for *P. falciparum*, 35.1 % for non-*P. falciparum* species, and 40% were mixed infections. A comparison of diagnosed *Plasmodium* species by microscopy and RDT is shown in Supporting material S1.

Vector composition, larval habitats and biting behaviour

A total of 2,549 *Anopheles* mosquitoes were collected with the HLC at the eight study sites. Of these, 2034 (79.8%) were *An. farauti,* 448 (17.5%) were *An. punctulatus,* 61 (2.4%) were *An. Longirostris*, 2 (0.08 %) were *An. Koliensis,* and 1 (0.04%) was *An. bancroftii*. There were 3 (0.1%) *Anopheles* mosquitoes collected whereby the species could not be ascertained by qPCR. Vector species across the eight study sites are shown in Figure 3A. Of note, there was only 1 Anopheline specimen collected in Masahet, and it was identified as *An. farauti.* Interestingly, the species distribution differed across geographic areas ($p<0.0001$), being *An. farauti* the predominant vector in the MIZ of Aniolam (94.2%, 95% CI: 88.0, 97.3), in Malie Island (100%, 95 % CI: 89.9, 100.0), and in Masahet Island (100%, 95% CI: 5.1-100). In contrast, *An. punctulatus* was the predominant species in the non-MIZ of Aniolam (75.7%, 95% CI: 72.1-79.0), followed by *An. farauti* (13.0%, 95% CI: 10.5-16.0) and *An. longirostris* (10.4 %, 95% CI: 8.2-13.2).

FIGURE CAPTION:

Figure 3. *Anopheles* species and larval habitats in the Lihir Islands.

Legend: (A) proportions of each species of *Anopheles* found by human landing catches reported by qPCR; and (B) proportion of *Anopheles* positive larval habitats after entomological identification of larvae grown into adults in the insectary.

Regarding *Anopheles* breeding sites, a total of 976 potential larval habitats were surveyed at ten entomological sites (the same eight sites than for the adult mosquito population plus two extra sites), of which 92 (9.4%) were positive for *Anopheles* larvae. Distribution of the larval habitats surveyed and those positive for *Anopheles* species is shown in Figure 3B. Proportion of habitats positive for *Anopheles* larvae varied across geographic areas ($p<0.0001$), with Malie Island having the highest (29.5%), followed by the non-MIZ of Aniolam (14.2%), the MIZ (5.9%) and Masahet Island (3.9%). The most frequently habitat for *Anopheles* larvae was the permanent groundwater, with 25% of the sampled wells positive for *Anopheles* species, followed by transient puddles (16.7%), and forest swamps (15.4%). *Anopheles* species present in each surveyed habitat is shown in Supplementary Table 2 (Supporting material S2).

Regarding biting behaviour of *Anopheles* mosquitoes, we analysed human biting rate (HBR), peak-biting time and whether they were biting indoors or outdoors, for the three main species identified. Overall HBR amongst all *Anopheles* collected (any species) varied significantly across areas ($p<0.0001$), with a mean of 46.5 bites per person-night in Malie Island, 6.5 bites per person-night in the non-MIZ, and a mean of 1.2 bites per person-night in the MIZ of Aniolam. Mean numbers of *An. farauti* captured across night hours in the different geographic areas of Lihir Islands are shown in Figure 4A. *An. farauti* HBR were also different across areas ($p<0.0001$), with a mean of 46.5 bites per person-night observed in Malie Island, 1.1 bites per person-night in the MIZ, and 0.8 bites per person-night in the non-MIZ of Aniolam. Interestingly, peak-biting time observed in Malie was 6-7pm with 34 bites per person-hour, and gradually decreased throughout the night. In contrast, *An. farauti* bitted later in the night

in the other areas, with a peak of 2.3 bites per person-hour at 10-11pm and at 2-3am in the non-MIZ of Aniolam, and of 1.5 bites per person-hour at 9-10pm in the MIZ. Finally, *An. farauti* fed predominantly outdoors, with an overall 73% of mosquitoes collected in the outdoor HLC. Biting behaviour of *An. farauti* in Masahet Island could not be studied given a single mosquito was collected. On the other hand, mean numbers of *An. punctulatus* and *An. longirostris* captured across night hours in the non-MIZ of Aniolam are shown in Figure 4B. These species were absent in the samples collected in Malie and Masahet Islands, or with a very low abundance in the MIZ, precluding the study of their behaviour in these areas. In the non-MIZ of Aniolam, HBR of *An. punctulatus* was 4.9 bites per person-night, with a peak of 6.1 bites per person-hour at 6-7pm and a similar peak at 9-10pm. On the other hand, *An. longirostris* showed the lowest HBR at 0.7 bites per person-night, with a peak of 3 bites per person-hour at 9-10pm, and another one of 4.7 bites per person-hour at 0-1am. Finally, *An. punctulatus* and *An. longirostris* also fed predominantly outdoors, with an overall 75% and 74% of mosquitoes collected in the outdoor HLC, respectively.

FIGURE CAPTION:

Figure 4. Anopheles species biting behaviour in Lihir Islands.

Legend: (A) mean human biting rate (HBR) per hour of *An. farauti* mosquitoes within the different geographical areas, and (B) mean human biting rate (HBR) An. punctulatus and An. longirostris within the non-Mine-impacted area of Aniolam.

Sporozoite rates and entomological inoculation rates

A total of 1085 *Anopheles* mosquitoes were tested for *Plasmodium* species by qPCR, which included 845 *An. farauti*, 196 *An. punctulatus*, 42 *An. longirostris*, and 2 *An. koliensis* specimens. From these, a total of 84 (7.7%) mosquitoes tested positive with qPCR for *Plasmodium* parasites. Overall, the sporozoite rate was 0.051 (95%CI: 0.038,0.064) for *P. vivax*, 0.022 (95%CI: 0.013,0.031) for *P. falciparum,* and 0.005 (95%CI: 0.001,0.009) for mixed

infections containing both *Plasmodium* species. Sporozoite rates across *Anopheles* species and geographical area are shown in Table 3.

Table 3. Sporozoite rates and entomological inoculation rates in each Lihir area.

Collection site	**Vector**	**All *Plasmodium* species**		***Plasmodium falciparum***		***Plasmodium vivax***	
		SR (95% CI)	**EIR (95% CI)**	**SR (95% CI)**	**EIR (95% CI)**	**SR (95% CI)**	**EIR (95% CI)**
Aniolam MIZ	*An. farauti*	0.183 (0.099, 0.267)	0.208 (0.113, 0.304)	0.037 (0.000, 0.077)	0.042 (0.000, 0.088)	0.134 (0.060, 0.208)	0.153 (0.069, 0.237)
	All *Anopheles*	0.183 (0.099, 0.267)	0.221 (0.120, 0.322)	0.037 (0.000, 0.077)	0.044 (0.000, 0.093)	0.134 (0.060, 0.208)	0.162 (0.073, 0.251)
Aniolam non-MIZ	*An. farauti*	0.187 (0.052, 0.323)	0.158 (0.044, 0.273)	0.031 (0.000, 0.091)	0.026 (0.000, 0.077)	0.156 (0.030, 0.282)	0.132 (0.026, 0.238)
	An. punctulatus	0.112 (0.068, 0.156)	0.552 (0.335, 0.770)	0 (0.000, 0.000)	0 (0.000, 0.000)	0.107 (0.064, 0.150)	0.527 (0.314, 0.741)
	An. longirostris	0.119 (0.021, 0.217)	0.081 (0.014, 0.147)	0 (0.000, 0.000)	0 (0.000, 0.000)	0.119 (0.021, 0.217)	0.081 (0.014, 0.147)
	All *Anopheles*	0.121 (0.082, 0.160)	0.789 (0.536, 1.041)	0.004 (0.000, 0.011)	0.024 (0.000, 0.071)	0.114 (0.076, 0.152)	0.741 (0.495, 0.986)
Malie	*An. farauti*	0.049 (0.034, 0.065)	2.289 (1.560, 3.018)	0.027 (0.016, 0.039)	1.272 (0.722, 1.821)	0.018 (0.008, 0.027)	0.827 (0.381, 1.272)
	All *Anopheles*	0.049 (0.034, 0.065)	2.289 (1.560, 3.018)	0.027 (0.016, 0.039)	1.272 (0.722, 1.821)	0.018 (0.008, 0.027)	0.827 (0.381, 1.272)

Legend: Sporozoite rate (SR, percentage of sporozoite infected mosquitoes) and entomological inoculation rate (EIR, percentage of infective bites per person-night) are shown in proportions and 95% confidence intervals (CI) per each of the species of *Anopheles* collected, *Plasmodium* species and geographical areas in Lihir Islands. Masahet was excluded from this analysis as only 1 *Anopheles* was collected. Abbreviations: MIZ = mine-impacted zone.

Regarding the distribution of malaria parasites across mosquito species, *An. farauti* carried a similar proportion of sporozoites from both *P. falciparum* and *P. vivax*, presenting a sporozoite rate of 0.034 (95%CI: 0.022,0.047) for *P. vivax*, 0.028 (95%CI: 0.017,0.040) for *P. falciparum*, and 0.005 (95%CI: 0.002,0.009) for mixed infections. In contrast, *An. punctulatus* carried mostly *P. vivax* parasites with a sporozoite rate of 0.107 (95%CI: 0.064, 0.150), and only 0.005 (95%CI: 0.000, 0.015) for mixed infections; no *An. punctulatus* carried *P. falciparum* only. Only

P. vivax sporozoites were detected in *An. longirostris* with a proportion of 0.119 (95%CI: 0.021, 0.217). No *Plasmodium* parasite was found in the *An. koliensis* tested. On the other hand, overall sporozoite rate varied across geographical areas (p<0.0001), with a proportion of *Anopheles* positive for any *Plasmodium* species of 0.183 (95%CI: 0.099, 0.267) in the MIZ of Aniolam, 0.121 (95%CI: 0.083, 0.160) in the non-MIZ, and 0.049 (95%CI: 0.034, 0.065) in Malie.

The EIR for *Plasmodium* species varied across geographic areas. The EIR of *Plasmodium* parasites by any *Anopheles* species was the highest in Malie Island, with 2.289 (95%CI: 1.560, 3.018) infective bites per person-night, followed by the non-MIZ with 0.789 (95%CI: 0.536, 1.041) infective bites per person-night, and the MIZ of Aniolam with 0.221 (95%CI: 0.120, 0.322) infective bites per person-night. Regarding parasite species, EIR of *P. falciparum* by any *Anopheles* species was also highest in Malie, with 1.272 (95%CI: 0.722, 1.821) infective bites per person-night, followed by the MIZ with 0.044 (95%CI: 0.000, 0.093) per person-night, and the non-MIZ of Aniolam with 0.024 (95%CI: 0.000, 0.071) per person-night. In contrast, the EIR of *P. vivax* by any *Anopheles* species was similar in Malie with 0.827 (95%CI: 0.381, 1.272) infective bites per person-night, and in the non-MIZ of Aniolam with 0.741 (95%CI: 0.495, 0.986) per person-night, and lower in the MIZ of Aniolam (0.162 per person-night; 95%CI: 0.073, 0.251). EIR across geographical areas, *Plasmodium* species and *Anopheles* species are shown in Table 3.

DISCUSSION

This study characterizes the human and vector determinants behind the different intensities of malaria transmission on the Lihir Islands of PNG, which are influenced by a gold mining operation. The MIZ of Aniolam, which previously exhibited lower prevalence (18), maintained a reduced burden with similar prevalence rates when assessed by microscopy. However, there has been a three-fold decrease in the incidence of the infection in this area in the last 10 years. In contrast, the non-MIZ of Aniolam and Malie Island exhibited significantly higher infection

559 rates, with prevalence and incidence comparable to the highest endemicity areas in PNG such
560 as the East Sepik (33). In fact, the north-western zone of Aniolam showed prevalence rates
561 similar to those observed in heavily malaria-burdened countries in Africa (5). In this context,
562 the study population, especially children, presented characteristics associated with high
563 malaria exposure such as moderate and severe anaemia, and splenomegaly (34). These clinical
564 characteristics were more common outside the MIZ and were associated with carrying malaria
565 parasites. Indeed, the strongest independent risk factor for carrying malaria parasites was the
566 geographic location of the participants' households.

567 Notably, as this study was performed at the household level, we were able to demonstrate
568 that after geographic location, sharing the household with a positive individual was the
569 strongest risk factor for malaria infection. This was also the case in other studies conducted in
570 Equatorial Guinea and Kenya (35, 36). In addition, living in a traditional house was also
571 associated with higher risk of infection, attributed to the open housing structures inherent in
572 the traditional dwellings, which are known to exacerbate malaria transmission (37). Of note,
573 use of LLIN was low across Lihir Islands, with only 37.2% of the participants sleeping under a
574 LLIN the previous night. This was a higher percentage compared to a previous study that
575 reported a mere 13.6% of LLIN use (19). However, despite the increased usage, LLINs remained
576 insufficient since we did not observe any association between their use and prevalence of
577 infection.

578 *P. falciparum* and *P. vivax* were the most prevalent species, both in the active and passive case
579 detection analyses, with mixed infections detected in a quarter of the positive cases when
580 using qPCR. These findings are similar to the reported in other places of PNG and in the Pacific
581 (38, 39). Minor species, *P. malariae* and *P. ovale*, were also present and better detected by
582 qPCR, like previously described in the Sepik Province, where prevalence of *P. ovale* increased
583 from 0% to 4.8%, and prevalence of *P. malariae* from 3.9% to 13.4% when using qPCR (40).

584 Interestingly, *P. vivax* and mixed infections uncovered with qPCR, were more frequent in
585 children. A previous study conducted in PNG demonstrated that this higher rate of *P. vivax*
586 sub-microscopic infections in this population is attributable to relapses (41). Of note, we found
587 few disagreements between microscopy and RDT in the prevalence survey. The excess in *P.*
588 *falciparum* infections detected by RDT were related to individuals undergoing antimalarial
589 treatment; while the lower non-*falciparum* infections were attributed to a lower RDT
590 sensitivity in detecting these species compared to microscopy (42). In addition, in the
591 incidence data, mixed infections were higher by RDT when compared to microscopy. Although
592 microscopists can miss few mixed infections in case of low *P. vivax* densities, false positivity of
593 RDT with *P. falciparum* high densities are well known (43).

594 The entomology survey confirmed an outdoor and early biting feeding behaviour for all species
595 found (15). Although a nationwide study showed no increase in the proportion of infective
596 bites occurring before 22:00 h (16), a posterior study conducted in Madang Province
597 demonstrated that earlier feeding behaviour of *An. farauti* was epidemiologically significant
598 (44). This finding challenges the effectiveness of universal coverage with LLINs as the main, and
599 often solely, vector control strategy in our setting; since people in PNG spend a significant
600 portion of their evenings and early nights engaged in outdoor activities without any mosquito
601 protection (37). This is an issue observed in other countries in the Pacific Region (45),
602 highlighting the need to innovate in vector control strategies to target these species bound to
603 cause residual transmission. In regards of the malaria species transmitted, the sporozoite rates
604 for the *Plasmodium* species were different in each geographic area. In Aniolam, both MIZ and
605 non-MIZ, the sporozoite rates for *P. vivax* infected mosquitoes were higher than for *P.*
606 *falciparum*, for all species of *Anopheles*. By contrast, in Malie, infected *An. farauti* showed
607 higher *P. falciparum* sporozoite rates. Although in PNG *P. falciparum* is more efficiently
608 transmitted than *P. vivax,* all the *Anopheles* species detected in Lihir are able to transmit both

609 species (46). Hence, these differences between areas, could be explained by the difference in
610 circulating parasites with infective capacity amongst the human population.

611 Moreover, vector composition, proportion of larvae colonized sites, and transmission
612 intensities were also different across the areas, and in accordance to the same variations seen
613 in malaria burden. Although the open-pit mine and the abundant human dwellings in the MIZ
614 of Aniolam, larvae colonized sites and HBR are low. This might be explained by the specific
615 vector control implemented by the mine (47), or alternatively by the better housing conditions
616 of this area (48). Interestingly, Malie Island has the highest HBR and EIR of Lihir Islands, and it
617 is the place with most breeding sites colonized by Anophelines. The numerous mangroves in
618 this area may explain the highest density of *An. farauti*, as this mosquito breads on brackish
619 water and coastal streams (49). On contrary, the non-MIZ of Aniolam exhibits a diverse
620 mosquito ecology with *An. punctulatus* as the main vector, which is considered a more
621 efficient (50) and highly anthropophilic vector (51). A study conducted in 2001 attributed this
622 finding to the presence of small temporary pools along the roads created by the mine (52). The
623 whole of Aniolam has suffered important environmental changes and the road has been
624 extended, which could justify the predominant presence of *An. punctulatus* out of the MIZ.
625 Surprisingly, Masahet Island, which only employs LLIN for vector control, showed the lowest
626 mosquito and larvae densities, with a lower human burden as well. All Masahet's villages are
627 located along the coastal line, and residents keep the cattle fenced several metres away from
628 the houses, a notable contrast to other villages in the Lihir Islands. A link between proximity of
629 cattle to human dwellings and a higher risk of infection has been showed before (53). Hence,
630 this distinct behaviour could explain the difference in vector densities, especially when the
631 only vector identified in this Island was *An. farauti*, a highly zoophilic mosquito (51).

632 Limitations for this study are those related to a cross-sectional survey, as the prevalence has
633 been captured only once and we did not consider climatic patterns; however, incidence

recorded in Lihir in 2019 did not shown variations during the year. Finally, sporozoite rates were only assessed in three of the entomological sites; and although this helped to understand differences in sporozoite rates and EIR between three different geographic areas, it may be insufficient to generalize the infective data to the whole of Lihir.

Identifying human and vector factors for malaria risk serves for locating areas of concentrated malaria transmission and directing specific measures towards reducing burden (54). The prevalence and incidence data on *P. vivax* infections, especially among children, highlight the need to improve the implementation of radical cure to prevent relapses and maximize the reduction of transmission, as suggested by a cohort study in East Sepik (55). In fact, targeting the paediatric population is crucial in moderate to high transmission settings like Lihir, as interventions aimed to reduce transmission would have the greatest impact (56). Additionally, specific measures aimed at reducing intrahousehold transmission could be considered, such as reactive focal mass drug administration at the household level and neighbouring households; even though it has been proven to be more effective in low-endemicity settings (57). Although the reported changes in *Anopheles* biting behaviours, improving usage of LLIN is still recommended; however, decreased bioefficacy of distributed nets in PNG in the last campaigns (58) encourage the need of frequent monitoring of this strategy (59). The evidence of lower malaria burden and lower entomological metrics in Masahet and the MIZ of Aniolam, support the use of specific vector control strategies across the Lihir Islands, such as segregating cattle from the human population (60), considering the implementation of endectocidal treatments like ivermectin in cattle (61), and expanding larviciding and environmental management to reduce breeding sites beyond the MIZ (62).

CONCLUSION

The present study unveils distinct transmission patterns within the Lihir Islands, emphasizing the need for tailored strategies directed to the particularities of each area. However, given the

high permeability of these areas, inside and outside the MIZ, strengthening of existent malaria control strategies and implementation of innovative strategies in the whole group of Islands is essential towards achieving a reduction in malaria burden. This extensive examination holds the potential to guide ongoing malaria control initiatives and offers a blueprint for addressing similar challenges in New Ireland Province and other high-transmission coastal zones across the Western Pacific.

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857

858 SUPPORTING MATERIAL CAPTIONS

859 **Supporting material S1:**

860 **Supplementary Figure 1. Malaria cases diagnosed at the Lihirian health facilities.**

861 Legend: (A) Total number of malaria cases by age groups (unspecified group are adults' patients
862 without identified age); (B): *Plasmodium* species diagnosed by light microscopy and Rapid
863 Diagnostic Test in patients presented at the health facilities.

864

865 **Supporting material S2:**

866 **Supplementary Table 1. Comparison of malaria incidence risks in Lihir Islands between age**
867 **groups and geographic areas.**

868 Legend: Abbreviations: CI (Confidence Interval), IR (Incidence Risk), IRR (Incidence Risk Ratio),
869 MIZ (Mine-impacted zone). Binomial regression model was used to estimate IRR with 95% CI
870 and p-values.

871 **Supplementary Table 2.** Type of habitats where Anophelines were found in the Lihir Islands.

872 **Legend:** Abbreviations: CI (Confidence Interval), N (number), Prop (proportion).

FIGURE 1

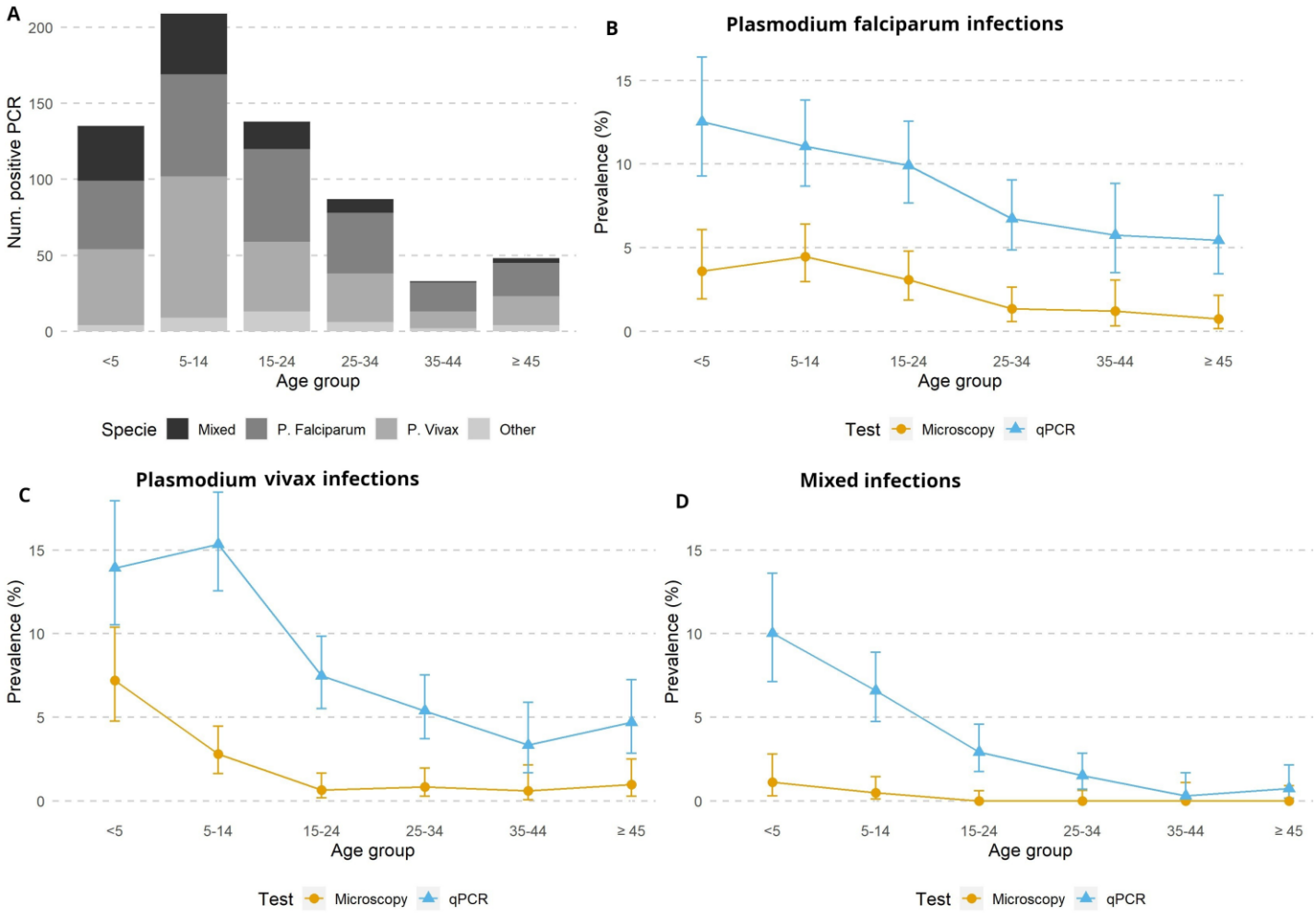

A
Num. positive PCR
200
150
100
50
0
<5
5-14
15-24
25-34
35-44
≥ 45
Age group
Specie
Mixed
P. Falciparum
P. Vivax
Other
B
Plasmodium falciparum infections
Prevalence (%)
15
10
5
0
Age group
Test
Microscopy
qPCR
C
Plasmodium vivax infections
D
Mixed infections

