EPIDEMIOLOGY AND CONTROL OF MALARIA IN PAPUA NEW GUINEA: FROM SMALL-SCALE HETEROGENEITY TO LARGE-SCALE SURVEILLANCE AND TARGETED RESPONSE

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To the people of Papua New Guinea

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ACT	Artemisinin based combination therapy
AQ	Amodiaquine
BCC	Behaviour Change Communications
CI	Confidence Interval
DDT	Dichlorodiphenyltrichloroethane
EIR	Entomological Inoculation Rate
eNHIS	Electronic National Health Information System
EOS	Earth Observing System
EVI	Enhanced Vegetation Index
FGD	Focus Group Discussion
GDP	Gross Domestic Product
GES DISC	Goddard Earth Sciences Data and Information Services Center
GFATM	Global Fund to Fight AIDS, Tuberculosis and Malaria
GPS	Global Positioning System
GTS	Global Technical Strategy for Malaria 2016-2030
Hb	Haemoglobin
IDI	In-depth Interview
IPTp	Intermittent preventive treatment in pregnancy
IQR	Interquartile Range
IRR	Incidence rate ratio
IRS	Indoor Residual Spraying
ITN	Insecticide Treated Net
LLIN	Long lasting insecticide-treated net
MGDs	Millennium Development Goals
MIS	Malaria Indicator Survey
MODIS	Moderate Resolution Imaging Spectroradiometer
NASA	National Aeronautics and Space Administration
NHIS	National Health Information System
NIP	New Ireland Province
NMCP	National Malaria Control Programme
NMSP	National Malaria Strategic Plan
OR	Odds ratio
Pf	*Plasmodium falciparum*

Pm	*Plasmodium malariae*
PNG	Papua New Guinea
PNGIMR	Papua New Guinea Institute of Medical Research
Po	*Plasmodium ovale*
PQ	Primaquine
Pv	*Plasmodium vivax*
RAM	Rotarians against Malaria
RDT	Rapid Diagnostic Test
SDGs	Sustainable Development Goals
SHF	Sentinel health facility
SP	Sulphadoxine (or Sulphametoxipyrazine)-Pyrimethamine
TRMM	Tropical Rainfall Measuring Mission
WHO	World Health Organization

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SUMMARY (ENGLISH)

Papua New Guinea (PNG), with a total estimated population of 8.8 million by 2019, has great environmental and cultural diversity which is mirrored by a complex malaria epidemiology. The geographic landscape in PNG is very diverse and in places extremely rugged. Malaria is endemic across most parts of Papua New Guinea and heterogeneous levels of endemicity characterize different areas of the country, from areas with intense transmission to unstable transmission areas with low levels of endemicity and even areas with "anophelism sans malaria". Heterogeneity in endemicity has been attributed to factors within the human, the vector and the parasite. For instance, it has been documented that abundance of alternative hosts such as dogs and pigs together with historic and current control have given rise to significant small-scale heterogeneities in morbidity.

In 2004, control efforts were re-intensified with funding from the Global Fund to Fight AIDS, Tuberculosis and Malaria. Countrywide campaigns distributed free LLINs at the household level and, starting late 2011, improved diagnosis by microscopy and RDTs together with the introduction of ACT have been provided progressively at more public and church-run health facilities. In addition the programme was complemented by advocacy and behaviour change campaigns. As a result, the prevalence of malaria decreased from 11.1% (95% confidence interval, CI: 8.5–14.3) in 2008–2009 to 5.1% (95% CI 3.6–7.4) in 2010–2011 and 0.9% (95% CI 0.6–1.5) in 2013–2014, an unprecedented reduction in PNG. In 2017, the latest national survey registered prevalence levels higher than those in 2010/11. In only three years, the estimated number of malaria infections across PNG increased 8.6-fold to 7.1% (95% CI 5.0, 10.1). Four different species of human malaria have been identified in PNG. Of these four, the two dominant species are *P. falciparum* and *P. vivax*. Overall, *Plasmodium falciparum* has remained the dominant species over *P. vivax*, but their distribution has not been even across the country. In addition, substantial heterogeneity in the prevalence of malaria across PNG has been consistently found over the years with marked differences even between nearby villages.

The aim of this work is to provide a better understanding of the heterogeneous malaria transmission and the dynamics of *Plasmodium*, humans and interventions rolled-out by the Papua New Guinea National Malaria Control Program. This work comprises two major components: 1) a retrospective analysis of incidence of malaria cases in selected sentinel health facilities including a visualization of trends over time in different Sentinel Health Facilities (SHFs), and 2) a cross-sectional malaria survey complemented by a community based qualitative behavioural study.

The retrospective analysis of incidence found that malaria incidence in different sites initially ranged from 20 to 115/1,000 population; subsequent trends varied by site. Overall, LLIN distributions had a cumulative effect, reducing the number of malaria cases with each round (incidence rate ratio range 0.12 to 0.53 in five sites). No significant reduction was associated with ACT introduction. *Plasmodium falciparum* remained the dominant parasite in all sentinel health facilities from 2010 to 2014. Resurgence was observed in one site in which a shift to early and outdoor biting of anophelines had previously been documented. LLINs distributions, but not ACT, were associated with reductions of malaria cases in a range of settings, but sustainability of the gains appear to depend on local factors. Malaria programmes covering diverse transmission settings such as PNG must consider local heterogeneity when choosing interventions and ensure continuous monitoring of trends.

The visualization of incidence trends and other information (net use and residence of patients) extracted from a routinely implemented surveillance system proved useful to inform local malaria control programs to better target interventions. The visualization approach added a geospatial component to health facility data in order to understand differences in malaria burden between villages and identify communities that would benefit from targeted interventions or investigations. However, a functional simple tool for calculating and mapping malaria case incidence at district or sub-district level (e.g. eNHIS or similar) is required to operationalize the approach, along with the capacity, policies, and mechanisms required to implement targeted response action at the respective operational level.

The qualitative behavioural investigation identified seven behavioural groups (or demographic groups exhibiting similar behaviours) and highlighted the substantial amount of time spent outdoors or in non-secure structures when 'indoors' as a major risk of exposure. Between 4pm and 8am, all age groups in both study sites were likely to be exposed to mosquito bites across all types of activities; sleeping under a LLIN was the exception. Such findings highlight the potential of 'outdoor biting' to hamper malaria control and elimination efforts if not addressed appropriately since people spent a remarkable amount of time outdoors without protection from mosquito biting. Targeting groups, places and activities in order to prevent outdoor biting in the early hours of the evening and the morning seems crucial towards elimination.

This work also reveals spatial heterogeneity in the prevalence distribution of malaria and LLIN use between study sites. Malaria prevalence in the Mugil area was 3.7 fold higher than in the Lemakot area. Interestingly, LLIN-use was 2.4 times higher in the Mugil area compared to the Lemakot area. Spatial heterogeneity of malaria was also observed at a village and households level. Prevalence between villages ranged from 0.8% to 19.5% and between households from

0% to 66.6%. In the Mugil area identified risk factor related to behavioural groups (adult women were at lower risk and school children at higher risk) and housing (screened windows and traditional houses were associated with lower exposure) while in the Lemakot area LLIN ownership was a predictor for infection. The identification of site-specific risk factors provides evidence to potentially inform complementary interventions in a local scale that target specific groups or areas.

Heterogeneity of malaria trends was consistent throughout this work. The retrospective analysis and the cross-sectional malaria survey identified: i) heterogeneous effects of malaria interventions across the country, and ii) a heterogeneous distribution of malaria cases over space and time. The cross-sectional malaria survey highlighted varying prevalence between study sites and between neighbouring villages within sites. These findings emphasize the need for locally informed strategies toward improved control. Some communities could still benefit from improved LLIN ownership and use, whereas others might need to complement control with alternatives to LLINs. Targeted interventions in areas of higher transmission has been proposed by modelling and some field studies as opposed to untargeted community-based approaches, but the evidence comparing their effectiveness is scarce. Future research in PNG could address this gap and compare the effect of different control strategies that combine targeted and untargeted interventions.

In addition, outdoor and earlier biting of *Anopheles* species has been identified as a threat to LLINs effectiveness in PNG and other settings. Studies in PNG have described a shift in mosquito biting to earlier hours following the first LLIN distribution (the peak exposure time to infective bites shifted from later than 9pm in 2008 to between 6 and 7 pm in 2011). Our results identified and increase in the number of cases in one site by 2014 despite consistently high LLIN ownership and use in the area. The behavioural investigation identified potential exposure to mosquito bites on the amount of time spent outdoors (when not asleep) or in non-protected structures. Therefore, it is possible that the reduced efficacy of LLINs in synergy with human behaviour and ACT stock outs led to the observed increase especially in places with historically high mosquito densities.

During the course of this work, malaria elimination from PNG by 2030 became less likely than when it was originally envisioned in the National Malaria Strategic Plan 2014-2018. The resurgence in malaria is likely to worsen unless malaria control is re-intensified and maintained. Structuring programmes in response to evidence of the local malaria burden together with an analysis of transmission will enable adapting the strategy to the local context and optimize the use of resources. However a strong and functional surveillance & response

system is needed to monitor the local burden and inform control efforts. Evidence in this study documented reasonable high LLIN ownership across study sites; however LLINs use can be improved in some areas. RDTs and ACTs were not always available in the health facilities therefore efforts need to be made to assure availability especially in areas with higher transmission. Since outdoor biting was consistently identified as an exposure risk and specific groups and areas at higher risk were also identified targeted complementary interventions could be explored and piloted in PNG. Further studies could address the current evidence gap on the effectiveness of targeted interventions.

SUMMARY (TOK PISIN)

Papua Niugini (PNG) em i wanpela kantri we long 2019, ol savemanmeri i tok igat sampela 8.8 million pipol. Em i wanpela kantri we igat planti kainkain environment na kalsa. Ron bilong sik malaria na ol peles we em i kamap em i wankain tu olsem ol dispela kainkain environment na kalsa. Planti kainkain giraun na maunten peles, we long sampela hap em i hat turu long igo long hap. Sik malaria em i bikpela steret insait long kantri, na em i sik i narakain long insait long wanwan peles. Sik malaria em i ken kamap bikpela long wanpela peles na long sampela peles, em ino inap kamap olgeta, maski sapos peles igat *Anopheles* natnat.

Dispela kainkain peles igat o nogat sik malaria em i asua bilong planti kainkain samting ikamap namel long ol manmeri, natnat i karim sik malaria na binatang i save givim sik malaria. Long sampela hap, ol savemanmeri i wok painim aut olsem sapos igat planti kainkain pig na dok i stap long peles na wantaim ol samting bilong banisim manmeri long kisim sik malaria, em i kamapim senis long strong bilong sik malaria insait long ol dispela kain peles.

Long 2004 ol wok bilong daunim sik malaria ibin kamap ken. Dispela em ibin kamap long wanem Global Fund to Fight AIDS, Tuberculosis and Malaria ibin givim moni bilong karim aut dispela wok. Planti taunam igat marasin long em ol ibin givim long ol manmeri long wanwan hauslain insait long PNG. Long 2011 ikam, wok bilong painim aut binatang bilong sik malaria we ol i usim ol microskop na RDT ikamap gutpela na wantaim ACT we ol iwok long givim moa long ol publik na sios helt senta. Bikos long dispela wok, sik malaria ibin go daun long 11% (95% confidence interval, CI: 8.5–14.3) long 2008–2009 igo long 5.1% (95% CI 3.6–7.4) insait long 2010–2011 na 0.9% (95% CI 0.6–1.5) long 2013–2014, we em ibin wanpela kain niupela samting ibin kamap insait long kantri. Tasol long ol yia ikam nau, sik malaria iwok long kamap bikpela ken. Laspela nesenal survey ibin kamap, ol savemanmeri i painim aut olsem namba bilong sik malaria iwok long kamap bikpela moa long namba bilong sik ol ibin painim long yia 2010-2011. Insait long tripela yia tasol na ol savemanmeri i gespaia olsem namba bilong sik malaria igo antap long sampela kain 8.6-fold igo long 7.1% (95% CI 5.0, 10.1) insait long 2017. Fopela kainkain species bilong sik malaria bilong man istap insait long PNG. Long dispela fopela species, *Plasmodium falciparum* na *P. vivax* em ol isave givim bikpela namba bilong sik insait long kantri. Tasol *P. falciparum* em i bikpela moa long *P. vivax*. Ol savemanmeri i painim aut olsem dispela tupela specie bilong sik malaria ino kamap long olgeta hap bilong kantri. Wankain tu, igat planti bikpela heterogeniti long namba bilong sik malaria insait long PNG, we ol savemanmeri i lukim olsem dispela difrens i kamap bikpela moa namel long ol peles i stap klostu klostu wantaim.

Astingting bilong dispela wok em i bilong kisim moa save long dispela heteroginas ron bilong sik malaria na pasin bilong binatang *Plasmodium*, ol manmeri na ol wok bilong daunim sik malaria we Papua Nuigini Nesenal Malaria Kontrol Program i wokim. Dispela wok em i gat tupela hap bilong en: 1) retrospective anelisis bilong namba bilong sik malaria kes insait long ol helt fesilitis mipela ibin makim long we mipela i lukluk long ol trens bihainim taim insait long ol dispela ol sentinel helt fesilitis (SHFs), na 2) cross-sectional malaria survey we mipela iwokim tu wanpela komuniti-bes qualitative behavioural stadi.

Retrospectiv anelisis bilong incidens bilong sik malaria i painim olsem insait long ol wanwan stadi peles istap namel long 20 na 115/1000 pipol, dispela em ino wankain namel long ol dispela peles. Distribusen bilong LLIN taunam ibin gat bikpela ifek, we em i daunim namba bilong sik malaria insait long wanwan raun (incidence rate ratio emi namel long 0.12 na 0.53 insait long faipela peles). Tasol dispela em i no wankain ifek mipela i lukim wantaim ACT. *P. falciparum* em i stap olsem namba wan binatang insait long ol SHF namel long 2010 na 2014. Insait long wanpela peles, mipela i lukim namba bilong sik malaria igo antap ken. Dispela peles em ol savemanmeri ibin lukim olsem ol *Anopheles* natnat iwok long kaikai manmeri long avinun iet na long autsait long haus. Bikos LLIN taunam em i luk olsem wanpela as bilong daunim namba bilong sik malaria insait long kainkain peles na ino ACT, sustenabiliti bilong dispela banis em i depen long ol local faktas. Ol malaria program i kamap insait long PNG we ron bilong malaria i gat kainkain rot i mas tingim dispela ol local heterogeniti taim ol i laik makim wanem kain ol banis bilong daunim sik malaria na i mas wokim moa wok long lukim ol trens bilong dispela sik.

Lukluk bilong ol insidens trens na ol narapela infomesen olsem taunam na peles bilong ol manmeri mipela kisim long ol rutin sevelens istap pinis em i gutpela wei bilong givim infomesen igo long ol local malaria kontrol program long wokim gutpela desisen long wanem banis bilong usim. Dispela wei mipela iwokim em i gat geospasol hap antap long ol helt fesiliti data na iwokim isi long lukim difrens insait long namba bilong sik malaria insait long ol wanwan peles na painim aut wanem ol peles em i gutpela long usim banis iken gat bikpela ifek long daunim sik malaria insait long dispela peles. Tasol, em imas gat wanpela tul bilong kauntim namba bilong sik malaria na peles we ol i kamap insait long distrik or sub-distrik level (e.g. eNHIS or wankain). Wantaim cepasiti, ol polisi na wei bilong ronim dispela ol respons eksen i mas stap long ol wanwan level.

Insait long dispela qualitative behavioural stadi mipela i lukim olsem igat sevenpela grup lain bikos long ol pasin bilong ol na taim we ol isave stap long autsait long haus o long ol peles we ol igat sans bilong kisim sik malaria. Namel long 4pm na 8am, olgeta manmeri long olgeta age grup

insait long tupela stadi peles i gat bikpela sans long natnat long kaikaim ol. Ino gat difrens long wanem samting ol wok, natnat bai igat sans long kaikaim ol, wanpela samting tasol em taim ol I silip aninit long LLIN taunam, natnat ino inap kaikaim ol. Dispela luksave em i soim olsem ol dispela pasin bilong ol natnat long kaikaim ol manmeri autsait long haus em iken bagarapim malaria kontrol na ol hatwok bilong rausim sik malaria olgeta, sapos dispela em i no wanpela hap we ol savemanmeri luksave na tingim long taim ol i wokim ol wok banis bilong daunim sik malaria. Lukluk long ol dispela grup we ol istap long bikpela sans bilong kisim sik malaria, ol peles we natnat i gat bikpela sans long kaikaim ol manmeri na ol wok we iken putim ol manmeri long sans bilong kisim sik malaria em i nambawan long ol wok bilong rausim sik malaria olgeta.

Dispela cross sectional malaria survey soim olsem malaria em i narakain insait long kainkain peles. Prevalens na LLIN em i difren namel long Mugil na Lemakot. Behavioural grup, ol haus na peles we ol wanwan manmeri istap long en em i ol risks bilong kisim malaria we mipela i lukim insait long Mugil. Tasol insait long Lemakot, mipela lukim olsem behavioural grup na LLIN ownership em ol risk faktas. Dispela wok painim aut i confirmim olsem dispela ol heterogeniti bilong distribusen sik malaria insait long PNG. Dispela ol heterogeniti mipela I lukim namel long ol stadi peles na long ol diferen risk bilong kisim sik malaria.

Mipela lukim olsem heterogeniti bilong malaria em i wanpela bikpela samting insait long dispela wok painim aut. Ol retrospective anelisis na cross sectional malaria survey i soim: i) heterogeniti bilong ol ifek bilong ol banis bilong daunim sik malaria na ii) heteroginas distribusen bilong sik malaria kes insait long ol peles na taim. Dispela cross sectional survey em i soim olsem prevalens bilong sik malaria em ino wankain namel long ol peles istap klostu klostu long ol iet insait long ol stadi peles. Dispela i soim olsem igat bikpela nid bilong wokim ol local desisen we ol i ken kamapim gut ol kontrol program insait long wanwan peles. Sampela kominiti ol i ken stil kisim banis long sik malaria sapos namba bilong LLIN ownership i go antap na long sampela kominiti, sampela narapela wei bilong banisim sik malaria igat nid long kamap antap long usim LLIN tasol. Dispela ol target intavensens insait long ol peles wantaim bikpela namba bilong sik malaria iken kamap sapos ol savemanmeri i wokim ol modelling wantaim sampela fil stadi na ino ol kominiti-bes wok tasol. Tasol evidens long skelim dispela tupela rot i sot. Ol behain wok insait long PNG imas lukluk long dispela gap na skelim ifek bilong ol kainkain kontrol strategis we ol bungim target intavensens o nogat.

Wankain tu, planti moa natnat species iwok long kaikaim ol manmeri autsait long haus na ol avinun igo nait iken bagarapim wok bilong ol LLIN taunam insait long PNG na ol narapela peles. Ol stadi insait long PNG i soim pinis olsem ol natnat i senisim taim bilong ol long kaikaim manmeri igo long early hour behain long ol ibin distributim ol fespela LLIN taunam (bikpela

exposa taim ibin senis long behain long 9pm long 2008 igo long namel long 6pm na 7pm long 2011). Wok painim aut bilong mipela i painim aut olsem namba bilong sik malaria igo antap insait long 2014 long wanpela peles, no meta ol ibin gat bikpela namba bilong LLIN taunam. Dispela behavioural wok painim aut i soim olsem igat bikpela sans long natnat kaikaim ol manmeri taim ol istap autsait long haus longpela taim. Mipela i lukim olsem stron bilong LLIN igo daun imas bikos long pasin bilong ol manmeri na tu sot long ACT marasin imas as bilong bikpela namba bilong sik malaria insait long ol peles we mipela isave olsem natnat i pulap long ol dispela hap.

Insait long taim mipela ibin wokim dispela wok painim aut, malaria eliminesen long PNG long 2030 i luk olsem em bai ino kamap olsem ol i tok insait long National Malaria Strategic Plan 2014-2018. Moa tu, namba bilong sik malaria igo antap moa na i luk olsem em I bai igo antap moa iet bikos long ol natnat kaikaim ol manmeri autsait long haus na iken daunim wok bilong ol LLIN taunam. Wankain tu, dispela bikpela heterogeniti i soim olsem igat bikpela nid bilong ol savemanmeri long lukluk long ol ron bilong sik malaria insait long wanwan peles na traim long daunim dispela sik wantaim ol narapela banis we iken halivim LLIN long daunim sik. Em I importen olsem malaria kontrol wok imas kamap bikpela na igo iet insait long ol peles we igat bikpela nid long wanem, em i wanpela samting we iken stopim sik malaria long kamap bikpela moa.

1. INTRODUCTION

1.1. MALARIA

Malaria is a life-threatening disease caused by unicellular protozoa of the genus *Plasmodium*. *Plasmodium* parasites are transmitted from human to human through the bite of infected female *Anopheles* mosquitoes. Five *Plasmodium* species are known to infect humans: *P. falciparum, P. vivax, P. malariae, P. ovale and P. knowlesi. P. falciparum* is the predominant parasite in Africa and is responsible for the main toll of malaria-associated mortality and morbidity (Bhatt et al., 2015; World Health Organization, 2018a). Geographically, *P. vivax* is more extensively distributed (Battle et al., 2015, 2012; Gething et al., 2011) and less responsive to standard control measures, such as sleeping under insecticide treated nets (ITNs) and case management with artemisinin based combination therapy (ACT). Therefore, *P. vivax* is considered to be more challenging parasite to eliminate (Cotter et al., 2013). Until recently, *P. vivax* was mistakenly considered "benign". A growing body of evidence associates *P. vivax* with severe malaria (Battle et al., 2012; Genton et al., 2008; Price et al., 2007). *P. malariae* has been observed in all major malaria-endemic areas. *P. ovale* distribution is limited to some areas of Africa, the Middle East, South-East Asia and the Pacific Islands (Mueller et al., 2007). Until today, rapid diagnostic tests (RDT) are unable to accurately detect *P. malariae and P. ovale*, hence their burden may be underestimated (Mueller et al., 2007). *P. vivax and P. ovale* are capable of developing a dormant liver stage (hypnozoites) (Mueller et al., 2009). Such forms can relapse after the initial infection (e.g. weeks, months, years) and such relapses are a major source of infection of these species (Betuela et al., 2012b; Robinson et al., 2015). Recently in Malaysia, *P. knowlesi* emerged as the fifth species. *P. knowlesi* is potentially life-threating and so far the only zoonotic *Plasmodium* species, infecting humans and macaques (Ahmed and Cox-Singh, 2015; Millar and Cox-Singh, 2015; Singh and Daneshvar, 2013).

1.2. MALARIA BURDEN

Malaria remains one of the main infectious diseases contributing to morbidity and mortality globally with nearly half of the world's population at risk (World Health Organization, 2018a). In 2017, it was estimated that 219 million malaria cases and 435,000 malaria-related deaths occurred worldwide (World Health Organization, 2018a). A widespread scale-up of coverage with the main malaria control interventions, insecticide-treated bed nets, indoor residual spraying (IRS), and malaria case management with artemisinin-based combination therapy, has reduced the malaria burden since 2000 (Bhatt et al., 2015). Compared to 2010, 2017 had 20

million fewer cases (World Health Organization, 2018a). However, data from 2015 to 2017 suggest the progress in reducing malaria cases has stalled (Bhatt et al., 2015; World Health Organization, 2018a). *P. falciparum* is the most prevalent malaria parasite in Africa, South-East Asia, the Middle East and the Western Pacific Islands while *P. vivax* is the dominant species in the Americas. Within areas of *P. falciparum* dominance pockets of intense stable transmission of *P. vivax* are often found in zones under intensive control (e.g. India and Myanmar) (Battle et al., 2012; World Health Organization, 2018a). Children under the age of five are the most vulnerable group carrying a death toll of 266,000 or 61% of global malaria deaths in 2017 (World Health Organization, 2018a).

1.3. Malaria transmission and ecology: Human, vector and parasite interactions

Malaria is transmitted through the bite of female *Anopheles* mosquitoes. Human malaria parasites enter the bloodstream in the form of sporozoites via an infected female *Anopheles* mosquito taking a blood meal. Sporozoites migrate to the liver, where they invade hepatocytes and multiply. The next stage of the parasite (merozoites) is then released into the bloodstream, where it invades a red blood cell (RBC) and initiates the asexual multiplication cycle. A fraction of merozoites released from infected RBCs forms gametocytes; the stage of the parasite infectious to *Anopheles* mosquitoes. Once ingested by mosquitoes, each gametocyte forms either one female macrogamete or up to eight male microgametes. Once in the mosquito midgut, the fusion of a female and a male gamete results in the formation of a zygote that develops into a motile ookinete that infiltrates the mid-gut wall to form oocysts. The oocyst expands over time, burst out and releases sporozoites that migrate to the mosquito salivary gland. Once the sporozoites reach the salivary gland, the mosquito becomes infectious to humans and the cycle starts again (Bousema and Drakeley, 2011). The intensity of transmission depends not only on the parasite and its cycle but also on the vector, the human host, and the environment.

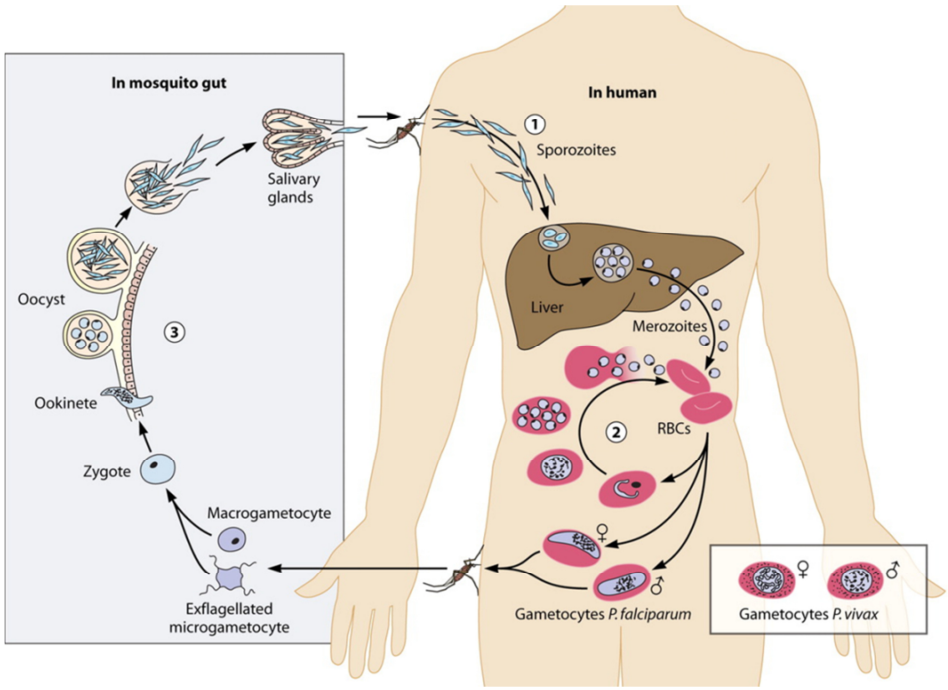

Figure 1.1. Malaria cycle of P. falciparum. Source: Adapted from Bousema and Drakely (2011).

1.3.1. THE VECTOR

Approximately 460 different species of *Anopheles* mosquito exist, approximately 70 of these species are physiologically competent vectors of malaria parasites and 41 have been identified as major malaria vectors (Killeen, 2014; Sinka et al., 2012). Each vector species holds a fluctuating vectorial capacity that can be defined by its distribution, abundance, host preference, ability to develop the parasite, association with humans, and longevity (Cooper et al., 2009). In general, malaria vectors bite between dusk and dawn (Killeen, 2014) and transmission depends upon an exacting set of conditions. A physiologically competent vector can only transmit malaria if in fact it bites humans and survives long enough for sporozoites to completely develop (Beier, 1998). The survival and reproduction of mosquitoes, as well as the *Plasmodium* development within their gut, are strongly dependent upon temperature, humidity and rainfall. Consequently, malaria transmission is most widespread and intense in warmer, more humid regions like the tropics (Gething et al., 2011; Killeen, 2014). In addition, species-specific behaviours exhibited by a vector population in a given area influence their vectorial capacity and their vulnerability to control (Killeen, 2014). Factors like variations in mosquito behaviour, insecticide resistance, behavioural avoidance, vector biodiversity, competitive and food web interactions among different vector species and environmental change affect transmission levels and hence interact with vector control (Ferguson et al., 2010).

1.3.2. *THE HUMAN HOST*

Human populations at risk of malaria infection vary in their exposure, susceptibility to infection and severity of illness (Breman, 2001; Heggenhougen et al., 2003). The immune status of the individual and population plays an important role in the clinical response to infection and transmission (Breman, 2001). Some populations are protected by their genetic makeup. The absence of the Duffy blood factor, hereditary ovalocytosis, α and β thalassemia, sickle cell and glucose-6-phosphate dehydrogenase (G6PD) deficiency among other genetic traits, have been associated with decreased susceptibility to malaria infection (Breman, 2001; Driss et al., 2011; Williams, 2006). In addition, population density (Clark et al., 2008), treatment-seeking, education and knowledge of protective measures, socio-economic status, housing type and co-morbidity have proven to be important factors of transmission (Carter and Mendis, 2002; Heggenhougen et al., 2003; Tusting et al., 2017). Human factors affecting malaria transmission also include a strong behavioural component related to economic development and social change, such as migration and conflict, use and perception of intervention, sociocultural practices, and human made ecological change (Dhiman, 2009; Heggenhougen et al., 2003; Martens and Hall, 2000; Messina et al., 2011). The human element of transmission varies greatly between settings and individuals. Understanding its complexity in a local level plays an important role for the success of interventions especially toward malaria elimination.

1.3.3. *THE ENVIRONMENT*

Transmission also depends on geographical and climatic conditions such as rainfall patterns, temperature and humidity, primarily due to its direct and indirect effect on the vector and the development of the parasite (Abeku et al., 2003; Gething et al., 2011; Midekisa et al., 2012). In some geographical areas, transmission is seasonal, in general with the peak during and just after the rainy season (Midekisa et al., 2012; Roca-Feltrer et al., 2010). Malaria epidemics have been associated with sudden changes in weather conditions that favour transmission in areas where people have little or no immunity to malaria (Pascual et al., 2008; Snow et al., 1993). Epidemics also occur with the move of susceptible populations into areas with intense malaria transmission, usually in order to find work, or as refugees (Heggenhougen et al., 2003; Martens and Hall, 2000). Infrastructure projects such as construction of dams, roads, and industrial or residential centres, often disrupt the terrain and increase the number of mosquito breeding sites (Breman, 2001). Natural and man-made variations in the environment affect malaria transmission therefore understanding its interactions with the parasite, the vector and the human host is relevant for control and prevention of malaria.

1.4. MALARIA CONTROL AND HETEROGENEITY

Malaria is a preventable and curable disease. Vector control approaches, such as the use of insecticide-treated mosquito nets (ITNs), especially long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS), together with case management (prompt access to diagnosis and effective treatment) have been critical for reducing malaria morbidity, mortality, and transmission (World Health Organization, 2015; World Health Organization and Global Malaria Programme, 2017).

1.4.1. MALARIA PREVENTION

The two core vector control interventions currently recommended by WHO are universal access to (and use of) LLINs and IRS for people at risk for malaria. Both interventions reduce human biting rate and vector survival, which in turn significantly reduce vectorial capacity and transmission. LLINs provide protection for individuals against biting *Anopheles* by constituting a physical barrier and killing the mosquitoes before or after they can take a blood meal (World Health Organization, 2015; World Health Organization and Global Malaria Programme, 2017). In general, reductions in prevalence follow patterns of increasing LLIN coverage. LLINs have certainly been the most important intervention across Africa, accounting for an estimated 68% (95% CI 62–72) decline in *P. falciparum* prevalence between 2000 and 2015 (Bhatt et al., 2015). IRS kills mosquitoes resting indoors after they have already taken a blood meal (World Health Organization, 2015; World Health Organization and Global Malaria Programme, 2017) and it was estimated its contribution reducing *P. falciparum* in Africa was 19% (15–24) over the same period (Bhatt et al., 2015). In addition, the first malaria vaccine known as RTS, S, entered pilot implementation in Kenya, Malawi and Ghana. These pilot implementation studies aim to inform the broader roll-out recommendation (van den Berg et al., 2019). The vaccine will be given to young children starting at 5 or 6 months of age and up to 2 years, in areas with a high burden of malaria where children under five years of age are at highest risk of dying (Adepoju, 2019). RTS,S has been the most effective in children aged 5–17 months, who received three doses of the vaccine followed by a booster at 20 months of age, reducing severe malaria cases by 36% (van den Berg et al., 2019).

1.4.2. MALARIA DIAGNOSIS AND TREATMENT

Malaria infection can result in asymptomatic parasitaemia, clinical malaria (febrile episodes with parasitaemia), severe malaria (anaemia, neurologic syndromes and other complications), and death. Symptoms typically become visible 10–15 days after the infective mosquito bite. Acute febrile illness, chronic effects, and pregnancy-related complications are common manifestations of clinical malaria (Breman, 2001; World Health Organization, 2010a). Children

developing severe malaria often exhibit at least one of the following symptoms: severe anaemia, respiratory distress (associated with metabolic acidosis), or cerebral malaria. In adults, multi-organ failure is common. In malaria endemic areas asymptomatic infections occur since people can build up immunity. If not treated within 24 hours from onset of visible signs and symptoms, a clinical attack of malaria could progress to severe illness, and death (Breman, 2001; World Health Organization, 2018a, 2010a).

Prompt diagnosis and treatment is the most effective way to prevent a mild case of malaria from developing into severe disease and death (World Health Organization, 2018a). Malaria infections in symptomatic cases are predominantly detected in blood by RDT or light microscopy (World Health Organization, 2015; World Health Organization and Global Malaria Programme, 2017). Malaria treatment should follow established guidelines (e.g. WHO or national). Treatment that entirely clears malaria infection is essential. Hence, when *P vivax* or *P. ovale* are detected, in addition to a drug clearing the blood-stage of the parasite (e.g. Artemisinin-based combination therapy), anti-relapse therapy (primaquine) is required to clear hypnozoites from the liver. For infections caused by *P. falciparum*, a gametocytocidal drug (primaquine) could be administered in addition to the blood-stage clearing drug in order to prevent further transmission (World Health Organization, 2015; World Health Organization and Global Malaria Programme, 2017).

1.4.3. MALARIA HETEROGENEITY (MICRO-EPIDEMIOLOGY)

As control efforts advance towards malaria elimination, it becomes progressively more important to understand the factors influencing the persistence of malaria transmission at finer spatial scales (Bannister-Tyrrell et al., 2018). The efficacy of interventions (individual or combined) varies by setting and is dependent on many local factors, including vector ecology, human behaviours, health systems, and coverage levels of core interventions (Bhatt et al., 2015). Implementation of standard control measures often result in persistent transmission in 'hot-spots' or particular population groups, even when transmission in the surrounding areas decreases (Bannister-Tyrrell et al., 2017; Bousema et al., 2010). The need to address persistent and heterogeneous transmission by targeting interventions suitable to specific local context seems crucial as countries draw closer to elimination. Thus, a profound understanding of local transmission dynamics is needed beforehand (Bannister-Tyrrell et al., 2018).

1.5. MALARIA SURVEILLANCE

Surveillance is "the continuous and systematic collection, analysis and interpretation of disease-specific data, and the use of that data in the planning, implementation and evaluation of public

health practice"(World Health Organization, 2018b, 2013). The objective of malaria surveillance is to support reduction of the malaria burden, eliminate the disease and prevent its re-establishment. The Global Technical Strategy for Malaria 2016-2030 (GTS) intends to transform malaria surveillance into a core intervention as depicted in Figure 1.2., Pillar 3 (World Health Organization, 2018b, 2015). This technical strategy offers a framework for the development of custom-made programmes to accelerate progress towards malaria elimination (World Health Organization, 2015).

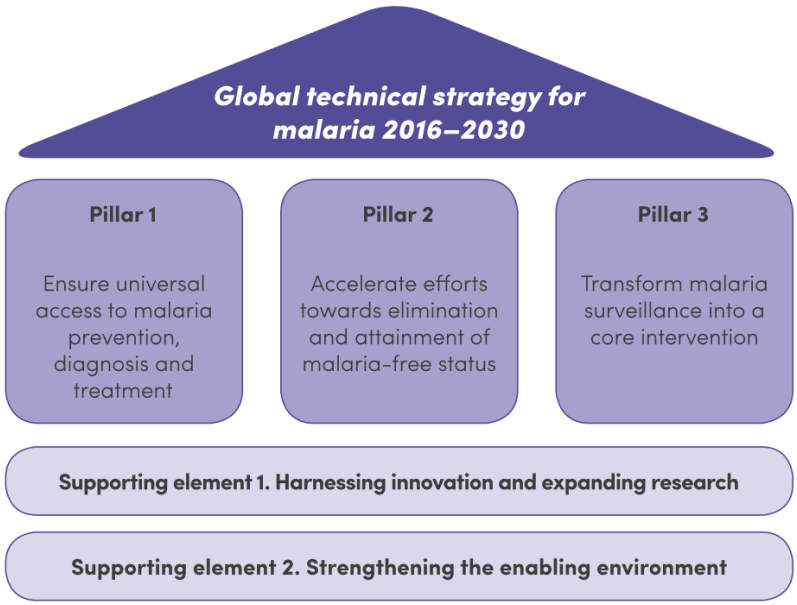

Figure 1.2. Global Technical Strategy for malaria 2016- 2030; framework, pillars and supporting elements.

Previous global and countrywide malaria strategies focused on reducing malaria morbidity and mortality through progressive scaling up of packages of interventions. Aggressive scale up and increase in coverage was the main focus of most programmes (Feachem and Sabot, 2008). The GTS increased the scope of the strategy and emphasizes the importance of surveillance. As a core intervention, surveillance becomes the basis of operational activities since it continually informs the programme efforts, directs targeted interventions and allows case-investigation and classification of cases into local and non-local transmission. The objectives of surveillance across the transmission continuum are to support reduction of the burden of malaria, to eliminate the disease and to prevent its re-establishment (World Health Organization, 2018b, 2015).

1.6. Malaria in Papua New Guinea

Papua New Guinea (PNG), with a total estimated population of 8.8 million by 2019 (United Nations and DESA /Population Divisions, n.d.), has great environmental and cultural diversity

which is mirrored by a complex malaria epidemiology. The geographic landscape in PNG is very diverse and, in places, extremely rugged. The central highlands extend over the length of New Guinea Island. Dense rainforests can be found in the highlands, lowlands and coastal areas and large wetlands surround the Sepik and Fly Rivers (Attenborough and Alpers, 1992; Pacific Climate Change Science Program, 2011). PNG is located within the tropics and mean temperatures are similar across the country but influenced by altitude. In contrast, rainfall shows no country-wide association with altitude (Attenborough and Alpers, 1992). PNG is known to experience irregular perturbations in temperature, humidity and rainfall associated with the El Niño/ Southern Oscillation (ENSO) phenomenon. ENSO related events include draughts, excessive rainfall and frost (Attenborough and Alpers, 1992; Carlowicz and Schollaert Uz, 2017).

Malaria is endemic across most parts of Papua New Guinea and heterogeneous levels of endemicity characterize different areas of the country (Betuela et al., 2012a; Mueller et al., 2003), from areas with intense transmission to unstable transmission areas with low levels of endemicity and even areas with "anophelism sans malaria" (Attenborough and Alpers, 1992). Heterogeneity in endemicity has been attributed to factors within the human, the vector and the parasite. For instance, it has been documented that abundance of alternative hosts such as dogs and pigs (Burkot et al., 1989; Cattani et al., 1986) together with historic and current control activities (Mueller et al., 2005) have given rise to significant small-scale heterogeneities in morbidity (Cattani et al., 1986).

1.6.1. MALARIA VECTORS IN PAPUA NEW GUINEA

PNG is home to at least eleven identified anopheline vectors and each species holds a fluctuating vectorial capacity (Cooper et al., 2009; Keven et al., 2019). The major vectors in PNG are members of the *Anopheles punctulatus* group and have been observed throughout the coast, low-inland areas, hills and even in the highlands (Mueller et al., 2003). Other important vectors include *An. farauti* in coastal villages and *An. koliensis* in lowland inland areas (Mueller et al., 2003). *Anopheles farauti* larvae usually breed in fresh or brackish water from coastal streams, swamps or temporary pools. *Anopheles punctulatus* prefers sunlit water, road tracks and drains. *Anopheles koliensis* larvae generally breed in temporary pools either in grasslands or around the edges of forests(Charlwood et al., 1986; Mueller et al., 2003). These species are anthropophilic and anthropophagic, but human feeding decreases considerably by availability of alternative hosts (Charlwood et al., 1986; Mueller et al., 2003). An interesting finding associated with *Plasmodium*-infected *An. punctulatus* in PNG has uncovered different feeding behaviour of the mosquito when infected with *P. vivax* or *P. falciparum* (Bockarie et al., 1996). More recently, it has been observed that even when high coverage of LLINs has been achieved, mosquitoes have

managed to maintain transmission due to one or more behaviours (e.g. avoiding contact with treated surface, outdoor biting) that reduce effectiveness of current interventions (Reimer et al., 2016; Thomsen et al., 2017).

1.6.2. THE HUMAN HOST IN PAPUA NEW GUINEA

Human factors such as immunity status, behaviour and social interactions have been shaping the epidemiology of malaria since it started its parasitic life in the human host (Carter and Mendis, 2002). In PNG, a variety of RBC traits are present and its geographical distribution appears to have developed in parallel with malaria endemicity (Attenborough and Alpers, 1992). Such traits include South Asian Ovalocytosis, the Gerbich (Ge)-negative blood group phenotype, α and β-thalassaemia, G6PD deficiency and the Duffy polymorphism (Mueller et al., 2003). In addition, the introduction of malaria to the highlands fringe has been attributed to the increased movement of people between lowland areas and the highlands especially due to recruitment of people from higher altitude to work on coastal plantations and the opening of the Highlands Highway in the 1950s (Attenborough and Alpers, 1992; Betuela et al., 2012a; Radford et al., 1976). Travel, nomadic habits, trade, inter-area marriages, collecting clay for pottery, paradise bird hunting, gardening, sago gathering, salt collection, road and transport development and even low intake of vitamin A and zinc have been identified as social and behavioural aspects within the malaria epidemiology of PNG (McMahon, 1974; Mueller et al., 2003; Radford et al., 1976). However, most of the recent research on social and behavioural aspects of malaria transmission in PNG has been centred on the uptake of interventions (Andrew et al., 2015; Pulford et al., 2018, 2012) whereas other aspects of livelihood and behaviour have not been re-explored in the last 30 years. An economic and social transformation is taking place in the country with the introduction of new technologies (e.g. mobile phones), infrastructure and the urbanization of some areas (Australian Government and Department fo Foreign Affairs and Trade, n.d.; The Commonwealth, n.d.; World Bank Group, 2019). Despite facing development challenges, per capita gross domestic product in the country has risen from $196 in 1967 to more than $2,268 in 2014. Life expectancy increased from 44 years to 63 years in the same period (Asian Development Bank, 2017). Such changes call for a re-exploration and revision of social and behavioural components in malaria transmission.