FIGURE 2

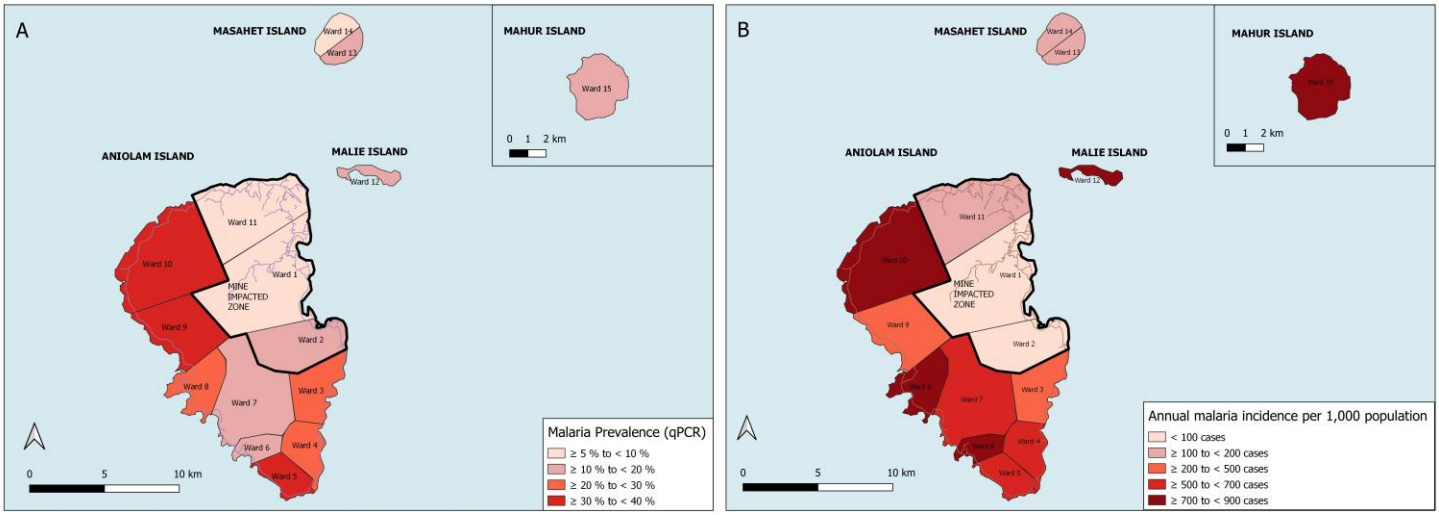


FIGURE 3

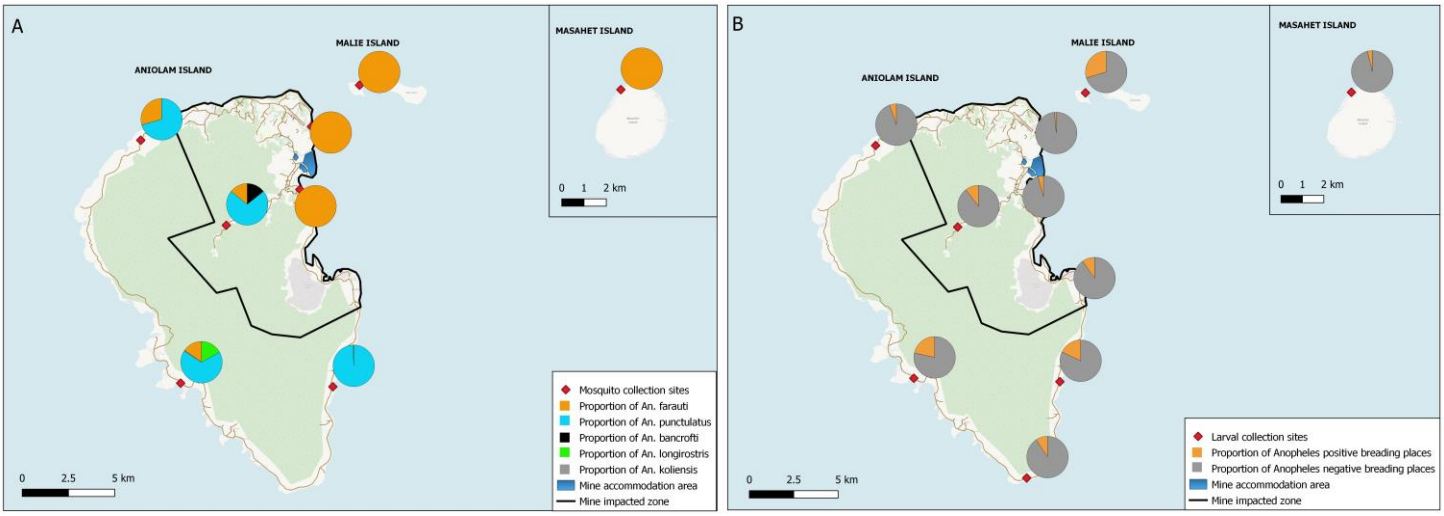
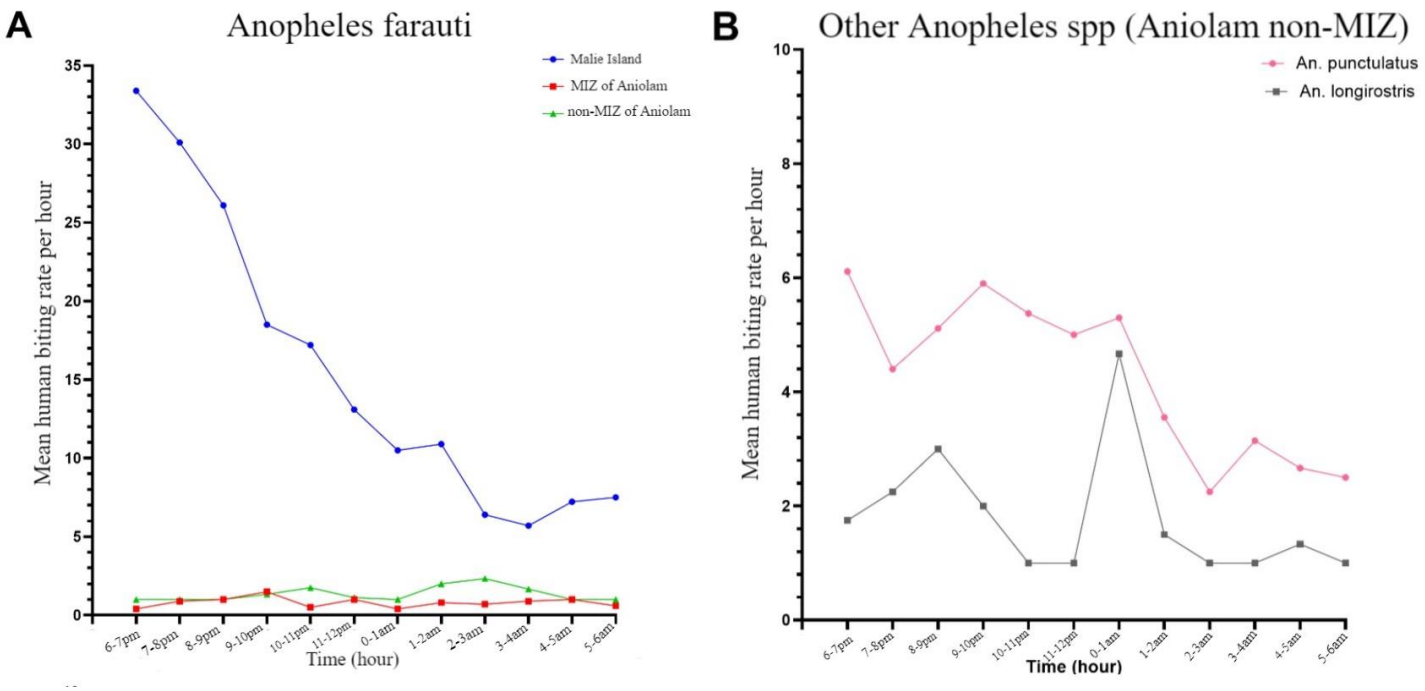


FIGURE 4


A
Anopheles farauti
Malie Island
MIZ of Aniolam
non-MIZ of Aniolam
Mean human biting rate per hour
Time (hour)
B
Other Anopheles spp (Aniolam non-MIZ)
An. punctulatus
An. longirostris
Mean human biting rate per hour
Time (hour)

Supplementary Table 1. Comparison of malaria incidence risks in Lihir Islands between age groups and geographic areas.

Variable		Incidence/1000 inhabitants	IR/1000 inhabitants (95% CI)	IRR (95% CI)	p-value
Age (years)	< 4	780	777.6 (760.4 to 794.2)	Reference	Reference
	≥ 4 to < 9	440	435.6 (420.8 to 450.4)	0.56 (0.54 to 0.58)	<0.001
	≥ 9 to < 15	450	460.0 (440.7 to 479.4)	0.59 (0.56 to 0.62)	<0.001
	≥ 15	250	249.0 (242.6 to 255.6)	0.32 (0.31 to 0.33)	<0.001
Geographic area	Aniolam MIZ	142	141.7 (135.8 to 147.7)	Reference	Reference
	Aniolam non-MIZ	596	596.4 (586.5 to 606.2)	4.21 (4.03 to 4.4)	<0.001
	Malie Island	828	827.9 (799.8 to 853.5)	5.84 (5.54 to 6.15)	<0.001
	Masahet Island	116	116.3 (101.6 to 132.2)	0.82 (0.71 to 0.94)	0.004
	Mahur Island	712	711.8 (682.0 to 740.2)	5.02 (4.74 to 5.32)	<0.001

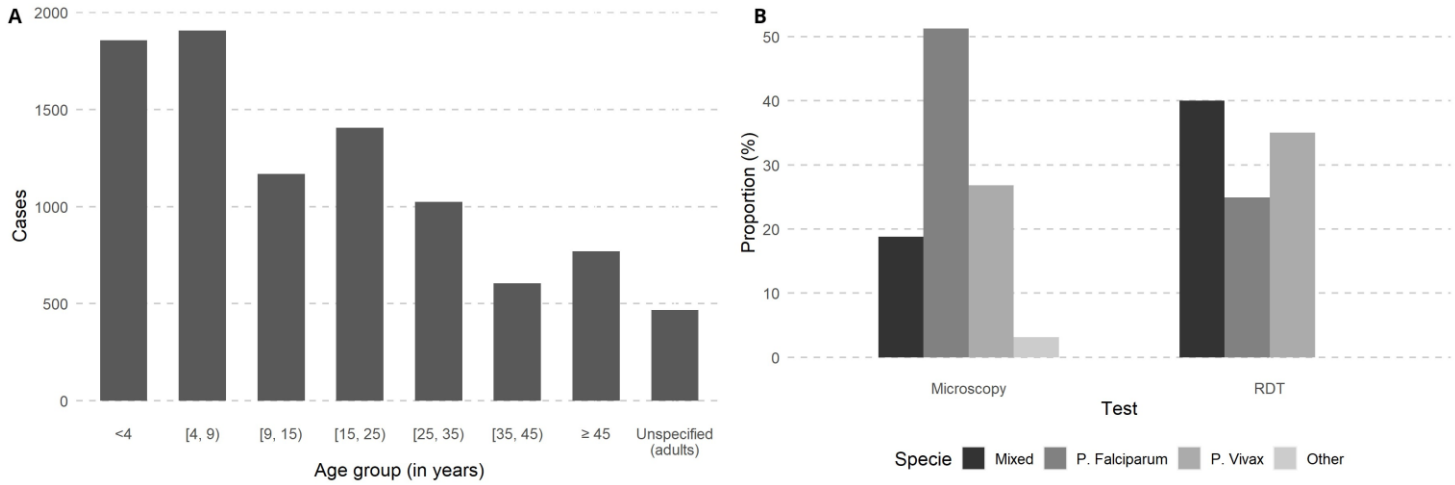
Abbreviations: CI (Confidence Interval), IR (Incidence Risk), IRR (Incidence Risk Ratio), MIZ (Mine-impacted zone). Binomial regression model was used to estimate IRR with 95% CI and p-values.

Supplementary Table 2. Type of habitats where Anophelines were found in the Lihir Islands.

Habitat type	Surveyed habitats (N)	An. farauti Prop (95% CI)	An. punctulatus Prop (95% CI)
Artificial containers	470	0.015 (0.006-0.030)	0.002 (0.0001-0.012)
Coconut shells	45	0.000	0.000
Pig wallows	45	0.022 (0.001-0.118)	0.022 (0.001-0.118)
Drainage channels	29	0.103 (0.029-0.242)	0.051 (0.006-0.173)
Forest swamps	13	0.154 (0.019-0.455)	0.000
Transient puddles	12	0.167 (0.021-0.484)	0.000
Rivers	11	0.000	0.000
Drainages	10	0.000	0.000
Permanent ground water	8	0.25 (0.032-0.651)	0.000
Tree holes	4	0.000	0.000
Coastal streams	3	0.000	0.000
Others	70	0.186 (0.103-0.297)	0.000
TOTAL	784	0.046 (0.032.-0.063)	0.024 (0.015-0.038)

Abbreviations: CI (Confidence Interval), N (number), Prop (proportion).

SUPPLEMENTARY FIGURE 1



8.2. Second article: Coverage, determinants of use and repurposing of long-lasting insecticidal nets two years after a mass distribution in Lihir Islands, Papua New Guinea: a cross-sectional study.

Malaria Journal

RESEARCH Open Access

Coverage, determinants of use and repurposing of long-lasting insecticidal nets two years after a mass distribution in Lihir Islands, Papua New Guinea: a cross-sectional study

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Abstract

Background: Universal coverage with long-lasting insecticidal nets (LLINs) is an essential component of malaria control programmes. Three-yearly mass distribution of LLINs in Papua New Guinea (PNG) has been successful in reducing infection transmission since 2009, but malaria prevalence ramped up from 2015 onwards. Although LLIN universal coverage is mostly achieved during these campaigns, it may not be related with net use over time. Uses given to LLINs and non-compliance of this strategy were evaluated.

Methods: A knowledge, attitude and practice (KAP) cross-sectional study was conducted in Lihir Islands, PNG, 2–2.5 years after the last LLIN mass distribution campaign. Data on bed net ownership, use and maintenance behaviour was collected using a household questionnaire administered by trained community volunteers. Logistic regression models were used to identify factors associated with owning at least one LLIN and sleeping under a LLIN the previous night.

Results: Among 2694 households surveyed, 27.4 % (95 % CI: 25.8–29.2) owned at least one LLIN and 8.7 % (95 % CI: 7.6–9.8) had an adequate LLIN coverage (at least one LLIN for every two people). Out of 13,595 individuals in the surveyed households, 13.6 % (95 % CI: 13.0-–4.2) reported having slept under a LLIN the preceding night. Determinants for sleeping under LLIN included living in a household with adequate LLIN coverage [adjusted OR (aOR) = 5.82 (95 % CI: 3.23–10.49)], household heads knowledge about LLINs [aOR = 16.44 (95 % CI: 8.29–32.58)], and female gender [aOR = 1.92 (95 % CI: 1.53–2.40)] (all p-values < 0.001). LLIN use decreased with older age [aOR = 0.29 (95 % CI: 0.21–0.40) for ≥ 15 year-olds, aOR = 0.38 (95 % CI: 0.27–0.55) for 5–14 year-olds] compared to < 5 year-olds (p-value < 0.001). Knowledge on the use of LLIN was good in 37.0 % of the household heads. Repurposed nets were reported serving as fishing nets (30.4 %), fruits and seedlings protection (26.6 %), covering up food (19.0 %) and bed linen (11.5 %).

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Conclusions: Two years after mass distribution, LLIN coverage and use in Lihir Islands is extremely low. Three yearly distribution campaigns may not suffice to maintain an acceptable LLIN coverage unless knowledge on maintenance and use is promoted trough educational campaigns.

Keywords: Bed net, Coverage, Long-lasting insecticidal net, LLIN, Malaria, Papua New Guinea, Repurposing, Vector control

Background

Universal coverage with insecticide-treated mosquito nets (ITNs) is recommended to achieve community-wide protection in malaria endemic areas [1]. In the last 3 years, more than 500 million ITNs have been distributed worldwide and undoubtedly contributed to malaria control efforts. However, coverage, maintenance and use of ITNs is heterogeneous and nets are sometimes misused or repurposed, reducing the efficiency of the overall strategy [2–4].

Long-lasting insecticidal nets (LLINs) have been massively distributed in Papua New Guinea (PNG) since 2004. Regular mass distribution campaigns were initiated in 2009 and are repeated every three years. According to a nationwide survey conducted in 2011 during the second mass distribution, 81.8 % of households retained at least one LLIN from the previous campaign [5]. Overall human malaria prevalence decreased from 15.7 % to 2009 to 4.8 % in 2011, although the reduction was more pronounced for *Plasmodium falciparum* than for *Plasmodium vivax* [6]. Moreover, a study conducted in selected sites in PNG, showed a steep decrease in malaria annual incidence: from 20 to 115 cases per 1000 population in 2010, to 1–79 cases per 1000 in 2014. This effect was attributed to LLIN mass coverage and use, rather than to the widespread use of highly effective artemisinin-based combination therapy [7]. Despite the initial success, PNG experienced a large increase of infections in the latest years, with an incidence that ramped up from 118.8 cases per 1000 inhabitants in 2015 to 184.5 cases per 1000 inhabitants in 2018 [8]. Consequently, in 2019, PNG accounted for 80 % of all malaria cases diagnosed in the Western Pacific Region [2, 9].

In view of the established efficacy of LLINs as a malaria control intervention, it is unclear why there was not more of an effect. There are several potential reasons, including insecticide resistance, vector behaviour change, incomplete campaign coverage and non-compliance to LLIN. *Anopheles* spp phenotypic resistance to pyrethroids, the insecticides commonly used in LLIN, is not yet present in PNG [10], ruling out a contribution of insecticide resistance to the changes observed in malaria epidemiology. In contrast, decreased bioefficacy of LLINs distributed between 2013 and 2019 has been described [11], as well as behavioural adaptation of local malaria vectors towards more frequent outdoor biting earlier in the evening [12, 13]. Lack of use, misuse or repurposing of LLINs may as well be related to the reduced efficacy of this particular malaria control strategy. Studies conducted in different countries have reported that repurposed nets served as fishing nets, gardening, or fencing [3, 4, 14–16]. The main reason for the lack of appropriate use and maintenance of LLIN are dislike and discomfort due to heat and perceived low mosquito density [15, 17, 18].

In this study LLIN ownership, maintenance and use in Lihir Islands were evaluated, two years after the 2016 mass distribution when 10,897 nets were issued and 97 % of individual coverage was achieved [19]. Misuse and misconception of villagers were also assessed as factors that may influence the effectiveness and sustainability of this vector control strategy over time.

Methods

Study setting

This study was conducted in Lihir Islands, located in the Bismarck archipelago, in New Ireland Province, PNG. New Ireland is among the PNG provinces experiencing a higher increase in malaria cases since 2015, with 426 cases per 1000 population in 2018 [20], although in previous years the province had achieved a reduction in cases comparable to other parts of the country [21, 22]. In addition, New Ireland is one of the provinces with lowest LLIN use despite high LLIN coverage according to a national indicator survey conducted in 2017 [23]. During the 2016 mass distribution campaign, 90,948 double nets were distributed by the national program in collaboration with Rotarians Against Malaria PNG (RAM), achieving a coverage ratio of 49 double-size LLINs per 100 population (97 % population coverage) [19].

Lihir islands is a group of four small islands: Aniolam (the largest, with an area of 200 km^2), and the smaller outer islands of Malie, Masahet, and Mahur. They are characterised by a tropical rainforest climate with extremely high precipitation figures all year round. There are 8 aid posts, 1 subhealth centre and 2 health centres in the islands. A gold mine located in Aniolam is the main source of employment. Employee migration from other parts of PNG, contributes to more than one third of the population on Aniolam [24]. In 2019, Lihir had a population of approximately 27,500 inhabitants and 6000

households [25]. Households can be permanent (built of bricks, with solid material in the roof and windows with glass), traditional (built of natural materials, especially wood and grass, with open windows) or makeshift (usually made with different kinds of materials from settlement areas, such as cardboard). Malaria is one of the main health issues in Lihir islands. In 2018, 11,267 confirmed malaria cases were reported (annual incidence of 478 cases per 1000 inhabitants) with minimal variation in number of cases per month.

Study design and procedures

A knowledge, attitude and practice (KAP) cross-sectional household survey was conducted between the 3rd of December of 2018 and the 25th of May of 2019 to assess ownership and use of LLIN distributed during 2016, before the following mass distribution campaign scheduled for late 2019.

The data collection was implemented by community volunteers, called Village Malaria Assistants (VMAs). They were selected by community leaders, and trained to implement malaria control activities at the village level, mainly awareness and education on malaria prevention, health-seeking behaviour and compliance with treatment. The VMA network in Lihir islands was established in 2018, after all VMAs were trained. A total of 40 VMAs worked in this study and were instructed to survey all households in their village or catchment area.

The survey was conducted in a convenient sample of 33 villages out of 40 located in the four islands of the Lihir group (see Fig. 1). Although the goal was set to survey all households in Lihir islands, reaching all households was not possible due to logistical constraints; hence, households were also selected using convenience sampling. A total of 2694 households were enrolled in the survey, which represents approximately half of the households on the Lihir islands [25].

Data collection and management

Interviews were conducted using structured questionnaires to household heads or, in its absence, to the partner or an adult permanently living in the house. A household was excluded from the study if the head of the household was not willing to participate or if no adults were available to answer the questionnaire after two separate visits.

Interviews included questions on household characteristics, demographic information of all household members (residents and long-term visitors sleeping in the same house), LLIN ownership, individual correct use of LLINs, alternative uses given to the nets, and behavioural questions related to malaria prevention. VMAs were instructed to enter the surveyed house to check the number of reported nets. Strategies to prevent malaria and alternative uses given to LLINs were asked with an open question, and household heads were prompt to list as many options as they considered relevant. After the questionnaires were collected, data were introduced into a database by two independent data clerks. See data collection tool in supplementary material, Additional file 1.

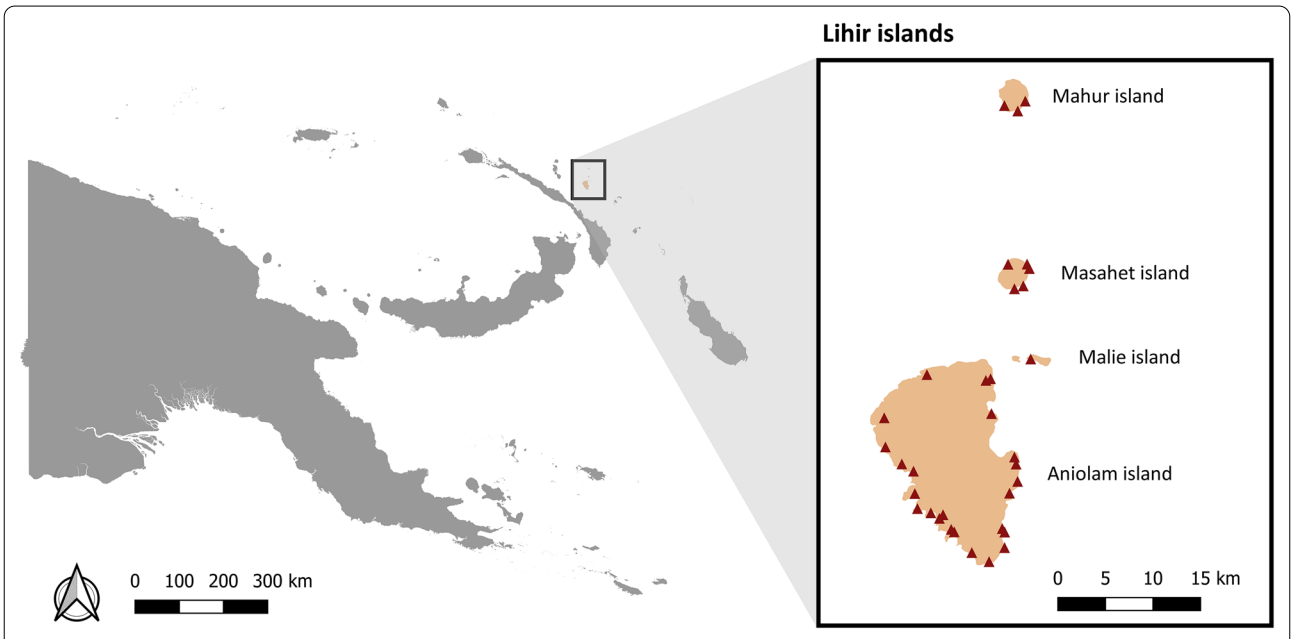

Fig. 1 Map of the surveyed villages in the Lihir islands, New Ireland province, Papua New Guinea

Statistical analysis

A sample size of 379 households was estimated to detect an unknown prevalence (50 %) of having at least 1 LLIN (conservative estimate with maximum imprecision), with a margin of error of ± 5 % and a confidence level of 95 %.

Basic characteristics of participating households and individuals were summarized using descriptive analyses. Different indicators of LLIN ownership and use were calculated following the recommendations of the Roll Back Malaria Monitoring and Evaluation Reference Group [26], including: (i) proportion of households with at least one LLIN, (ii) proportion of households with at least one LLIN for every two people, (iii) proportion of population with adequate access (≥ 1 LLIN/2 individuals) to a LLIN in their household, (iv) proportion of the population that slept under a LLIN the previous night, and (v) proportion of children under five years of age who slept under a LLIN the previous night. Formulae used are shown in supplementary material, Additional file 2. Knowledge of malaria prevention tools and alternative uses given to LLIN revealed by the head of the household were reported using descriptive analyses.

Univariable and multivariable logistic regression analyses were used to identify factors associated with maintaining at least one LLIN in the household. Both models were adjusted for village as a fixed effect parameter. Similarly, univariable and multivariable mixed-effects logistic regression analyses were used to identify factors associated with sleeping under a LLIN the previous night among individuals living in a household with at least one LLIN. These two models were adjusted for household and village as random effects parameters. We have considered village as a fixed effect in the first models and random effect in the second type of models following the recommendations of Green and Tukey [27]. Analysis results are presented as odds ratios (ORs) with 95 % confidence intervals (CIs). The strength of the evidence for the different associations was calculated using likelihood-ratio tests.

Data were analysed using Stata 16 software [28]. All graphs were drawn using Stata 16 software, and a map of the surveyed villages was created using QGIS Desktop v3.16 Hannover software [29].

Ethical considerations

The research protocol was approved by the PNG Medical Research Advisory Committee (MRAC No. 18.07). Permission to conduct this survey was also obtained from village leaders. All household heads orally consented before recording data of each household. No biological samples were collected.

Results

Study population

A total of 2694 households, with 13,595 individuals, were visited and enrolled in the survey. Characteristics of participating households and their residents are described in Table 1. Half of the houses (50.1 %) were permanent, 39.7 % were traditional, and 10.2 % were makeshift. The median number of people per household was 5 (interquartile range [IQR]: 3–7), and a large proportion of the household heads were male (76.6 %). Approximately half of the individuals living in the surveyed households were male (51.9 %), and 62.4 % of the population were aged 15 years and older. Most school-age children attended a local school (78.0 %), and only 22.6 % of the adults were employed.

LLIN ownership and use

Among the 2694 households responding the survey, 27.4 % (95 % CI: 25.8–29.2) owned at least one LLIN, 2–2.5 years after the mass distribution that took place

Table 1 Household and study population characteristics

Variable	N (%)
Household characteristics (n = 2694)	
Type of household	
Permanent	1349 (50.1)
Traditional	1069 (39.7)
Makeshift	276 (10.2)
Number of individuals per household	
Median (IQR)	5 (3–7)
Sex of the household head	
Male	2063 (76.6)
Female	631 (23.4)
Individual chracteristics (n = 13,595)	
Gender[a]	
Male	7056 (51.9)
Female	6537 (48.1)
Age [years][b]	
< 5	2088 (15.5)
5–14	2979 (22.1)
≥ 15	8432 (62.4)
Employed [if ≥ 15 years old][c]	
No	5411 (77.4)
Yes	1584 (22.6)
Studying [if 5–14 years old][d]	
No	639 (22.0)
Yes	2267 (78.0)

[a] n = 13,593 (0.01 % missing)

[b] n = 13,499 (0.7 % missing)

[c] n = 6995 (17.0 % missing)

[d] n = 2906 (2.5 % missing)

in the islands. A total of 416 households had one LLIN, 117 had two LLINs, 183 had three LLINs, and 23 had four or more LLINs. Only 8.7 % (95 % CI: 7.6–9.8) of households had at least one LLIN for every two individuals (adequate household coverage). Similarly, the percentage of people with adequate access to a LLIN within their household was estimated to be 6.7 % (95 % CI: 6.2–7.1).

Regarding LLIN use, a total of 1851 individuals [13.6 % (95 % CI: 13.0–14.2)] reported to have slept under a LLIN the previous night. Among individuals living in a household with at least one LLIN, a 46.3 % (95 % CI: 44.7–47.8) slept under a LLIN the previous night. Among individuals living in a household with adequate LLIN coverage, a 66.7 % (95 % CI: 63.6–69.8) did so. A detailed breakdown of individuals using LLIN by age and sex according to household LLIN coverage is shown in Fig. 2. Among the key population of children under five years of age, 19.9 % (95 % CI: 18.2–21.7) reported to have slept under a LLIN the previous night. The percentage of individuals reporting to have slept under a LLIN increased with LLIN availability within a household (Fig. 3).

Determinants of owning at least one LLIN

Factors associated with LLIN ownership are shown in Table 2. In the multivariable analysis, owning at least one LLIN was associated with having at least one resident aged < 5 years-old [adjusted OR (aOR) = 1.55 (95 % CI: 1.17–2.06), p-value = 0.002], having at least one resident being an adult woman [aOR = 1.82 (95 % CI: 1.04–3.16), p-value = 0.029], and with the head of the household knowing that sleeping under a LLIN prevents malaria [aOR = 30.32 (95 % CI: 21.25–43.27), p-value < 0.001].