1.6.3. HISTORY OF MALARIA CONTROL IN PAPUA NEW GUINEA

Four different species of human malaria have been identified in PNG (Genton et al., 2008; Hetzel, 2009; Kazura et al., 2012). Of these four, the two currently dominant species are *P. falciparum* and *P. vivax*. Of the remaining two species, *P. malariae* was reported in the sixties as the dominant species in the Sepik and was equally distributed with *P. falciparum* and *P. vivax* in

the highlands. Following introduction of control interventions in 1968 in the area *P. malaria* was permanently reduced (Desowitz and Spark, 1987; Mueller et al., 2003). Currently *P. malariae* distribution is irregular and is mainly found in East Sepik Province while *P. ovale* is very rarely found (Mueller et al., 2003).

PNG species composition is closely related to historical attempts to control and eradicate malaria. It has been generally agreed that before the introduction of vector control programmes*, P. vivax* was the predominant species, followed by *P. falciparum* and *P. malariae* (Attenborough and Alpers, 1992; Hairston et al., 1947). In 1953, spraying with dichlorodiphenyltrichloroethane (DDT) started, followed by mass drug administration with mainly chloroquine across the country (Parkinson, 1974). At first, spraying appeared to increase *P. vivax* dominance, but the spraying was abandoned in the late 1970s. As a consequence, the long dominance of *P. vivax* shifted to *P. falciparum* immediately after spraying interruption (Cattani et al., 1986; Genton et al., 2008; Mueller et al., 2003). Changes in drug use patterns and drug resistance to chloroquine by *P. falciparum* might also have intensified the malaria species shift (Mueller et al., 2003). The era of the global malaria eradication campaign lead by the World Health Organization (WHO) concluded before elimination was achieved and malaria resurged in the 1990s (Attenborough and Alpers, 1992; Hetzel et al., 2015; Nájera et al., 2011). After eradication efforts stop, no control efforts were implemented on a large scale in PNG until the early 2000's.

In 2004, control efforts were re-intensified with funding from the Global Fund to Fight AIDS, Tuberculosis and Malaria (Hetzel et al., 2014c). Countrywide campaigns distributed free LLINs at the household level and, starting late 2011, improved diagnosis by microscopy and RDTs together with the introduction of ACT have been provided progressively at more public and church-run health facilities. In addition the programme was complemented by advocacy and behaviour change communications (Hetzel et al., 2017a, 2014c). As a result, the prevalence of malaria decreased from 11.1% (95% confidence interval, CI: 8.5–14.3) in 2008–2009 to 5.1% (95% CI 3.6–7.4) in 2010–2011 and 0.9% (95% CI 0.6–1.5) in 2013–2014, an unprecedented reduction in PNG. However, malaria prevalence dramatically increased across PNG in the recent years. The latest national survey registered prevalence levels higher than those in 2010/11. In only three years, the estimated number of malaria infections across PNG increased 8.6-fold to 7.1% (95% CI 5.0, 10.1) in 2017 (Hetzel et al., 2018). Overall, *Plasmodium falciparum* has remained the dominant species over *P. vivax*, but their distribution has not been even across the country (Hetzel et al., 2017a). In addition, substantial heterogeneity in the prevalence of malaria across PNG has been consistently found over the years with marked differences even between nearby villages (Hetzel et al., 2017a, 2015).

The resurgence in malaria coincided with a reduction in the Global Fund support to the PNG National Malaria Control Programme (NMCP) and a decline in PNG public expenditure in the health sector that likely resulted in a decrease in the availability of ACTs and RDTs across PNG (Hetzel et al., 2018). In addition, regular outdoor biting of local *Anopheles* species and a shift in peak biting times to earlier in the evening may contribute to reducing the effectiveness of LLINs (Reimer et al., 2016; Thomsen et al., 2017). Experiences from previous campaigns evidently show that relaxing of control leads to malaria resurgences in environments that are conducive for malaria transmission, such as most areas of PNG below 1600m of altitude (Cohen et al., 2012; Mueller et al., 2005).

2. AIMS OF THE THESIS AND SPECIFIC OBJECTIVES

The overall aim of this thesis is to provide a better understanding of the heterogeneous malaria transmission and the dynamics of *Plasmodium*, humans, vectors and interventions rolled-out by the Papua New Guinea National Malaria Control Program.

Specific objectives include the following:

2.1. *To use health facility surveillance data to assess changes in malaria case incidence since the roll-out of interventions and compare the malaria burden between sites from 2010 to 2014.*

- To describe changes in the number of malaria cases between sites and over time
- To compare heterogeneous distributions of cases between sites and over time.
- To identify drivers of change in the number of malaria cases over time

2.2. *To use health facility surveillance data and investigate the usefulness of the spatial disaggregation of routine data for informing targeted interventions.*

- To identify any administrative clusters (hamlets, village and/or ward level) showing higher or singular patterns in malaria cases within each sentinel health facility and through the years (2010 to 2014).

2.3. *To investigate the distribution of malaria infection across spatial clusters and population sub-groups in order to identify the extent of residual malaria at the time of study.*

- To investigate local drivers of prevalence
- To map household-prevalence of malaria for selected villages.

2.4. To better understand the role of human behaviour in relation to malaria transmission and transmission heterogeneities in selected sites.

- To identify the range of activities that could potentially expose individuals to outdoor biting during *Anopheles* biting times.
- To identify behavioural groups relevant to transmission based on the range of activities carried out during *Anopheles* biting times.
- To quantify behaviours relevant to intervention use and malaria prevention.
- To identify aspects of livelihood that might be relevant to malaria transmission in selected study sites.

3. METHODS

This work comprises two mayor components. To address specific objectives 2.1. and 2.2., a retrospective analysis of incidence of malaria cases in selected sentinel health facilities was conducted. To address specific objectives 2.3. and 2.4., a cross-sectional malaria survey complemented by a community based qualitative behavioural study was used. The following paragraphs describe in more detail each component of this work.

3.1. RETROSPECTIVE ANALYSIS OF INCIDENCE OF MALARIA CASES IN SELECTED SENTINEL HEALTH FACILITIES

Since 2004, the Global Fund to Fight AIDS, Tuberculosis and Malaria has been the main donor to the PNG NMCP. An integral part of the Global Fund support was an evaluation and operational research program developed by the Papua New Guinea Institute of Medical Research (PNGIMR) (Hetzel et al., 2014c). The evaluation included a complementary set of studies including national surveys and sentinel surveillance. Sentinel surveillance sites were established to follow morbidity and mortality trends alongside intervention coverage indicators in the same known population over the entire period of the Global Fund grant (Round 8, 2009 to 2014). The sentinel site activities included (i) morbidity surveillance in Sentinel Health Facilities (SHF), (ii) demographic surveillance and repeated household surveys in the catchment area, and (iii) entomological surveys. A total of 7 sentinel surveillance sites were established. Two sentinel sites were set up in the Southern (East Cape and Balimo), Momase (Sausi and Dreikikir) and Islands Regions (Lemakot and Arawa) and one in the Highlands Region (Karimui). One site per region (East Cape, Sausi, Lemakot and Karimui) was dedicated to comprehensive surveillance including SHF- and community-based morbidity and mortality surveillance; the remaining 3 sites (Balimo, Dreikikir and Arawa) were considered complementary with only SHF-based activities (Hetzel et al., 2014c). Part of this work (Chapter 4 and 5) was built around data collected in the established SHF from 2010 to 2014.

At the SHFs all outpatients and admissions were routinely screened for history of fever in the previous three days. A capillary blood sample for diagnosis of malaria by RDT alongside thick and thin blood films for microscopic diagnosis were collected by a trained PNGIMR nurse. Demographic details (age, sex, pregnancy status, village of residence), clinical signs and symptoms, previous visit to the health facility, recent use of antimalarials, use of LLIN, haemoglobin (Hb) level, body temperature, RDT result and final diagnosis were recorded on a paper case report form (CRF) for each identified fever case.

Microscopy slides were examined twice independently by two microscopists following WHO guidelines (World Health Organization, 2010b). Discordant reads were confirmed with a third read by a senior microscopist. Microscopy results and CRFs were double entered at PNGIMR into a digital database. Both data sets were merged and the resulting data set was cleaned and analysed retrospectively after data collection for Round 8 ended in all SHF. To address specific objective 2.1., (Chapter 4) data from all seven SHF was used. To address specific objective 2.2., (Chapter 5) data from the four SHFs with community based demographic data was used since the size of the studied population at a village level and the geo-location of the villages was essential for the analysis. Chapter 5 was developed using an innovative video-communication format that combine the traditional manuscript in a shorter version with a video.

3.2. CROSS-SECTIONAL MALARIA SURVEY COMPLEMENTED BY A COMMUNITY BASED QUALITATIVE BEHAVIOURAL STUDY

In order to address objective 2.3., a cross sectional malaria prevalence survey was conducted in two study sites. The survey was complemented with a community based behavioural study in order to address specific objective 2.4. These two components were part of a larger project addressing multiple aspects of residual malaria transmission in PNG.

According to WHO malaria terminology (Global Malaria Programme and World Health Organization, 2016) residual malaria transmission (also referred to as “ongoing transmission”) is the transmission of malaria that persists even after core malaria measures have been implemented. A clearer understanding of the magnitude and drivers of residual malaria transmission could help to develop and optimize tools and strategies towards malaria elimination. Following this rationale, five research projects around the globe (Greater Mekong Sub region, South America, South Pacific and East and West Africa) were selected and supported to provide the baseline evidence of residual malaria in selected settings. Financial and technical support was offered by TDR, the Special Programme for Research and Training in Tropical Diseases (vbd-environment, n.d.).

The selected project in the South Pacific, i.e. in PNG, aimed to determine the prevalence of on-going malaria transmission in two sites in Papua New Guinea and to understand how humans, vectors and their interactions influence human infection by malaria (Figure 3.1.). Part of this work was built around the *Plasmodium* prevalence survey and human behavioural component of this study (vbd-environment, n.d.).

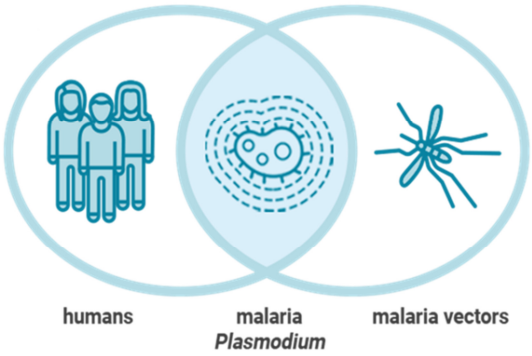

Figure 3.1. Scope of the TDR funded study on residual malaria transmission in PNG

3.2.1. Assessment of ultra-sensitive malaria diagnosis

In order to maximize resources in such resource-limited setting and promote cooperation between research projects, a study assessing ultra-sensitive malaria diagnosis versus standard molecular diagnostics was embedded into the cross sectional malaria survey. Collection of blood samples was adapted in two villages (Mirap and Megiar) in order to accommodate this parallel study that required venous blood samples from 300 participants. The study, which was funded by the Swiss National Science Foundation, resulted in a published manuscript (Appendix 1).

3.3. THE STUDY SITES

The study sites for the two components of this work differ between chapters. The data for the retrospective analysis (objectives 2.1. and 2.2.) was collected in the four geographical regions of PNG in seven sites whereas the cross-sectional malaria survey and community based behavioural study (objectives 2.3. and 2.4.) were conducted in two sites, one in Madang Province and one in New Ireland Province. The following map (Figure. 3.2.) depicts the location of all sites used for data collection throughout the entirety of this work. More study-specific details on the study sites can be found respectively in the following results chapters.

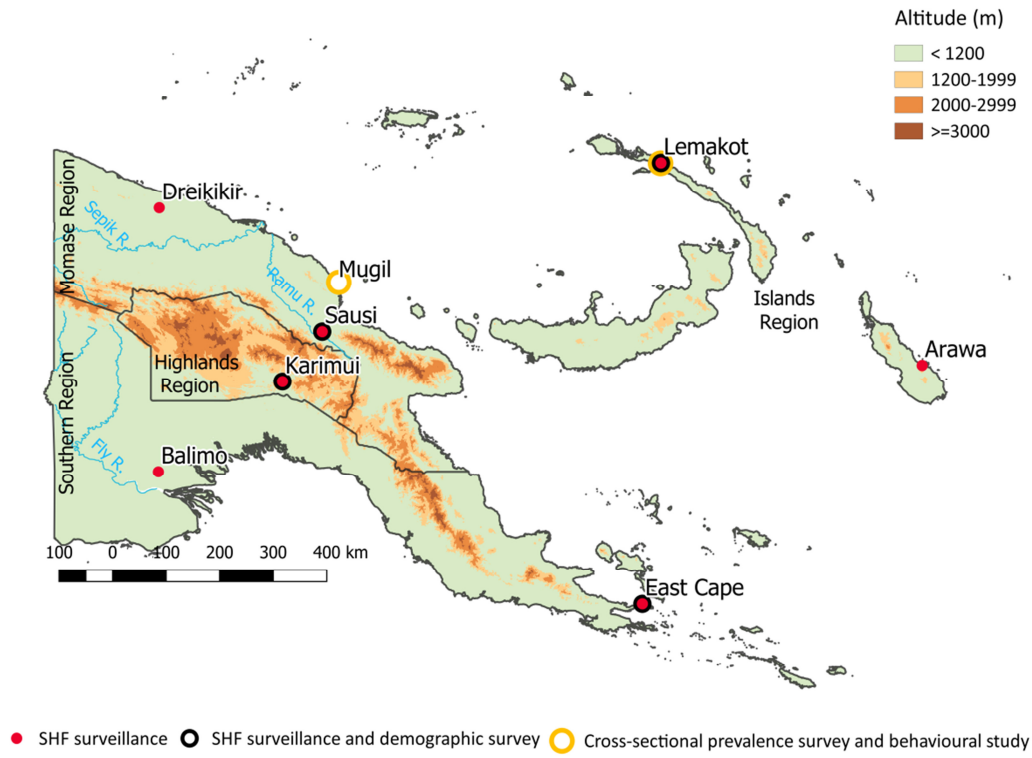

Figure 3.2. Map of Papua New Guinea with all study sites locations and the type of data collection implemented in each site.

4. Repeated mosquito net distributions, improved treatment, and trends in malaria cases in sentinel health facilities in Papua New Guinea

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4.1. Abstract

Background

Long-lasting insecticidal nets (LLIN), improved diagnosis and artemisinin-based combination therapy (ACT) have reduced malaria prevalence in Papua New Guinea since 2008. Yet, national incidence trends are inconclusive due to confounding effects of the scale-up of rapid diagnostic tests, and inconsistencies in routine reporting.

Methods

Malaria trends and their association with LLIN and ACT roll-out between 2010 and 2014 in seven sentinel health facilities were analysed. The analysis included 35,329 fever patients. Intervention effects were estimated using regression models.

Results

Malaria incidence initially ranged from 20 to 115/1,000 population; subsequent trends varied by site. Overall, LLIN distributions had a cumulative effect, reducing the number of malaria cases with each round (incidence rate ratio range 0.12 to 0.53 in five sites). No significant reduction was associated with ACT introduction. *Plasmodium falciparum* remained the dominant parasite in all sentinel health facilities. Resurgence occurred in one site in which a shift to early and outdoor biting of anophelines had previously been documented.

Conclusions

LLINs, but not ACT, were associated with reductions of malaria cases in a range of settings, but sustainability of the gains appear to depend on local factors. Malaria programmes covering diverse transmission settings such as PNG must consider local heterogeneity when choosing interventions and ensure continuous monitoring of trends.

Keywords

Malaria, Incidence, Vector control, Artemisinin-based combination therapy, *Plasmodium falciparum*, *Plasmodium vivax*

4.2. BACKGROUND

Malaria in Papua New Guinea (PNG) was described by Koch in 1900 (Attenborough and Alpers, 1992; Ewers, 1972) and to date malaria transmission remains endemic in PNG especially in areas below 1,400 m altitude (Betuela et al., 2012a; Hetzel et al., 2015). Over the last century, the epidemiology of malaria in PNG has been influenced by control and elimination efforts (Attenborough and Alpers, 1992; Hetzel et al., 2015; Mueller et al., 2003; Parkinson, 1974). In the 1950s to 1980s, PNG had joined efforts of the Global Malaria Eradication Programme with spraying of dichlorodiphenyltrichloroethane (DDT) and mass drug administration (primarily chloroquine) (Parkinson, 1974). The programme concluded before elimination was achieved and malaria resurged in the 1990s (Attenborough and Alpers, 1992; Hetzel et al., 2015). In 2004, control efforts were re-intensified with funding from the Global Fund to Fight AIDS, Tuberculosis and Malaria to the PNG national malaria control programme (NMCP). Since then, the NMCP has promoted: 1) the country-wide free distribution of long-lasting insecticidal nets (LLIN); 2) behaviour change campaigns (BCC); and, 3) the scaling-up of parasitological diagnosis by rapid diagnostic test (RDT) or microscopy, together with the introduction of artemisinin-based combination therapy (ACT), specifically artemeter-lumefantrine (Hetzel et al., 2014c).

Since this last scale-up, the malaria burden in PNG has steadily decreased as reflected in declining prevalence of infection (Hetzel et al., 2017b), incidence (Hetzel et al., 2016) and transmission (Reimer et al., 2016). However, malaria control efforts are intrinsically affected by the great environmental and socio-cultural diversity across the country, including a major mountain range over the length of the main island, dense rainforests in the highlands, lowland and coastal areas, and large wetlands surrounding major rivers (Attenborough and Alpers, 1992). This diversity has influenced human population distribution, human behaviour and mosquito ecology. All this, and the presence of four human pathogenic malaria parasites (*Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale*), results in a complex malaria epidemiology with heterogeneous levels of endemicity (Attenborough and Alpers, 1992; Betuela et al., 2012a; Cattani et al., 1986; Mueller et al., 2003).

While changes in malaria prevalence have been consistently investigated since 2008 (Hetzel et al., 2017a), national trends in malaria incidence are inconclusive and difficult to interpret due to confounding effects of the scale-up of RDTs, changes in health facility reporting forms, and inconsistencies in routine reporting (Hetzel et al., 2014c).

This study aimed to estimate malaria trends over time (2010-2014) in seven sentinel health facilities (SHF) and assess the effect of repeated household-level distributions of LLINs and the introduction of ACT in distinct epidemiological settings across PNG.

4.3. METHODS

4.3.1. STUDY DESIGN

A health facility based longitudinal study established surveillance of malaria cases, severity of symptoms, net use and parasite species composition in seven purposively selected sentinel health facilities from 2010 to 2014 (Figure 4.1.). Intervention roll-out was recorded for each site. In four sites a population census was conducted in the catchment area of the SHF at the beginning of the surveillance period. In addition satellite data was extracted for each site and for the duration of the surveillance period to complement clinical data with environmental data.

4.3.2. STUDY SITES

SHFs were functioning health centres and one sub-centre (Sausi), accessible by road or air, with a catchment population of at least 5,000 people that regularly reported malaria cases. The catchment area defined by the local authorities was adopted for the surveillance. Surveillance activities were established as part of the continuous independent evaluation of the NMCP (Hetzel et al., 2015, 2014c). Seven SHFs were selected from all geographical region of PNG (Southern, Highlands, Momase, Islands) two per region except for the Highlands where only one was selected due to a lower malaria burden at higher altitudes(Betuela et al., 2012a). A description of each site is provided in Additional file 1.

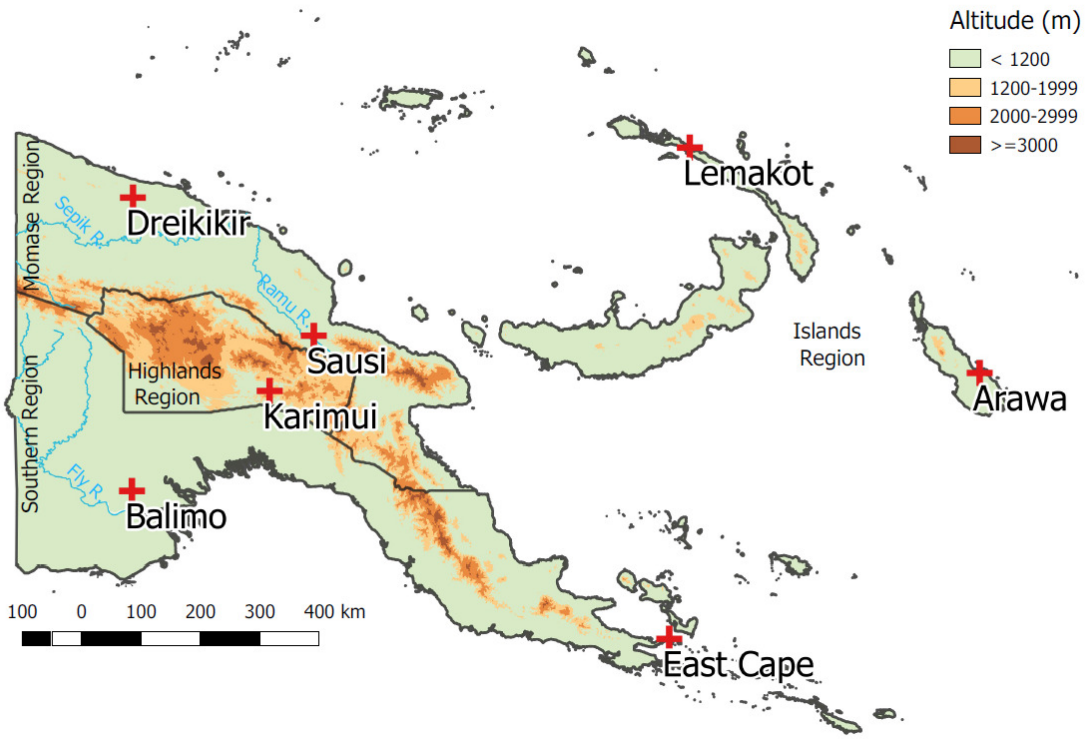

Figure 4.1. Location of sentinel health facilities in Papua New Guinea (red crosses). Dark lines indicate regional boundaries

4.3.3. DATA COLLECTION

Clinical data at the sentinel health facilities

The surveillance period in each SHF and the timing of LLIN distribution rounds and introduction of ACT as first-line treatment are provided in Additional file 2.

All outpatient cases attending a SHF were routinely screened for a history of self- reported fever in the previous three days ('fever case'). Study-related procedures were performed by registered nursing officers or community health workers ("study nurses") trained in the proper performance of capillary blood sample collection, the use and reading of RDT test kits according to the manufacturer's guidelines, the disposal of bio-hazardous waste, and the recording or results according to the study protocol. The study nurses were based full-time at the facility and collected a capillary blood sample by finger-prick from all consenting fever patients for: 1) point-of-care diagnosis of malaria by RDT; 2) thick and thin blood smear for malaria diagnosis by light microscopy; and, 3) measurement of haemoglobin (Hb) concentration. All RDT-positive cases were considered 'malaria cases' in this analysis. Severe malaria was defined as RDT-positive cases presenting with at least one of the following danger signs: impaired consciousness (including coma or convulsions), difficulty breathing, or severe anaemia (Hb <8 g/dl or <7 g/dl for children under 5 years old and pregnant women). Demographic details of the patients (age, sex, pregnancy status) and self-reported mosquito net use the previous night

were recorded on paper case report forms (CRF) alongside selected clinical indicators, including axillary temperature, Hb measurement and RDT results. Patients were then transferred to a health facility clinician for further examination. The final diagnosis as determined by the health facility clinician and prescribed treatment were recorded in the same CRF. The study team ensured availability of RDTs during the surveillance period.

RDTs (ICT Malaria Combo HRP2/aldolase, ICT Diagnostics, South Africa) were used following manufacturer's guidelines. A sub-sample was further examined by light microscopy at the Papua New Guinea Institute of Medical Research (PNGIMR) to identify the *Plasmodium* species (Additional file 3). Microscopy slides were fixed with methanol (thin smear), stained with Giemsa (thin and thick smear) and read independently by two microscopists. Discordant reads were confirmed with a third read by a senior microscopist (WHO level 1 or 2). The number of parasites was counted for 200 white blood cells and a slide was declared negative after reading a minimum of 200 thick film fields. Hb concentration was measured using a HemoCue Hb 201+ Analyser (HemoCue AB, Sweden) and axillary temperature with a digital thermometer.

Demographic composition

A population census was conducted in the catchment area of East Cape, Karimui, Sausi and Lemakot at the beginning of the surveillance period. The available funding was insufficient to conduct a baseline census in the three remaining SHFs. For the census village leaders assisted in the identification of households and enumeration of household members. The variables captured for each household included: household size, and age and sex of each household member. The annual population growth rate of 3.1% was obtained from the National Statistics Office (National Statistical Office, 2013).

Environmental data

Rainfall (product 3B43) and Enhanced vegetation index (EVI; products MOD13Q1 and MOD13A3) data were extracted from remote-sensing databases by the Tropical Rainfall Measuring Mission (TRMM) and the Earth Observing System (EOS) respectively. Rainfall data were accessed using the Mirador system in the NASA Goddard Earth Sciences Data and Information Services Center (GES DISC) website (Dinku et al., 2007; Midekisa et al., 2012; Precipitation Processing System Tropical Rainforest Measuring Mission, 2017). EVI data were accessed using the NASA Earth Data Search website(Didan et al., 2015). In addition the annual occurrence of the El Niño/La Niña phenomena was extracted from the NASA Earth Observatory (Carlowicz and Schollaert Uz, 2017). Key environmental variables are available in Additional file 4.

4.3.4. DATA ANALYSIS

Statistical data analysis was conducted using Stata/IC v.13.1 (Stata Corp LP., College Station, USA). Monthly data were graphically displayed to visualize trends in the numbers of fevers and malaria cases by SHF. Monthly accumulated rainfall in mm (product 3B43), was included as proxy of site-specific seasonality (Dinku et al., 2007; Midekisa et al., 2012; Precipitation Processing System Tropical Rainforest Measuring Mission, 2017). Missing periods in surveillance data reflect temporary unavailability of study nurses due to leave or staff change and were not related to particular times of the year.

The annual proportions of RDT-positive fever cases (RDT positivity) were calculated with 95% exact confidence interval (CI). The *Plasmodium* species composition was estimated from the light microscopy results. Details are provided in Additional file 3.

For the four sites with available census data, malaria incidence (all cases with a positive RDT) and 'severe malaria' incidence were calculated per 1,000 population per year. Population denominators were adjusted for an annual growth rate of 3.1%.

The association between the roll-out of interventions (each of the three rounds of LLIN distribution and of the introduction of ACT as first-line treatment for test-confirmed malaria) and the number of malaria cases was assessed using regression models. The number of cases was used as the outcome since denominators were only available in four sites. To investigate the effect of LLIN distribution rounds, malaria cases were disaggregated by age groups for each LLIN distribution round. Regression models were used to assess the effect of both interventions simultaneously in the seven sites. The LLIN distribution variable had three different values, one for each period between LLIN distributions. The ACT variable was binary, with value zero before the introduction of ACT and value one thereafter. In preliminary analyses, time since the intervention was included as a variable. However, due to the limited number of observations for each site and the need for simplicity for interpretation these variables were not included in the final model.

Negative binomial regression was used to estimate the effect of the interventions on the monthly aggregate number of malaria cases. Fixed effects were included for the interventions and further covariates. Separate models were applied for each SHF after first establishing that the effects of the LLIN rounds were significantly different between sites using interactions terms. Due to convergence limitations, Poisson regression was used for the model with interactions.

The use of environmental variables such as rainfall (with and without time-lag) and enhanced vegetation index (EVI) was explored. Initially rainfall was included in the model as a monthly mean per day and alternatively as accumulated monthly aggregate. Introduction of these variables in the model was explored with and without time-lags (1 month and 2 months). EVI variables were similarly explored in the model. The monthly averages of two different EVI products were introduced in the model with and without time-lag. These variables were later omitted from the model due to poor predictive ability. Finally the estimates were adjusted for El Niño and La Niña annual occurrence. El Niño/La Niña variable was introduced in the model as a categorical variable with 3 possible values for annual occurrence (El Niño in 2010, La Niña in 2011 and 2012 and none in 2013 and 2014).

4.4. RESULTS

During the surveillance period, a total of 35,329 fever cases were recorded across all SHFs. RDT results were available for 98% (range: 94-99%) of all cases (Table 4.1.). The pooled RDT positivity was 32%. Site-specific RDT positivity ranged from 4% in Balimo to 49% in East Cape.

Table 4.1. Number of fever cases and rapid diagnostic test result by sentinel health facility

Health facility	**Fever cases N**	**RDT positive N (%, 95% CI)**	**RDT not done N (%, 95% CI)**
Balimo	1,596	57 (4, 3-5)	100 (6, 5-8)
East Cape	7,311	3,601 (49, 48-50)	137 (2, 1.6-2.2)
Karimui	3,166	583 (18, 17-20)	34 (1, 0.7-1.5)
Dreikikir	3,869	876 (23, 21-24)	27 (0.7, 0.5-1.0)
Sausi	6,450	1,654 (26, 25-27)	238 (4, 3-4)
Arawa	3,183	439 (14, 13-15)	61 (2, 1-2)
Lemakot	9,754	4,111 (42, 41-43)	77 (0.7, 0.6-1.0)
Total	**35,329**	**11,321 (32, 32-33)**	**674 (2, 1.8-2.1)**

RDT = Rapid diagnostic test CI = Confidence interval

The pattern of fever and malaria cases varied over the surveillance period and between SHFs (Figs. 4.2., 4.3., 4.4. and 4.5.). All sites displayed monthly variations but there was no clear relationship with rainfall patterns. The number of fever and malaria cases decreased over the surveillance period in all sites except in Dreikikir and Sausi, where after an initial decrease an increase was noted in 2014. Malaria cases initially increased in Lemakot (2012), but decreased steadily thereafter. Annual RDT positivity decreased steadily over the surveillance period in most sites but fluctuations were observed especially in sites with low numbers of cases. A substantial increase in RDT positivity in Lemakot (from 35 to 68%) in the year 2012 with proportional increase in P. vivax (Figures 4.2. and 4.5.) and a spike of cases in women aged 15-20 (Additional file 6) suggests a local epidemic.

Plasmodium falciparum was the predominant species in all sites and all years even though species composition fluctuated over time and differed between sites. Balimo was the only SHF in which no infections with *P. vivax* were detected. Proportional increases of *P. vivax* were observed in Lemakot (2012) and Sausi (2014) at a time when the total number of malaria cases also increased (Figures 4.2. and 4.5.). Over the entire surveillance period, only 0.2% of malaria cases were diagnosed with *P. malariae* and 0.02% with *P. ovale*.

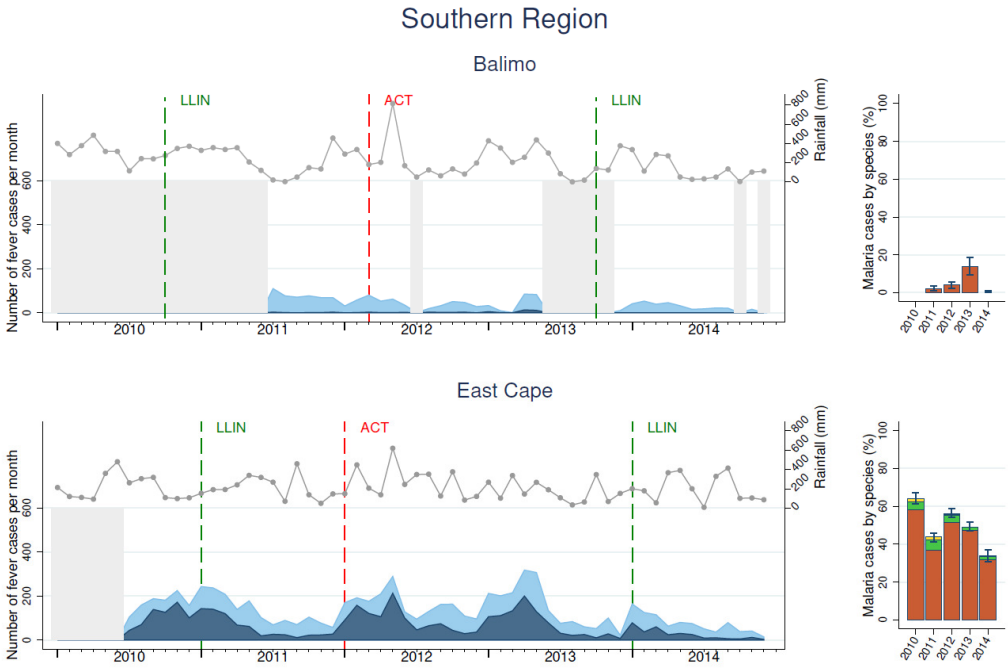

Figure 4.2. Malaria cases in Southern Region (Balimo and East Cape) sites.

Left of each panel: monthly number of fever cases RDT negative (bright blue) and RDT positive (dark blue); cumulative monthly rainfall (grey line); timing of LLIN distribution and introduction of ACT (vertical dashed lines). Missing data is indicated by light grey shaded background. Right of each panel: annual RDT positivity (bar total) by species: P. falciparum (orange), P. vivax (green), mixed infections (yellow), no species data available (white).

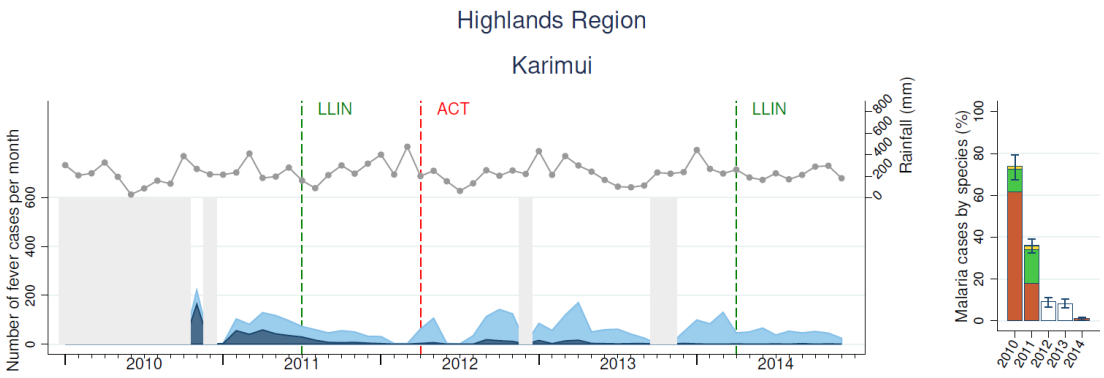

Figure 4.3. Malaria cases in Highlands Region (Karimui) site

Left of each panel: monthly number of fever cases RDT negative (bright blue) and RDT positive (dark blue); cumulative monthly rainfall (grey line); timing of LLIN distribution and introduction of ACT (vertical dashed lines). Missing data is indicated by light grey shaded background. Right of each panel: annual RDT positivity (bar total) by species: P. falciparum (orange), P. vivax (green), mixed infections (yellow), no species data available (white).

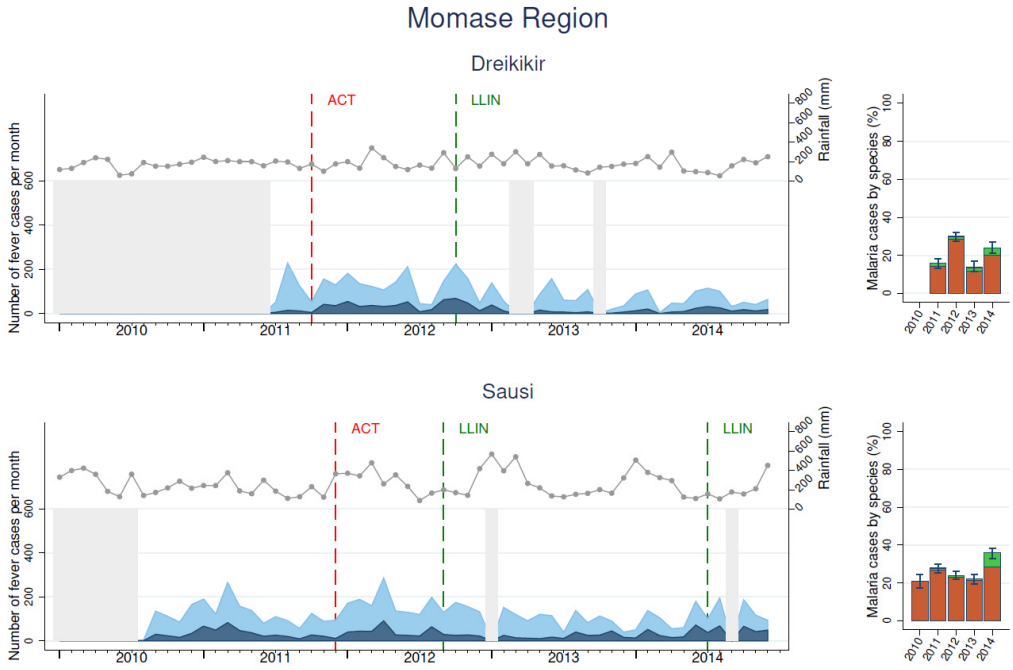

Figure 4.4. Malaria cases in Momase Region (Dreikikir and Sausi) sites

Left of each panel: monthly number of fever cases RDT negative (bright blue) and RDT positive (dark blue); cumulative monthly rainfall (grey line); timing of LLIN distribution and introduction of ACT (vertical dashed lines). Missing data is indicated by light grey shaded background. Right of each panel: annual RDT positivity (bar total) by species: P. falciparum (orange), P. vivax (green), mixed infections (yellow), no species data available (white).

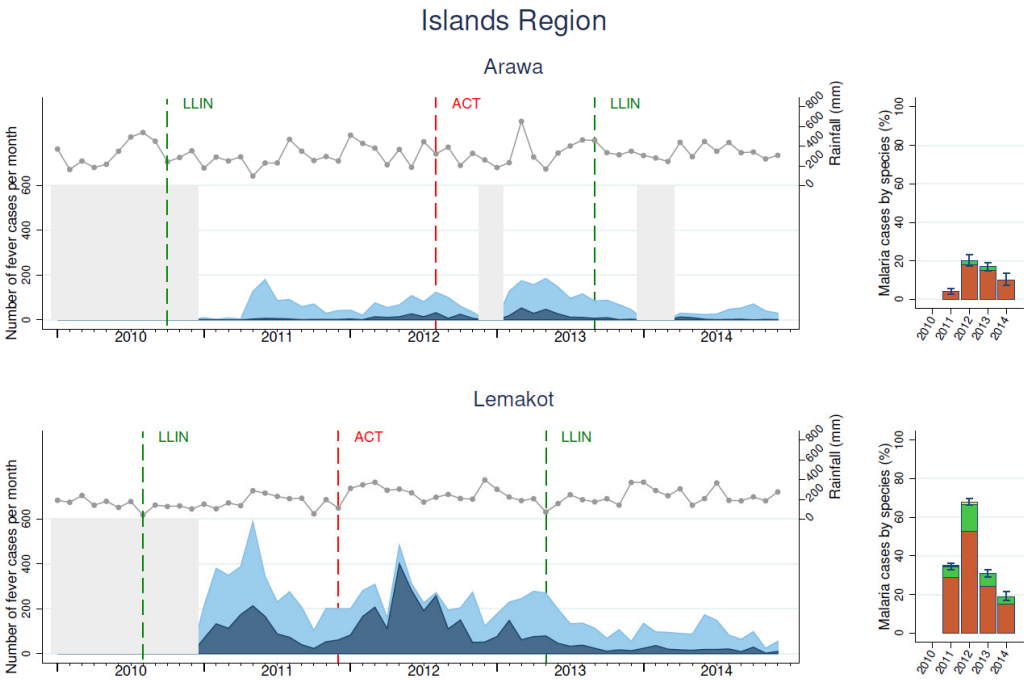

Figure 4.5. Malaria cases in Islands Region (Arawa and Lemakot) sites

Left of each panel: monthly number of fever cases RDT negative (bright blue) and RDT positive (dark blue); cumulative monthly rainfall (grey line); timing of LLIN distribution and introduction of ACT (vertical dashed lines). Missing data is indicated by light grey shaded background. Right of each panel: annual RDT positivity (bar total) by species: P. falciparum (orange), P. vivax (green), mixed infections (yellow), no species data available (white).

The annual incidence rate of malaria calculated for four sites ranged from 1/1,000 population in Karimui in 2014 to 187/1,000 in Lemakot in the peak year 2012. Incidence rates were highest in East Cape and Lemakot, except in 2014, when Sausi exhibited significantly higher incidence than the other sites. Severe malaria incidence ranged from 0.4/1,000 in Karimui in 2014 to 28/1,000 in Lemakot in 2011. In general, severe malaria incidence was highest in 2011 and lowest in 2014, except in Sausi, where a 2.6-fold increase was observed after 2013 (Table 4.2.). The annual proportion of malaria cases being severe malaria ranged from 4% in East Cape in 2013 to 67% in Karimui in 2014. In Balimo and Arawa all severe malaria cases were attributed to *P. falciparum.* The greatest proportion of severe malaria with *P. vivax* (39%) was observed in Karimui in 2011 but by 2014 all cases were *P. falciparum* (Additional file 5). Since the number of severe malaria cases is very low in Karimui some of the variations might be attributed to chance fluctuations.

Table 4.2. Annual incidence of malaria and 'severe malaria' per 1,000 population in four sentinel health facilities

Health facility	2010		2011		2012		2013		2014	
	N	95% CI	N	95% CI	N	95% CI	N	95% CI	N	95%CI
Malaria incidence										
East Cape	115	(107, 124)	117	(109, 125)	179	(170, 189)	141	(133,150)	47	(42, 52)
Karimui	19	(16, 22)	34	(31, 38)	6	(4, 8)	6	(5, 8)	1	(0.2 , 1)
Sausi	20	(17, 24)	79	(72, 86)	79	(72, 87)	43	(38, 48)	79	(72, 86)
Lemakot	-	-	113	(107,119)	187	(180, 194)	55	(51, 59)	19	(17, 22)
'Severe malaria' incidence										
East Cape	7	(5, 9)	24	(20, 28)	10	(8, 13)	6	(4, 8)	3	(2, 5)
Karimui	2	(1, 4)	14	(12, 17)	3	(2, 4)	2	(1, 3)	0.4	(0.1 , 1)
Sausi	1	(1, 3)	18	(15, 22)	13	(10, 16)	8	(6, 10)	21	(17, 25)
Lemakot	-	-	28	(25, 31)	27	(24, 30)	6	(5, 8)	4	(3, 5)

Only sites with available census denominator data were included.