Determinants of sleeping under LLIN

Out of 4,002 individuals that lived in a household with at least one LLIN, 46.3 % (95 % CI: 44.7–47.8) reported sleeping under a LLIN the preceding night. Table 3 shows the factors associated with LLIN use. In the multivariable analysis, sleeping under a LLIN the previous night was associated with living in a household with adequate LLIN coverage [adjusted OR (aOR) = 5.82 (95 % CI: 3.23–10.49)], head of household knowing that sleeping under a LLIN prevents from malaria [aOR = 16.44 (95 % CI: 8.29–32.58)], being female [aOR = 1.92 (95 % CI: 1.53–2.40)] and decreasing age [aOR = 0.38 (95 % CI: 0.27–0.55) for 5–14 year-olds, and aOR = 0.29 (95 % CI: 0.21–0.40)

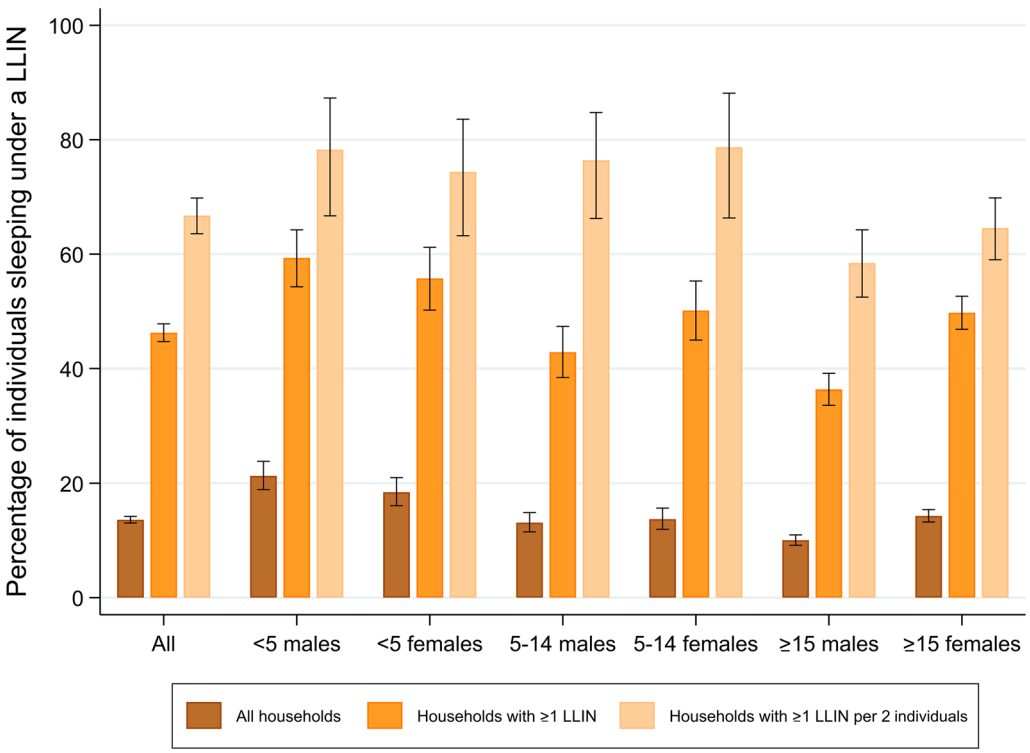

Fig. 2 Percentage of individuals reported to sleep under a LLIN the previous night by sex and age groups, among (1) all households, (2) households that have at least 1 LLIN, and (3) households with at least 1 LLIN per 2 individuals

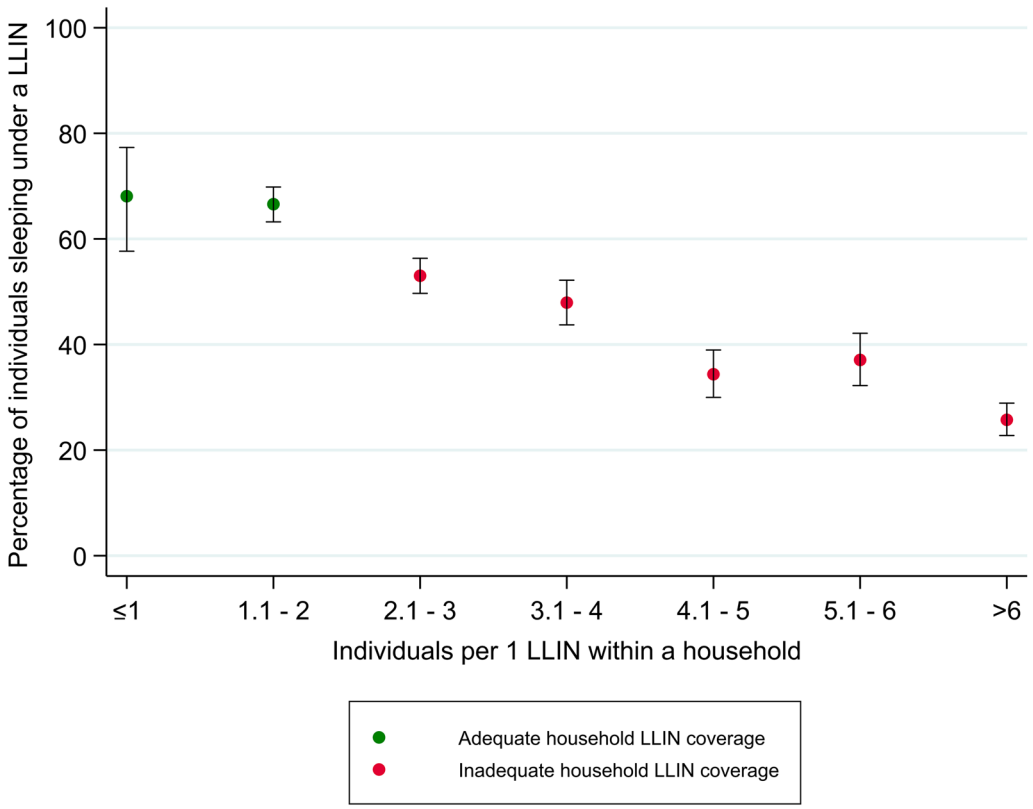

Fig. 3 Percentage of individuals reported to sleep under a LLIN the previous night by the ratio individuals:LLIN of the household, among the 739 households with at least 1 LLIN

for ≥15 year-olds, compared both to <5 year-olds] (all p-values <0.001).

Knowledge of malaria prevention tools

When the head of the household was asked about strategies to prevent malaria, 37.0% (997/2694) responded that sleeping under a LLIN was effective to prevent malaria. Most of them (1620; 60.1%) answered that strategies of environmental management are effective, including cleaning the house and removing water from gardens, and/or cleaning villages. Another frequent answer (1528; 56.7%) was that creating smoke by burning bush material was useful to prevent malaria, while the use of mosquito repellents as a malaria prevention tool was pointed out by only 12.9% of the household heads. Figure 4 shows the knowledge on malaria prevention tools by household heads.

Alternative uses of LLIN

When asked about use of LLINs, a total of 1170 (43.4%) household heads reported to have used LLINs only for the purpose of sleeping under. Many gave alternative uses (either misuse or repurposing) to the LLINs: 937 (34.8%) households provided one alternative use, 401 (14.9%) households provided two alternative uses, 127 (4.7%) provided three, and 59 (2.2%) provided four or more. The most common alternative use for LLINs was fishing (818; 30.4%), followed by protecting fruits and seedlings in gardens (716, 26.6%). Other uses given to the LLINs included covering food in the house (512, 19.0%) and using them as bed linen (310, 11.5%). Figure 5 shows the alternative uses given to the LLINs.

Discussion

This study shows a very large reduction over time on the adequate LLIN coverage in Lihir Islands, decreasing from 97% of individuals having access to LLIN after 2016 mass distribution [19] to less than 7% in 2–2.5 years (one year prior to the next mass distribution campaign). This reduction in LLIN coverage is rather striking, and significantly different to the relatively high bed net coverage maintained in previous distributions campaigns in the country. As an example, a 2011 study in selected sites in PNG showed that 88.8% of people still had access to LLIN in villages where the distribution had been conducted during the 2 years preceding the survey, whereas

Table. 2 Factors associated with household ownership of at least one LLIN (n = 2694 households)

Variable	Total households	Owning ≥ 1 LLIN (%)	Univariable aOR (95 %CI)	p-value	Multivariable aOR (95 %CI)	p-value
Type of household						
Permanent	1349	360 (26.7)	1	0.099	1	0.405
Traditional	1069	289 (27.0)	0.91 (0.74–1.13)		0.90 (0.67–1.21)	
Makeshift	276	90 (32.6)	1.34 (0.97–1.85)		1.21 (0.79–1.86)	
Number of individuals per household						
Median (IQR)	5 (3–7)	5 (4–7)	1.07 (1.03–1.12)	0.001	1.02 (0.96–1.09)	0.529
Household head knows that sleeping under a LLIN prevents malaria						
No	1697	117 (10.4)	1	< 0.001	1	< 0.001
Yes	997	562 (56.4)	24.08 (17.96–32.30)		30.32 (21.25–43.27)	
Gender of the household head						
Male	2063	555 (26.9)	1	0.690	1	0.908
Female	631	184 (29.2)	1.05(0.83–1.32)		0.98 (0.71–1.35)	
At least 1 resident aged < 5 years[a]						
No	1320	288 (21.8)	1	< 0.001	1	0.002
Yes	1347	443 (32.9)	1.70 (1.39–2.06)		1.55 (1.17–2.06)	
At least 1 resident an adult woman [> 15 years old]						
No	241	38 (15.8)	1	< 0.001	1	0.029
Yes	2453	701 (28.6)	2.35 (1.56–3.53)		1.82 (1.04–3.16)	
At least 1 resident employed [> 15 years old][b]						
No	1129	268 (23.7)	1	0.022	1	0.579
Yes	1122	340 (30.3)	1.29 (1.04–1.61)		1.08 (0.82–1.43)	
At least 1 resident studying [5–14 years old][c]						
No	1342	334 (24.9)	1	0.108	1	0.167
Yes	1315	391 (29.7)	1.17 (0.97–1.43)		0.81 (0.61–1.09)	

[a] n = 2667 (1.0 % missing)

[b] n = 2251 (16.4 % missing)

[c] n = 2657 (1.4 % missing)

coverage decreased to 67.6 % in those villages where the distribution had been done more than 2 years before the survey [5]. Lack of LLIN maintenance is a common issue in other tropical areas: in Uganda, LLIN population coverage decreased from 65 to 18 % three years after distribution [30]; and in Tanzania, households with at least one LLIN for every two people were below 30 % two years after LLIN distribution [31]. Accordingly, only less than 14 % of the total population surveyed slept under LLIN the previous night; and adequate LLIN coverage was strongly associated with LLIN use as previously reported [5, 32], confirming the importance to achieve high coverage and maintenance to sustain LLIN use.

Interestingly, while adequate LLIN coverage in PNG has improved over time, especially in the islands' region where Lihir Islands are located (increasing from 46 % to 2009 to 82 % in 2011), use of LLIN still remained poor: 25 % of individuals in this region reported sleeping under LLIN in 2009, which increased to only 40 % in 2011 despite the substantial improve in LLIN coverage [33]. In fact, the villages in the PNG islands' region are those with better LLIN coverage but worse LLIN use [23]. Hence, achieving adequate LLIN coverage during mass distribution is clearly not sufficient to ensure their use. A qualitative study in PNG found that many environmental, human and nets factors could be linked to the impediments of the adequate use of nets; however, the most important factor that reduced the use was a lack of fear of malaria infection [34]. Promoting LLIN maintenance over time is also key to enhance their use and impact on malaria transmission. Mass LLIN distribution campaigns in PNG, similar as to many other countries, achieve a high coverage in the minimum time possible using strategies that are proven to work such as use of coupons or training of distributors [35]. Including additional strategies to target issues affecting long-term coverage could enhance maintenance and use of LLIN until the following mass distribution.

Households with at least one child under 5 years-old and households with at least one adult woman had a higher odds of owning at least one LLIN similar to other studies in PNG [33]. The scale up of antenatal

Table 3 Factors associated with sleeping under a LLIN among individuals residing in households owning at least one LLIN (n = 4002 individuals)

Variable	Total individuals	Slept under a LLIN the previous night (%)	Univariable aOR (95 %CI)	p-value	Multivariable aOR (95 %CI)	p-value
Gender						
Male	2093	879 (42.0)	1	< 0.001	1	< 0.001
Female	1909	972 (50.9)	1.99 (1.64–2.41)		1.92 (1.53–2.40)	
Age [years][a]						
< 5	721	416 (57.7)	1	< 0.001	1	< 0.001
5–14	867	399 (46.0)	0.37 (0.27–0.51)		0.38 (0.27–0.55)	
≥ 15	2373	1021 (43.0)	0.32 (0.24–0.41)		0.29 (0.21–0.40)	
Type of household						
Permanent	2168	917 (42.3)	1	0.021	1	0.200
Traditional	1402	738 (52.6)	2.08 (1.26–3.42)		1.44 (0.82–2.52)	
Makeshift	432	196 (45.4)	0.99 (0.47–2.06)		0.66 (0.29–1.53)	
Household LLIN coverage						
Inadequate (< 1 LLIN per 2 individuals)	3097	1247 (40.3)	1	< 0.001	1	< 0.001
Adequate (≥ 1 LLIN per 2 individuals)	905	604 (66.7)	6.61 (3.99–10.96)		5.82 (3.23–10.49)	
Household head knows that sleeping under a LLIN prevents malaria						
No	982	211 (21.5)	1	< 0.001	1	< 0.001
Yes	3020	1640 (54.3)	20.84 (11.48–37.85)		16.44 (8.29–32.58)	
Gender of the household head						
Male	3081	1443 (46.8)	1	0.696	1	0.252
Female	921	408 (44.3)	0.89 (0.52–1.53)		0.68 (0.36–1.28)	
At least 1 resident employed [> 15 years old][b]						
No	1147	623 (54.3)	1	0.028	1	0.082
Yes	2044	924 (45.2)	0.54 (0.32–0.90)		0.62 (0.37–1.04)	
At least 1 resident studying [5–14 years old][c]						
No	1399	695 (49.7)	1	0.150	1	0.670
Yes	2528	1124 (44.5)	0.70 (0.45–1.11)		0.89 (0.53–1.49)	

[a] n = 3961 (1.0 % missing)

[b] n = 3191 (20.3 %missing)

[c] n = 3927 (1.9 %missing)

services in the country, where pregnant women are targeted for prevention strategies and receive LLIN [36], has likely contributed to increase LLIN ownership in their households and probably enhanced maintenance. Antenatal services provide awareness on the importance of pregnant women and children below 5 years old to sleep under LLIN to prevent malaria. Interestingly, gender and age were associated with LLIN use, with women being more likely to sleep under LLIN as well as the younger members of the household.

However, the key factor for LLIN ownership and use was the head of the household's knowledge about LLIN preventing from malaria infection. Only 37 % of the heads of the households reported that sleeping under a LLIN was effective to prevent malaria. These results suggest that education campaigns on malaria prevention tools targeting the heads of the households could further promote LLIN maintenance and use, as shown in previous studies [37]. Such education campaigns could be included as part of the mass LLIN distribution strategy, which could also look for the support of community leaders, pastors and other influential community members to deliver key awareness messages. In Lihir islands, the VMAs deployed an extensively education campaign at village and hamlet level during the LLIN distribution in 2019, targeting the heads of the households and involving community and church leaders. This intensive education campaign had not been

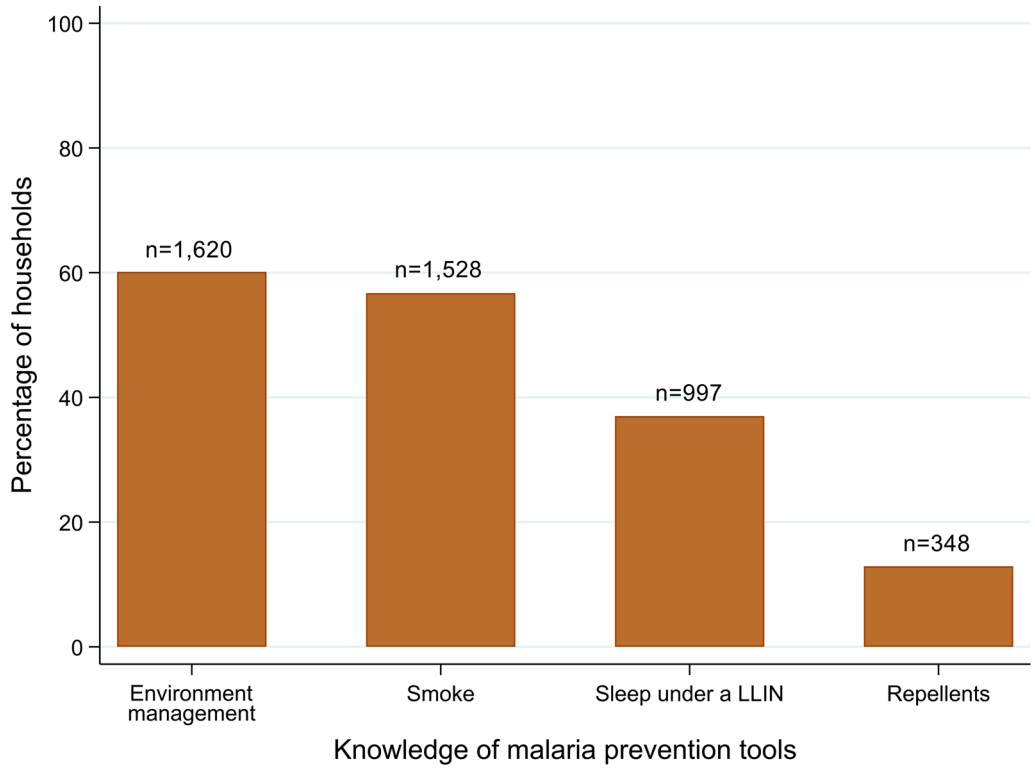

Fig. 4 Knowledge of malaria prevention tools reported by the household head (n = 2694 households)

conducted previously and it may have improved LLIN coverage, use and maintenance.

In addition, although an association between a household having at least one resident attending school and increased odds of owning one LLIN or sleeping under a LLIN was not shown, half of the households had at least one child between 5- and 14- years old attending school. Consequently, there is also a big opportunity to promote LLIN maintenance and use through frequent education campaigns in schools. In Tanzania, for example, nets were provided annually to children attending primary and secondary school, which resulted in high level of maintenance (50–75% of nets given) over the first four years of distribution, even in the absence of a mass distribution campaign [38].

On the other hand, addressing alternative uses and repurposing of LLIN could also enhance maintenance and use. In this study, half of the surveyed households admitted using LLIN for alternative purposes. The study was unable to determine if LLIN used for alternative purposes were those provided in the distribution campaign in 2016 or those remaining from the 2013 campaign. However, since most households did not retain a single LLIN, it is likely that most nets used for other purposes were those given in 2016. The most common alternative use given to LLIN was fishing, which could be related to the low LLIN maintenance and use observed in the PNG islands' region, among other factors. Another common alternative use was to protect seedlings and food. These common misuses have been also described in sub-Saharan Africa [4, 12–14] and all relate to basic needs like ensuring food supply. LLIN distribution using mass campaigns are proven to increase LLIN ownership [39–41]; however, especially when health education might not be sufficient to reduce alternative uses of LLIN when other basic needs are involved, some creative solutions could be used during mass LLIN distribution campaigns, such as facilitating access of target communities to suitable and without insecticide materials for fishing and gardening. In addition, ensuring high LLIN bioefficacy and teaching communities about the impact of LLIN in reducing mosquito population could further motivate communities to better maintain LLIN.

This study used a community approach that allowed a massive outreach to the Lihirian population but also had some limitations. Although the goal of VMAs was to survey their entire village or assigned part of a village, full coverage of all villages was not possible due to logistical constraints. Hence, it could be subjected to selection bias. However, all statistical models were adjusted

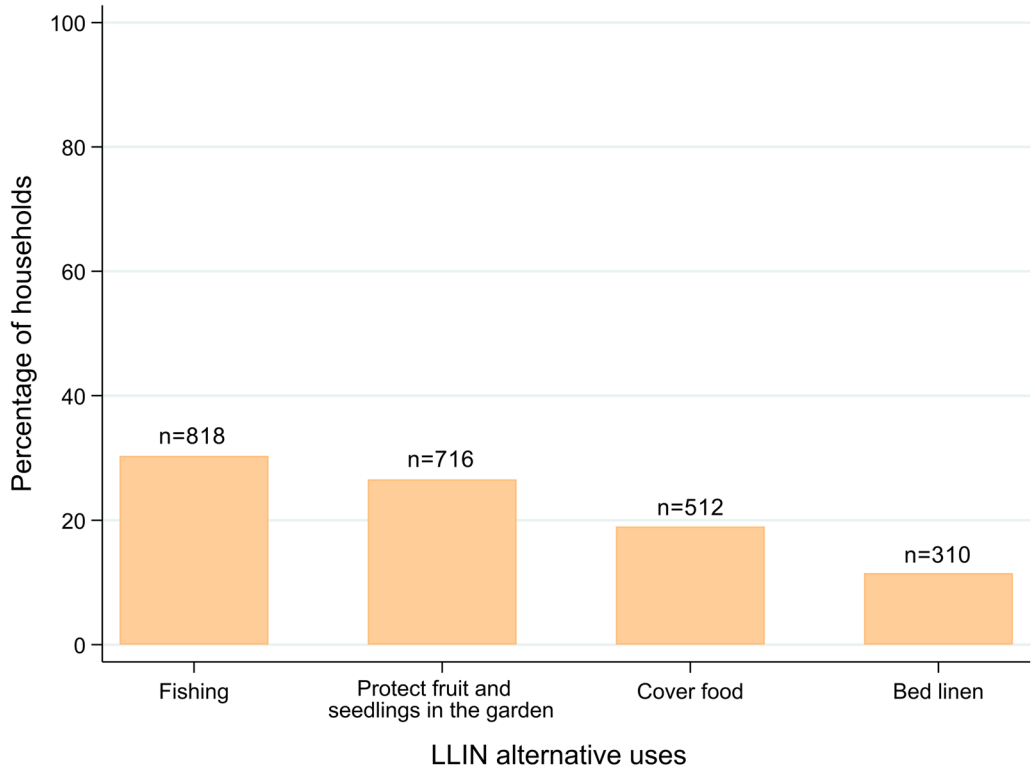

Fig. 5 Alternative uses of LLIN reported by the household head (n = 2694 households)

for village in order to minimize bias arising from the non-representativeness of a few villages. In addition, our sample size was very large and spatio-geographically and demographically representative. Because data collection was conducted by the VMAs who are living in the same village, sensitive questions such as socioeconomic factors or enquiring about pregnancy status were avoided. These two pieces of information could have given important insight, such as LLIN use during pregnancy or the role of socioeconomic status contributing to repurposing of LLIN. It was observed that, while less than 7% of individuals had adequate access to LLIN, close to 14% were reported to sleep under them, which could be due to more than two individuals sharing a double net (as commonly seen for young children) and due to the social desirability bias inherent in self-reported measures. Finally, in order to maximize quality of the results, VMAs received an intensive training and close supervision, and data were carefully reviewed to recode impossible values and minimize missing values.

Conclusions

Although mass LLIN distribution campaigns are a proven health intervention to promote LLIN ownership and use, and reduce the malaria burden, distribution every three years does not seem to be sufficient to maintain an adequate LLIN coverage in the Lihir islands, PNG. Knowledge on malaria prevention tools by household heads is a determinant factor for retention and use of LLINs, as well as strategies targeting risk groups like pregnant women and children below 5 years of age. Thus, it is extremely important to ensure education of local communities in how to use and maintain the LLINs distributed to sustain the achieved high coverage during mass distribution for as long as possible and maximize impact for malaria control. Community approaches to gather information through trained community volunteers are useful to understand and deploy public health strategies. Lack of maintenance and use of LLIN, together with reduced LLIN bioefficacy and changes in mosquito biting behaviour, might altogether explain the recent increase in malaria cases observed in PNG.

Abbreviations

aOR: Adjusted odds ratio; CI: Confidence interval; IQR: Interquartile range; ITN: Insecticide-treated net; KAP: Knowledge, attitude and practice; LLIN: Long-lasting insecticidal nets; OR: Odds ratio; PNG: Papua New Guinea; RAM: Rotarians Against Malaria; VMA: Village malaria assistant.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12936-021-03867-z.

Additional file 1: Data collection tool used by the village malaria assistants (VMA) tocollect information for this survey.

Additional file 2: Definitions of the LLIN ownership and use indicators, used for thedata analysis.

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Authors' contribution
PMM: conceptualization, methodology, supervision, data curation, writing-original draft; RG: conceptualization, methodology, supervision; NB: data curation, formal analysis, writing-review and editing; SL: methodology, supervision; SS: formal analysis; SR: investigation; AE: investigation; CW: investigation; BP: investigation; JD: investigation; ST: investigation; SK: conceptualization, methodology, writing-review and editing; ML: conceptualization, methodology, writing-review and editing; WP: conceptualization, methodology, writing-review and editing; OM: conceptualization, methodology, writing-review and editing; BB: Conceptualization, methodology, supervision, writing-review and editing, project administration; QB: Conceptualization, writing-review and editing, project administration. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Our research protocol was given ethics clearance at the PNG Medical Research Advisory Committee (MRAC No. 18.07). Permissions for this survey were also obtained from village leaders, and head of households orally consented before recording data of each household.

Consent for publication
This manuscript does not contain personal data from any individual person, hence the consent for publication is not applicable.

Competing interest
The authors declare that they have no competing interests.

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Publisher's Note

8.3. Third article: Electrocardiographic Safety of Repeated Monthly Dihydroartemisinin-Piperaquine as a Candidate for Mass Drug Administration.

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CLINICAL THERAPEUTICS

Electrocardiographic Safety of Repeated Monthly Dihydroartemisinin-Piperaquine as a Candidate for Mass Drug Administration

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ABSTRACT Mass drug administration (MDA) of sequential rounds of antimalarial drugs is being considered for use as a tool for malaria elimination. As an effective and long-acting antimalarial, dihydroartemisinin-piperaquine (DHA-PQP) appears to be suitable as a candidate for MDA. However, the absence of cardiac safety data following repeated administration hinders its use in the extended schedules proposed for MDA. We conducted an interventional study in Lihir Island, Papua New Guinea, using healthy individuals age 3 to 60 years who received a standard 3-day course of DHA-PQP on 3 consecutive months. Twelve-lead electrocardiography (ECG) readings were conducted predose and 4 h after the final dose of each month. The primary safety endpoint was QT interval correction (QTc using Fridericia's correction [QTcF]) prolongation from baseline to 4 h postdosing. We compared the difference in prolongations between the third course postdose and the first course postdose. Of 84 enrolled participants, 69 (82%) participants completed all treatment courses and ECG measurements. The average increase in QTcF was 19.6 ms (standard deviation [SD], 17.8 ms) and 17.1 ms (SD, 17.1 ms) for the first-course and third-course postdosing ECGs risk difference, −2.4 (95% confidence interval [95% CI], −6.9 to 2.1; $P = 0.285$), respectively. We recorded a QTcF prolongation of >60 ms from baseline in 3 (4.3%) and 2 (2.9%) participants after the first course and third course ($P = 1.00$), respectively. No participants had QTcF intervals of >500 ms at any time point. Three consecutive monthly courses of DHA-PQP were as safe as a single course. The absence

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of cumulative cardiotoxicity with repeated dosing supports the use of monthly DHA-PQP as part of malaria elimination strategies.