CI = Confidence interval

The effect of the LLIN distribution on age-specific malaria incidence was assessed in the four sites with available age-specific population data. Malaria incidence was reduced with each LLIN distribution round in East Cape, Karimui and Lemakot. The greatest decrease was observed in the age groups 0-4 years and 5-9 years (Figure 4.6.). Incidence in Sausi initially decreased but increased again after the third distribution. When disaggregated by age group and gender, females in some sites and age groups appeared to have a higher incidence of malaria, e.g., in Lemakot (age group 15-19) and Sausi (age group 30-39), and differences in incidence rates between distribution rounds did not always affect males and females equally (Additional file 6).

Self-reported LLIN use increased in general, with each distribution round and then gradually decreased over the subsequent years. Net use was highest in Sausi (90-100%), Balimo (95-100%) and Dreikikir (77-86%) and lowest in the two Islands sites of Arawa (21-69%) and Lemakot (41-48%), confirming data of the 2010/2011 national malaria indicators survey (Additional file 7) (Hetzel et al., 2014a). The treatment of malaria patients with ACT was consistently high (>80% each year) after the introduction of the drug in Balimo, East Cape, Dreikikir, Sausi, and Lemakot. The previous treatment combination of amodiaquine or

chloroquine plus sulphadoxine-pyrimethamine (SP) was phased out over the same period. The opposite trend was observed in Karimui, where ACT was gradually substituted by the previous treatment regimen one year after its introduction and in Arawa, where in 2014, most patients were treated neither with the old, nor with the new regimen (Additional file 8). Annually, less than 1.3% of negative cases were treated with ACT in all SHFs. Information on the use of Primaquine is available in the Additional file 9.

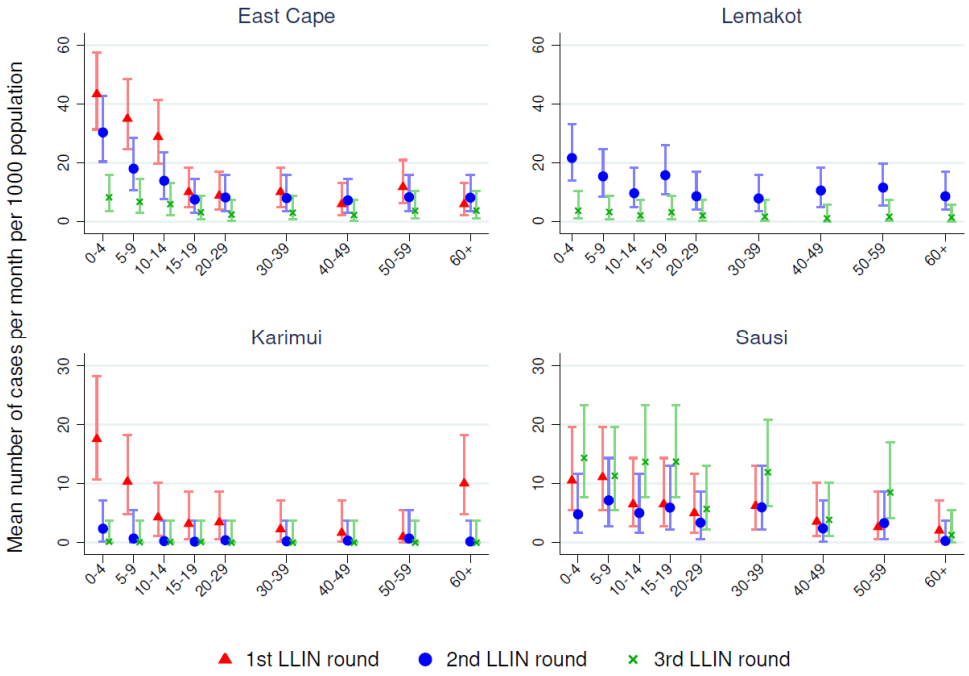

Figure 4.6. Malaria incidence rate by age group after each LLIN distribution round in four sites
LLIN = long-lasting insecticidal-treated bed net

Due to significant heterogeneity in the estimated effects of LLIN distribution rounds on the number of malaria cases between sites ($p<0.001$, interaction test), the effects of interventions were estimated for each SHF individually and adjusted for El Niño/La Niña years. In general, subsequent distribution of LLINs led to cumulative reductions in the number of malaria cases (Table 4.3.). The greatest reductions were in settings where the number of cases was the lowest (Balimo, Karimui, Arawa) rather than in places with high net use but high case load. In Sausi, where an earlier study had suggested significant impact of the first LLIN distribution (Hetzel et al., 2016), the second distribution reduced the number of cases by 57% (95% CI: 11% - 79%) but a two-fold increase was observed after the third distribution.

Table 4.3. Estimated effects of each round of LLIN distribution and ACT introduction on the number of malaria cases by sentinel health facility

Health facility	2nd *vs.* 1st LLIN distribution		3rd *vs.* 2nd LLIN distribution		Introduction of ACT	
	IRR	**95% CI**	**IRR**	**95% CI**	**IRR**	**95% CI**
Balimo	-	-	1.32E-09	(0.00, -)	1.21	(0.47, 3.14)
East Cape	0.53	(0.20, 1.41)	0.34	(0.18, 0.64)	1.50	(0.79, 2.85)
Karimui	0.19	(0.06, 0.58)	0.12	(0.03, 0.43)	0.95	(0.32, 2.80)
Dreikikir	1.15	(0.54, 2.46)	-	-	3.95	(1.89, 8.25)
Sausi	0.43	(0.21, 0.89)	2.08	(1.16, 3.72)	1.28	(0.76, 2.16)
Arawa	-	-	0.15	(0.06, 0.36)	1.79	(0.57, 5.64)
Lemakot	-	-	0.26	(0.14, 0.50)	1.73	(1.09, 2.75)

IRR = Incidence rate ratio

CI = Confidence interval

All estimates were adjusted for La Niña or El Niño year.

The change of treatment from amodiaquine or chloroquine plus SP to ACT did not appear to significantly affect the number of malaria cases except in Drekikir and Lemakot where an increase was observed.

4.5. Discussion

Malaria surveillance in SHFs revealed varying trends in the number of malaria cases and the magnitude of their association with control interventions between 2010 and 2014. In general, reductions in the number of malaria cases were observed with each of three rounds of household-level LLIN distribution while no substantial reductions followed the change of treatment to ACT. The number of malaria cases was found to increase in one site after the third distribution round. The findings disclose a substantial sub-national heterogeneity in the epidemiology and control of malaria in PNG.

After the first large-scale LLIN distribution in PNG, data from six sentinel surveillance sites indicated a drop in the average monthly malaria incidence rate from 13/1,000 population to

2/1,000 (incidence rate ratio = 0.12; 95% CI: 0.09-0.17) and reductions in prevalence and transmission confirming significant short-term effect of LLIN in the absence of ACT (Hetzel et al., 2016). Previous modelling studies suggested that the effect of LLIN may fade over time as acquired immunity in the population is reduced, particularly in areas with a high pre-intervention entomological inoculation rate (EIR) (Briët and Penny, 2013). The situation observed in Dreikikir and Sausi is consistent with this prediction. On the other hand, considering LLIN coverage was consistently high (self-reported use 90-100% in Sausi, 77-89% in Dreikikir, Additional file 5) (Hetzel et al., 2016), other factors such as aging of nets may fuel ongoing transmission. In the absence of insecticide resistance, early and outdoor biting of *Anopheles* mosquitoes has been identified as a threat to the effectiveness of LLINs and entomological studies in Sausi have described a shift in mosquito biting to earlier hours following the first LLIN distribution (the peak exposure time to infective bites shifted from later than 9pm in 2008 to between 6 and 7 pm in 2011) resulting in decreased protection against mosquito bites (Reimer et al., 2016; Thomsen et al., 2017).

Gender differences, or anomalies in the age-specific incidence rates, (e.g., higher incidence rates in females age 15-19 in Lemakot; Additional file 6) might suggest gender-specific risk. In Lemakot, the substantial increase in malaria cases in 2012 was disproportionately due to cases in teenage girls, suggesting a local outbreak. Age or gender-specific behaviour (e.g., evening activities, division of household chores) or other social or cultural determinants including location and quality of houses may result in different levels of exposure. Such factors have been well investigated in settings in which most of the transmission is limited to specific population groups (e.g., in Southeast Asia (Gryseels et al., 2015; Liwang et al., 2012; Thanh et al., 2015)). In highly diverse settings such as PNG, one challenge will be to identify risk factors that disproportionately affect particular population groups, and to translate such knowledge into targeted control action. A mixed methods approach that captures behavioural patterns alongside prevalence and incidence data and linked with entomological investigations in settings with ongoing transmission will be an important first step.

Most of the evidence of the impact of insecticide-treated nets originates from African settings and is limited to the effect on *P. falciparum* (Pryce et al., 2018). While multiple studies demonstrated the effect of LLIN programmes on *P. falciparum* incidence (e.g. (Aregawi et al., 2017, 2014)), evidence from settings with several *Plasmodium* species is scarce. Although it has been suggested that the impact of vector control on *P. vivax* may be delayed due to the parasite's biology, this study only found short-term transient increases in the proportion of *P. vivax* by light microscopy, suggesting that at intermediate to high transmission, both species are affected by vector control and both species may resurge. A modelling study found that in such areas

LLINs alone could not lead to interruption of *P. vivax* transmission and additional tools are required to accelerate to elimination (White et al., 2018). Considering the abundance of low level parasitaemia, particularly in *P. vivax* infections as transmission is reduced, more sensitive diagnostic tools may need to be applied to monitor progress and species composition (Hofmann et al., 2018).

A number of field and modelling studies have shown a reduction in malaria cases and/or transmission after the introduction of ACT alone and in combination with LLINs (Barnes et al., 2005; Bhattarai et al., 2007; Bretscher et al., 2017; Nosten et al., 2000; Okell et al., 2008; Shah et al., 2013). In this study, the change in first-line treatment from amodiaquine or chloroquine plus SP to ACT did not lead to a decrease in the number of cases. Neither did it result in an increase in the proportion of *P. vivax* cases despite the higher susceptibility of *P. falciparum* to artemeter-lumefantrine (Karunajeewa et al., 2008) and the low consistent use of primaquine as radical cure of *P. vivax*. In general, the previous treatment was used widely (though not always strictly according to guidelines) before the introduction of ACT (Additional file 7) and the regimen had retained approximately 82% efficacy (chloroquine plus SP in 2005-2007 (Karunajeewa et al., 2008)) limiting the increase in efficacy after ACT roll-out to 13%. Additionally, while an efficacious drug can improve clinical outcomes (Bhatt et al., 2015) a community-wide effect on transmission (and hence incidence) is, among other factors, a function of treatment seeking and prevalence of asymptomatic and sub-microscopic infections. Individuals with asymptomatic infections fuel ongoing transmission and do not seek treatment (Bousema et al., 2014; Hetzel et al., 2017a; Robinson et al., 2015). In PNG, only 43% of fever cases were found to attended a health facility (2013/14) (Hetzel et al., 2014b), thus the increase in efficacy might have been insufficient to translate into reduced transmission and incidence, as already demonstrated in an African high-transmission setting (Huho et al., 2012). In the two sites in which an increase in the number of malaria cases was observed following the introduction of ACT, overall facility attendance did not suggest an availability effect resulting from the introduction of free intervention, as documented elsewhere (Ansah et al., 2009; Heinmüller et al., 2013). A recent study in PNG even suggests that the shift to ACT may have negatively impacted treatment seeking and patient satisfaction, possibly related to low perceived quality of care provided to patients with non-malarial illness (Pulford et al., 2018). Differences in the length of post-intervention periods used for the regression model may have affected the reliability of estimates for Dreikikir as the period preceding ACT introduction was only three months (Additional file 2), most likely not enough to gain reliable estimates for both interventions.

Different surveillance starting points and lack of pre-LLIN data were limitations to this study but data from previous surveillance activities and national prevalence surveys provide

supporting evidence of the short-term effect of LLIN (Hetzel et al., 2017a, 2016, 2015). Fluctuations in treatment seeking or facility attendance may have influenced incidence estimates to a certain degree, but data from repeat national surveys suggests that the proportion of fever patients attending a formal health facility has remained largely unchanged since the first assessment in 2008/09 (Hetzel et al., 2018).

Despite previous validation and use in other setting(Ceccato et al., 2005; Midekisa et al., 2012), none of the available site-specific satellite weather variables (EVI: MODIS products MOD13Q1 and MOD13A3; and rainfall: TRMM product 3B43) could explain variations in malaria incidence over time which supports historical descriptions of an intricate and complex environment driving malaria epidemiology across in PNG (Attenborough and Alpers, 1992; Hetzel et al., 2017a; Mueller et al., 2005, 2003; Radford et al., 1976). Particularly in an environment with high overall rainfall, weather variables may not be a good single predictor of malaria incidence. In contrast the El Niño/La Niña phenomena appeared to be a useful and more stable environmental predictor since it affects larger areas for a longer period of time than site-specific weather data.

Differences in pre-intervention malaria transmission and in the impact of interventions between sites are a function of the diverse social and ecological settings which lead to differences in vector abundance, vector behaviour and human-vector interaction. Multi-disciplinary studies on a smaller scale are required to broaden the understanding of malaria transmission dynamics on a sub-national level and identify regional factors driving the observed heterogeneity. Insights from such investigations should be translated into response strategies that take into consideration sub-national heterogeneity in the drivers of ongoing malaria transmission. A robust surveillance system reporting case incidence should be supported by monitoring of entomological and immunological parameters to explain differences in the impact of interventions.

4.6. Conclusions

Subsequent household level distributions of LLINs had a cumulative effect on reducing the number of malaria cases in the SHFs but the magnitude of the association varied between sites and over time. Changing treatment to ACT had no apparent effect. Malaria programmes covering diverse transmission settings such as PNG must consider local heterogeneity when choosing interventions and ensure continuous monitoring of trends.

4.7. DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Fever patients were asked for verbal consent prior to sample collection. For children below 16 years of age consent was obtained from the parent or caregiver. Ethical clearance was granted by the Institutional Review Board of the PNGIMR and the PNG Medical Research Advisory Committee (MRAC No. 10.12).

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIAL

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

FUNDING

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AUTHORS' CONTRIBUTIONS

MWH, IM, PMS and JP conceived of the study. SM, MWH and JP established and supervised data collection systems. LL and LJR were in charge of malaria diagnosis by light microscopy. DRR analysed the data with support from AR and drafted the manuscript with major inputs from MWH, AR and JP. All authors read and approved the final manuscript

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kindly provided support to explore remote-sensing data bases for the environmental variables used in the study.

4.8. ADDITIONAL FILES

ADDITIONAL FILE 1: SENTINEL SITES DESCRIPTION[1]

Health facility	Region	Province	Altitude (m)	Population (villages)	Health facility surveillance	Census
Balimo	Southern	Western	15	NA	06/2011-12/2014	NA
East Cape	Southern	Milne Bay	15	5,684* (509)	02/2010-12/2014	2010
Karimui	Highlands	Chimbu (Simbu)	1140	8,506* (24)	11/2010-12/2014	2010
Dreikikir	Momase	East Sepik	420	24,359** (NA)	06/2011-12/2014	NA
Sausi	Momase	Madang	170	5,158* (48)	08/2010-12/2014	2010
Arawa	Islands	Bougainville	10	15,447** (NA)	01/2011-12/2014	NA
Lemakot	Islands	New Ireland	15	10,384* (33)	01/2011-12/2014	2010

NA Not available data **Study census* ***Provided by District Health Authorities 2014*

Details of each site

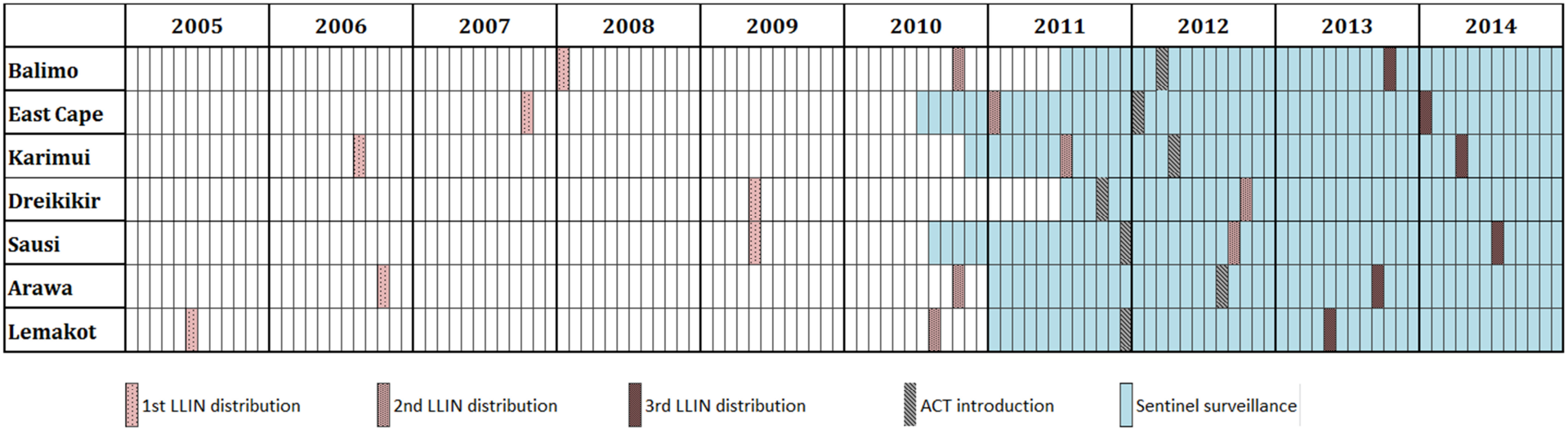
East Cape is located in Milne Bay Province which comprises only 14% of land mass with the rest being ocean; 25% of its landmass are islands and atolls[2]. Karimui is in a highland fringe area in Simbu Province. About 70% of Simbu Province lies at altitudes between 1000-2600 m above sea level and normally has a very wet and non-seasonal climate [3],[4] Lemakot is located on the main island of New Ireland Province, which is long (~200 km), narrow (~8 km) and mountainous with a wet tropical climate [5]. Sausi is located in Madang Province in the Ramu Valley where industrial sugar cane and oil palm estates have been established. Nearby areas are regularly flooded by the Ramu River and its branches. Arawa is located in the centre of the Autonomous Region of Bougainville. The climate in Bougainville is tropical with consistent temperature, rainfall and humidity throughout the year[6]. Balimo is located in Western Province along the

[1] Manuel W Hetzel, Justin Pulford, and others, 'Evaluation of the Global Fund-Supported National Malaria Control Program in Papua New Guinea, 2009-2014', *PNG Med J*, 57.1–4 (2014), 7–29.
[2] R M Bourke, M G Allen, and J G Salisbury, 'Food Security for Papua New Guinea', in *Proceedings of the Papua New Guinea Food and Nutrition 2000 Conference, PNG University of Technology, Lae* (Lae, Papua New Guinea, 2000), pp. 1–882.
[3] Robert D. Attenborough and Michael P. Alpers, *Human Biology in Papua New Guinea : The Small Cosmos* (Oxford University Press, 1992).
[4] Bourke, Allen, and Salisbury.
[5] David K. Holdsworth, Chris L. Hurley, and Sue E. Rayner, 'Traditional Medicinal Plants of New Ireland, Papua New Guinea', *Quarterly Journal of Crude Drug Research*, 18.3 (1980), 131–39.
[6] CM Yule, 'Trophic Relationships and Food Webs of the Benthic Invertebrate Fauna of Two Aseasonal Tropical Streams on Bougainville Island, Papua New Guinea', *Journal of Tropical Ecology*, 12.4 (1996), 517–34.

Aramia River and the surrounding lowland forest. Lagoon systems with seasonal flooding surround villages situated on land and islands points[7],[8]. Dreikikir is located in East Sepik Province in hilly terrain (~200 m). The climate in East Sepik is seasonal with most abundant rainfall between December and June[9,10,11,12].

[7] Jeffrey Mitchell Warner, 'The Epidemiology of Melioidosis in Papua New Guinea. PhD Thesis, James Cook University', 2004.
[8] Jeffrey Mitchell Warner and others, 'Melioidosis in a Rural Community of Western Province, Papua New Guinea', *Trans R Soc Trop Med Hyg*, 101 (2007), 809–13.
[9] Moses J Bockarie and Henry Dagoro, 'Are Insecticide-Treated Bednets More Protective against Plasmodium Falciparum than Plasmodium Vivax-Infected Mosquitoes?', *Malaria Journal*, 5 (2006), 15.
[10] Manuel W Hetzel, Susan Paul, and others, 'Proportion of Fevers Attributable to Malaria Varies Significantly between Sites in Papua New Guinea', *PNG Med J*, 57.1–4 (2014), 39–51.
[11] Moses J Bockarie and others, 'Randomised Community-Based Trial of Annual Single-Dose Diethylcarbamazine with or without Ivermectin against Wuchereria Bancrofti Infection in Human Beings and Mosquitos', *Lancet*, 351.9097 (1998), 162–68.
[12] James W Kazura and others, 'Parasitologic and Clinical Features of Bancroftian Filariasis in a Community in East Sepik Province, Papua New Guinea', *American Journal of Tropical Medicine and Hygiene*, 33.6 (1984), 1119–23.

ADDITIONAL FILE 2: TIMELINE OF IMPLEMENTATION OF MALARIA CONTROL INTERVENTIONS AND SURVEILLANCE IN EACH SENTINEL HEALTH FACILITY, 2005-2014



ADDITIONAL FILE 3: RAPID DIAGNOSTIC TEST AND LIGHT MICROSCOPY RESULTS

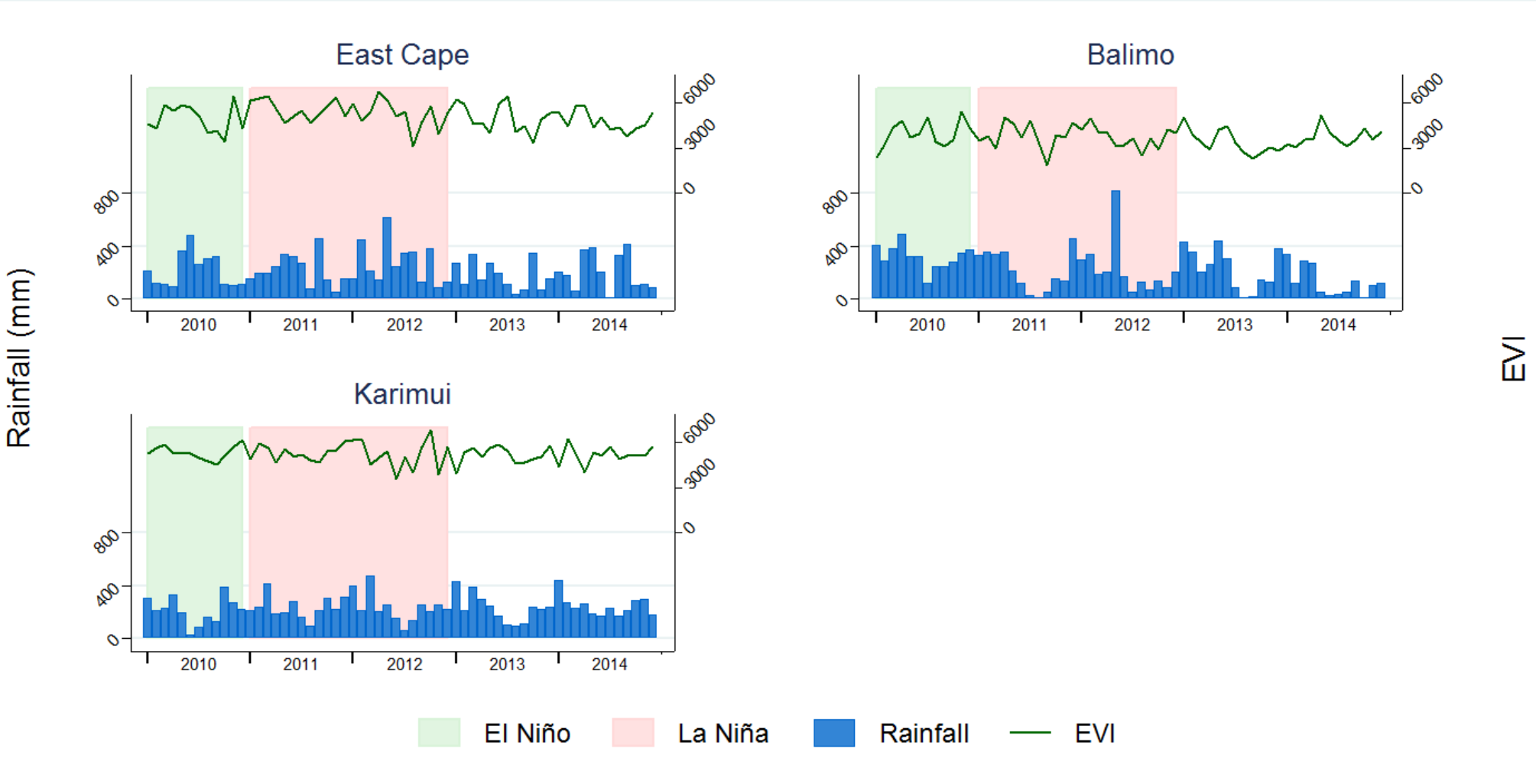
Number of RDT-positive and RDT-negative cases and proportion of RDT results read by microscopy (for species identification)

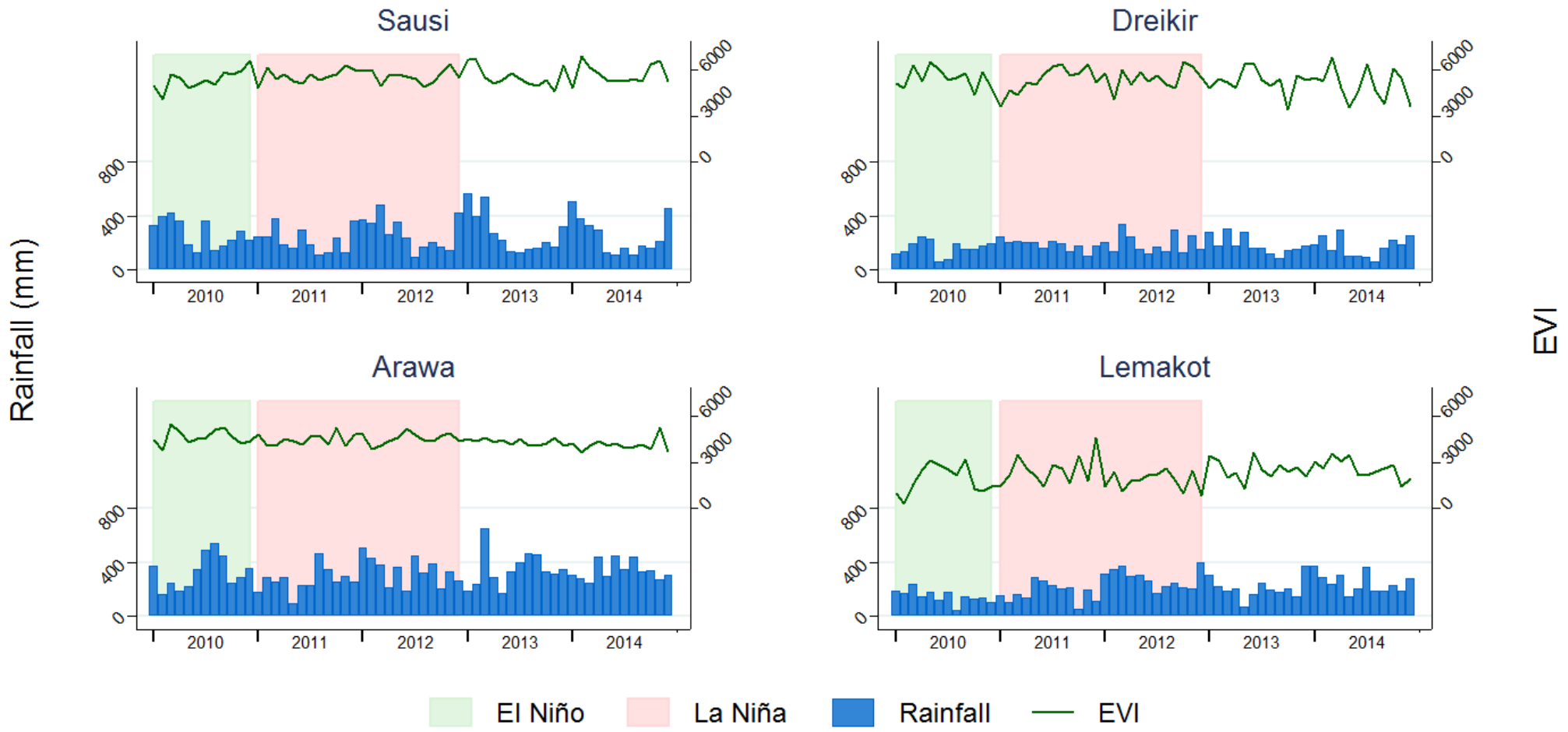
	2010		2011		2012		2013		2014	
Health facility	N positive RDT (LM%)	N negative RDT(LM%)	N positive RDT(LM%)	N negative RDT(LM%)	N positive RDT(LM%)	N negative RDT(LM%)	N positive RDT(LM%)	N negative RDT(LM%)	N positive RDT(LM%)	N negative RDT(LM%)
East Cape	655(26)	364(54)	685(51)	888(83)	1083(29)	837(70)	879(15)	902(45)	299(19)	582(62)
Karimui	163(86)	58(84)	301(35)	542(89)	54(0)	567(56)	59(0)	657(50)	6(17)	725(38)
Lemakot	NA	NA	1205(65)	2276(96)	2062(21)	975(66)	623(11)	1387(53)	221(29)	928(48)
Sausi	104(25)	397(37)	418(51)	1101(89)	434(30)	1375(59)	241(17)	861(49)	457(29)	824(54)
Arawa	NA	NA	29(24)	682(99)	156(7)	617(47)	219(30)	1071(55)	35(9)	313(73)
Balimo	NA	NA	9(33)	465(99)	18(17)	479(80)	30(3)	191(42)	0(0)	304(66)
Dreikikir	NA	NA	116(64)	629(98)	465(44)	1099(71)	104(26)	631(45)	191(26)	607(39)

RDT = Rapid diagnostic test

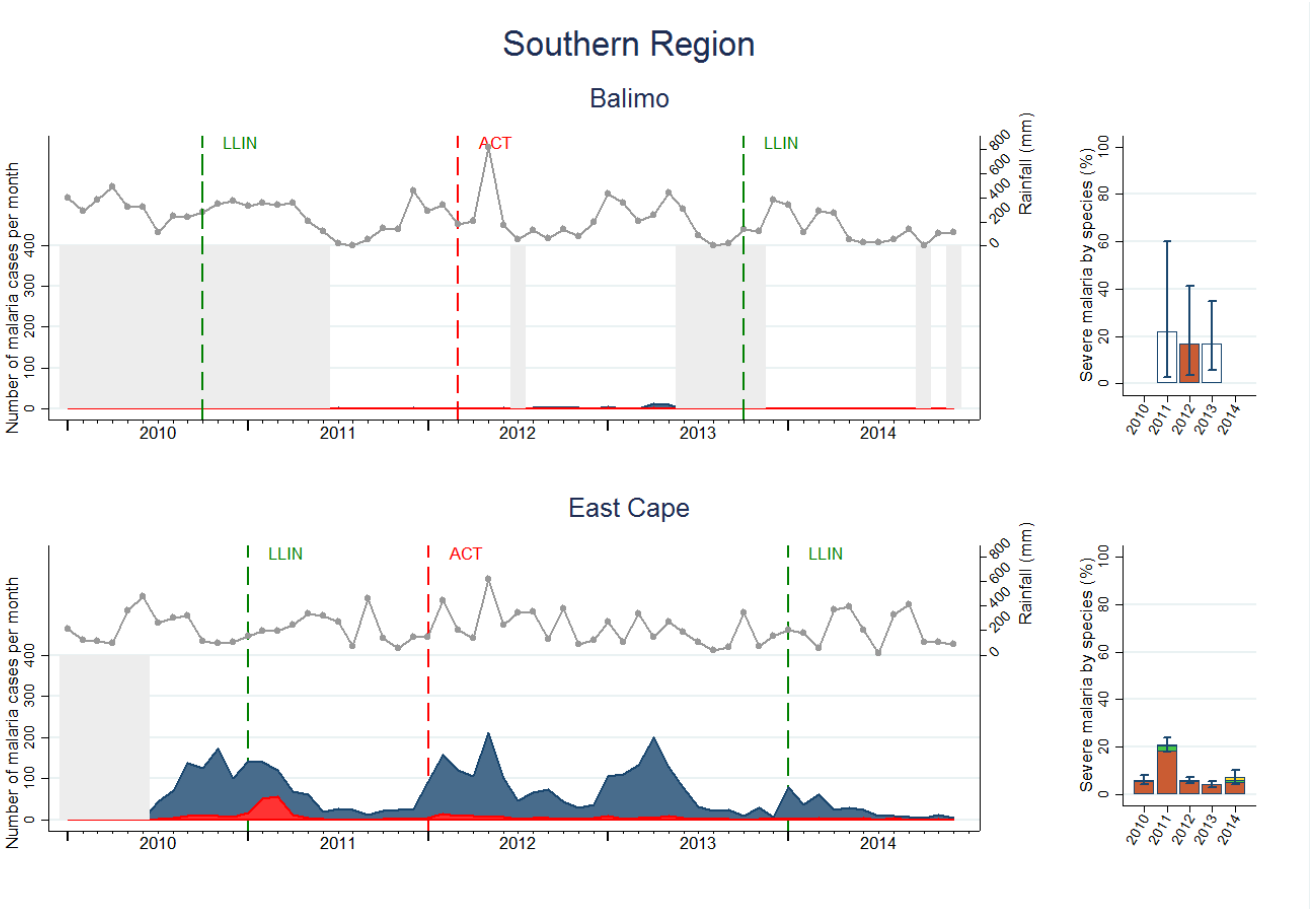
LM% = Proportion of RDT-result read by light microscopy

ADDITIONAL FILE 4: KEY ENVIRONMENTAL VARIABLES BY SITE

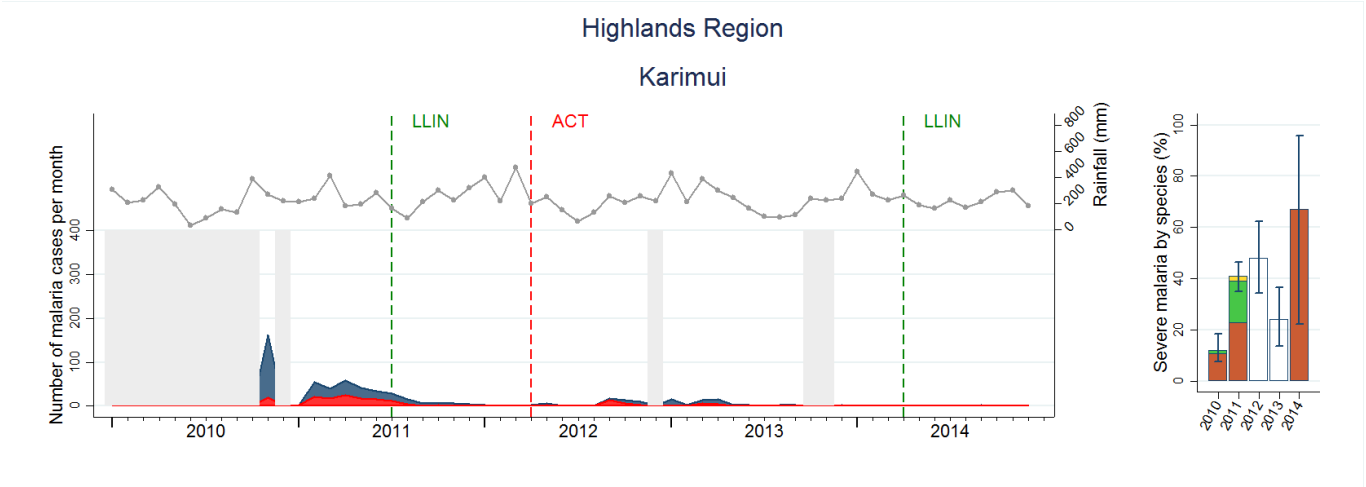



Sausi
Dreikir
Arawa
Lemakot
Rainfall (mm)
EVI
0
400
800
0
3000
6000
2010
2011
2012
2013
2014
El Niño
La Niña
Rainfall
EVI

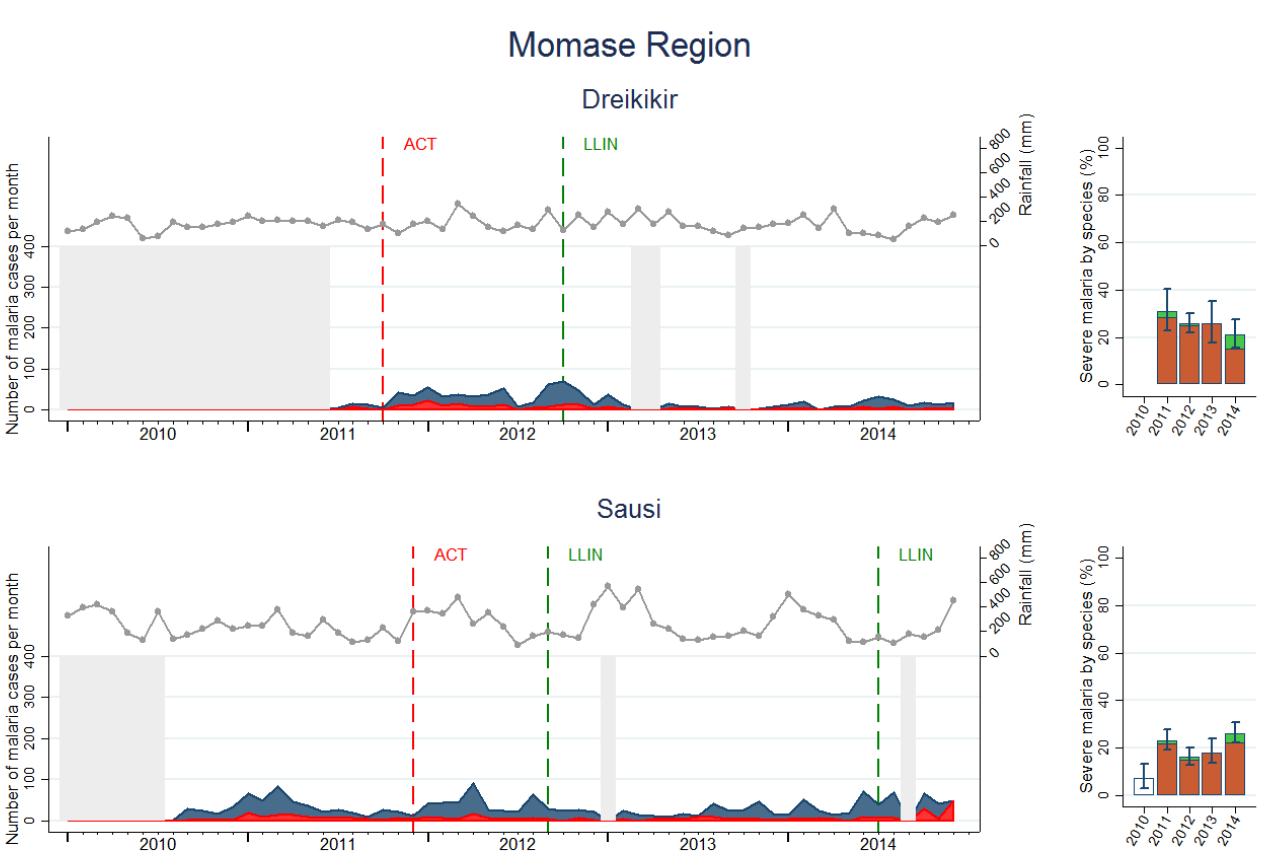
ADDITIONAL FILE 5: SEVERE MALARIA IN SENTINEL HEALTH FACILITIES



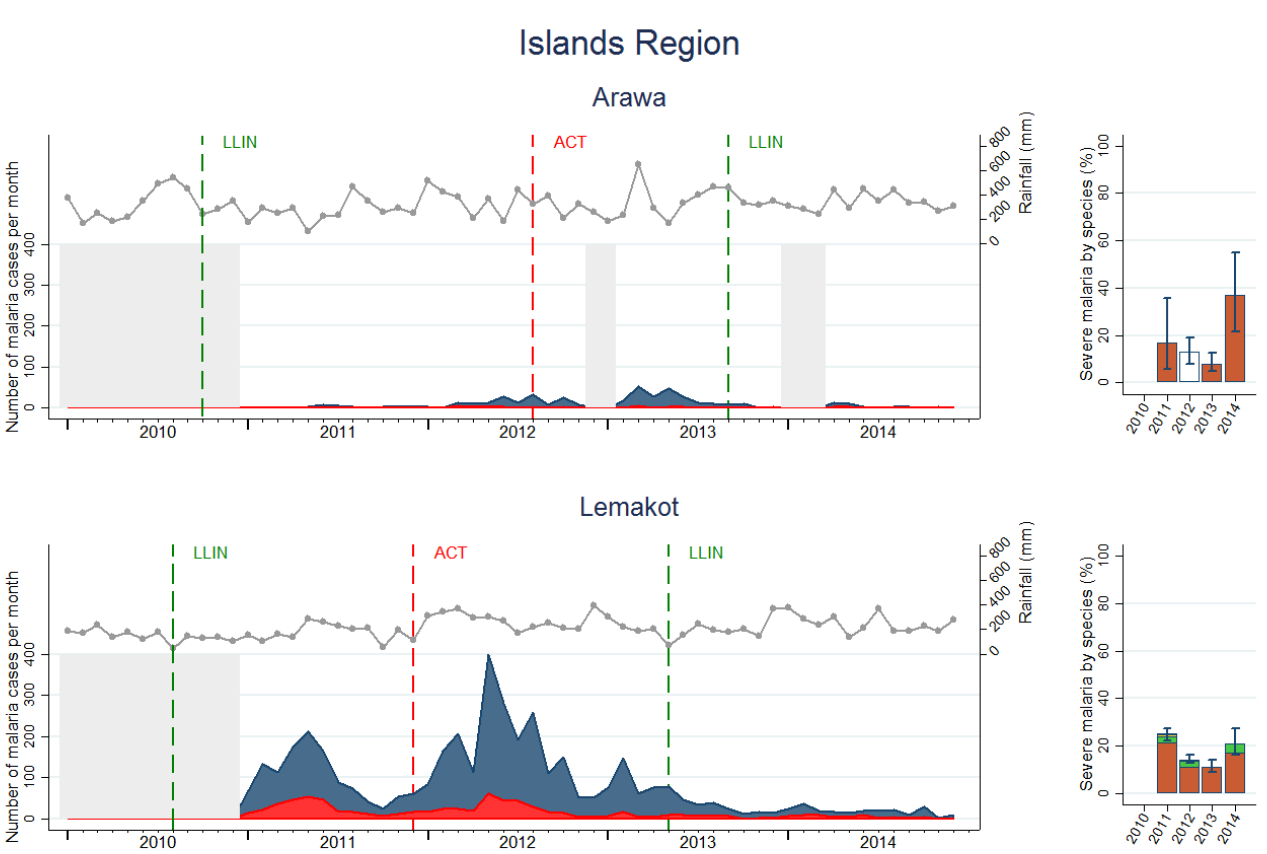
Severe malaria cases in Southern Region (Balimo and East Cape) sites. Left of each panel: monthly number of malaria cases; uncomplicated malaria (dark blue) and severe malaria (red); accumulated monthly rainfall (grey line); timing of LLIN roll-out and introduction of ACT (vertical dashed lines). Right of each panel: Percentage of malaria cases identified as severe (bar total) and Plasmodium species composition: P. falciparum (orange), P. vivax (green), mixed infections (yellow), no species data available (white)