KEYWORDS cardiac safety, cardiotoxicity, dihydroartemisinin-piperaquine, electrocardiography, malaria

Malaria elimination is set to be a global health priority in coming years, and ambitious plans for scaling up from malaria control to elimination already exist in many areas of endemicity worldwide. New strategies being actively considered for interrupting malaria transmission include mass drug administration (MDA) (1). This strategy requires the treatment of entire populations with effective antimalarial drugs to reduce the human reservoir of high and low parasite density blood-stage infections, in addition to conferring posttreatment prophylaxis that prevents reinfection and relapse over a time period that significantly exceeds the life span of the anopheline vector (2). Because most individuals treated in an MDA program are asymptomatic parasite carriers or uninfected, the ideal antimalarial for MDA must (i) have a prolonged duration of effect that optimizes the period of prophylaxis against reinfection and relapse and (ii) be demonstrated to be safe when delivered in sequential repeated treatment courses in the manner proposed for MDA deployment (2).

Dihydroartemisinin-piperaquine (DHA-PQP) is a good candidate for MDA. The rapid-acting artemisinin-derivative reduces the parasite biomass of existing infections, and the long-acting PQP component exerts an especially long posttreatment prophylactic effect (3). Repeated monthly exposure to standard 3-day treatment courses of DHA-PQP over 3 consecutive months should theoretically prevent new infections for a period of at least 90 to 120 days. In a meta-analysis of 11 studies, monthly DHA-PQP for high-risk populations was associated with an 84% reduction in the incidence of malaria parasitemia (4–6).

Nevertheless, the feasibility of DHA-PQP for MDA has been questioned because PQP can cause dose-dependent prolongation of the electrocardiographic QT interval. As such, PQP is contraindicated in patients with congenital long-QT syndrome (about one in 2,500 children) or those taking other drugs that prolong the QT. Mild QT prolongation is clinically silent, but extreme prolongation can cause fatal arrhythmias, such as torsades de pointes (TdP). Prior studies have demonstrated that QTc prolongation associated with DHA-PQP is predominantly mild and not associated with clinical adverse cardiac outcomes (4, 7). However, PQP is eliminated slowly (elimination half-life, ~23 days), and the risk of QT prolongation may be exacerbated when repeated doses are given, especially if given monthly (8). Therefore, the drug manufacturer and the European Medicines Agency (EMA) recommend that repeated treatment courses of DHA-PQP (brand name Eurartesim) in 160-mg/20-mg film-coated tablets should not be administered within 2 months of initial treatment (9). Unfortunately, this is unlikely to be optimally effective if DHA-PQP is given as part of MDA; mathematical models suggest a maximum interval of 1 month between treatment rounds in order to interrupt transmission (10).

The current study aims to assess the cardiac safety of three repeated monthly courses of DHA-PQP in Lihir Island, Papua New Guinea (PNG), a population of endemicity for malaria likely to be targeted in future MDA activities.

RESULTS

Of 110 individuals screened, 84 (76.3%) individuals met the inclusion criteria and consented to participate in the study. Eighteen individuals declined consent, and 8 individuals were ineligible (first trimester pregnancy, $n = 2$; uncontrolled hypertension, $n = 2$: asymptomatic arrhythmia, $n = 2$; clinical heart failure, $n = 1$; and congenital heart disease, $n = 1$). Of the 84 participants who were initially enrolled, 69 (82.2%) participants completed all treatment courses and follow-up appointments (per-protocol population) and were included in subsequent analysis.

The mean age of participants in the per-protocol population was 27.4 years (standard deviation [SD], 12.6 years); 7 (10%) participants were children (age 3 to 15 years),

TABLE 1 Comparison of baseline characteristics of participants that completed follow-up and participants that were lost to follow-up[a]

Characteristic	Participant status			*P* value[b]
	Finished study	Lost to follow-up	Total	
Demographic variables				
Sex				
Male	35 (51)	11 (73)	46 (55)	0.111
Female	34 (49)	4 (27)	38 (84)	
Age (mean [SD]) (yr)	27.4 (12.6)	29.6 (12.8)	27.8 (12.6)	0.546
<15	7 (10)	1 (7)	8 (10)	1.000
≥15	62 (90)	14 (93)	76 (90)	
Clinical variables				
Hypertension				
No	68 (99)	15 (100)	83 (99)	1.000
Yes	1 (1)	0 (1)	1 (1)	
Diabetes				
No	69 (100)	15 (100)	84 (100)	
Yes	0 (0)	0 (0)	0 (0)	
Chronic treatment				
No	68 (99)	14 (93)	82 (98)	0.327
Yes, not related to risk of QT enlargement	1 (1)	1 (7)	2 (2)	
Temp (mean [SD]) (°C)	35.5 (0.9)	35.2 (1.1)	35.4 (0.9)	0.238
Cardiac auscultation				
No alterations	64 (93)	15 (100)	79 (94)	0.580
Presence of murmur	5 (7)	0 (0)	5 (6)	
Splenomegaly				
No	64 (93)	15 (100)	79 (94)	0.580
Yes	5 (7)	0 (0)	5 (6)	
Blood tests				
Malaria test (blood slide)				
Negative	63 (91)	15 (100)	78 (93)	1.000
Positive *Plasmodium falciparum*	4 (6)	0 (0)	4 (5)	
Positive *Plasmodium vivax*	1 (1)	0 (0)	1 (1)	
Positive *Plasmodium malariae*	1 (1)	0 (0)	1 (1)	
Concn (mean [SD])				
Glucose (mmol/liter)	4.8 (3.7)	5.4 (1.4)	4.9 (3.4)	0.564
Creatinine (μmol/liter)	70.7 (26.5)	79.6 (26.5)	70.7 (26.5)	0.370
Sodium (mmol/liter)	139.9 (3.8)	141.7 (1.4)	140.2 (3.5)	0.083
Potassium (mmol/liter)	4.0 (0.3)	4.3 (0.3)	4.1 (0.3)	**0.018**
Hemoglobin (g/dl)	12.5 (2.1)	13.1 (2.0)	12.6 (2.1)	0.309
ECG parameters (mean [SD])				
Heart rate (bpm)	68.4 (12.4)	73.8 (11.6)	69.4 (12.3)	0.127
PR segment (ms)	166.9 (18.7)	165.1 (22.0)	166.6 (19.2)	0.749
QRS segment (ms)	90.9 (9.5)	92.7 (9.2)	91.2 (9.4)	0.525
QTcF segment (ms)	397.3 (17.2)	391.6 (18.8)	396.3 (17.5)	0.255
Conclusion				
ECG normal	65 (94)	12 (80)	77 (92)	0.104
Minor nonpathological abnormalities	4 (6)	3 (20)	7 (8)	

[a]Results are described as number (%), unless otherwise specified.
[b]*P* value corresponds to the results of the paired *t* test comparing both groups. Statistically significant results are reflected in bold.

and 34 (49%) participants were female. One female participant was pregnant (confirmed second trimester by dates and clinical examination). At baseline, the mean QT interval with Fridericia's correction (QTcF) was 397.3 ms (SD, 17.2 ms). Sixty-five (94%) participants had a normal baseline ECG, and 4 (6%) participants had minor abnormalities of no clinical relevance (i.e., 3 with nonpathological T-wave inversion and 1 with poor R-wave progression). A comparison of baseline demographic, clinical, and electrocardiographic characteristics of participants who completed follow-up and those who were lost to follow-up were not significantly different (Table 1).

The mean (SD) ΔQTcF (from 0 h to 52 h) was 19.6 ms (17.8 ms) in course 1, 23.6 ms (15.3 ms) in course 2 (difference, 4.0 ms [95% confidence interval {95% CI}, 0.1 to 8.0 ms]; $P = 0.043$), and 17.1 ms (17.1 ms) in course 3 (difference, −2.4 [95% CI, −6.9 to 2.1] $P = 0.285$; Table 2 and Fig. 1 and 2).

TABLE 2 Electrocardiographic measurements of QTcF and ΔQTcF at 52 h postdose during the second and third monthly course with dihydroartemisinin-piperaquine compared with the first month[a]

			Course 2			Course 3		
ECG parameter	0 $h_{course1}$ (mean ± SD) (ms)	52 $h_{course1}$ (mean ± SD) (ms)	52 $h_{course2}$ (mean ± SD) (ms)	*P* value	Risk difference (95% CI)	52 $h_{course3}$ (mean ± SD) (ms)	*P* value	Risk difference (95% CI)
QTcF	397.3 ± 17.2	416.8 ± 23.4	421.0 ± 19.8	**0.040**	**4.1 (0.2 to 8.1)**	414.3 ± 23.6	0.270	−2.5 (−7.1 to 2.0)
ΔQTcF		19.6 ± 17.8	23.6 ± 15.3	**0.043**	**4.0 (0.1 to 8.0)**	17.1 ± 17.1	0.285	−2.4 (−6.9 to 2.1)

[a]The 52-h readings were conducted 4 h after administration of the third dose. The parameter measurements are the arithmetic mean of measurements from triplicate readings. Statistically significant results are in bold type. *P* values are by paired *t* test for comparison with measurement of 52 $h_{course1}$.

In subgroup analyses (Table S1), the mean (SD) ΔQTcF was higher in females (25.8 ms [20.5 ms]) than in males (13.5 ms [12.2 ms]) after course 1 ($P = 0.003$). However, there was no significant difference in ΔQTcF values between males and females after the second and third courses (22.7 ms [15.9 ms] in males and 24.5 ms [14.9 ms] in females, $P = 0.623$ for the second course; and 14.3 ms [15.5 ms] in males and 20.1 ms [18.4 ms] in females, $P = 0.158$ for the third course). The mean QTcF segment and ΔQTcF values did not differ significantly according to age group (data not shown).

Figure 1 shows the evolution of QTcF at all time points (days 0, 2, 7, 28, 30, 35, 56, 58, and 63). Table 3 shows the resolution in ECG parameters at 7 days after the start of each DHA-PQP treatment course and prior (at 0 h) to the following course. The QTcF at 0 h for course 3 (QTcF 0 $h_{course3}$) was shorter than QTcF 0 $h_{course1}$ (−4.0 ms, $P < 0.001$), which represents a full resolution of the QTcF prolongation 28 days after the start of treatment of course 2. The QTcF and ΔQTcF parameters on 7 $days_{course2}$ and 7 $days_{course3}$ showed no differences compared to those at 7 $days_{course1}$ (Table S2).

No participant had cardiac-related severe adverse effects (SAEs) during the study period. Table 4 shows the recording of cardiac adverse effects of special interest (AESIs) occurring any time during the 63-day study observation period. A ΔQTcF of >60 ms was observed in 3 (4.3%), 1 (1.4%, $P = 0.50$), and 2 (2.9%, $P = 1.00$) participants after the first, second, and third courses of treatment, respectively. None of the participants

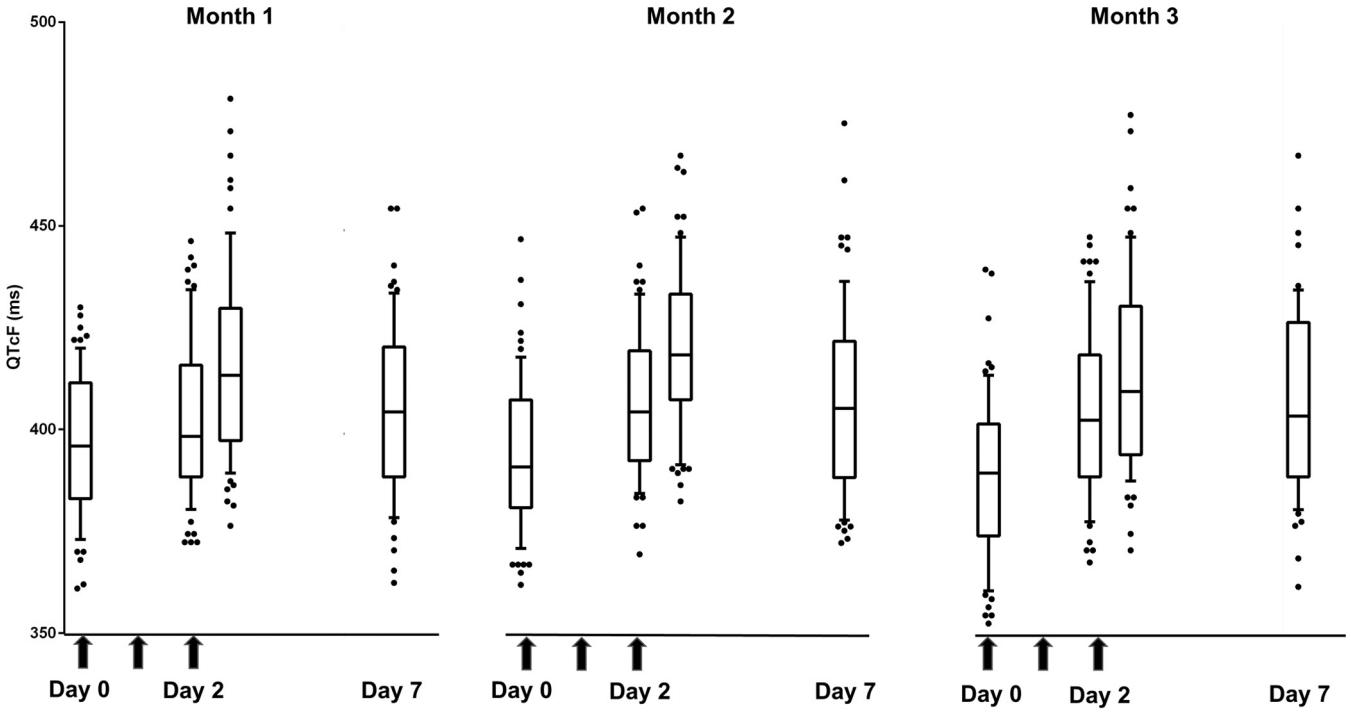

FIG 1 Electrocardiographic QTcF measurements over the study period. Results are median and interquartile ranges (IQR) of all QTcF measurements over the 63 days of study. Arrows are the DHA-PQP doses. Day 0 corresponds to the 0-h value of each month, day 2 values correspond to 48-h (predose) and 52-h (4 h postdose) measurements, and day 7 value is the day 7 measurement of each month.

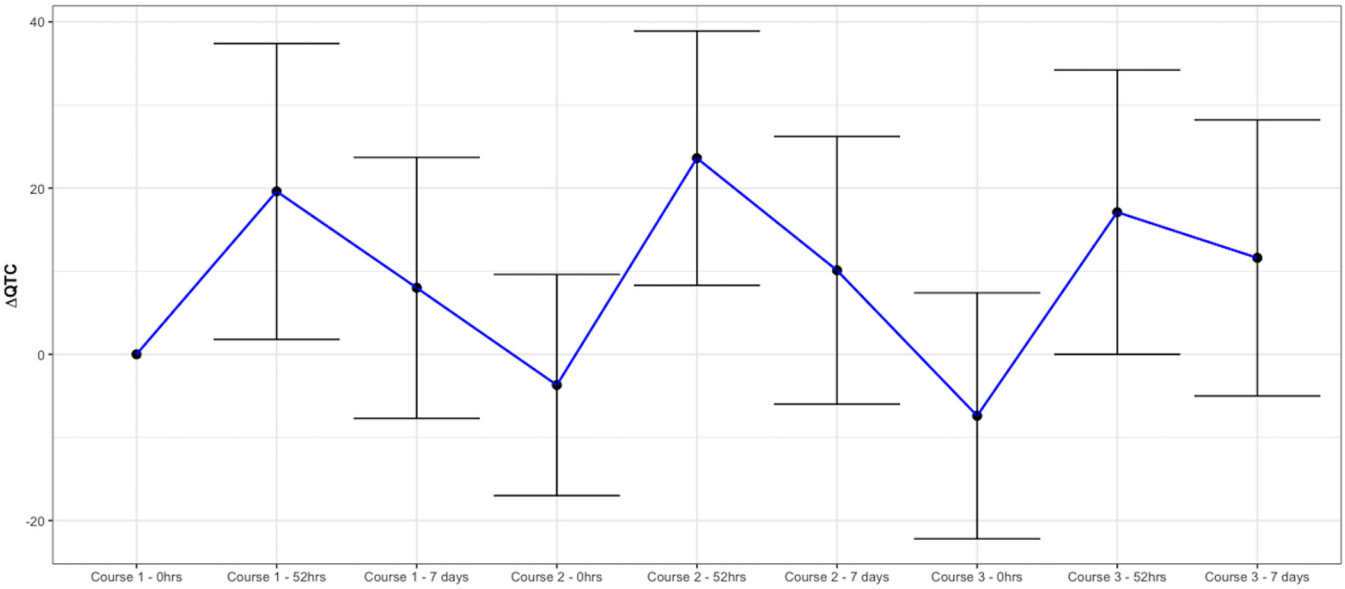

FIG 2 Changes in the QTcF measurements (ΔQTcF) over the study period. Results are mean and standard deviation (SD) of the ΔQTcF (difference in QT corrected by Fridericia's correction between baseline and each point of measurement) over the 63 days of study. 0 $h_{course1}$ corresponds to day 0 predose, 52 $h_{course1}$ corresponds to day 2 postdose, day $7_{course1}$ corresponds to day 7 postdose, 0 $h_{course2}$ corresponds to day 28 predose, 52 $h_{course2}$ corresponds to day 30 postdose, day $7_{course2}$ corresponds to day 35, 0 $h_{course3}$ corresponds to day 56 predose, 52 $h_{course3}$ corresponds to day 58 postdose, and day $7_{course3}$ corresponds to day 63.

had QTcF readings of more than 500 ms. QTcF readings of ≥450 to <500 ms were noted in 6 (8.9%), 5 (7.2%, $P = 1.00$), and 5 (7.2%, $P = 1.00$) participants after the first, second, and third courses of treatment, respectively. Other reported cardiac AESIs included sinus bradycardia (<40 beats per minute [bpm]) in 1 (1.4%) participant in each treatment course, and 2 (2.9%) participants with changes in T-wave morphology after the second courses of treatment. No AESIs were accompanied by clinical symptoms. Other previously defined AESIs (new bundle branch block and arrhythmia of new appearance) were not reported by any participant at any time during the study.

Noncardiac AEs occurred in 29 (14.0%) of 207 patient visits. All reported AEs were mild and transient, with no participant requiring specialized treatment. The most commonly reported AE were abdominal pain ($n = 12$ [5.8%]), followed by headache (3.8%), cough (2.4%), and nausea (1.9%). All rapid diagnostic tests (RDTs) and microscopy smears collected at 0 $h_{course2}$ and 0 $h_{course3}$ were negative for malaria parasitemia.

Other ECG measurements (HR, ΔHR, PR, ΔPR, QRS, and ΔQRS) along the 63 days of evolution are described in Tables S3 and S4.

DISCUSSION

In this study, three monthly repeated courses of DHA-PQP resulted in our primary study endpoint, a change in the mean post-final-dose QTcF between the first and final courses, 17.7 ms; this is a magnitude generally similar to those described after a single course and lying below the U.S Food and Drug Administration's 20-ms threshold of high level of concern (11). Our findings that QTcF prolongation did not increase in a cumulative manner with repeat courses was supported by the observation that the

TABLE 3 Electrocardiographic measurements of QTcF and ΔQTcF at 7 days after the start of each course of DHA-PQP and before the start of the following monthly course compared with 0-h measurements on day 0 first course[a]

ECG parameter	0 $h_{course1}$ measurement	7 $d_{course1}$		0 $h_{course2}$		7 $d_{course2}$		0 $h_{course3}$		7 $d_{course3}$	
		Measurement	*P* value	Measurement	*P* value	Measurement	*P* value	Measurement	*P* value	Measurement	*P* value
QTcF	397.3 ± 17.2	405.7 ± 20.9	<0.001	393.9 ± 18.1	0.043	407.7 ± 22.7	<0.001	389.9 ± 18.9	<0.001	407.7 ± 22.4	<0.001
ΔQTcF		8.0 ± 15.7		−3.7 ± 13.3		10.1 ± 16.1		−7.4 ± 14.8		11.0 ± 16.9	

[a]Results of measurements (mean ± SD, in ms) and *P* values for the paired *t* test for comparison with baseline 0-$h_{course1}$ measurements. All *P* values shown are statistically significant.

TABLE 4 Adverse events of special interest recorded on 52 h of each treatment course[a]

AESI description[b]	No. (%)		*P* value for difference with course 1[c]	52 $h_{course3}$ (no. [%])	*P* value for difference with course 1[c]
	52 $h_{course1}$	52 $h_{course2}$			
QTcF prolongation >60 ms	3 (4.3)	1 (1.4)	0.50	2 (2.9)	1.00
QTcF ≥450 ms and <500 ms	6 (8.7)	5 (7.2)	1.00	5 (7.2)	1.00
Sinus bradycardia (<40 bpm)	1 (1.4)	1 (1.4)	1.00	1 (1.4)	1.00
T-wave morphologic changes	0 (0.0)	2 (2.9)	0.50	0 (0.0)	

[a]Results are described in the number of cases and percentage (%) in the per-protocol study population.
[b]AESI, adverse event of special interest. No new cases of bundle branch block or arrhythmia were described.
[c]*P* value results are shown using the exact McNemar significance probability test.

QTcF returned to normal prior to each subsequent course of treatment. Interestingly, the QTcF difference increased slightly (23.6 ms) after the second but not the third course. This result is intriguing and will require correlation with drug concentrations once the results related to the drug's pharmacokinetics of the current study become available. In addition, it is reassuring that no individual had any QTcF of >500 ms and that the number of cases with an absolute increase of >60 ms were limited to 6 individuals evenly spread throughout each of the monthly courses. This therefore does not add to concerns raised by a recent large multicentric clinical trial about the possibility of increased incidence of this event after a repeated dose (7). Our results support previous findings that QT/QTc prolongation following DHA-PQP administration is consistently lower than that caused by other commonly used antimalarial drugs, such as quinine (12, 13) or chloroquine (14). Previous studies assessing the cardiotoxicity of DHA-PQP have shown minimal QTc prolongation following a single 3-day course. For example, in Cambodia, the mean (corrected by Bazett's formula [QTcB]) prolongation in 62 individuals was 11 ms at 24 h after first dose (15), and the mean QTcF prolongation in 56 adults in Thailand was 29 ms at 52 h after the start of treatment (16). In a cohort of 1,002 malaria patients from a study conducted in four African countries, only 3 (0.3%) patients had a QTcF of >500ms after a standard 3-dose treatment (1-month course), and fewer than 10% of participants had an increase in QTcF more than 60 ms from baseline (17). A recent meta-analysis of 11 studies involving repeated exposures of DHA-PQP for seasonal malaria chemoprevention or treatment of clinical malaria found no increased incidence of AEs with repeated dosing; however, only one of these (a study of 13 pregnant women [G. Dorsey, unpublished data]) performed electrocardiographic assessments of QTc prolongation. The authors called for more studies incorporating electrocardiogram measurements (4). Recent MDA programs using DHA-PQP in large populations have shown that this drug combination is safe to use due to the minimal occurrence of serious adverse events; however, electrocardiographic assessments were not performed to monitor cardiac side effects (18–20).

Several secondary observations are worth noting, including sex-related differences in the measurements of QT-related parameters. The study showed an increase in QTcF among females at all time points that is consistent with previous studies (21, 22). Regarding AEs, less than one-fifth of patients reported mild abdominal pain, headache, cough, or nausea. All appeared to be self-limiting, and none required specific treatment or intervention. Although this trial was not designed to evaluate the efficacy of DHA-PQP, all participants remained parasite negative for the duration of the study. This lack of breakthrough infections, in an area where malaria transmission is high (5, 6, 23) and endogenous *Plasmodium vivax* relapses are common, reassuringly suggested that posttreatment prophylaxis was satisfactory.

This study, however, does present certain limitations. The first one includes the lack of a control group, which may hinder some of the interpretation of our results. Another limitation was the relatively high attrition rate that saw 18% of enrolled participants fail to complete the scheduled follow-up appointments, and these were therefore excluded from our per-protocol analysis. If those lost to follow-up had been prone to greater QTc prolongation, this could have been a source of bias. However, there were no statistically significant differences in baseline characteristics between participants who were lost to

follow-up and those who completed follow-up. We also acknowledge an issue of generalizability of our findings regarding the way we managed food coadministation in this study. Administration of DHA-PQP with food, particularly fat, increases bioavailability, leading to increased drug concentrations and greater degree of QT prolongation (8). We carefully controlled this by advising all participants to avoid food intake for the 3 h before and after drug administration. However, while feasible in the context of a tightly controlled research study, this level of compliance may be difficult to achieve in a real MDA campaign delivered on a very large scale. Hence, it is possible that individuals not following this dietary advice could be prone to greater drug absorption, higher cumulative doses, and greater QTc prolongation than seen in our study.

Our primary endpoint was to examine changes in mean QTcF values consistent with guidelines by the U.S. FDA and other regulatory bodies. However, in terms of population risk, the way in which values are distributed across populations is perhaps more important than the measures of centrality reported here. It is those individuals who lie on the upper edge of the population distribution who are most important. For example, if an MDA intervention is deployed in 100,000 people, presuming that QTc distribution has a normal population distribution, 2,500 people will have QTc prolongations that are 2 SD above the population mean. These would be the individuals at greatest risk of a serious cardiac event. Our clinical study, like all others performed to date, did not have anywhere near the sample size required to define the population distribution accurately enough to define risk in this group. In practice, the only way this will ever be further clarified is by robust pharmacovigilance employed during large-scale MDA implementation programs.

In this study, all patient QTc measurements after three courses of DHA-PQP treatment were within approved safety margins outlined by U.S. drug regulatory institutions. The data do not suggest that the known risk of QT prolongation increases cumulatively with repeated monthly courses out to the 3rd course. However, the observation of slightly increased QT prolongation after two courses of treatment requires further investigation. At this stage, data from this study, together with the lack of reported adverse cardiac events in repeated-course MDA intervention programs elsewhere (5, 23), suggest that DHA-PQP can be used safely as MDA delivered using conventional dosing in up to 3 monthly rounds as a tool for malaria elimination. Cautions need to be articulated in relation to the antimalarial major drawbacks. First, multidrug resistance could reduce the impact; therefore, monitoring the drug's efficacy will be required (24). Second, the three daily doses required for each round of MDA result in a major logistic burden and stretch the tolerance of the target population. Nevertheless, the absence of a single-dose antimalarial with safety data for repeated dosing support DHA-PQP as one of the best available options for MDA.

MATERIALS AND METHODS

Participants and study setting. From 21 September to 21 December 2015, we conducted a prospective single-arm intervention study with healthy volunteers who resided in Lihir Island, New Ireland Province, Papua New Guinea (PNG). Eligible participants were male or female individuals age 3 to 60 years with good general health as determined by medical history, physical examination, baseline electrocardiographs, and laboratory tests. Participants were excluded if they (i) had a QT interval (adjusted using Fridericia's correction [QTcF]) greater than 450 ms or clinically significant abnormalities of rhythm at screening, (ii) had a known history of additional risk factors for TdP, (iii) had a family history of long-QT syndrome or sudden cardiac death, (iv) were using concomitant medications known to prolong the QT/QTc interval, or (v) had a history of relevant allergic reactions. All female participants in reproductive age were tested for pregnancy (urinary beta-human chorionic gonadotropin [βHCG] dipstick) and excluded if they were in the first trimester.

The study protocol was approved by the PNG Institute of Medical Research institutional review board (number 15.01), the PNG Ministry of Health Medical Research Advisory Committee (number 15.14), and the ethics committee of Barcelona's Hospital Clinic in Spain (HCB/2014/0424). Written informed consent was obtained from all adult participants or, for children, from parents or guardians.