Severe malaria cases in Highlands Region (Karimui). Left of each panel: monthly number of malaria cases; uncomplicated malaria (dark blue) and severe malaria (red); accumulated monthly rainfall (grey line); timing of LLIN roll-out and introduction of ACT (vertical dashed lines). Right of each panel: Percentage of malaria cases identified as severe (bar total) and Plasmodium species composition: P. falciparum (orange), P. vivax (green), mixed infections (yellow), no species data available (white)

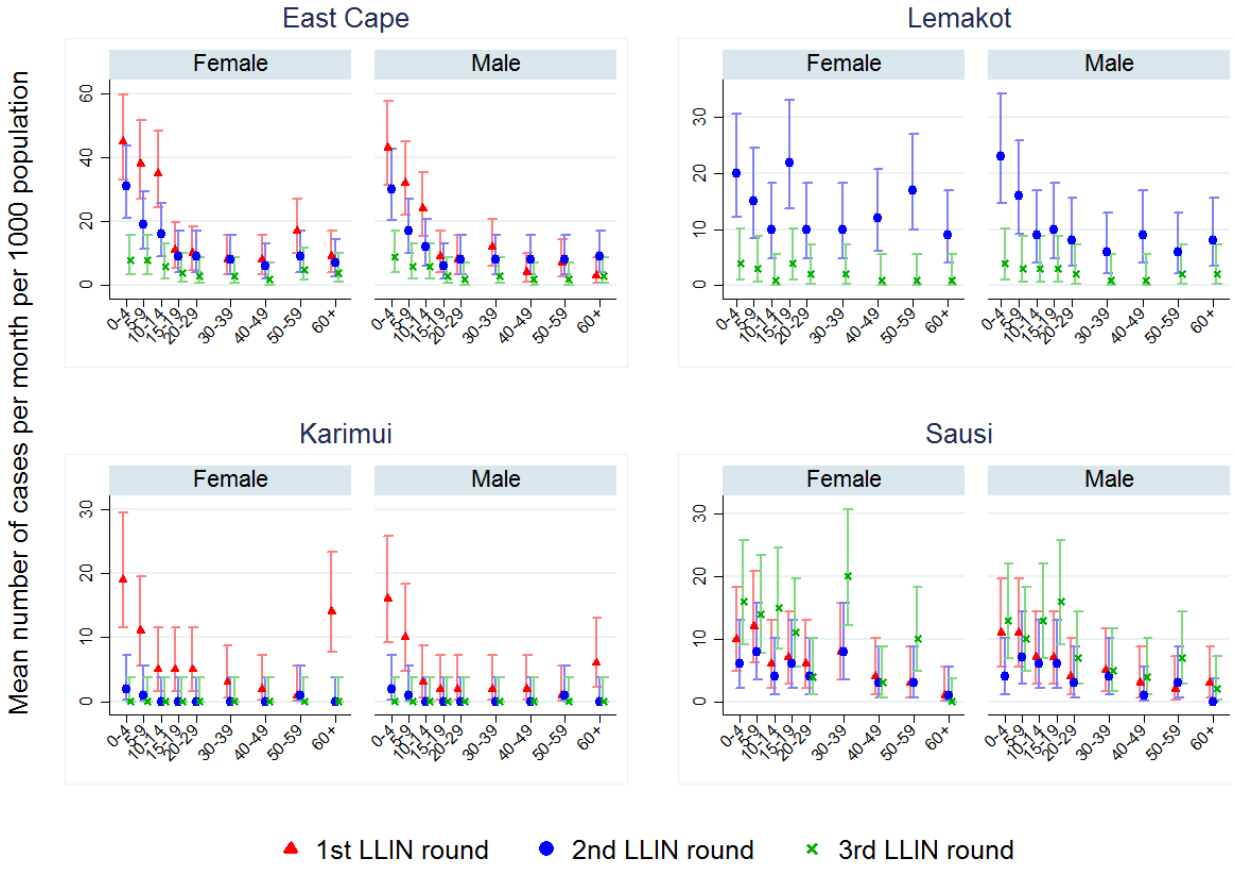


Severe malaria cases in Momase Region (Dreikikir and Sausi) sites. Left of each panel: monthly number of malaria cases; uncomplicated malaria (dark blue) and severe malaria (red); accumulated monthly rainfall (grey line); timing of LLIN roll-out and introduction of ACT (vertical dashed lines). Right of each panel: Percentage of malaria cases identified as severe (bar total) and Plasmodium species composition: P. falciparum (orange), P. vivax (green), mixed infections (yellow), no species data available (white)



Severe malaria cases in Islands Region (Arawa and Lemakot) sites. Left of each panel: monthly number of malaria cases; uncomplicated malaria (dark blue) and severe malaria (red); accumulated monthly rainfall (grey line); timing of LLIN roll-out and introduction of ACT (vertical dashed lines). Right of each panel: Percentage of malaria cases identified as severe (bar total) and Plasmodium species composition: P. falciparum (orange), P. vivax (green), mixed infections (yellow), no species data available (white)

ADDITIONAL FILE 6: MALARIA INCIDENCE BY AGE GROUP AND SEX



Malaria incidence by age group and sex for each LLIN distribution round in four sites.

LLIN = long-lasting insecticidal nets

Self-reported net use by year and LLIN distribution round (red: after 1st round, blue: after 2nd round, green: after 3rd round).

For comparison, 2010/11 national malaria indicator survey net use results:

Southern Region 67% (95% CI: 57, 76)

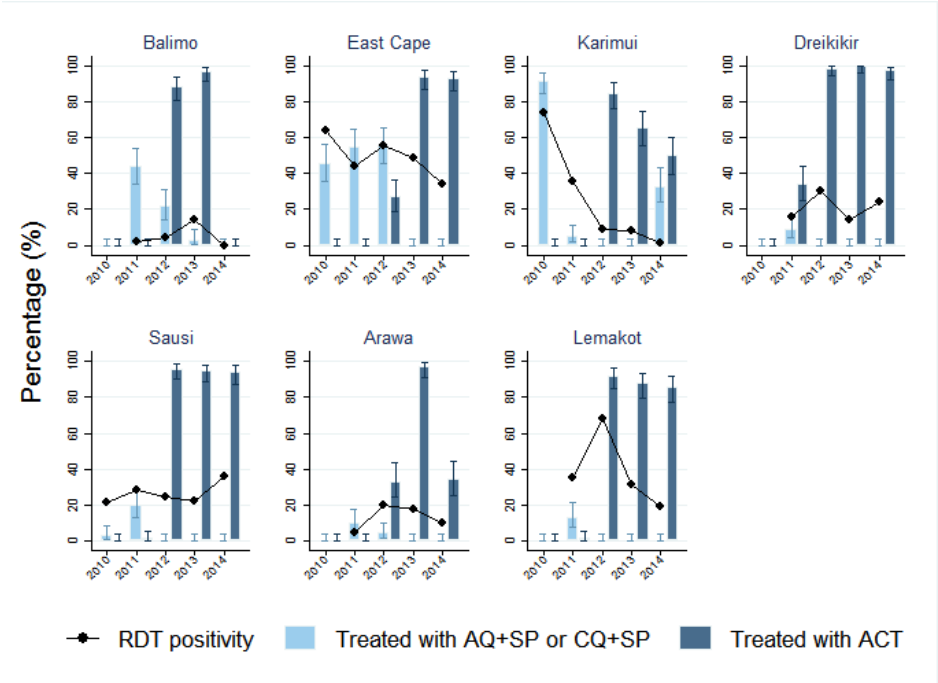
Highlands Region 40% (95% CI: 29, 51)

Momase Region 49% (95% CI: 36-62)

Islands Region: 40% (95% CI: 33-48)[13]

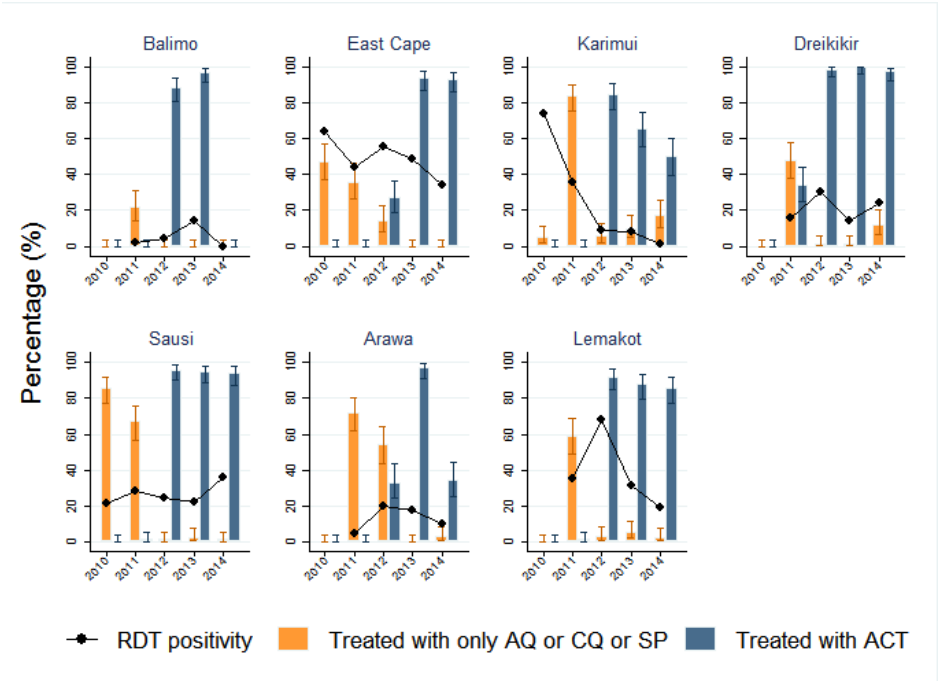
[13] Manuel W Hetzel, Adnan A K Choudhury, and others, 'Progress in Mosquito Net Coverage in Papua New Guinea', *Malaria Journal*, 13 (2014), 242.

ADDITIONAL FILE 8: PERCENTAGE OF MALARIA CASES TREATED WITH THE PREVIOUS FIRST LINE TREATMENT OR PARTIAL TREATMENT (MONO-THERAPY), AND WITH ARTEMISININ-BASED COMBINATION THERAPY



Annual malaria positivity and percentage of malaria cases treated with AQ+SP or CQ+SP, or with ACT.

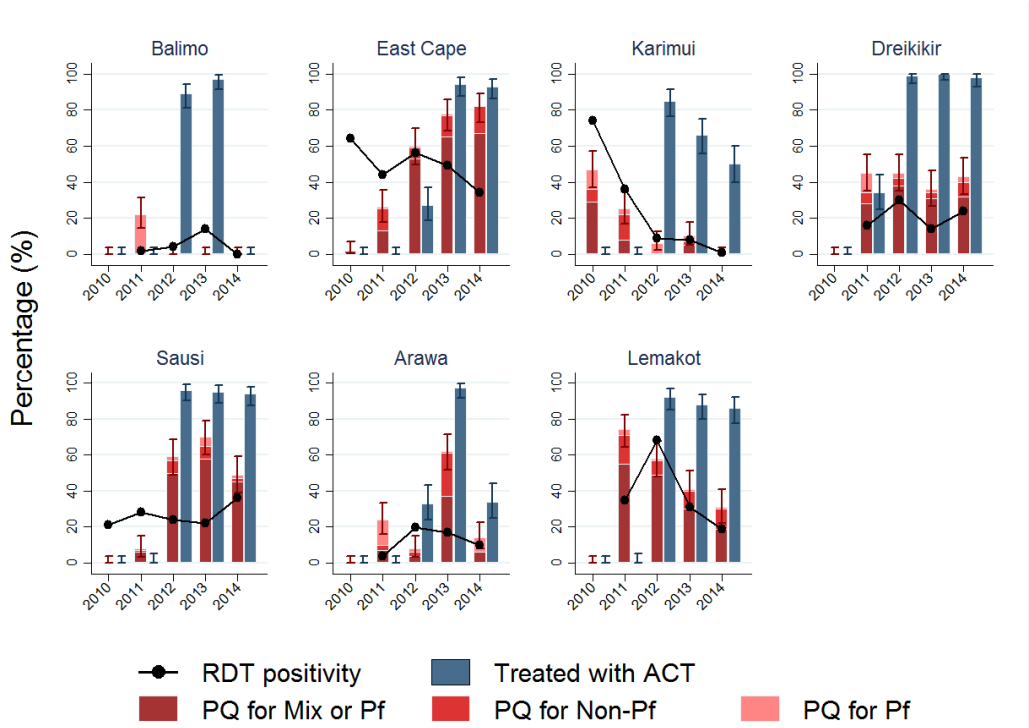
AQ =amodiaquine, CQ = chloroquine, ACT = artemisinin-based combination therapy



Annual malaria positivity and percentage of malaria cases treated with monotherapy (AQ or CQ or SP), or with ACT.

AQ =amodiaquine, CQ = chloroquine, ACT = artemisinin-based combination therapy

ADDITIONAL FILE 9: PERCENTAGE OF MALARIA CASES TREATED WITH PRIMAQUINE (FOR DIFFERENT RDT RESULTS), AND WITH ARTEMISININ-BASED COMBINATION THERAPY



Annual malaria positivity and percentage of malaria cases treated with primaquine and RDT result, or with ACT.

PQ =primaquine, ACT = artemisinin-based combination therapy, Pf = Plasmodium falciparum

5. MAPPING ROUTINE MALARIA INCIDENCE AT VILLAGE LEVEL FOR TARGETED CONTROL IN PAPUA NEW GUINEA

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5.1. Abstract

Malaria surveillance and response-systems are essential for identifying the areas most affected by malaria and for targeting interventions and optimizing resources. This study aimed to assess whether the visualization of routinely collected health facility data linked to village of residence provides evidence for targeting control interventions in four sentinel health facilities (SHF) in Papua New Guinea. A video format was used to visualize the dynamics in case incidence over time and space alongside photographs illustrating the context of the data collection in the study sites. Incidence changes overtime were illustrated in animated maps. Despite limitations, this approach appeared useful in sites with very few remaining cases or with increasingly marked heterogeneity. Villages that could benefit from targeted interventions or investigations were identified.

5.2. Background Section

Variation in the risk of malaria prevalence and incidence between villages in regions with on-going transmission has long been recognized (Bousema et al., 2012; Greenwood, 1989). Such variations become more evident in regions with moderate and low transmission, e.g. after scale-up of malaria control (Bousema et al., 2012). Malaria control efforts in Papua New Guinea (PNG) were re- intensified in 2004. Differences in malaria burden, transmission, and impact of interventions have subsequently been identified even between neighbouring villages (Hetzel et al., 2016, 2014c), confirming earlier findings of small-area heterogeneity(Cattani et al., 1986). An analysis of routine health-facility data from seven sentinel sites found that reductions in malaria incidence were associated with Long Lasting Insecticidal Nets (LLIN) distributions but the effect varied between sites (Rodríguez-Rodríguez et al., 2019c). As malaria transmission decreases, and resources remain limited, targeting of interventions becomes increasingly important (Bousema et al., 2012; Mendis et al., 2009).

Malaria surveillance and response-systems are essential for identifying the areas or population groups that are most affected by malaria and for targeting resources for maximum impact (World Health Organization, 2018a). The Global Technical Strategy for Malaria 2016-2030 proposes the use of a comprehensive approach that includes vector control measures and early diagnosis and treatment, especially at the village level (World Health Organization, 2018a, 2015). Identifying villages with on-going transmission and monitoring changes over time is therefore of utmost importance to effectively target interventions. Particularly in settings with weak health systems this information should be generated by simple approaches. Routine data collected at health facilities may provide a viable option if the relevant information is captured

and readily analysed. However, research on how to best apply geospatial analyses of simple routine data to identify heterogeneity at village level and support targeting malaria interventions is scarce (Kelly et al., 2012).

This study aimed to assess whether the visualizations of health facility data linked to village of residence of patients provides evidence for targeting malaria control interventions. It used malaria incidence data linked to the self-reported village of residence of the patients, collected routinely in four sentinel health facilities (SHF) in PNG. If found to be a valid approach, the operational feasibility of targeting malaria control at district or sub-district level would have to be investigated within the frame of existing capacities and resources of the local health system.

Sentinel surveillance was established in the health centres of East Cape (Southern Region), Sausi (Momase), Karimui (Highland) and Lemakot (Islands) – one SHF per geographical region of PNG. Surveillance was established as part of the continuous independent evaluation of the National Malaria Control Program (NMCP) (Hetzel et al., 2015, 2014c, 2012). Details of the sites are provided elsewhere (Hetzel et al., 2014c; Rodríguez-Rodríguez et al., 2019c).

All outpatient cases attending the SHF were routinely screened for a history of fever during the previous three days. A study nurse at the facility collected a capillary blood sample from all consenting fever patients of all ages for diagnosis of malaria by Rapid Diagnostic Test (RDT; ICT Malaria Combo HRP2/aldolase, ICT Diagnostics, South Africa). Demographic details including village of residence and self-reported mosquito-net use the previous night were recorded on paper case report forms alongside RDT results. Paper forms were then double entered at the Papua New Guinea Institute of Medical research (PNGIMR). The study team ensured availability of RDTs throughout the surveillance period.

Data was collected from 2010 to 2014 to characterize annual incidence variations between villages in the catchment areas of the SHFs and identify patterns that could guide malaria control efforts. Recently, a paper-based “Malaria Register” has been implemented by the PNG National Department of Health and an electronic National Health Information System (eNHIS) is being piloted (Rosewell et al., 2017). The register and eNHIS routinely collect malaria indicators linked to the village of residence of the patient, which is comparable to this analysis, allowing the scale-up of the approach if it proves useful.

The delineation of the SHF catchment areas was defined by local health authorities. A population census conducted by the PNGIMR in the SHF catchment areas at baseline, during which all houses were identified and each household member was listed, was used as source of

village coordinates and population denominator data (Hetzel et al., 2014c). The geo-referenced year 2000 National Census database was used to complement the PNGIMR census, particularly to identify villages outside the catchment area. During the surveillance period 25,097 fever cases were tested for malaria across all SHF, 38% (95% CI: 37.6-38.8) were RDT-positive. Table 5.1. details the number of malaria cases diagnosed at the SHF residing within and outside the SHF catchment area. The analysis includes only the cases within the catchment area. It is important to note that a large number of patients from outside the catchment area were diagnosed and treated in East Cape and Lemakot SHFs. Both health centres are located in areas of constant transit of people.

Table 5.1. Total number of malaria cases (all ages) and malaria cases in children under five years of age residing within and outside the catchment area by site.

		Malaria cases residing in the catchment area	**Malaria cases residing outside the catchment area**	
Site	Age	N (%)	N (%)	Total N (%)
East Cape				
	All ages	3,265 (93)	250 (7)	3,515 (100)
	Children <5 years	1,076 (93)	82 (7)	1,158 (100)
Sausi				
	All ages	1,532 (99)	13 (1)	1,545 (100)
	Children <5 years	305 (98)	6 (2)	311 (100)
Karimui				
	All ages	545 (99)	8 (1)	553 (100)
	Children <5 years	260 (98)	5 (2)	265 (100)
Lemakot				
	All ages	2,831 (71)	1,142 (29)	3,973 (100)
	Children <5 years	818 (73)	299 (27)	1117 (100)
Total	**Total cases in all ages**	**8,173 (85)**	**1,413 (15)**	**9,586 (100)**
	Total cases in children <5 years	**2,459 (86)**	**392 (14)**	**2,851 (100)**

The video format allowed us to visualize the dynamics in case incidence over time and space alongside photographs illustrating the context of the data collection in the study sites. The main focus of the visualization was on the animated maps illustrating incidence changes overtime. The audio-visual format is easily accessible to a range of stakeholders with the potential to better communicate geospatial relationships in an understandable format (Krieger et al., 2012).