Study procedures. We recruited a nonprobabilistic convenience sample of healthy volunteers through group presentations by trained staff in 11 local communities, followed by one-to-one interviews. The volunteers were admitted at the hospital for a period of 72 h to facilitate drug administration under direct observation and fasting, as well as measurement of ECG traces. Arterakine (Pharbaco Central Pharmaceutical, Vietnam), registered product in PNG by the national authorities, was given daily for 3 days (i.e., at times 0, 24, and 48 h) according to the dosing schedule of the PNG National Malaria

Treatment Protocol, DHA-PQP at 2.1/17.1 mg/kg of body weight (25). Patients fasted for the 3 h before and after each DHA-PQP dose.

Detailed physical examination, routine clinical laboratory tests, pregnancy test for women of reproductive age, a rapid diagnostic test (RDT) for malaria, and a malaria blood slide were performed at baseline (time 0 h) prior to treatment. Participants were assessed for adverse events (AEs) with a structured questionnaire and examination every 8 h throughout day 3 (i.e., at time 48, 56, 64, and 72 h) of each course. Examination included measurement of blood pressure, pulse, respiratory rate, cardiac auscultation, respiratory auscultation, and abdominal palpation for all participants. Blood samples to analyze drug levels were collected at 52 h (4 h after third dose) of each administration course.

Electrocardiography and electrocardiographic endpoints. Twelve-lead ECG readings were conducted using an ELI 150 cardiograph at a speed of 25 mm/s at predose (at time 0 h, in triplicate), immediately prior to administration of the third dose (at time 48 h, single trace), and 4 h after the third dose (at time 52 h, in triplicate). For ECGs taken in triplicate, parameter measurements for the final analysis were based on the arithmetic mean of measurements from the 3 readings. Participants were discharged 24 h after the last dose of treatment (at time 72 h). In addition, ECGs were conducted at 7 days after the start of treatment and before (at time 0 h) the following treatment course. The same procedure was repeated for the second and the third monthly treatment courses. Throughout the study period, ECG readings were conducted at 12 time points over the 63-day follow-up period: day 0 predose (0 $h_{course1}$), day 2 predose (48 $h_{course1}$), day 2 postdose (52 $h_{course1}$), day 7 (7 $days_{course1}$), day 28 predose (0 $h_{course2}$), day 30 predose (48 $h_{course2}$), day 30 postdose (52 $h_{course2}$), day 35 (7 $days_{course2}$), day 56 predose (0 $h_{course3}$), day 58 predose (48 $h_{course3}$), day 58 postdose (52 $h_{course3}$), and day 63 (7 $days_{course3}$).

The QT interval (i.e., distance from the Q wave to the end of the T wave) was corrected for heart rate using Fridericia's correction formula (QTcF). This was defined as the measured QT interval divided by the cube root of the RR interval. The autocalculated QTcF measurements were manually verified by the study clinician for safety purposes. All ECG results were electronically transferred to a cardiac core lab (Banook/Cardiabase, France) where independent and centralized interpretation of the tracings were repeated by a certified cardiologist blinded to each participant's details. The following parameters were obtained: RR (in milliseconds), HR (beats per minute), PR (in milliseconds), QRS (in milliseconds), QTcF (in milliseconds), and ΔHR (beats per minute), ΔPR (in milliseconds), ΔQRS (in milliseconds), ΔQTcF (in milliseconds) by comparison with baseline ECG reading (time 0 h) for each course of treatment.

A data safety monitoring board (DSMB) was established to which the site clinician (PM) was responsible for reporting any AE classified as either adverse events of special interest (AESIs) or severe adverse events (SAEs) according to whether they met prespecified criteria. We defined AESI as QTcF prolongation from baseline of >60 ms, QTcF at any time >450 ms, T-wave morphologic changes during therapy, bundle branch block, or any new arrhythmia. We defined cardiologic SAEs as a QTcF of >500 ms (sensitivity is 94% and specificity 97% for prediction of malignant arrhythmia in overdose schedules), any malignant ventricular arrhythmia (e.g., TdP), or any episode of sudden death (26). We established a predetermined threshold for study cessation as the occurrence of three episodes meeting any of our criteria for a cardiologic SAE.

Statistical analysis. The primary endpoint of the study was ΔQTcF, calculated as the difference between QTcF measured at the 0-h recording to 52 h of each of the three courses. Secondary endpoints were (i) occurrences of AESIs and SAEs and (ii) QTcF resolution. For analysis of the primary endpoint, we estimated the risk difference and two-sided 95% CIs in ΔQTcF between the ECG reading at 52 $h_{course3}$ and the ECG reading at 52 $h_{course1}$ using a paired t test for comparisons. For analysis of the secondary endpoints, we estimated the risk difference and two-sided 95% CI for the difference in QTcF between the ECG reading at 0 $h_{course3}$ and the ECG reading at 0 $h_{course1}$, and we also looked at the difference between the ECG readings at 7 $days_{course3}$ and the ECG reading at 7 $days_{course1}$. We counted and summarized the number of AESIs, and we used the McNemar test to compare the difference in occurrence of AESIs between the third and first courses of treatment. Data analysis was performed using the Stata 15.1 software (Stata Corporation, College Station, TX, USA).

We calculated that 73 individuals would be required for the primary analysis to estimate a 5-ms difference in mean QTcF prolongation between the first and third treatment courses (assuming a prolongation in mean QTcF of 20 ms for the first month and 25 ms for the third month), with a confidence level of 95% and a power of 80%. Assuming that 10% of participants would be lost to follow-up, the target recruitment was 82 individuals.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.01153-18.

TEXT S1, PDF file, 0.1 MB.

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O.M. and Q.B. conceived the study and developed the analysis plan. P.M.-M. and R.I. conducted the study, including patient enrollment and data collection. H.A. and K.P. conducted the laboratory tests. P.M.-M. and S.S. conducted the statistical analyses. O.M., P.M.-M. and Q.B. prepared the first draft of the article with important intellectual input and revisions from R.I., M.L., L.R., H.K., L.M., and B.M. All authors approved the final version of the article.

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8.4. Fourth article: Piperaquine pharmacokinetic and pharmacodynamic profiles in healthy volunteers of Papua New Guinea after administration of three-monthly doses of dihydroartemisinin-piperaquine.

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PHARMACOLOGY

Piperaquine Pharmacokinetic and Pharmacodynamic Profiles in Healthy Volunteers of Papua New Guinea after Administration of Three-Monthly Doses of Dihydroartemisinin–Piperaquine

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ABSTRACT Mass drug administration (MDA) with monthly dihydroartemisinin-piperaquine (DHA-PQP) appears useful in malaria control and elimination strategies. Determining the relationship between consecutive piperaquine phosphate (PQP) exposure and its impact on QT interval prolongation is a key safety consideration for MDA campaigns. Healthy volunteers from Papua New Guinea received a 3-day course of DHA-PQP (2.1/17.1 mg/kg) monthly for 3 consecutive months in a single arm longitudinal study. Plasma PQP concentrations were measured after the third dose of each course (at 52–54 h) and at 0 h of course 3. Twelve-lead electrocardiographic readings were conducted at 0 h, 48 h, 52 h, and day 7 of each course. QT interval corrected by Fridericia's formula (QTcF) was measured at each time point. A pharmacokinetic–pharmacodynamic model using nonlinear mixed effects models was developed to correlate PQP concentrations with QTcF. Ten thousand female and 10,000 male individuals were simulated at each treatment course. Eighty-two participants were included; mean age was 28.3 years (standard deviation [SD] ±12.3 years), and 36 (44%) were female. Pharmacokinetic–pharmacodynamic models were determined with 290 PQP concentrations and 868 QTcF observations. The average baseline QTcF was 392 ms with a between-subject variability SD ±14.4 ms and between-occasion variability SD ±3.64 ms. From the population modeled, only 0.08% of males and

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0.45% of females would be at risk of an absolute QTcF of >500 ms. DHA-PQP is safe at standard doses in consecutive months, and the likelihood of severe cardiac events occurring during an MDA campaign is very low. This study has been registered at ClinicalTrials.gov under identifier NCT02605720.

KEYWORDS cardiac safety, malaria, piperaquine, pharmacokinetics, QT interval

Dihydroartemisinin–piperaquine (DHA-PQP) is one of the five artemisinin-based combination therapies recommended for the treatment of uncomplicated malaria (1, 2). It has also been administered in mass drug administration (MDA) programs aiming to reduce malaria epidemics or as a tool to accelerate malaria elimination (3–7). The short half-life artemisinin-derivative component (DHA) rapidly reduces the parasite load. By contrast, the PQP component is characterized by a large distribution volume and reduced rates of excretion after multiple doses (8). PQP has an estimated prolonged posttreatment prophylactic effect of 28 days (9), which could potentially prevent new infections for a period of at least 90 to 120 days if administered during three consecutive monthly courses. These characteristics make PQP an ideal candidate drug for MDA programs.

In preparation for MDA programs, the safety of DHA-PQP in healthy populations needs to be assessed, with particular attention to potential cardiovascular toxicity (3). Although DHA-PQP has been shown to be well tolerated and effective in mass treatments and as an intermittent preventative therapy for high-risk populations, one of the undesirable risks of the PQP component is the potential to prolong QT interval, reflecting delayed cardiac repolarization that, if severe, may put patients at risk of polymorphic ventricular tachycardia, Torsade de Pointes, and sudden cardiac death (10, 11). Previous studies have demonstrated this QT corrected interval prolongation is mild in most cases and not associated with severe cardiac adverse events (12–16). However, additional cardiovascular safety data are desirable to recommend the extended use of DHA-PQP in repeated monthly doses, particularly among healthy populations targeted for MDA programs (17).

Understanding the relationship between PQP exposure (pharmacokinetics [PK]) and the effect on the QT interval (pharmacodynamics [PD]) is critical to inform and optimize safe MDA regimens with DHA-PQP. One PK-PD study of single courses of DHA-PQP in predominately male participants suggested a 3-day regimen was safe (18). A PK study of monthly DHA-PQP treatment as intermittent preventive therapy in pregnancy (IPTp) reported that PQP concentrations are suitable to protect from malaria infection, but did not examine cardiotoxicity specifically (19). Finally, simulations of MDA extrapolated from single doses in a small number of healthy volunteers indicate that dangerous QT prolongation is unlikely (20). While these studies provide some reassurance that MDA with DHA-PQP is likely to be safe, no study has examined the effect of monthly DHA-PQP on QT interval in a healthy population where MDA is being considered.

To fill this knowledge gap, we conducted an interventional study of a standard 3-day course of DHA-PQP administered monthly for 3 consecutive months in healthy volunteers. PQP concentrations and QT corrected by Fridericia's formula (QTcF) observations were integrated into a nonlinear mixed effects PK-PD model to describe the relationship between PQP exposure and QTcF prolongation.

RESULTS

Participant characteristics. Eighty-two participants were included in the PK study. During the 3 months of trial duration, 10 participants were lost to follow-up after the first treatment course, and 2 were lost to follow-up after the second treatment course; the last treatment course was undertaken by 70 participants of the original 82 included (Fig. S1 in the supplemental material). Baseline characteristics of participants are summarized in Table 1. The mean age of participants was 28.3 years (standard deviation [SD] ±12.3 years, range 3–59); 7 (9%) participants were children (age 3 to <15 years), and 36 (44%) participants were female. The mean participants' weight was 61.5 kg (SD ±14.3 kg). One female participant was pregnant in her second trimester of pregnancy.

TABLE 1 Baseline characteristics for the participants included in the pharmacokinetic study

Characteristic	*n* = 82	Results
Demographics		
Female (%)		36 (44)
Age (mean (SD))		28.3 (± 12.3)
<15 yrs old (%)		7 (9)
15–<30 yrs old (%)		42 (51)
30–<45 yrs old (%)		24 (29)
≥45 yrs old (%)		9 (11)
wt (mean (SD))		61.5 (± 14.3)
Clinical variables		
Pregnancy (%)		1 (1)
Hypertension (%)		1 (1)
Splenomegaly (%)		5 (6)
Laboratory results[a]		
Malaria blood slide negative (%)		76 (93)
Positive *P. falciparum* (%)		4 (5)
Positive *P. vivax* (%)		1 (1)
Positive *P. malariae* (%)		1 (1)
Blood glucose (mean (SD))		5.0 (± 3.5)
Blood creatinine (mean (SD))	*n* = 81	74.3 (± 23.9)
Blood sodium (mean (SD))		140.3 (± 3.4)
Blood potassium (mean (SD))		4.1 (± 0.3)
Blood haemoglobin (mean (SD))	*n* = 80	12.6 (± 2.1)

[a]Laboratory reference ranges: glucose 3.9–5.6 mmol/L; creatinine 53.0 to 114.5 μmol/L; sodium 135.0 to 147.0 mmol/L; potassium 3.5 to 5.0 mmol/L; hemoglobin 14.0–18.0 g/dL (males) and 12.0–16.0 g/dL (females).

At baseline, 6 (7%) participants had a positive malaria blood test without showing clinical signs of acute malaria, and 5 (6%) had palpable splenomegaly.

Population pharmacokinetic model and QTcF model. From the 82 individuals included in the present analysis, there were 290 PQP plasma concentrations taken from venous blood available for the determination of individual PK parameters (81 samples taken at 52–54 h of treatment course 1, 71 samples taken at 52–54 h of treatment course 2, 70 samples taken at 0 h of treatment course 3, and 68 samples taken at 52–54 h of treatment course 3) and 868 QTcF observations for the PD model (Fig. S1).

Individual predicted PQ concentrations resulting from the PK Bayesian forecasting step correlated well with observed concentrations (Fig. S2), with only a small bias underestimating higher concentrations. A direct linear model did not demonstrate significant bias, with a clear positive linear relationship between QTcF and individually estimated PQP concentrations. Between-subject variability was estimated for both baseline QTcF (ΔOFV –644.557) as well as SLOPE (ΔOFV –7.174), with between-occasion variability also estimated for baseline QTcF (ΔOFV –8.157). Gender was found to be a significant covariate for baseline QTcF (ΔOFV –18.414).

Results of the PD model are presented alongside the bootstrap results (Table 2). Bias from the median bootstrap result was lower than 7% for all model parameters. Goodness of fit plots are presented (Fig. 1) with visual predictive checks (Fig. 2). No significant bias was identified with these plots. The population average baseline QTcF was 392 ms with a between-subject variability (BSV) standard deviation of 14.4 ms (coefficient of variation [CV] based on population average of 3.6%) and between-occasion variability standard deviation of 3.64 ms (CV based on population average 0.9%). Females had a higher baseline QTcF (+15.2 ms). The SLOPE of the model was 0.0479 ms per μg/L of PQP with a BSV standard deviation of 0.0132 ms/(μg/L) (CV based on population average 27%). There was a slight negative correlation between the two additive BSV terms for Baseline QTcF and SLOPE (−0.0864); however, the bootstrap 95% interval crossed zero. This correlation was maintained in the final model given the intent to use the model for simulations. Standard deviation of residual variability (RV) was 10.7 ms for the additive error model.

TABLE 2 Final model population pharmacodynamic estimates and final model bootstrap results for QTcF in individuals receiving piperaquine[a]

Final model			
Parameter	**Mean**	**RSE %**	**Bootstrap median [95% CI]**
Objective function value (OFV)	5,329.485		5313.673 [4978.344–5606.316]
Baseline QTcF (ms)	392	1	392 [387–397]
SLOPE (ms/(μg/L))	0.0479	6	0.0479 [0.0419–0.0539]
Gender effect on baseline QTcF (female, ms)	15.2	22	15.2 [8.85–21.5]
BSV in baseline QTcF (ms) [shrinkage%][b]	14.4 [4]	16	14.1 [12.0–16.4]
BSV in SLOPE (ms/[μg/L]) [shrinkage%][b]	0.0132 [49]	47	0.0128 [0.0061–0.0181]
BSV baseline QTcF ~BSV SLOPE	0.0864	150	–0.0443 [–0.48831–0.999]
BOV in baseline QTcF (ms) [shrinkage%][b]	3.64 [54,61,60]	56	3.52 [1.29–5.35]
RV additive error (ms) [shrinkage%][b]	10.7 [9]	7	10.8 [10.0–11.6]

[a]RSE, relative standard error; BSV, between-subject variability; BOV, between-occasion variability; RV, residual variability.
[b]The standard deviation of the untransformed BSV and BOV terms and the additive error term are provided as $\sqrt{variance}$.

Results from the simulations based on dosing from this study are presented in Table 3. Based on 10,000 simulations of each gender with Papua New Guinea (PNG) regimen dosing, over all three dosing periods only a very small number of simulated data have absolute QTcF of >500 ms (0.08% in simulated males and 0.45% in simulated females) or ΔQTcF (difference [delta] between a time point and the baseline of QTc corrected interval by Fridericia's formula) of >60 ms (1.63%), with up to the 99% prediction interval not meeting these thresholds, even when taking into consideration the higher baseline QTcF in females and that simulations include extremes that are unlikely to be seen in real life. When repeated using WHO recommended dosing, similar results were obtained, with slightly higher percentages with QTcF of >500 ms over all three doses (0.15% in simulated males and 0.42% in simulated females) owing to higher mg/kg doses in some weight groups (Table S1). No trends in maximum simulated QTcF were seen according to the weight of the simulated participants, relating in part due to the variability present in the PD model (data not shown) and the mg/kg dosing.

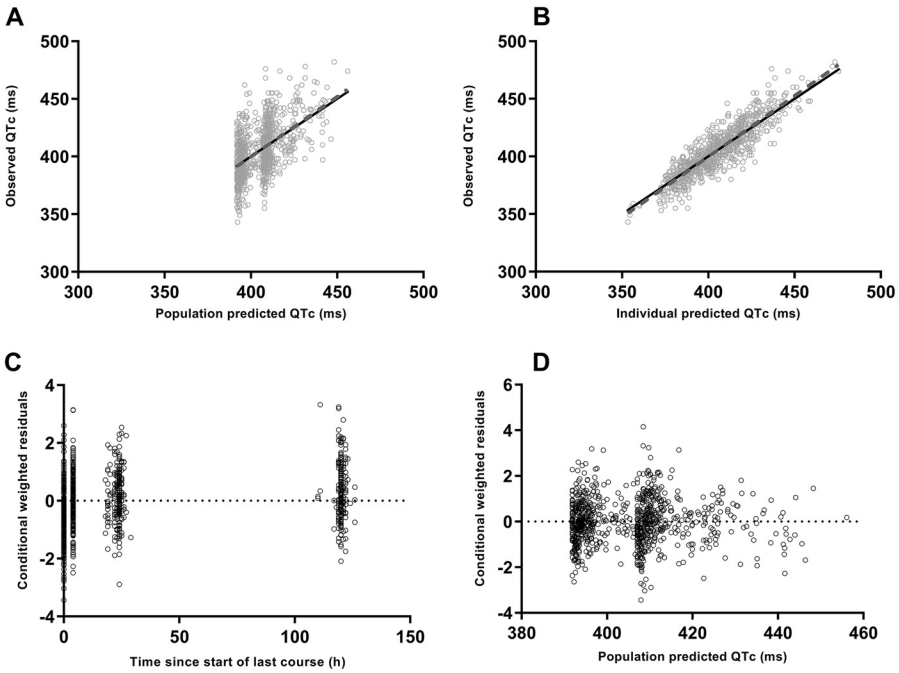

FIG 1 Goodness-of-fit plots for piperaquine including observed concentration against population (A) and individual (B) predicted concentrations, and conditional weighted residuals against time after previous dose (C) and population predicted concentrations (D).

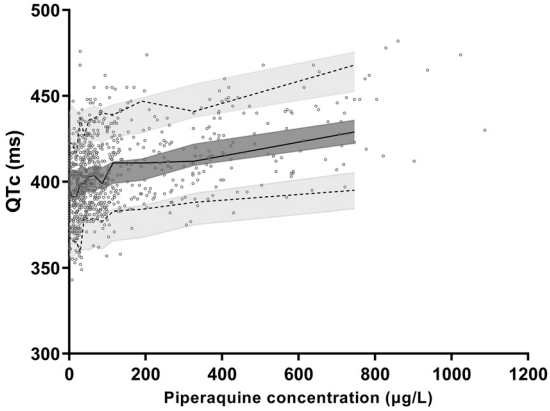

FIG 2 Prediction corrected visual predictive checks for QTcF for individuals receiving piperaquine with observed 50th (solid line) and 5th and 95th (dotted lines) percentiles within their simulated 95% CI (gray shaded areas) are shown with overlying data points (○).

DISCUSSION

This population PK-PD model linking PQP exposure and its effect on QTcF shows minimal accumulation affecting QTcF, and posed minimal QTcF prolongation risk in a healthy population when DHA-PQP was given monthly, mimicking an MDA schedule. In this study, very few individuals in this population would be expected to have an absolute QTcF of >500ms or ΔQTcF of >60 ms, noting that simulations can include extreme cases that are unlikely to be seen.

TABLE 3 Results of QTcF simulations of 10,000 female and 10,000 male individuals receiving three daily doses of PQP at 17.1 mg/kg[a]

	PNG dosing regimen		WHO dosing regimen	
Parameter	Male	Female	Male	Female
Course 1				
Peak PQP concn (μg/L)	231 (83–754)		248 (88–820)	
Peak QTcF (ms)	405 (373–442)	420 (388–458)	406 (374–445)	421 (389–460)
QTcF >500 ms	1 (0.01%)	10 (0.10%)	2 (0.02%)	12 (0.12%)
Peak ΔQTcF	10.7 (3.1–40.0)		11.5 (3.3–43.2)	
ΔQTcF >30 ms	557 (5.57%)		694 (6.94%)	
ΔQTcF >60 ms	48 (0.48%)		67 (0.67%)	
Course 2				
Peak PQP concn (μg/L)	250 (100–789)		268 (107–857)	
Peak QTcF (ms)	405 (375–443)	421 (391–459)	407 (376–446)	422 (391–461)
QTcF >500 ms	2 (0.02%)	9 (0.09%)	5 (0.05%)	14 (0.14%)
Peak ΔQTcF	11.5 (1.0–39.6)		12.5 (1.1–43.4)	
ΔQTcF >30 ms	640 (6.40%)		794 (7.94%)	
ΔQTcF >60 ms	46 (0.46%)		65 (0.65%)	
Course 3				
Peak PQP concn (μg/L)	258 (109–823)		276 (114–875)	
Peak QTcF (ms)	407 (376–445)	422 (391–460)	408 (377–447)	423 (392–463)
QTcF >500 ms	5 (0.05%)	13 (0.13%)	8 (0.08%)	16 (0.16%)
Peak ΔQTcF	11.8 (1.3–42.2)		12.7 (1.5–45.3)	
ΔQTcF >30 ms	712 (7.12%)		881 (8.81%)	
ΔQTcF >60 ms	69 (0.69%)		90 (0.90%)	
Over all three courses				
Peak PQP concn (μg/L)	375 (172–1,048)		403 (182–1,114)	
Peak QTcF (ms)	414 (384–456)	430 (399–471)	416 (385–460)	431 (400–475)
QTcF >500 ms	8 (0.08%)	45 (0.45%)	15 (0.15%)	42 (0.42%)
Peak ΔQTcF	17.8 (5.8–54.0)		19.1 (6.4–59.4)	
ΔQTcF >30 ms	1,692 (16.92%)		2,027 (20.27%)	
ΔQTcF >60 ms	163 (1.63%)		216 (2.16%)	

[a]Data presented as median (95% interval) or *n* (%).

In a previous study conducted by the same team, a three-monthly repeated full course of DHA-PQP resulted in a change in the mean post-final-dose QTcF between the first and final course of 17.7 ms (16); a similar magnitude prolongation was observed after a single course (16, 21–23). The simulations based on the observed data in this study demonstrate that it is highly unlikely to see QTcF prolongation of concern when DHA-PQP is given monthly with standard doses. While female gender was associated with a longer QTcF interval at baseline as previously described (24, 25), gender did not influence the relationship of concentration with QTcF (i.e., the SLOPE of the PD model), and therefore females did not have a significantly increased risk of cardiotoxicity compared to males.

The results of this study align with previous reports. Although PQP administration has been unambiguously related to QTcF interval prolongation (10, 11, 26), available data from different studies show that this prolongation is not a cause of concern in most clinical settings. In a large African cohort, only 0.3% of the participants who received single course treatment with DHA-PQP had registered a QTcF of $>$500 ms (23), similar to the percentage in the present simulations. Previous MDA campaigns with DHA-PQP in thousands of participants, despite not conducting regular electrocardiograph (ECG) assessments, have not observed serious cardiac adverse events with monthly courses (6, 27–29).

The methods used in this study are comparable to a population PD model derived from a small number of healthy volunteers who received single doses (20). The present study used QTcF without adjustment for baseline (rather than a "double-delta QTcF"), which enabled simulation of absolute values of QTcF and changes in QTcF within an individual without consideration of the effect of adjustment with baseline values. Despite these methodological differences, the size of the PK-PD effects observed in both studies were similar (0.0479 ms/(μg/L) in the present study and 0.0417 ms/(μg/L) in the previous model). These data also align, with the value obtained from simple linear regression after a single course being 0.05 ms/(μg/L) (18). Given that between-subject variability standard deviation was characterized (0.0132 ms/[μg/L]), simulations based on the present model would include individuals who are more susceptible to PQP QTcF prolongation and therefore at greatest risks. Another strength of the method used in this study is that the Bayesian forecasting for PK parameters followed by a PD model has allowed the use of PD observations that were not directly paired with PK observations.

While this is one of the largest reported cohorts of its kind, there are some limitations in extrapolating to a general population. Firstly, very few children and older adults were included, with none of the participants being older than 60 years of age. Indeed, baseline QT in extreme age groups (children and older population) can differ from that in young adult populations and impact the QTc prolongation values (30, 31); moreover, older participants are more likely to be on other medications affecting QT. These vulnerable groups should be considered populations of greater concern if MDA is conducted. They should be further monitored in such programs, with the need to collect additional safety data given there is not enough available evidence. Food intake is recommended to be avoided 3 h before and after the DHA-PQP intake as, particularly, high fat meals are associated with increased PQP concentrations and therefore a greater degree of QT prolongation (17). The food intake recommendations were followed by the participants in this study; however, this may present an additional challenge when implementing MDA campaigns. If DHA-PQP is given at a large scale, the role of food intake in the increase of PQP levels should specifically be taken into consideration. There was a small bias underestimating higher concentrations in the Bayesian forecasting step, which would result in the model suggesting lower concentrations causing the same degree of QTcF prolongation. Given that the simulations were based on the same PK model, this potential bias, which possibly overestimates QTcF, would not influence the final conclusions.

Conclusion. There is a positive linear relationship between PQP concentration and QTcF prolongation; however, the overall effect of this relationship is small and unlikely to generate safety concerns. If DHA-PQP is to be given as part of an MDA campaign, it is unlikely that severe cardiac events would occur in the general population.

MATERIALS AND METHODS

Study design and participants. Healthy volunteers from Lihir Island, Papua New Guinea (PNG), from a single arm longitudinal clinical study of electrocardiographic safety assessment for DHA-PQP (16), were included in this population PK study. Briefly, all participants received a 3-day fixed oral combination of DHA-PQP 2.1/17.1 mg/kg of body weight (Arterakine Pharbaco Central Pharmaceutical; 40 mg DHA and 320 mg PQP tetraphosphate per tablet) under fasting conditions, following PNG malaria treatment guidelines, which introduce slight modifications to the doses recommended by WHO (see Table S1 for comparison between PNG recommended doses and WHO) (32). Each drug administration was supervised by a health worker. Venous blood specimens for PQP concentrations were collected at 0 h of course 3 (before the month 3 treatment) and at 4–6 h after the third dose of each treatment course (at 52–54 h), to align with the expected peak concentrations (Cmax) (20, 21). Twelve-lead electrocardiograph (ECG) readings were performed using an ELI 150 cardiograph at a speed of 25 mm/s at predose (at time zero h, in triplicate), immediately prior to administration of the third dose (at time 48 h, single trace), at 4 h after the third dose (at time 52 h, in triplicate), and on day 7 (single trace) of each treatment course. All ECG readings were electronically transferred to a cardiac core lab (Banook/Cardiabase, France), and a centralized interpretation of the tracings was conducted by a blinded cardiologist. For ECGs taken in triplicate, parameter measurements were based on the arithmetic mean from the 3 readings. A QT interval was measured at each time point and adjusted by Fridericia's correction formula (QTcF).

Ethical approval was granted by the PNG Institute of Medical Research Institutional Review Board (15.01), the PNG Ministry of Health Medical Research Advisory Committee (15.14), and Barcelona's Hospital Clinic Ethics Committee in Spain (HCB/2014/0424). Written informed consent was obtained from all adult participants or, for enrolled children, from parents or guardians. The study was registered in ClinicalTrials.gov with identifier NCT02605720.

Laboratory procedures. Venous blood (3 mL) was collected into lithium heparin tubes, and immediately centrifuged at 1,800 $\times$ *g* for 5 min. Separated plasma was stored at −20°C until time of shipment (Perth, Australia; shipped on dry ice). Samples were then stored at −80°C until time of analysis.

Plasma PQP concentrations were measured using a triple quadrupole mass spectrometer (LCMS/MS-8060; Shimadzu, Kyoto, Japan). Plasma samples were extracted by protein precipitation. The precursor–product ion pair was *m/z* 535.3 $>$ 288.2 for PQP and *m/z* 541.3 $>$ 294.1 for PQP–d6, which was used as the internal standard. Chromatographic separation was performed on a Kinetex biphenyl column (50 $\times$ 2.0 mm, 2.6 μm; Phenomenex, Lane Cove West, Australia). The mobile phase comprised methanol and water with 0.1% wt/vol formic acid pumped at 0.4 mL/min in a gradient mode. The limits of quantification and detection were 2 μg/L and 1 μg/L, respectively. The intraday and interday variability for the concentrations 5–2,000 μg/L ranged from 2.4–6.5% and 4.1–8.7%, respectively. The accuracy calculated from the quality control samples for the concentrations 5–2,000 μg/L were within the analytical range (97.4–110.5%).

Population pharmacokinetic and QTcF modeling. NONMEM (v 7.2.0, ICON Development Solutions, Ellicott City, MD, USA) with an Intel Visual FORTRAN 10.0 compiler was utilized for nonlinear mixed effects modeling of the QTcF data. The first order conditional estimates method with interaction (FOCE INTER) estimation was used with the minimum value of the objective function value (OFV), goodness of fit plots, condition number ($<$1000), and predictive checks used to arrive at suitable models during the model-building process. A significance level of $P < 0.05$ (ΔOFV −3.841, df = 1) was set for comparison of nested models. An additive residual variability (RV) model was utilized, given QTcF measurements are within a small range. The modeling of the pharmacodynamic (PD) data proceeded in two stages. Firstly, PK parameters were obtained for each participant using a Bayesian forecasting, without re-estimation of the original model parameters (33), using their measured concentrations and a previously developed population model of PQP in nonpregnant women within NONMEM (34). This model included three compartments for distribution and transit compartment absorption, and included allometric scaling based on weight. The selected model also included between-occasion variability for bioavailability of piperaquine. The individual PK parameters obtained from this process were then used to individually predict PQP concentrations at the times of the PD (QTcF) observations. Once the individual concentrations were determined, QTcF was modeled as a PD measure with a direct-linear relationship to the predicted PQP concentration:

$$\mathrm{QTcF(t)} = \mathrm{Baseline\,QTcF} + \mathrm{[PQP](t)} \times \mathrm{SLOPE},$$

where QTcF(t) is the model predicted QTcF at time t, Baseline QTcF is the model-predicted QTcF at baseline, [PQP](t) is the PK model derived concentration at time t, and SLOPE is the effect of PQP concentration on QTcF. This approach mirrors a previous report in healthy volunteers where instead of QTcF itself, a "double-delta-corrected" QT was utilized (20). In early modeling, a review of this relationship did not suggest any model misspecification; therefore, more complex models were not progressed.

Between-subject and between-occasion (differences for each treatment course) variability as untransformed normal distributions were assessed for both baseline QTcF and SLOPE parameters, including correlation between variability terms. All potential covariate–parameter relationships were tested within NONMEM using a stepwise forward and backwards approach ($P < 0.10$, ΔOFV −2.706, df = 1 for forward steps; and $P < 0.05$, ΔOFV −3.841, df = 1 for backwards steps). Covariates available for assessment were age, gender, weight, and mg/kg dose.

Final models were evaluated using goodness-of-fit plots including observed versus individual and population predicted values, conditional weighted residual plots against time from last dose, and population predicted values. A nonparametric bootstrap (*n* = 1,000) was performed with the 95% empirical confidence interval, and bias from the median for each parameter determined to facilitate evaluation of final estimates. Additionally, visual predictive checks (*n* = 1,000), including those stratified according to gender, were performed with the observed 5th, 50th, and 95th percentiles plotted with their respective

simulated 95% confidence intervals (CIs) to assess the predictive performance of the model and to assess for any major bias (Fig. S3).

Simulations. From the final obtained PD model, supported by a published PK model for nonpregnant women in PNG (34), 10,000 female and 10,000 male individuals were simulated. To align with the population observed, a uniform distribution of weight from 15 to 100 kg was used. Three monthly courses of 3 doses of PQP, prescribed in accordance with the dose schedule for the present study as well as standard WHO dosing (1), was applied (Table S1). Simulations were conducted at 6-min intervals after the final dose to capture the peak of QTcF for each simulated individual with the corresponding ΔQTcF. The simulated data were summarized to identify peak simulated ΔQTcF greater than 30 ms and 60 ms, as well as peak QTcF values of greater than 500 ms. The data were summarized for each course and over all three doses, noting that the population PK model included between-occasion variability for bioavailability (F), and therefore a simulated individual's highest concentration may have not occurred with the final course.

Data availability. The raw data sets used and analyzed during the current study are uploaded as supplemental material.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

SUPPLEMENTAL FILE 2, XLSX file, 0.02 MB.

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O.M., Q.B., L.J.R., M.L., H.K. and L.M. designed the study; P.M.-M. enrolled the participants and collected the data; S.S., B.R.M., M.P.-S. and K.T.B. did the PK analysis and modeling of data; P.M.-M. and S.S. wrote the manuscript. All authors interpreted the data and critically revised the manuscript for important intellectual content. All authors have seen and approved the final version of the manuscript.

We declare that we have no conflicts of interest.

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8.5. Fifth article: A cross-sectional study to ascertain malaria prevalence among asymptomatic travellers arriving on the Lihir Group of Islands, Papua New Guinea: implications for elimination efforts

A cross-sectional study to ascertain malaria prevalence among asymptomatic travellers arriving on the Lihir Group of Islands, Papua New Guinea: implications for elimination efforts

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ABSTRACT

Background: The Lihir Islands of Papua New Guinea host a mining operation that has resulted in a mine-impacted zone (MIZ) with reduced malaria transmission and a substantial influx of mine employees, informal cross-country traders, returning locals, and visitors. Prevalence of malaria parasites was assessed in travellers arriving on the Lihir Group of Islands to evaluate the risk of parasite importation**. Methods:** In 2018, a cross-sectional study at the airport and main wharf was conducted, targeting asymptomatic travellers who had been away from Lihir for at least 12 days. Microscopy, rapid diagnostic tests (RDTs), and quantitative PCR (qPCR) were used to determine *Plasmodium* parasite prevalence, employing logistic regression models to identify factors associated with qPCR positivity. **Results:** 398 travellers arriving by plane and 402 arriving by boat were included. Both cohorts were significantly different. Mean age among travellers arriving by plane was 40.1 years (SD±10.1), 93% were male and 96% were employed at the mine. In contrast, among travellers arriving by boat, the mean age was 31.7 years (SD±14.0), 68% were male and 36% were employed at the mine. The prevalence of malaria infection among travellers arriving by plane was 1% by RDT and microscopy, and increased to 5% by qPCR. In contrast, those arriving by boat showed a prevalence of 8% by RDT and microscopy, and 17% by qPCR. Risk factors for infection were arriving by boat (OR 4.2;

95%CI: 2.45,7.21), arriving from nearby provinces with high malaria incidence (OR 5.02; 95%CI: 1.80, 14.01), and having been away from Lihir for 91 days or more (OR 4.15; 95%CI: 2.58, 6.66). Being mine worker staying at the mine accommodation was related with less infection risk (OR 0.24; 95% CI: 0.14, 0.43); while Lihirian residents returning from a trip, VFRs, or people with trading unrelated to mining had higher risks (p=0.0066). **Conclusions:** Travellers arriving by boat faced increased risk of malaria infection than those arriving by plane. This subpopulation poses an import risk to the MIZ and the rest of Lihir Islands. Screening of high-risk groups at wharfs, and collaboration with nearby Islands, could sustain reduced transmission and facilitate malaria elimination strategies.

Keywords: imported, islands, malaria, Pacific, Plasmodium, travellers.

INTRODUCTION

In 2020, Papua New Guinea (PNG) reported over 750,000 confirmed malaria cases (1), accounting for 87% of all cases and 94% of all malaria-related deaths in the WHO's Western Pacific region (2). Malaria transmission in PNG exhibits geographical heterogeneity, with low endemicity in the high-altitude inland areas, and high transmission levels in the coastal areas (3). The provinces most affected, including New Ireland, East and West New Britain, Sandaun (West Sepik), and Milne Bay, exhibit an incidence of over 200 yearly cases per 1,000 inhabitants (4). The most prevalent *Plasmodium* species are *P. falciparum* and *P. vivax* with an overall prevalence by microscopy of 2.1% and 0.5%, respectively (5); however, *P. malariae* and *P. ovale* are also present (6).

Lihir Islands, located in New Ireland province, host a gold mining operation of Newcrest Mining Ltd on their largest island, Aniolam. Due to the high malaria transmission rates in the area and

71 the additional risks posed by deforestation and open-pit mining (7), the company collaborates
72 in a public-private partnership to provide essential services such as electricity, improved
73 sanitation, and healthcare services to its employees and part of the general public. This
74 partnership with the local government aims to reduce the burden of communicable diseases in
75 their operational setting. The Mine-Impacted Zone (MIZ) includes the communities
76 surrounding the open pit, the main town Londolovit, the mining accommodation (a well-
77 equipped housing camp within a 2 km^2 area), and the airport, all located in the north-east of
78 Aniolam. Similar to other public-private partnerships in regions with high endemicity of vector-
79 borne diseases, efforts are also directed towards implementing vector control strategies (8).
80 Since 2006, Newcrest finances a vector control program involving larval source management
81 such as drainage of potential smaller larval habitats and the application of larvicides to larger
82 water bodies within the MIZ (9). The rest of the Lihir Islands depend on universal coverage
83 with long-lasting insecticidal nets (LLINs) for vector control, which are distributed free of
84 charge through mass campaigns every three years. Despite achieving coverage rates of 97%-
85 98%, most of the population does not consistently use or maintain the LLINs over time (10).

86 In 2010, a cross-sectional study was conducted in Aniolam to evaluate malaria prevalence and
87 the impact of the implemented vector control programs (9). While there was a marked
88 reduction in malaria positive children detected by microscopy within the MIZ (from 31.5% in
89 2006 to 5.8% in 2010), the reduction was substantially smaller in the non-MIZ (from 34.9% in
90 2006 to 26.9% in 2010). Despite these efforts, malaria remains a common diagnosis, even
91 among mine employees and contractors. In 2019, there were 784 malaria cases diagnosed
92 among the workforce, of which 488 were identified in employees staying at the mine
93 accommodation (unpublished, data provided by the Lihir Medical Center, funded by Newcrest
94 and located within the MIZ). Of note, there are approximately 3,000 mobile mine employees
95 and contractors arriving by plane or boat from other areas within the same province or from
96 other provinces. Beyond the mobile population related to mine activities, additional

97 population movements occur between Aniolam and the neighbouring islands for trading
98 purposes, along with an unspecified number of visiting friends and relatives (VFRs) between
99 nearby islands and provinces.

100 Travellers are common sources of imported malaria. For instance, a prevalence survey in the
101 low-burden island of Bioko, Equatorial Guinea, showed that malaria infection was highly
102 related with having a history of travel to mainland Equatorial Guinea (11). In PNG, the
103 haplotypes of *P. vivax* isolates from different regions revealed patterns of transmission
104 following major human migration routes, especially within the 'Islands region' of PNG (12).
105 These findings suggests that improved diagnosis and treatment of travellers could be critical
106 for the success of malaria control and elimination strategies (13). Even after achieving malaria
107 elimination, the frequency of infected individuals entering non-endemic areas remains a
108 primary risk factor for malaria re-establishment (14). Cabo Verde serves as an example, where
109 malaria resurged after interruption of local transmission in two occasions within the last 50
110 years, due to the imported infections by those travellers arriving from mainland Africa (15).

111 Herewith, a cross-sectional study was conducted to assess the prevalence of *Plasmodium*
112 parasites in travellers arriving to the Lihir Islands by plane or boat, and to evaluate the
113 importation risk they pose to the ongoing transmission within the MIZ and in the rest of Lihir.
114 Parasite prevalence was determined using microscopy, RDT and quantitative PCR (qPCR), and
115 factors associated with qPCR positivity were identified. Estimating the burden of malaria
116 among travellers to Lihir can provide valuable insights for refining current control strategies
117 and guiding the sustainability of future interventions aimed at malaria elimination on these
118 islands.

METHODS

Ethical considerations

This study obtained ethical clearance from the national ethical committee in PNG, the Medical Research Advisory Committee (PNG-MRAC), with MRAC No.18.07. Written informed consent was collected from all individuals in the study. Children under the age of 18 were verbally consented and parents or legal guardians signed the consent form on their behalf. Those participants unable to read and/or write were verbally consented and an impartial witness countersigned the consent form.

Study setting

Lihir Islands are located 900 km northeast of Port Moresby in the New Ireland Province of PNG. Lihir consists of a group of four islands: Aniolam (the largest, with an area of 200 km^2), and the smaller outer islands of Malie, Masahet, and Mahur. They are characterised by a tropical rainforest climate with extremely high precipitation figures all year round, a limited public health infrastructure, and a near-inaccessible geography in some areas. Londolovit is the main town and is the centre for most of the local business and those related with the mining activities. A population census, conducted between 2018 and 2020, estimated 26,528 inhabitants living in the Lihir Islands. Mining employees migrating from other parts of PNG, contribute to more than one third of the population on Aniolam (16).

Study design and study population

Between the 30th of May and the 7th of December of 2018, a cross-sectional survey was conducted at the main points of entry for travellers, the Lihir airport and the Londolovit wharf. All individuals arriving at the wharf and airport were considered potential candidates for recruitment. Individuals, both male or female, aged 6 months or older; who were not residents of Lihir or had been away from the islands for more than 12 days; and did not show clinical symptoms of malaria at arrival were eligible for inclusion in the study. Exclusion criteria were

individuals unwillingness to provide informed consent or withdrawal of consent, as well as arrival from a non-endemic country.

Data and sample collection

After signing the informed consent, a questionnaire was obtained from all participants, which included demographic and clinical data, information on LLIN usage, and mobility/travel information. Data collection was conducted using a paper questionnaire in the field, and subsequently, two data clerks independently entered the data into a database upon their return from the field. For each participant, a health practitioner performed a finger prick to collect blood drops for a malaria RDT, a blood slide (thin and thick smear) for light microscopy examination, and 2 dry blood spots in filter paper for qPCR. Results of the RDT were immediately interpreted by a clinician who provided antimalarial treatment according to PNG treatment guidelines in case of a positive result.

Laboratory procedures

Upon return from the field, only thin smear was fixed with methanol and blood slides were stained with 10% Giemsa during 10min at the local laboratory following the MM-SOP-07A from WHO. Blood slides were examined under x1000 magnification by two independent level 1-2 microscopists who had completed WHO quality assurance courses. A sample was considered negative after examining one hundred fields of view. When a parasite was observed, counts of white cells and parasites were conducted until 300 white cells were counted. The parasite density in parasites per µL was then calculated, assuming a white cell count of 8,000 white cells/µL. The results for this first reading were cross-checked with the Institute of Medical Research (IMR) Vector-borne Diseases Unit at Madang (PNG). Any discrepancies were addressed at IMR with the involvement of a third WHO-certified level 1 microscopist.

Filter papers with dry blood spots were dried in the field, placed into separate zip-lock bags and stored at -20 ºC. Subsequently, these samples were sent to the IMR Vector-borne Diseases

Unit in Madang (PNG) for further processing. DNA was extracted using FavorPrepTM 96-well Genomic DNA kit (FAVORGEN®) and performed according to the manufacturer's instructions to obtain genomic DNA from blood. Following DNA extraction, a generic qPCR 'QMAL' assay that amplifies a conserved region of the 18S rRNA gene was run on all samples (17); and for all positive samples, a species-specific qPCR for *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale* were performed as previously described (18). Finally, in cases where qPCR QMAL positive samples yielded negative results in the species-specific qPCR, ultra-sensitive qPCRs targeting Pf-varATS for *P. falciparum* and Pv-mtCOX1 for *P. vivax* were conducted (19, 20).

Sample size and statistical analysis

The minimum sample size was 385 individuals arriving by plane and 385 individuals arriving by boat, considering a 5% precision and a 95% bilateral normal confidence interval (CI), and assuming an unknown prevalence of *Plasmodium spp* carriage in travellers (50%).

Data were analysed using the statistical software STATA (21) and described as frequencies and mean (standard deviation, SD) for categorical and continuous variables, respectively. For typically skewed quantitative variables, the median and interquartile range (IQR) were also considered. Chi-squared test (or Fisher's exact test) and t-test were performed to assess differences between groups for categorical and continuous variables, respectively. Spearman's rank correlations were used to estimate the association of continuous variables. Logistic regression models were used to determine the factors that were associated with qPCR positivity. For this model, the variable of origin or place stayed while being away from Lihir was stratified according to level of incidence following the 2019 National Department of Health report (3) as; low-incidence provinces (0 to 75 cases per 1,000 population: National Capital District, Bougainville, Hela, Enga, Western Highlands, Southern Highlands, Jiwaka, Chimbu, and Eastern Highlands); medium incidence provinces (76 to 225 cases per 1,000 population: East Sepik, Western, Morobe, Central, Madang, Gulf and Milne Bay); and high-incidence provinces

(> 225 cases per 1,000 population: West Sepik, Oro, West New Britain, East New Britain, Manus and New Ireland). All significance levels were set at 0.05.

RESULTS

Demographic and other characteristics of travellers arriving on Lihir

A total of 800 travellers arriving at the main points of entry in Lihir were recruited: the airport and the main wharf, located in Londolovit Town. Smaller wharfs outside the MIZ observe considerably fewer arrivals are were not included for sampling. The map in Figure 1 shows the boundaries of the mine accommodation area, the MIZ, and the points of arrival for travellers in the Lihir Islands.

Our study population comprised 398 participants arriving by plane and 402 participants arriving by boat. Table 1 presents the demographic and travel characteristics of both cohorts. These two groups were significantly different across nearly all recorded variables: sex, age, duration outside Lihir, purpose of the visit and place of stay while on Lihir (all $p<0.0001$).

Travellers arriving by plane were predominantly male (93%) with a mean age of 40.1 (SD ± 10.1) years, who were employed at the mine (96%) and intended to stay at the mine accommodation facilities (99%). Given the mine's standard 14-day work roster (14 days on Lihir, 12 days off), the majority of these fly-in fly-out employees (87%) had spent less than a month away from Lihir.

In contrast, travellers arriving by boat displayed greater diversity. While males still formed the majority, females (32%) and children (12%) were present, with a mean age of 31.7 (SD ± 14.0) years old. Their travel purposes also varied widely, with only 36% being mine workers. Many travellers arriving by boat were either Lihir residents returning home or engaging in VFRs (30%), trading or business outside the mine (23%) or visiting Lihir for other reasons (11%). Consequently, only 35% of this cohort planned to stay at the mine's accommodation facilities,

218 with most opting for either one of the Lihirian villages (42%) or Londolovit Town (23%). They
219 also had spent longer periods outside Lihir prior to travelling, with 41% of them being away for
220 3 months or more.

221 There was a clear association between the place of stay on Lihir and the purpose of the visit,
222 both in travellers arriving by plane (p=0.0019) and in those arriving by boat (p<0.0001).
223 Conversely, despite variations in the place of stay and the intention of the visit, both cohorts
224 shared a similar mean length of stay on Lihir (around 3-weeks).

225 **Table 1**. Demographic, travel data and prevention measures used by the study participants.

Variables			**Plane (N = 398)**	**Boat (N = 402)**	**Total**	**p-value***
Demographic characteristics						
Sex	Male	n (%)	369 (93)	273 (68)	642 (80)	<0.0001
	Female	n (%)	29 (7)	129 (32)	158 (20)	
Age (years)		mean (SD)	40.1 (10.1)	31.7 (14.0)	35.9 (12.9)	<0.0001
Age category	<15 years old	n (%)	0 (0)	47 (12)	47 (6)	<0.0001
	15 to 29 years old	n (%)	54 (14)	112 (28)	166 (21)	
	≥30 years old	n (%)	344 (86)	243 (60)	587 (73)	
Travel information						
Time away from Lihir[a]	>12 to 31	n (%)	348 (87)	213 (53)	561 (70)	<0.0001
(days)	32 to 90	n (%)	18 (5)	25 (6)	43 (5)	
	91 or more	n (%)	32 (8)	163 (41)	195 (24)	
Place while on Lihir	Mine accommodation	n (%)	395 (99)	140 (35)	535 (67)	<0.0001
	Londolovit Town	n (%)	3 (1)	94 (23)	97 (12)	
	Villages of Lihir	n (%)	0 (0)	168 (42)	168 (21)	
Intention of the visit	Returning resident/VFR	n (%)	0 (0)	122 (30)	122 (15)	<0.0001
	Mine worker	n (%)	383 (96)	144 (36)	527 (66)	
	Trading unrelated to mining	n (%)	1 (0)	92 (23)	93 (12)	
	Other purpose	n (%)	14 (4)	44 (11)	58 (7)	
Time intended to stay on Lihir (in days) [b]		mean (SD)	20.2 (10.3)	22.1 (40.9)	21.1 (29.1)	0.3707
Malaria episodes and prevention measures						
Received malaria treatment last year	Yes	n (%)	320 (80)	382 (95)	702 (88)	<0.0001

Last malaria episode (in months) [a]		mean (SD)	3.7 (0.7)	3.6 (0.9)	3.6 (0.8)	0.0888[c]
Frequency of sleeping under net while away from Lihir [a]	Never	n (%)	217 (55)	221 (56)	438 (55)	0.0807
	Sometimes	n (%)	93 (24)	108 (27)	201 (25)	
	Most nights	n (%)	28 (7)	33 (8)	61 (8)	
	Always	n (%)	57 (14)	34 (9)	91 (12)	
Slept under net while away from Lihir[a]	Yes	n (%)	178 (45)	175 (44)	353 (45)	0.8834

Abbreviations: LLIN = Long-lasting insecticidal net, SD = Standard Deviation, VFR = Visiting Friends and Relatives. [a] missingness <1.1%, [b] n = 740 (7.5% missing); [c] Chi squared test; *significance level set at 0.05.

Origin of travellers, last malaria episode and use of LLINs

The origin of travellers arriving to Lihir differed significantly between those arriving by plane and those arriving by boat (p<0.0001). Figure 2 illustrates the 22 provincial-level divisions of PNG and the origin of travellers arriving on Lihir by plane or boat. Travellers arriving by plane came from various provinces in PNG, including regions of high, moderate and low malaria transmission. Almost all PNG provinces were represented in these cohort, with East New Britain and the National Capital District being the most prominent, accounting for 26% and 19% of the travellers, respectively. In contrast, the majority of travellers arriving by boat came from the nearby islands of New Ireland (56%) or East New Britain (39%); where malaria transmission is high.

Despite the different origin of travellers in both cohorts, most reported having received a full course of antimalarial treatment during the preceding 12 months. However, this proportion was higher among travellers arriving by boat (95%) compared to those arriving by plane (80%) (p<0.0001). The reported time of the last malaria episode was between 3 and 4months prior to enrolment in the study for both cohorts. In addition, the use of LLIN was similar, with most travellers not utilizing them while being away from Lihir. No participant had fever, or any active symptom compatible with malaria upon recruitment.

Prevalence of *Plasmodium* parasites in travellers arriving on Lihir

Regarding infection status of travellers arriving by plane, only 3 cases (1%) were positive by RDT and microscopy. Microscopy showed one case of *P. falciparum* infection and two of *P. vivax*. When using qPCR to detect *Plasmodium* parasites, 18 (5%) of these travellers were positive, 16 (4%) were male and 2 (7%) females. Most of the positive cases arrived from provinces where malaria transmission is high or moderate, including East New Britain (6), East Sepik (3), Morobe (3), New Ireland (2), and Western Province (1). Unfortunately, the amount and/or quality of the extracted DNA from these samples was not sufficient to successfully determine *Plasmodium* species in the subsequent specification reaction, with 77% of the positive qPCR samples yielding a negative result.

Conversely, among travellers arriving by boat, 33 (8%) were positive by RDT and microscopy. Microscopy showed 22 (67%) cases of *P. falciparum* infection, 5 (15%) of *P. vivax*, 1 (3%) of *P. malariae,* and 5 (15%) of mixed infections, which included *P. falciparum* and *P. vivax*. In contrast, RDT diagnosed 14 (42%) cases of *P. falciparum* infection, 10 (30%) of non-*P. falciparum* species, and 9 (27%) potential mixed infections. When using qPCR, 67 (17%) of travellers arriving by boat were positive; 43 (64%) were male and 24 (36%) were female. Most of them (98%) arrived from either New Ireland (43 individuals) or East New Britain (23 individuals), and 1 arrived from the Autonomous Region of Bougainville. In this cohort, DNA extraction was optimized and 63% of the positive qPCR samples yielded a positive result for the subsequent specification reaction. Among those, 21 were positive for *P. falciparum*, 6 for *P. vivax*, 2 for *P. malariae*, 11 for both *P. falciparum* and *P. vivax*, and 3 for *P. falciparum* and *P. malariae*. There were no *P. ovale* positive samples.

Factors associated with malaria infection

The uni- and multivariate logistic regression models including both cohorts of travellers are shown in Table 2. In the univariate analysis, there was an increased risk of qPCR confirmed

malaria infection among those arriving by boat (OR 4.2; 95% CI: 2.45, 7.21), in those arriving from PNG provinces with high malaria incidence (OR 5.02; 95% CI: 1.80, 14.01), and in individuals that had been away from Lihir more than 91 days (OR 4.15; 95% CI: 2.58, 6.66). Being mine worker offered protection against carrying malaria parasites (OR 0.24; 95% CI: 0.14, 0.43) compared to other intention of the visit such as Lihirian resident returning from a trip or VFRs, or people with intention to trading out of the mine business (OR 1.27; 95% CI: 0.67, 2.43). Travellers intended to stay at Londolovit Town (OR 5.93; 95% CI: 3.33, 10.56) or at the villages of Lihir (OR 3.24; 95% CI: 1.88, 5.58) had higher risk compared to those planning to stay at the mine accommodation. From all this assessed travel characteristics, none except had been away from Lihir more than 91 days (aOR 2.53; 95% CI: 1.25, 5.10) showed to be related with malaria infection detected by qPCR. Additionally, the univariate logistic regression model showed that being male (OR 0.52; 95% CI: 0.31, 0.85) and age (OR 0.57; 95% CI: 0.25, 1.30 for 15-29 years-old; OR 0.37; 95% CI: 0.17, 0.78) being protective factors for infection; however, these findings could not be confirmed in the multivariate model. Finally, there was no association between having slept under LLIN while away from Lihir (OR 1.28; 95% CI: 0.81, 2.02) and malaria infection.

Table 2. Logistic regression analyses for *Plasmodium* qPCR positive results in all the travellers.

Variable		**Univariate model**		**Multivariate model**	
		OR (95% CI)	**p-value**	**aOR (95% CI)[#]**	**p-value**
Sex	Female	reference group	0.0092	reference group	0.6828
	Male	0.52 (0.31, 0.85)		1.13 (0.62, 2.06)	
Age (years)	< 15	reference group	0.0191	reference group	0.9384
	15 to 29	0.57 (0.25, 1.30)		0.92 (0.38, 2.24)	
	≥ 30	0.37 (0.17, 0.78)		0.87 (0.37, 2.01)	
Arriving by	Plane	reference group	<0.0001	reference group	0.5655
	Boat	4.20 (2.45, 7.21)		1.69 (0.68, 4.21)	
Origin (arriving from)[a]	Low incidence PNG provinces	reference group	0.0004	reference group	0.3703

	Medium incidence PNG provinces	1.58 (0.43, 5.73)		1.74 (0.47, 6.50)	
	High incidence PNG provinces	5.02 (1.80, 14.01)		2.34 (0.72, 7.62)	
Time away from Lihir (days)[b]	>12 to 31	reference group	<0.0001	reference group	0.0308
	32 to 90	1.45 (0.49, 4.28)		1.28 (0.40, 4.09)	
	91 or more	4.15 (2.58, 6.66)		2.53 (1.25, 5.10)	
Place while on Lihir	Mine accommodation	reference group	<0.0001	reference group	0.1834
	Londolovit Town	5.93 (3.33, 10.56)		1.80 (0.43, 7.48)	
	Villages of Lihir	3.24 (1.88, 5.58)		1.00 (0.24, 4.17)	
Intention of the visit	Returning resident/VFR	reference group	<0.0001	reference group	0.4856
	Mine worker	0.24 (0.14, 0.43)		0.82 (0.19, 3.45)	
	Trading unrelated to mining	1.27 (0.67, 2.43)		1.04 (0.53, 2.04)	
	Other purpose	0.45 (0.17, 1.16)		0.48 (0.17, 1.30)	
Slept under net while away from Lihir [b]	No	reference group	0.2830	reference group	0.6750
	Yes	1.28 (0.81, 2.02)		1.11 (0.68, 1.82)	

Abbreviations: LLIN = Long-lasting insecticidal net, aOR = adjusted odds ratio, OR = odds ratio, PNG = Papua New Guinea, VFR = Visiting Friends and Relatives. [a]n = 777 (2.6 % missing), [b]missingness <1.1%. *significance level set at 0.05. [#]multivariate analysis conducted with 767 observations.

To further explore factors associated with malaria infection and their respective risk, separate analyses for each cohort were conducted. For travellers arriving by plane, no variable was found to be significantly associated with testing positive by qPCR or any of the utilized techniques (Supplementary table, Additional file 1). In contrast, among travellers arriving by boat, a significant association was observed between testing positive by qPCR and the duration of time spent outside Lihir (p=0.0003), the purpose of the visit (p=0.0066), and the place of stay on Lihir (p=0.0011) (Table 3). The univariate logistic regression model showed that travellers spent three months or more away from the Island had a 3-fold higher risk of malaria infection (OR 3.01; 95% CI: 1.71, 5.30). Those travellers intending to stay at Londolovit town (OR 3.74; 95% CI: 1.80, 7.74) or at the villages (OR 1.95; 95% CI: 0.97, 3.94) had also higher risk of infection. On contrary, mine workers had less risk of infection (OR 0.39; 95% CI: 0.19, 0.79).

303 The only independent risk factor shown after the multivariate logistic analysis was the time
304 spent outside Lihir, with aOR 2.35 (95%CI: 1.07, 5.18) for those travellers being 91 days or
305 more outside the Islands. There were no significant associations between sex, age, point of
306 origin or having slept under LLIN, and malaria infection as detected by qPCR.

307 **Table 3.** Variables and their associations with *Plasmodium* qPCR positive results and logistic
308 regression models for the boat cohort

Variable		**qPCR positive n (%)**	**Association p-value***	**Univariate OR (95 % CI)**	**Multivariate aOR (95 % CI)#**
Sex	Female	24 (19)	0.4736	reference group	reference group
	Male	43 (16)		0.82 (0.47, 1.42)	1.34 (0.71, 2.54)
Age (years)	< 15	10 (21)	0.4341	reference group	reference group
	15 to 29	21 (19)		0.85 (0.37, 1.99)	1.01 (0.40, 2.56)
	≥ 30	36 (15)		0.64 (0.29, 1.41)	0.88 (0.36, 2.13)
Origin (arriving from)[a]	Low incidence provinces	0 (0)	0.3983	reference group	reference group
	Medium incidence provinces	0 (0)		1 (-)	1 (-)
	High incidence provinces	67 (17)		1.21 (0.14, 10.23)	1.99 (0.19, 17.27)
Time away from Lihir	>12 to 31	22 (10)	0.0003	reference group	reference group
(days)[a]	32 to 90	3 (12)		1.18 (0.33, 4.28)	1.20 (0.29, 4.89)
	91 or more	42 (26)		3.01 (1.71, 5.30)	2.35 (1.07, 5.18)
Place while on Lihir	Mine accommodation	13 (9)	0.0011	reference group	reference group
	Londolovit Town	26 (28)		3.74 (1.80, 7.74)	1.64 (0.28, 9.63)
	Villages of Lihir	28 (17)		1.95 (0.97, 3.94)	0.87 (0.15, 5.07)
Intention of the visit	Returning resident/VFR	25 (20)	0.0066	reference group	reference group
	Mine worker	13 (9)		0.39 (0.19, 0.79)	0.59 (0.10, 3.50)
	Trading unrelated to mining	23 (25)		1.29 (0.68, 2.46)	1.07 (0.54, 2.13)
	Other purpose	6 (14)		0.61 (0.23, 1.61)	0.55 (0.20, 1.53)
Frequency of sleeping	Never	32 (14)	0.6155	reference group	reference group
under net while away	Some nights	20 (19)		1.34 (0.73, 2.48)	1.23 (0.65, 2.34)
from Lihir [a]	Most of the nights	4 (12)		1.05 (0.38, 2.93)	0.72 (0.24, 2.19)
	Always	7 (21)		2.13 (0.91, 4.97)	2.14 (0.84, 5.48)
	No	32 (14)	0.1894	reference group	

Slept under net while away from Lihir [a]	Yes	34 (9)	1.42 (0.84, 2.42)	not considered for this analysis

Abbreviations: aOR = adjusted odds ratio, OR = odds ratio, qPCR = quantitative polymerase chain reaction, VFR = visiting friends and relatives. [a] all missingness <1.5%. *significance level set at 0.05. [#]multivariate analysis conducted with 393 observations.

DISCUSSION

This study characterized the prevalence of malaria parasites in travellers arriving on Lihir Islands to estimate the risk of malaria importation. Our study revealed a substantial four-fold increase in the risk of malaria infection among travellers arriving by boat compared to those arriving by plane. Demographic and travel characteristics of the two cohorts had distinct profiles that likely contribute to differing risks of malaria infection.

The travellers arriving by plane predominantly consisted of adult male mine workers, who were on a 14-day work roster and stayed at the mine accommodation facilities. This group exhibited a lower prevalence of malaria parasites, likely due to their diverse origins from provinces with varying transmission intensities, and possibly in relation to better protection practices related to improved living conditions. In contrast, the cohort of travellers arriving by boat exhibited greater diversity, with the presence of females and children, and were mostly locals or residents in neighbouring islands. This group had a notably higher prevalence of malaria parasites, indicating increased risk of infection associated with their demographic and travel characteristics, including longer stays away from Lihir. Notably, qPCR-detected infections in boat-traveling individuals (17%) closely paralleled the mean prevalence in the local Lihirian population (15%) reported in 2019 (Millat-Martinez et al., under revision). In contrast, malaria prevalence in travellers arriving by plane (5%) was lower than any prevalence found in Lihir Islands.

Furthermore, when looking for risk factors associated with malaria infection in the whole cohort and the boat cohort, the only independent risk factor identified was an extended absence from Lihir, specifically over 91 days. This further supports their increased exposure and subsequent elevated infection rates. Although arriving from high incidence PNG provinces emerged as a risk for infection, there was no independent association between point of origin and malaria infection, probably because most of the travellers by boat arrived from high incidence provinces. Interestingly, the univariate logistic regression analysis in the boat travellers and when both cohorts were analysed together showed an association between infection and visit intent. Lihirian residents returning home, VFRs and traders had higher risk of infection compared to mine employees and contractors. These findings contrast with other mining settings, especially illegal operations, where mine workers presented higher malaria burden than the indigenous population (22, 23). This divergence might be attributed to the relatively higher socioeconomic status, better living conditions, sanitation, and awareness of malaria prevention among Lihir mine employees and contractors. Stay location in Lihir also influenced infection risk, as mine workers exclusively used the well-conditioned mine accommodations within the MIZ.

Despite differing malaria parasite prevalence and province origins, the large majority of both cohorts had experienced at least one malaria episode in the last 12 months. Our hypothesis is that travellers from low transmission provinces could have been exposed during their work roster in Lihir; while those coming from nearby islands might have been infected locally or in Lihir. In addition, PNG's population, having been exposed to *P. vivax,* is likely to be carriers of hypnozoites, contributing to a high annual relapse burden (24). On the other hand, both cohorts showed low LLIN usage, although it exceeded the local population figures (10). Surprisingly, LLIN usage away from Lihir did not correlate with malaria infection. This may be explained by the exhibited decline in bioefficacy of distributed nets in PNG (25, 26), or by the early biting of infected *Anopheles* when people are still engaged in outdoor activities (27).

This study included participants who had been away from Lihir for a minimum of 12 days, with the aim of capturing mine workers following the 14-day on-site and 12-day off-site work roster. Notably, while *P. falciparum* typically manifest an incubation period of 9 to 14 days, other species can have longer incubation periods (28). Additionally, in PNG up to 80% of *P. vivax* active cases are estimated to be relapses (29), usually relapsing 3-6 weeks apart from the primary infection (30). Thus, despite not being able to ensure that all infections were contracted away from Lihir, these infections represent the parasite load that could potentially contribute to local transmission after travellers' arrival. Another limitation of our study is that 77% of the qPCR positive samples from the plane cohort, and 37% from the boat cohort, yielded negative results for the species specification reaction. The extracted DNA was of low amount and quality, which may be explained by possible disruptions in the cold chain interfering with DNA stability (31), or by degradation, considering that DNA extraction occurred 3 years after sample collection.

In the last decade, Newcrest Mining ltd has considered the possibility of malaria elimination in the Lihir Islands by implementing a pilot program that integrates diverse strategies to reduce the malaria burden and ultimately halt local transmission. However, the amount of imported malaria parasites could challenge these efforts (32). For instance, in Zanzibar, Tanzania, a stochastic model studying local transmission estimated up to 18% of imported cases and 25% of introduced (locally transmitted after an imported case), which noteworthy contributed to the local malaria burden and transmission (33). Targeting travellers in low and moderate transmission settings have the potential to reduce local malaria burden, if encompassed with robust surveillance and response systems, intensive vector control, awareness programs, healthcare training, and frequent epidemiological and entomological monitoring (34, 35). In Lihir, the primary risk of importing malaria stems from individuals arriving by boat, particularly returning residents, VFRs, and traders. In islands regions like Lihir, where entry points are well identified, an effective approach for avoiding transmission of imported cases involves

integrating passive and active case detection with travellers' test and treat at borders (13, 36). In accordance to other prevalence studies in PNG (37, 38), our study found an important number of submicroscopic infections. Ultra-sensitive RDTs and loop-mediated isothermal amplification could offer potential solutions for tackling these infections in travellers (39, 40), albeit with substantial factors to consider. Hence, other strategies could be higher feasible in Lihir. For instance, in Sri Lanka, two screening strategies for travellers have proven effective: the utilization of standard RDTs upon arrival followed by a repeat test within two weeks (41), and targeting high-risk travellers (42). Finally, islands do not have a guaranteed success in control and elimination programs (43), and collaboration with neighbouring areas is just as essential as it is in settings with land borders adjacent to endemic areas (44, 45). Newcrest Mining Ltd's engagement with local and provincial governments, extending strategies beyond the MIZ, could yield sustainable, cost-effective results in curbing malaria amidst a mobile population in Lihir Islands.

CONCLUSIONS

Travellers arriving by boat to Lihir Islands exhibit a significantly higher risk of malaria infection compared to those arriving by plane, thus posing a higher risk for parasite importation. Lihirian residents returning home, VFRs, and traders face a heightened risk for malaria infection in contrast to mine employees. Implementing screenings among high-risk travellers arriving by boat could potentially prevent transmission from importation. In the long-term, particularly if malaria transmission decreases across the Lihir Islands, improved control endeavours could greatly benefit from integrating actions in the neighbouring islands. Failing to proactively address imported malaria cases among travellers to the island could pose challenges to ensuring the sustainability and impact of malaria control or elimination efforts.

LIST OF ABBREVIATIONS

CI: confidence interval

IMR: Institute for Medical Research

LLIN: Long-lasting insecticidal net

MIZ: mine-impacted zone

MRAC: Medical Research Advisory Committee

PNG: Papua New Guinea

qPCR: quantitative Polymerase Chain Reaction

RDT: rapid diagnostic test

SD: standard deviation

VFR: visiting friends and relatives

WHO: World Health Organization

DECLARATIONS

Ethics approval and consent to participate

The research protocol for this study was given ethics clearance at the PNG Medical Research Advisory Committee with MRAC No.18.07. Written informed consent was individually collected from all participants in the study before any data or procedure was undertaken.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contribution

PMM, BB, SK, OM, ML, WP and QB designed the study; PMM, BB, CW, SR and AE collected the data and samples for all participants; SS conducted the statistical analysis; BK conducted the molecular work and TK and MKO supervised the molecular work; BB, LL, CW, SR, and AE conducted the microscopy analysis; PMM and BB wrote the manuscript. All authors interpreted the data and critically revised the manuscript for important intellectual content. All authors have seen and approved the final version of the manuscript.

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FIGURE TITLES AND LEGENDS

Figure 1. The Lihir Islands of Papua New Guinea.

Legend: map showing the main points of entry for travellers, the limits of the mine impacted zone and the mine accommodation areas in the Lihir Islands.

Figure 2. Origin of travellers arriving on the Lihir Islands.

Legend: maps showing the percentage of travellers arriving to the Lihir Islands from each of the 22 provincial divisions of Papua New Guinea, in (A) the plane cohort; and (B) the boat cohort.

ADDITIONAL MATERIAL

Additional file 1.

File format: word document.

Title of data: Supplementary Table 1.

Description of data: Variables and their associations with *Plasmodium* qPCR positive results and logistic regression models for the plane cohort.

FIGURE 1

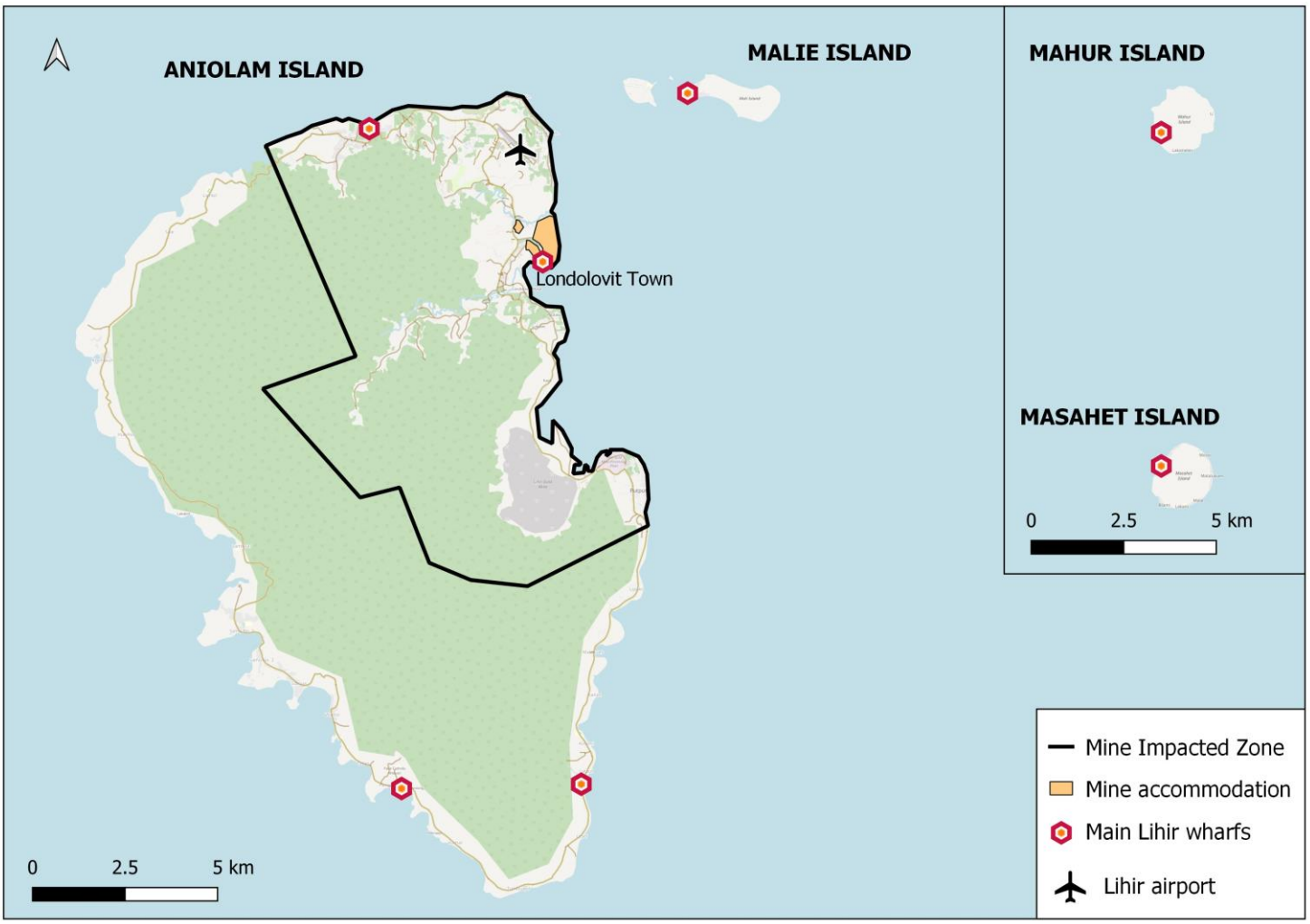
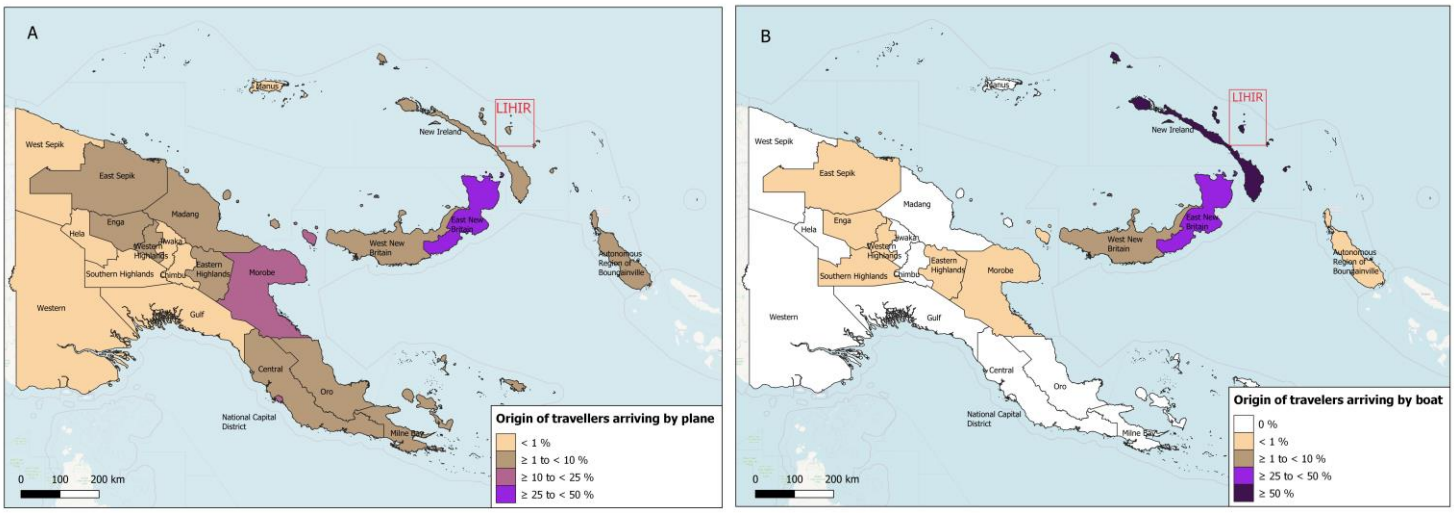

ANIOLAM ISLAND
MALIE ISLAND
MAHUR ISLAND
MASAHET ISLAND
Londolovit Town
0
2.5
5 km
Mine Impacted Zone
Mine accommodation
Main Lihir wharfs
Lihir airport

FIGURE 2


A
LIHIR
Manus
New Ireland
West Sepik
East Sepik
Madang
Enga
Hela
Jiwaka
Western Highlands
Eastern Highlands
Southern Highlands
Simbu
Morobe
West New Britain
East New Britain
Autonomous Region of Bougainville
Western
Gulf
Central
Oro
National Capital District
Milne Bay
0 100 200 km
Origin of travellers arriving by plane
< 1 %
≥ 1 to < 10 %
≥ 10 to < 25 %
≥ 25 to < 50 %
B
LIHIR
Manus
New Ireland
West Sepik
East Sepik
Madang
Enga
Hela
Jiwaka
Western Highlands
Eastern Highlands
Southern Highlands
Simbu
Morobe
West New Britain
East New Britain
Autonomous Region of Bougainville
Western
Gulf
Central
Oro
National Capital District
Milne Bay
0 100 200 km
Origin of travelers arriving by boat
0 %
< 1 %
≥ 1 to < 10 %
≥ 25 to < 50 %
≥ 50 %

ADDITIONAL FILE 1

Supplementary Table 1. Variables and their associations with Plasmodium qPCR positive results and logistic regression models for the plane cohort.

Variable		qPCR positive n (%)	Association p-value*	Univariate OR (95 % CI)	Multivariate aOR (95 % CI)[#]
Sex	Female	2 (7)	0.6324	reference group	reference group
	Male	16 (4)		0.62 (0.13, 2.82)	0.54 (0.11, 2.66)
Age (years)	15 to 29	1 (2)	0.4876	reference group	reference group
	≥30	17 (5)		2.77 (0.36, 21.27)	2.20 (0.28, 17.37)
Origin (arriving from)[a]	Low incidence PNG provinces	3 (2)	0.2964	reference group	reference group
	Medium incidence PNG provinces	7 (6)		2.02 (0.49, 8.26)	2.15 (0.49, 9.42)
	High incidence PNG provinces	8 (6)		2.48 (0.64, 9.56)	2.62 (0.65, 10.61)
Time away from Lihir	>12 to 31	15 (4)	0.6004	reference group	reference group
(days)[b]	32 to 90	1 (6)		1.30 (0.16, 10.44)	1.43 (0.17, 12.07)
	91 or more	2 (6)		1.53 (0.33, 7.00)	3.19 (0.62, 16.35)
Place while on Lihir	Mine accommodation	18 (5)	1	reference group	not considered
	Londolovit Town	0 (0)		1 (-)	for this analysis
Intention of the visit[c]	Mine worker	18 (5)	1	reference group	not considered
	Trading unrelated to mining	0 (0)		1 (-)	for this analysis
	Other purpose	0 (0)		1 (-)	
Frequency of sleeping	Never	10 (5)	0.8312	reference group	reference group
under net while away	Some nights	3 (3)		0.69 (0.19, 2.57)	0.65 (0.17, 2.50)
from Lihir [d]	Most of the nights	2 (7)		1.59 (0.33, 7.67)	1.31 (0.25, 6.83)
	Always	3 (5)		1.19 (0.32, 4.50)	0.63 (0.12, 3.29)
Slept under net while	No	10 (5)	0.9764	reference group	not considered
away of Lihir [d]	Yes	8 (4)		0.99 (0.38, 2.55)	for this analysis

Abbreviations: aOR = adjusted odds ratio, OR = odds ratio, PNG = Papua New Guinea, qPCR = quantitative polymerase chain reaction. [a]n = 376 (5.0% missing), [b]n = 392 (1.0 % missing), [c]n = 381 (3.8% missing), [d]n = 393 (0.7% missing). *significance level set at 0.05. [#]multivariate analysis conducted with 358 observations.

9. DISCUSSION

The studies included in this doctoral thesis offer a comprehensive analysis of malaria transmission in the Lihir Islands, examining both human and vector populations. They also provide valuable insights into various aspects that are key for malaria elimination purposes, including the prevalence of imported malaria on Lihir, the effectiveness of mass LLIN distribution as the primary vector control strategy, and the toxicity and cardiac safety of DHA-PQP when administered over three consecutive months. This section places these results in context, and outlines recommendations for shaping a future malaria control and elimination program in the Lihir Islands based on these findings.

The investigations conducted in the first article of this doctoral thesis revealed a high malaria burden in the Lihir Islands, which was among the highest in the country for the year 2019 (104, 107). Our study showed very high incidence in the non-MIZ of Aniolam, with 596 cases per 1,000 inhabitants, with some areas exhibiting malaria burdens comparable to those seen in countries such as Guinea, Liberia or Sierra Leone in Africa (6). Although the period comprised between May and August detected the highest number of infections, incidence rates did not significantly differ throughout the year. Differences in incidence and prevalence of infections were significant between the geographic areas of Lihir, being lower in the MIZ of Aniolam and in Masahet Island compared to the other areas. In consequence, living in the non-MIZ of Aniolam or in Malie were identified as risk factors for infection. Furthermore, malaria-associated morbidities, such as splenomegaly and severe to moderate anaemia, were also common in Lihir, detecting higher prevalence in the geographic areas most affected by malaria. Both of these conditions were more prevalent in the paediatric population than in older age groups, as observed in similar endemic settings (127). The assessed vector metrics, such as the mosquito and larval densities and the EIRs, also indicate high malaria transmission in Lihir with high variation across zones, being significantly lower in the MIZ and Masahet.

Although the prevalence of microscopic parasitaemia in children remained stable in the MIZ of Aniolam over the past decade, this area has seen a significant threefold decrease in incidence, with 142 cases per 1,000 inhabitants in 2019 versus 437 cases in 2011

(121). This is probably reflecting a change in diagnostics and treatment availability across the health facilities of Aniolam. In 2011, febrile patients were more frequently attended at the LMC (the only health facility of the MIZ) than in the rest of the facilities. On the other hand, prevalence of microscopic infections in children within the non-MIZ of Aniolam decreased from 27% in 2012 to 16% in 2019. Comparison with incidence data outside the MIZ was not possible, as we are the first to report incidences in all the geographic areas of the Lihir Islands. Hence, it is likely that a modest, progressive decrease of malaria transmission did occur in the non-MIZ of Aniolam, due to the introduction of LLIN through mass distribution campaigns, as well as improved access to diagnostics and ACTs across the aid posts located in this area. However, malaria transmission remains high, emphasizing the limited effectiveness of these basic control measures.

Regarding the *Plasmodium* species causing malaria in Lihir, the majority of infections are caused by *P. falciparum* and *P. vivax,* with a high number of mixed infections only uncovered after using qPCR. This aligns with studies conducted in other parts of PNG, which have shown a 17.5-fold increase in the frequency of mixed infections when molecular techniques are used (128). *P. ovale* infections were only revealed after using qPCR, and *P. malariae* infections were much higher when this technique was utilized, as previously reported in the Sepik province (108). The current diagnostic techniques are unable to measure hypnozoites, and previous research in PNG has attributed the vast majority of new *P. vivax* clinical infections (approximately 80%) to arise from pre-existing hypnozoites (70). Hence, the actual number of people infected with *P. vivax* and mixed infections (and probably with *P.ovale* too), in a highly co-endemic setting such as Lihir, is probably higher than reported. In addition to that, we also identified children and young adults (aged <25 years) as the demographic groups at highest risk of malaria infection, especially revealed with qPCR. Children and young adults, accounting for more than 50% of the population living in Lihir, could potentially contribute to significant transmission, given the large number of submicroscopic infections detected. The same risk groups for submicroscopic infections have been identified in other areas of the Pacific region (129) and in Africa (130). Hence, they are important drivers of malaria transmission and should be especially targeted in malaria elimination programs (23).

Altogether, we consider the Lihir Islands a high-endemic setting with stable transmission and co-endemicity of *P. vivax* and *P. falciparum* (and to a lesser extent of *P. malariae* and *P. ovale*), with the young population being the most affected. The co-endemicity of these two main species is common in most of the country and is a distinctive characteristic of malaria epidemiology in PNG and nearby endemic countries (131, 132). On the other hand, the high malaria burden is only found in specific areas of PNG (101, 120) and in Sub-Saharan Africa (1).

Embarking on malaria elimination efforts without reducing the current malaria transmission levels in Lihir may not be the ideal approach. On the contrary, the deployment of multi-approach strategies to reduce the malaria burden to a level where elimination can be more feasible is recommended (12). Large collaborative malaria elimination initiatives are underway in many regions, both in areas with extremely low malaria burdens, such as the Mesoamerican Malaria initiative (133), and in areas with high endemicity, such as the Elimination Eight Initiative in Sub-Saharan Africa (134). However, efforts to address elimination in areas where *P. vivax* is highly endemic and coexists with other common malaria species have not been comprehensively approached. This is why Lihir can still be an ideal location for a pilot program in PNG, capable of generating relevant information on different malaria control approaches with the ultimate aim of elimination.

The LMEP preparation phase for elimination was progressively implemented from 2016 to 2020, and it involved a package of activities aimed at reducing the malaria burden just before the elimination phase. The main pillar activities were health system strengthening, community engagement and awareness, and improvement of coverage of LLIN distributed every three years. We provided training for the health staff in diagnosis and treatment as well as supplies to prevent stock-outs of ACTs and RDTs, which resulted in widespread use of ACT treatments in all health facilities. In addition, we implemented awareness campaigns on malaria transmission and basic prevention measures in the 100% of the communities. Despite all these interventions, overall malaria incidence and prevalence in Lihir Islands remained high, with only a modest progress from 2016 to 2020, compared to the progress achieved between 2005 and 2012 (121). One discernible effect in 2019, when compared to the period of 2017-2018, was a

small decrease in incidence identified through the analysis of passive case detection data. This likely reflects the impact of more frequent and effective awareness activities at communities to enhance health seeking behaviour and compliance to treatment, probably resulting in improved patient treatment. Nevertheless, these slight variations in incidence could also be attributed to changes in rainfall or other environmental factors that we did not study. Overall, the malaria burden in Lihir during 2019 did not differ significantly from the burden seen in rural areas of the nearby islands of New Ireland (104), despite improved control measures in Lihir. While the higher number of new infections reported at the health facilities during the first year of the LMEP program could be explained by an improvement in the reporting system, other factors explaining a rise in incidence should be taken into consideration for the next years. For example, it would be worth studying the effectiveness of LLIN to prevent malaria in Lihir, as well as monitor the emergence of resistances, both of *Anopheles* mosquitoes to insecticides, and of malaria parasites to ACTs.

We have not assessed parasite resistance to artemether-lumefantrine or DHA-PQP, the commonly used ACTs in PNG. However, a recent surveillance from different sites in PNG (including isolates from New Ireland Province) reported a very low prevalence of mutations in the *P. falciparum* kelch13 gene, which is involved in delayed parasite clearance after treatment with artemisinin derivatives (135). Hence, it is highly improbable that parasite resistance to ACTs played a role in the high malaria burden in the Lihir Islands.

In contrast, the limited effectiveness of LLINs may have contributed to the lack of progress in malaria control. The only ongoing strategy in the Lihir Islands, beyond the diagnosis of clinical malaria cases and their prompt treatment, is the distribution of LLINs every three years. In previous studies, this strategy was proven to be effective, as it was considered the main reason for the decline in incidence between 2010 and 2014 in PNG (101, 102). The second article of this doctoral thesis showed that, despite initial high coverage (98%), LLIN use over time is poor. Only 28% of the households preserved at least one LLIN and only 14% of the population slept under a LLIN two years after distribution. Distributed LLINs in Lihir were used for other purposes such as fishing, protecting seeds and fruits, or covering food, like seen in other settings (31, 35).

Our findings reveal that families where the head of household knew that LLIN prevents malaria were 30 times more likely to conserve at least one LLIN. People living in those households were also 16 times more likely to have used the LLINs for malaria prevention. Consequently, if awareness and education on malaria prevention measures are widely disseminated, there may be room for improved maintenance and use of LLIN. The results of a systematic review on information and education interventions showed that those interventions based on combined mass-media and person-to-person approaches resulted in positive outcomes (136). Mass-media information and education included radio messages, posters, slogans, workshops and village demonstrations; while person-to-person monitoring and evaluation included house-to-house visits, village bed net committees, community debates, or training of local health leaders. Current LLIN distribution campaigns in Lihir, and in PNG, are focused on achieving universal coverage at the time of distribution rather than increasing their use and maintenance overtime. Considering all the above, we propose that mass distribution campaigns of LLINs in Lihir (and in other coastal areas of PNG) should be conducted together with frequent awareness campaigns on use and maintenance. A way forward for Lihir could be to utilize the existent network of VMAs to deliver intensive awareness on malaria protective measures before, during, and after the distribution campaigns. The VMAs are local people who understand the particularities of each village, are well-trained in malaria prevention tools, and have the capacity to implement programs house-to-house, targeting the entire population within Lihir.

Nevertheless, effectiveness of LLINs does not rely only on coverage and usage. It is important to consider the biting behaviours of the local mosquitoes. The first article of this thesis reported that *Anopheles* in Lihir have an outdoors feeding behaviour and an early peak biting time, especially in the non-MIZ of Aniolam and in Malie Island. In consequence, even if LLINs were correctly conserved and used in all communities, their effectiveness might not be as high as expected. For instance, a study conducted in 2016-2017 in the Madang Province found that after 7 years of LLIN-based control program in communities with >80% of coverage, effectiveness of LLIN was limited (24) They found vector abundance and transmission intensities similar to the reported in 2009 just after the first LLIN distribution. This was attributed to an earlier and outdoor biting behaviour in the local Anophelines population after repeated LLIN distributions.

Similar results in mosquito behavioural changes are reported from other areas of the Pacific, such as in the Solomon Islands (137).

On the other hand, vector resistance to insecticides could also threaten effectiveness of LLINs. Although it has been reported in PNG, insecticide resistance is not widely spread in the country (113). This study included mosquitoes captured in the Lihir Islands during the 2019 survey, and they did not show any resistance to deltamethrin, the insecticide used within the distributed LLINs. However, low bio-efficacy of the distributed LLINs in 2013, 2016 and 2019 has been reported (138), suggesting defects in the treatment of LLIN with the insecticide or its preservation. Only 17% of LLINs distributed in PNG after 2013 met the required WHO bio-efficacy standards (115). This concerning issue, together with the outdoor and early biting behaviours of Anophelines, and the low use of LLINs, explain the limited effectiveness of their mass distribution to control malaria.

In Lihir, improved coverage and use of high quality LLINs could decrease malaria burden; however, our studies suggest their impact will be limited, discouraging their implementation as the sole vector control strategy.

In consequence, universal coverage with LLINs in Lihir would benefit to be complemented with other vector control strategies. For example, IRS with a different insecticide could be used as a complementary measure, like recommended by the WHO (3). However, IRS has shown limited effectiveness when the vector possesses a highly outdoor biting behaviour such as in Lihir (139, 140). Hence, its cost-effectiveness could be compromised and it should be assessed before implementation. A different vector control strategy that could complementarily work in Lihir is larviciding of breading places. Lihir contains many aquatic habitats suitable for Anopheline breeding, and they are typically small, often less than 1 km² in size. This size can enhance the effectiveness of this strategy (38). Larviciding with *Bacillus thuringiensis israelensis* is already implemented close to the mine employees' accommodation, and it is one of the factors that could explain a lower malaria burden in the MIZ. As reported in the first study of this thesis, there are less Anopheline-positive breeding places within the MIZ compared to the other areas. Another strategy is to implement environmental alteration programs reducing the number of breeding sites (141). This strategy is costly, and could be

unaffordable in a place like Lihir, where torrential rains are frequent, leading to the constant formation of pools and puddles. However, it could be used for specific geographical areas close to populations at highest risk (around schools and boarding schools for example), or in areas close to the open-pit mine, where deforestation and roads create the perfect environment for new breading places (142).

Additional strategies seeking to reduce contact between vector and human populations could impact malaria transmission in Lihir. In the first article of this doctoral thesis, we showed that living in a traditional house was associated with higher risk of infection. This is common in other areas of PNG, and has been attributed to the open housing structures inherent in the traditional dwellings that allow higher contact human-vector-human (114). A systematic literature review on environmental management effects on reduction of malaria burden, showed that modifications of human habitation could reduce the risk of malaria by 79.5% (143). Making houses and tanks impervious to mosquitoes, and modifications of house design like sealing edges of the roofs, closing stilts, or installing mosquito screens in windows, are some examples of these strategies. This strategy could be more cost-effective compared to the previously proposed, and it probably could be well accepted amongst the Lihirian population.

Finally, another complementary intervention in Lihir to reduce mosquito-human interaction could be recommending cattle fencing far from human dwellings. Cattle could be used as a bait for mosquitoes when the species present has an important zoophilic behaviour like *An. farauti* (144), the only vector found in Malie and Masahet, and the main vector in the MIZ of Aniolam. Malaria transmission in Masahet Island was very low, similar to the MIZ, despite there are no additional control strategies deployed. Factors that are different compared to the other areas of Lihir are improved health seeking behaviour and village cleanliness, geographic characteristics creating fewer breading places, and that pigs are fenced away from the human dwellings and close to the forest. This could theoretically decrease mosquitoes feeding on humans as they would encounter the animals first and feed on them, several metres away of the human dwellings.

Aside of vector control measures, there are other strategies aimed at reducing malaria burden that could work towards achieving elimination in Lihir, and which should be considered in parallel. MDA with antimalarials could halt transmission as seen in many examples reported in the introduction of this doctoral dissertation. It is difficult to predict the likelihood of success of an MDA program targeting elimination in a setting like Lihir, where transmission is high and malaria re-establishment after elimination is likely. Repeated campaigns of high coverage MDA targeting both *P. falciparum* and *P. vivax* malaria would likely achieve a significant reduction in malaria burden and transmission (estimated at around 80%), as observed in other settings (55-57). However, before embarking into consecutive MDA campaigns, it would be ideal to have reduced the malaria burden, and to secure enough funds for implementing these campaigns and deploying a surveillance and response program and other necessary following steps to maintain transmission at very low levels (51, 145).

In case that an MDA in Lihir could be implemented in the future, the third and the fourth articles of this doctoral thesis showed that DHA-PQP given in standard 3-day treatment courses over 3 consecutive months would be a safe drug regimen. In the third article, we showed that DHA-PQP is well tolerated and has a favourable cardiac safety profile when used for 3 consecutive monthly courses. There were no SAEs in our clinical trial, and self-limited adverse events occurred in less than one-fifth of participants, with the most frequent being mild abdominal pain, headache, cough, or nausea. All of these adverse events are reported as common in the product safety profile (61). The mean QTcF prolongation after three consecutive months of intake did not differ to the showed after a single course. Similar findings were reported in other studies analysing the effect on QT prolongation after a single 3-day course (146, 147). Although there are very few studies assessing ECG determinations in individuals receiving consecutive monthly courses of DHA-PQP, the findings of a meta-analysis assessing adverse events in close to 20,0000 people receiving DHA-PQP show the safety profile of our proposed regimen (64). We have shown our support with the results and recommendations shed by this metanalysis in an opinion piece attached in the supplementary material contained in the Introduction of this doctoral dissertation (148). Our results could also help clarify that this regimen is safe enough to be given in MDA campaigns.

On the other hand, the PK-PD simulations included in the fourth article showed that 0.08% of males and 0.45% of females in Lihir could be at risk of having an absolute QTcF >500 ms if standard DHA-PQP 3-day treatment courses over 3 consecutive months are given. Considering the current population census, this would mean that up to 65 cases of QTcF> 500 ms could occur with this MDA schedule. It is worth mentioning that a significant association between female gender and higher QTcF at baseline was found and reported in both articles, which aligns with previous findings (149). The third article reported that although QTcF was significantly higher in females at all time points, none of the participants reached QTcF >500 ms. In the fourth article, sex did not influence the relationship of PQP concentration with QTcF, and females did not have a significantly increased risk of cardiotoxicity compared to males. Despite this, in the simulated model with 20,000 individuals, there were slightly more QTcF determinations above 500 ms in the female population. Our simulations included extreme populations (individuals more susceptible to QTcF prolongation) which are unlikely to be seen in real life. Hence, we would expect a smaller number of people with QTcF prolongation of concern if an MDA with DHA-PQP is deployed in Lihir. Importantly, a QTcF >500 ms does not necessarily result in a sudden cardiac event or death, so it is more appropriate to consider it as an adverse event of special interest than a SAE per se.

The results of the fourth article showed that although we would expect none or very few SAEs with this MDA schedule in Lihir, a pharmacovigilance program should be planned. This program could include passive detection of adverse events for the general population, and closer monitoring of individuals at higher risk of QT prolongation, such as the elderly (150). Additionally, individuals under treatment with other QTcF prolonging medications or with history of cardiac disease may be excluded from the intervention. On the other hand, it is recommended that DHA-PQP be taken under fasting conditions because food increases its bioavailability, leading to higher drug concentrations (61). Although this would add complexity to an MDA campaign, it could be recommended for individuals or population groups at higher risk of QT prolongation.

The design of the clinical trial reported in the third and fourth articles did not allow for the analysis of PQP plasma concentrations at long term (i.e., 14, 28 or 42 days after intake), as we only had plasma determinations at 0 hours of course 3 and at 52-54 hours

of each treatment course (after the third dose). This timing was chosen to align with the expected peak concentrations and calculate the greatest impact on the QT interval, as described elsewhere (68). Despite this limitation, we did not find breakthrough infections during the follow-up period, which reassures us that DHA-PQP is an efficacious treatment with a good post-treatment prophylaxis effect. Our findings are consistent with results from other studies. For example, a meta-analysis assessing *P. vivax* recurrence at day 42 post-treatment showed that the risk of recurrence was significantly lower in the DHA-PQP group than in patients treated with artemether-lumefantrine, reflecting the long post-treatment prophylactic period (151). Also, in a study conducted in PNG, the clearance rates at day 42 of 174 positive *P. falciparum* and *P. vivax* patients after a 3-day course of DHA-PQP were 100% and 92.3%, respectively (152).

Another aspect to consider when antimalarial mass treatment is used is the potential for accelerating parasite resistance, driven by the selective pressure on the parasite. This is particularly relevant in *P. falciparum* endemic settings, and when the intervention coverage is low (51). A recent stochastic model demonstrated that the risk of *P. falciparum* resistance due to kelch13 mutations could increase when DHA-PQP is used in four consecutive monthly rounds with population coverages between 75% and 85% (153). In this model, resistances were more likely to emerge when three key factors were met: a limited number of parasite positive individuals in the targeted population, creating a strong genetic bottleneck; the importation of artemisinin-resistant genotypes; and the sustained selection pressure resulting from use of the same ACT for treating new cases after the MDA. In contrast, in Mozambique, where two rounds of MDA with DHA-PQP were conducted with a 4–6-week interval over two consecutive years, no kelch13 mutations were detected. In addition, prevalence of pfpm2 mutations, associated with piperaquine resistance, remained stable compared to before the MDA (154). In the Mozambican program, the coverage for all of the monthly rounds reached <75% of the population. In PNG, the first-line treatment for malaria infections consists of a three-day course of artemether-lumefantrine, with DHA-PQP being reserved for a second-line treatment in case of failures. A therapeutic efficacy study conducted at two sites of PNG found a high rate of parasite clearance after a course of artemether-lumefantrine, with only 1.9% of *P. falciparum* and 4.3% of *P. vivax* infections showing

late parasitological failure (152). Hence, failures of first-line treatment are rare in PNG, and health workers do not typically make use of the second-line treatments. On the other hand, mutations affecting kelch13 and pfmdr1, both associated with resistance to artemisinin-derivatives, have been identified in PNG, albeit without widespread distribution (135, 155). Consequently, a surveillance system for treating failures and monitoring molecular markers of resistance would be advisable to be in place during and after an MDA campaign in Lihir.

Importantly, MDA with blood stages parasiticidal drugs alone is not recommended in a setting where *P. vivax* is endemic (49, 72, 129). The first article of this dissertation, showed a high burden of *P. vivax* in Lihir; thus, we would recommend adding 8-aminoquinolines (tafenoquine or primaquine at full-treatment doses) to the MDA with DHA-PQP. This approach raises concerns about the safety of treating G6PD deficient people with 8-aminoquinolines. An unpublished study conducted in Lihir showed that 0.4% of the tested males were severely deficient (G6PD activity of 0-10%) and another 5.5% were moderate-to-severely deficient (10-30% activity). Based on these findings, it was estimated that 3.5% of the population in Lihir (including males and females) would have a G6PD activity of below 30%, hence being at risk of experiencing clinically significant haemolysis. Thus, we would recommend G6PD testing for the targeted population in case of adding 8-aminoquinolines to the MDA schedule. Due to its lower half-life, and without disposing of tools to revert the effect of a possible haemolysis with tafenoquine, it would be safer to use primaquine in our setting, albeit adding operational complexity. Another consideration when adding primaquine to an MDA schedule is determining the appropriate dosage for radical cure (78). In views of the findings of two recent meta-analysis studying effectiveness and safety of different doses (80, 81), we would recommend to use the higher dose of 0.5 mg/kg/day for 14 consecutive days in participants without G6PD deficiency. In an opinion piece attached in the supplementary material contained in the Introduction of this doctoral dissertation, we reassure that the use of primaquine at this dose is effective and safe for the radical cure (156).

On the other hand, given the main vectors found in Lihir possess a high zoophagic rate, especially *An. farauti*, one possibility could be to add an endectocidal treatment to the

MDA schedule with DHA-PQP + primaquine. This could be particularly useful in Lihir, as human population share common spaces with cattle (pigs mainly) in most of the villages. Adding ivermectin to the antimalarial MDA and/or include mass ivermectin treatment for cattle, could reduce *Anopheles* populations due to its endectocidal properties still preserved when mosquitoes feed on human or animal blood (157-159). However, trials assessing such an approach are ongoing in different malaria endemic areas, and the relative contribution of ivermectin remains to be determined (160, 161).

Finally, if this tremendous effort for reducing malaria burden and transmission in Lihir is to be done, there should be a surveillance and response system for halting transmission from any remanent autochthonous case, relapses, and imported cases (162). The fifth article included in this thesis shows that malaria importation into Lihir is frequent, with 5% of people arriving by plane and 17% of those arriving by boat carrying malaria parasites. Risk of re-establishment in Lihir has not been assessed with mathematical models, and we have not considered other factors such as the climatological conditions. However, the first article of this thesis demonstrated a high receptivity (capacity of a given territory to transmit malaria), and the fifth article a high vulnerability (risk of parasite importation), resulting in a high risk of malaria re-establishment in Lihir. Consequently, in case that local malaria burden is reduced, and more importantly, in the event that elimination is achieved, continuation of strategies to decrease receptivity and prevent transmission of residual and imported cases will be key in Lihir.

A surveillance and response system consisting of passive case detection, and adding active case detection in settings with high re-establishment risk, has demonstrated to be one of the most effective methods for prevention of re-establishment (163, 164). In Lihir, a robust vigilance program could be deployed and integrated into the health system, if it is reinforced with health workers and the intensive training program for the health staff is continued. The network of VMAs could also perform important tasks in the surveillance and response program, helping in the management of outbreaks, recognizing febrile individuals and helping them get to the nearest aid post, identifying and locating potential mosquito breeding places around the houses of positive cases, and

assisting in the implementation of vector control strategies as part of the control of local transmission foci.

Part of this surveillance and response system should be focused on travellers arriving to Lihir, as there is a high influx of arrivals from nearby Islands with high malaria transmission. Patterns of flow following major human migration routes between PNG provinces have already been studied with haplotypes of *P. vivax* isolates (165). An important pattern identified has been the flow between neighbouring Islands of PNG. Nevertheless, the fifth article included in this doctoral dissertation showed that risk of re-establishment from people arriving to the airport was low, as these travellers were mostly mine workers staying at the mine accommodation, associated with less infection risk. The larviciding and removal of breading places are not the only preventative activities conducted around the mine accommodation. The employees' rooms host only one or two individuals, they have air-conditioning and doors and windows are covered with mosquito screens, altogether preventing malaria transmission. Also, many of these workers arrived from low and medium transmission provinces with less risk of importation. In consequence, aside of the usual passive case detection and increased awareness for travellers, specific surveillance strategies for travellers arriving by plane may not be necessary in Lihir because they may not be cost/effective (166).

On the contrary, travellers arriving by boat would face a significantly higher risk of triggering local malaria outbreaks. According to the findings of the fifth article, individuals arriving at the MIZ of Aniolam were three times more likely to be infected compared to those residing near the main wharf, as reported in the first article. Many of these travellers arriving by boat came from nearby areas with a high malaria burden, and they mostly were Lihirian residents returning from a trip, people visiting friends and relatives, and traders. These profiles were all associated with higher infection risk. Furthermore, most of these travellers stayed in the Lihirian villages or in Londolovit town, where people usually share living and resting spaces within a household. In addition, particularly in the villages out of the MIZ, there is a higher percentage of traditional houses as well as higher rates of human biting rates and *Anopheles* densities. Therefore, implementing a robust passive case detection system for these travellers is indispensable. This could be complemented with active case detection at the wharfs.

While the WHO recommends (conditionally) against routine testing and treatment at points of entry (145), the Island condition of Lihir with well-identified points of entry may warrant a different approach. We propose to add screening and treatment upon arrival at Aniolam's wharfs for those travellers arriving from non-Lihirian Islands. An example of a success program of testing upon arrival can be seen in Sri Lanka. This country has implemented active case detection at points of entry for specific risk groups, detecting and treating a high number of imported cases that could have been at risk of transmitting to the local population (167). In Lihir, in case of an active case detection program at the wharfs, a reasonable approach would be to do it with RDTs upon arrival, followed by a repeat test within two weeks. This methodology could improve cost-effectiveness, because some initial low-density parasitaemia infections not seen upon arrival could be later diagnosed (168). Also, for targeting individuals with submicroscopic parasitaemia, molecular techniques such as loop-mediated isothermal amplification could be implemented at these testing points (169), albeit they are much more expensive.

Finally, there are other topics not assessed in the articles included in this dissertation, which are relevant to malaria elimination efforts. If the proposed strategies are implemented in Lihir, they would likely reduce the malaria burden and transmission initially. However, there is no guarantee of long-term success (170). All these strategies are complex and need of a huge collaborative effort from the affected communities. In consequence, communities' engagement is key for the success of any elimination pilot program, like reported in other settings (51, 171-174). Elimination programs in Lihir will fail without early community involvement, strong population sensitization and mobilization, and bidirectional feedback. In addition, collaboration with neighbouring areas is just as essential as it is in settings with land borders adjacent to endemic areas, so importation of infections can be reduced (175, 176). Hence, better engagement with the local, provincial and national government will be necessary. This would allow improving the existent health system, and implementing important advocacy plans for overseeing activities like the pharmacovigilance program or the surveillance and response systems. Elimination programs have had different degrees of success, and collaborative ones, engaging public-private partnerships with strong government involvement have had better results (177-179). Lastly, a big economic investment is key

for a successful program. Pilot programs like the one recommended in this discussion need better economic investments sustained over time. Studies assessing the cost-effectiveness of MDA combined with antimalarials and vector control showed their effectiveness, albeit initially spending large costs. For example, in Zambia, MDA with DHA-PQP showed superior cost-effectiveness in number of infections averted when deployed in areas of high transmission (180). Also, the Magude project in Mozambique was considered to be cost-effective, despite the initial high costs and volume of resources associated to MDA. The Magude project averted a total of 3,171 disability-adjusted life years at an incremental cost of $2.89 million and an average yearly cost of $20.7 per targeted person (181).

10. CONCLUSIONS

1. Malaria transmission is high in the Lihir Islands, and the malaria burden outside of the mine-impacted zone is among the highest in Papua New Guinea. Hence, strengthening malaria control activities is critical before embarking on elimination strategies.

2. The main vector control strategy deployed in Lihir is insufficient to effectively protect the population from malaria infections. Mass distribution of long-lasting insecticidal-treated nets every three years is not sufficient to maintain adequate coverage and usage, especially if it is not accompanied by strong educational campaigns.

3. Even if long-lasting insecticidal-treated nets coverage and usage were high and maintained over time, it should be combined with other strategies to halt malaria transmission in Lihir.

4. If mass drug administration is considered as part of a strategy to accelerate progress towards elimination, the administration of three consecutive monthly courses of dihydroartemisinin-piperaquine does not pose a cardiac risk to the residents of Lihir. However, the implementation of a pharmacovigilance system is recommended.

5. Importation of malaria infections into Lihir is significant, especially among travellers arriving by boat. It is advisable to implement a surveillance and response system to prevent malaria re-establishment targeting travellers arriving by boat, with a focus on high-risk groups for importation.

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