Aggregated annual malaria incidence was calculated for every village with at least one malaria case reported by the SHFs. Annual incidence in children under five years of age was calculated and mapped as a proxy of local transmission since young children are the least immune age group and compared to adults are less likely to travel (Bousema et al., 2012). Self-reported mosquito-net use in all fever patients during the last year of surveillance (2014) was mapped by village and reported net use by all fever cases was graphed by year as an indicator of trends over time.

Following a general declining trend, clear differences in incidence between villages were found in some sites. Mapping of village-level incidence appeared most useful in settings with very few malaria cases (Karimui and Sausi) or with pronounced spatial clustering of cases (Lemakot). In such settings, villages that could benefit from targeted interventions could be identified. However, further investigations in some of the identified local foci are required to understand local heterogeneity. Unequal access to health facilities, availability of other health care providers and treatment seeking behaviour may confound village-level incidence particularly if data is only originating from one facility. In addition, in some communities, village, hamlet and ward names may be used inconsistently by both patients and health workers. It is possible that cases in small communities are attributed to larger nearby villages. Furthermore, case-reporting becomes inaccurate in areas with constant transit of people. Uniform surveillance across all health facilities and a harmonized use of village names could optimize the current approach. Villages have been used as operational units for household-level net distributions in PNG. Villages may also be the smallest feasible unit to target interventions making village level data highly relevant.

Since effective management of malaria programmes requires geo-spatial components to inform response-systems, a next step could devise a simple standardized approach to generate spatial data on malaria risk that can be easily translated into response action can complement universal coverage campaigns in a meaningful way. Since 2015, eNHIS has been piloted in 184 health facilities in PNG. The platform includes a geo-referencing feature for mapping malaria cases at a village level and automated data analysis, reporting and identification of outbreaks

(Rosewell et al., 2017). If proven successful eNHIS could considerably strengthen malaria surveillance in PNG. A similar Spatial Decision Support System building upon and extending existing data collection systems and exploiting current geo-spatial tools has been validated in nearby Vanuatu and Solomon Islands in areas of very low transmission (Kelly et al., 2012). Areas in PNG that have reached a low level of transmission with clear foci may benefit from a similar approach. However, local (sub-national) capacity to further investigate local foci and implement targeted response action would be required as much as sufficient and sustained funding for these activities.

5.3. Outlook

- Age-specific malaria data collected routinely at health facilities and linked to the village of residence of patients may direct programmes to local foci of transmission.
- This approach appears most useful in settings with few cases or marked heterogeneity, where it may direct further investigations or complementary interventions.
- A simple tool for calculating and mapping malaria case incidence at district or sub-district level, as is currently included in the eNHIS, is required to operationalize the approach, along with the capacity, policies, and mechanisms required to implement targeted response action at the respective operational level.
- The chosen audio-visual format is easily accessible to a range of stakeholders with the potential to better communicate geospatial relationships in an understandable format.

5.4. Box 1 Overall aim

To assess weather, the visualization of health information (malaria incidence, net use and residence of patients) extracted from a routinely implemented surveillance system can inform local malaria control programs to better target interventions. Malaria surveillance systems are crucial for identifying the areas that are most affected by malaria. The proposed approach adds a geospatial component to health facility data in order to understand differences in malaria burden between villages and identify communities that would benefit from targeted interventions.

5.5. Box 2 Software used

Maps were generated using the open-source software QGIS (version 3.0 Girona). The video was edited using Adobe Premier Pro CC (version 13.0.2, Adobe Systems Incorporated, San Jose, CA, USA)

5.6. Declarations

Acknowledgements

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Key words

Surveillance, malaria, Papua New Guinea, malaria control, surveillance-response, visualization

Contributions

MWH, JP, IM, LM and PMS conceived of the study. SM, SJM, AT, MWH and JP established and supervised data collection systems and LM facilitated access to the study sites. DRR analysed the data and drafted the manuscript and video concept with inputs from MWH. All authors reviewed and approved the final manuscript and video.

Conflict of interests

The authors declare no conflict of interest.

FUNDING

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VIDEO LINK

https://www.youtube.com/watch?v=KISbow_E07I

5.7. SCRIPT –VHEALTH COMMUNICATION

Video link: https://www.youtube.com/watch?v=KISbow_E07I

5.7.1. INTRODUCTION

- Title and authors
- PNG map and images of people, LLIN distributions, Mala 1, LLINs.
    - 94% of the 8.2 million people of Papua New Guinea are at high risk of malaria infection.
    - Since 2004, the global fund to fight AIDS, Tuberculosis and Malaria has supported the PNG national malaria control programme. The program has promoted 1) the free distribution of long-lasting insecticidal nets; 2) behaviour change campaigns, and 3) the scaling-up of malaria diagnosis by rapid diagnostic test (RDT), together with the introduction of artemisinin-based combination therapy (ACT).
- Insert Image of PNGIMR
    - In order to monitor trends in malaria incidence the Papua New Guinea Institute of Medical Research intensified malaria surveillance in selected health facilities.
- Indicate the location of the Sentinel Sites and mention PNG geographical areas.
    - The four sentinel sites: East Cape, Sausi, Karimui and Lemakot; covered different geographical areas of the country.
- Show pictures of malaria testing
    - All outpatient cases attending the SHF were routinely screened for a history of fever during the previous three days. A study nurse at the facility collected a finger-prick blood sample from all patients for diagnosis of malaria by Rapid Diagnostic test.
    - Overall, 25,097fever cases were screened for malaria and 38% tested positive in all sentinel sites [Display numbers]
    - Demographic details including village of residence and self-reported mosquito-net use were recorded for all cases.

- The study addressed the following research question: "Do health facility data linked to village of residence provide useful evidence to target [malaria] interventions?"
- For the analysis three populations of interest were defined:
    - 1-The catchment area population
    - 2-The population of villages with malaria cases
    - 3-The population of villages with malaria cases in children under 5 years of age: This group was used as a proxy for local transmission since young children are the least immune age group and compared to adults they are less likely to travel.

5.7.2. STORY OF MALARIA INCIDENCE IN EACH SITE

- Show map of PNG
    - Each of the four sentinel sites represents a different malaria transmission setting.

East Cape

- East Cape pictures
    - The East Cape Sentinel site comprises the catchment area of East Cape Health Centre. It is located at the tip of mainland Papua New Guinea in Milne Bay province and is a port of entry to the mainland for surrounding island groups.
- Display map with the location of ALL villages and names (Catchment area population-blue)
    - The catchment area includes 13 villages and almost 4,200 (4,176) inhabitants; 14% are children under 5.
    - In addition patients from 68 villages outside the catchment area were diagnosed and treated in East Cape HC. Their villages are displayed in red on this map.
- Display map with the location of villages reporting malaria cases 2010-2014 (orange); size of the bubble corresponds to village incidence)
    - The following analysis includes only cases within the catchment area.

- This map displays in orange bubbles villages with malaria cases; the size of the bubble corresponds to village incidence per one hundred.
- Between 2010 and 2014 malaria cases were diagnosed from all villages in the area.

- Display map with the location of villages reporting malaria in children under 5 from 2010-2014 (red); size of the bubble corresponds to under-5 village incidence).
  - This map displays in red villages with malaria cases in children under 5. The size of the bubble corresponds to incidence per 100 children in this age group. All villages except one reported malaria cases in children suggesting that there is malaria transmission everywhere. The only village without cases has a functional aid post and it is possible that children were diagnosed locally and were not recorded at the SHF.

- Display map animation of annual village-incidence variation in children under 5 for each year from 2010 to 2014 (red), size of the bubble corresponds to village-incidence in children under 5
  - When disaggregated by year the annual incidence and proportion of villages with malaria in children varied. Overall, malaria transmission decreased across the area but it was not interrupted. Fewer cases were reported furthest from the health facility and closer to the aid post and to the provincial capital Alotau.

- Display map with net use by village in all fever cases during the last year of surveillance and overall proportion of net-use (purple).
  - This map displays in purple the proportion of fever cases sleeping under a mosquito net the previous night.
  - Net-use varies across villages.
  - Overall reported net-use remained below 75% every year during the surveillance period.

- Conclusion

- In conclusion: transmission in the area remains too high to use this method to target interventions but some useful information can be extracted from the analysis:
    - There is room for improvement of net -use in the area
    - Further investigations in villages with higher incidence in children under 5 could disclose the reason behind persistent local transmission
    - Reducing efforts in areas with lower incidence is not advisable since the reason for the decrease is not clear. Transmission in these villages could indeed be interrupted or malaria cases from this area are simply not treated in East Cape health centre.

Sausi

- Map of PNG and pictures of Sausi
    - The Sausi Sentinel site comprises the catchment area of Sausi Health Centre. Sausi is located in the Ramu Valley of Madang Province. Industrial sugar and oil palm estates are well established here. Areas around the local villages are regularly flooded by the Ramu River and its branches.
- Display map with the location of ALL villages and names (Catchment area population-blue)
    - The catchment area includes 33 villages and around 4,400 (4,406) inhabitants; 14% are children under 5.
    - 2% of the fever cases in the SHF were from outside the catchment area.
- Display map with the location of villages reporting malaria in children under 5 (red) 2010-2014; size of the bubble corresponds to average annual incidence by village)
    - Between 2010 and 2014 malaria cases in children under 5 were reported in 23 villages. No malaria cases were reported in four villages. (PSY block- Mauno, Bundi block, Markham block 49 and Kesowai 2). These four villages, represented by blue dots, are not clustered and all four have good road access to the HF like the neighbouring villages which have malaria cases. Agricultural pest control might lead to lower malaria transmission in some places. However, it is also possible that village-names were used inconsistently by patients and health

workers. Malaria cases in small communities could have been erroneously attributed to close by larger villages.

- Display map with the location of villages reporting malaria in children under 5 each year from 201 to 2014 (red); size of the bubble corresponds to annual under-5 village incidence.
    - Interestingly, when disaggregated by year after an initial decrease in 2013, malaria incidence increased in 2014; particularly in six villages south east of the HF. In contrast, the incidence around the health centre remained stable.
    - Some remote villages may have difficulty accessing the HF.
- Display map with net use by village in all fever cases during the last year of surveillance and overall proportion of net-use (purple).
    - Self-reported net-use in all fever cases has been high since the beginning of the surveillance period and is consistent with household surveys done in the area. (REFERENCE ON SCREEN: Hetzel MW, Gideon G, Lote N *et al.* Ownership and usage of mosquito nets after four years of large-scale free distribution in Papua New Guinea. *Malar J* **2012**;11:192.)
- Conclusion
    - In conclusion, an increase in a specific area was identified with this analysis. Further studies could disclose the reason behind the increase and propose control measures complementary to mosquito-nets.

Karimui

- Map of PNG and pictures of the area
    - The Karimui Sentinel site comprises the catchment area of Sigimaru Health Centre. It is located in Simbu province, an isolated area without road access, approximately 1000 m above sea level.
- Display map with the location of ALL villages and names(Catchment area population-blue)
    - The catchment area includes 25 villages and almost 7,700 (7697) inhabitants; 16% are children under 5.

- During the surveillance period 2% of fever cases came from outside the catchment area.

- Display map with the location of villages reporting malaria in children under 5 2010-2014 (Population of villages with malaria cases in children under 5 (red); size of the bubble corresponds to under-5 village incidence).
    - Between 2010 and 2014 malaria cases in children under 5 years of age were reported from 22 villages. Only two villages on opposite sides of the catchment area had no cases.

- Display map animation of average annual village-incidence variation from 2010 to 2014 (red), size of the bubble corresponds to village-incidence.
    - When disaggregated by year we observed only 5 villages with malaria cases in 2014 and only 2 villages reported malaria cases in children under 5.

- Display map with net use by village in all fever cases during the last year of surveillance and overall proportion of net-use (purple).
    - Reported net-use in all fever cases was relatively high in 2014
    - Overall reported net-use has significantly improved over the years.

- Conclusion:
    - In conclusion, what looked much like a general epidemic with local transmission at the beginning changed into a situation with very focal transmission. Remaining foci could probably be eliminated with a targeted approach. Simple mapping of cases proved useful in this setting and effectively identified areas to target with complementary interventions.

Lemakot

- Map of PNG and Lemakot pictures
    - The Lemakot Sentinel site comprises the catchment area of Lemakot Health Centre. It is located in New Ireland, a long, narrow, and mountainous Island province. A well-maintained highway along the East Coast connects North and South of the province.

- Display map with the location of ALL villages and names(Catchment area population-blue)
    - The catchment area includes 28 villages and over 8400 (8423) inhabitants; 16% are children under 5.
    - In addition patients from 52 villages outside the catchment area were diagnosed and treated in Lemakot HC.
- Display map with the location of villages reporting malaria in children under 5 2010-2014 (red); size of the bubble corresponds to under-5 village incidence.
    - Between 2011 and 2014 malaria cases in children under 5 were reported from 26 villages.
    - The highest incidence is observed in two clusters on the East Coast.
- Display map animation of annual village-incidence variation from 2010 to 2014 (red), size of the bubble corresponds to village-incidence)
    - When disaggregated by year we observed an initial increase in 2012.
    - In 2014 only 7 villages reported malaria cases in children under five. Most cases concentrated in one specific area.
- Display map with net use by village in all fever cases during the last year of surveillance and overall proportion of net-use (purple).
    - This reduction was observed even though net-use in the area remained rather low.
    - Overall, net-use in all fever patients has been below 55% since 2011.
- Conclusion
    - In conclusion:
        - There is room for improvement of net -use in the area
        - A decline in transmission can be observed over the years with most cases concentrated in one area the last year of surveillance.

- Further investigations in this area could disclose the reason behind such highly focused transmission and propose alternative control interventions.

5.7.3. OVERALL CONCLUSION

- Display text on the screen
    - Coming back to the research question: "Do health facility data linked to village of residence provide useful evidence to target interventions?"
    - Malaria incidence measured at village level may be influenced by factors like:
        - Treatment seeking
        - Unequal levels of access to sentinel health facilities
        - Availability of other treatment sources
    - Despite limitations, this approach appeared useful in sites with very few remaining cases, such as Karimui; or with increasingly marked heterogeneity, such as Lemakot and Sausi. In these sites, specific villages that could benefit from targeted interventions or investigations were identified.

5.7.4. CREDITS

- Display picture in the background, roll credits and play music.
    - Concept and Script
        - Daniela Rodríguez-Rodríguez
    - Scripts supervision
        - Manuel Hetzel
    - Editing
        - Andreas Weber
    - Maps
        - Daniela Rodríguez-Rodríguez

- Voice
    - Daniela Rodríguez-Rodríguez
- Music
    - Marvellous Maprik by Sharzy ft. Avisat (2016)
- Images in order of appearance (excluding maps)
    1. Papua New Guinea location – Source: google.com/maps
    2. Rural life in remote Papua New Guinea - Source: © Olga Fontanellaz for Swiss Malaria Group
    3. Rainy East Cape - Source: Manuel Hetzel
    4. Goroka overview - Source: Daniela Rodríguez-Rodríguez
    5. Traditional dance in Madang Show - Source: Daniela Rodríguez-Rodríguez
    6. Every family must have a net - Source: © Olga Fontanellaz for Swiss Malaria Group
    7. Net Distribution in Puas- Source: Tim Freeman and Madelline Balu for Rotarians Against Malaria
    8. Bed nets in Inu Village, Papua New Guinea - Source: © Tim Siegenbeek van Heukelom for Swiss Malaria Group
    9. Marasin Stoa Kipa Fotome testing for malaria in Papua New Guinea - Source: © Tim Siegenbeek van Heukelom for Swiss Malaria Group
    10. Mala-1 (Artemeter-Lumefantrine 5 to 15 kg dose) – Source: Daniela Rodríguez-Rodríguez and Manuel Hetzel
    11. Baby in Bilum and PNGIMR logo – Source: Daniela Rodríguez-Rodríguez
    12. Nurse and patient in New Ireland – Source: Daniela Rodríguez-Rodríguez
    13. Finger prick for RDT testing – Source: Manuel Hetzel
    14. RDT for a child in Sausi – Source: Diana Timbi, PNGIMR

15. Rapid Diagnostic Test – Source: Manuel Hetzel
16. Community in Madang Province- Source: Leanne J. Robinson, Burnet Institute
17. Finger prick on child in Lemakot – Source: Daniela Rodríguez-Rodríguez
18. Children playing in Madang Province – Source: Daniela Rodríguez-Rodríguez
19. Island and reef in East Cape – Source: Manuel Hetzel
20. East Cape Health Centre – Source: Manuel Hetzel
21. East Cape sign – Source: Manuel Hetzel
22. Sausi valley – Source: Manuel Hetzel
23. Sausi Health Centre – Source: Manuel Hetzel
24. Man climbing a palm tree – Source: Tim Freeman for Rotarians Against Malaria
25. Karimui Health Centre – Source: Tim Freeman for Rotarians Against Malaria
26. Unloading a plane for net distributions – Source: Tim Freeman for Rotarians Against Malaria
27. Net distribution in Karimui –Source: Tim Freeman for Rotarians Against Malaria
28. Lemakot road and island in the Background – Source: Manuel Hetzel
29. Boluminski highway, street vendor and public transport in New Ireland East Coast – Source: Manuel Hetzel
30. New Ireland Province Malaria Program – Source: Daniela Rodríguez-Rodríguez
31. Plantation road in New Ireland West Coast – Source: Daniela Rodríguez-Rodríguez

32. Mosquito feeding – Source: Mayeta Clark Walter and Eliza Hall Institute of Medical research 2015

33. Sunset in New Ireland Province – Source: Daniela Rodríguez-Rodríguez

6. HUMAN BEHAVIOUR, LIVELIHOOD AND MALARIA TRANSMISSION IN TWO SITES OF PAPUA NEW GUINEA

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6.1. ABSTRACT

BACKGROUND

Malaria transmission is currently resurgent in Papua New Guinea (PNG) following historic lows reported in 2013/14 and sub-national heterogeneities in malaria trends have been observed Social and cultural patterns, the response of people to interventions, and local political and economic realities have previously been responsible for changes in the epidemiology of malaria in PNG. This study aims to better understand the role of human behaviour in relation to current malaria transmission and transmission heterogeneities.

METHODS

A mixed-method design was used to investigate human behavioural patterns with potential relevance for malaria transmission in two sites in PNG. In-depth interviews, focus group discussions (FGD), a cross-sectional malaria indicator survey and a population census were implemented in parallel.

RESULTS

This study identified seven distinct population groups based on demographics and behavioural patterns within the study population. The study highlights the substantial amount of time spent outdoors or in non-secure structures when "indoors" as a major risk of exposure to anophelines bites. Between 4pm and 8am, all groups in both sites are likely to be exposed to mosquito bites across all types of activities; sleeping under a LLIN was the exception.

CONCLUSIONS

Our findings highlight the potential of 'outdoor biting' to hamper malaria control and elimination efforts if not addressed appropriately since people spend a remarkable amount of time outdoors without protection from mosquito biting. Targeting groups, places and activities in order to prevent outdoor biting in the early hours of the evening and the morning seem crucial to advance towards elimination.

KEYWORDS

Malaria, Residual transmission, Outdoor transmission, Papua New Guinea, Human behaviour, Outdoor mosquito exposure, Human-vector contact, LLINs

6.2. INTRODUCTION

Residual malaria transmission (also referred to as persistent or ongoing transmission) after high coverage has been achieved with core interventions such as long lasting insecticidal nets (LLINs) presents a challenge to malaria control and elimination efforts (Sherrard-Smith et al., 2019). To address residual transmission, an understanding of when and where vector and human behaviour intersect is necessary. Since social patterns and human behaviours that could modify exposure to *Anopheles* biting have been identified as drivers of transmission, it becomes increasingly important to understand such behaviours in local settings (Bannister-Tyrrell et al., 2018; Cotter et al., 2013; Monroe et al., 2019b).

In 2017, 881,697 malaria cases were confirmed in Papua New Guinea (PNG) and it was estimated that 94 % of the population was at high risk of malaria infection (World Health Organization, 2018a). Since 2004, much progress has been made in reducing the malaria burden and high coverage of LLINs has been achieved. The PNG National Malaria Control Program (NMCP) has been financially supported by The Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM), and malaria prevalence below 1600 m altitude decreased from 11% in 2008/09 to <1% in 2013/14 (Hetzel et al., 2018, 2017a, 2014a; Pulford et al., 2013). However, despite this success, malaria resurged dramatically across PNG by 2016/2017 with an estimated 8.6-fold increase in prevalence in only three years (Hetzel et al., 2018). While certain shortfalls in the NMCP coincided with this increase, use of LLINs had remained stable at around 50% since 2011 (Hetzel et al., 2018). Yet, early and outdoor biting of *Anopheles* mosquitoes had been identified as a threat to the effectiveness of LLINs. The peak exposure time to infective bites shifted from later than 9 pm in 2008 to between 6 and 7 pm in 2011 (Reimer et al., 2016; Thomsen et al., 2017). In combination with these behavioural changes in the local vector population, human behaviour such as sleeping patterns and early evening and night-time social, cultural and economic activities could increase exposure to infective mosquito bites.

Alongside the recent resurgence in malaria, variations in malaria trends have been observed in a number of different sites (Rodríguez-Rodríguez et al., 2019c). Considering the within-country heterogeneity in malaria (Betuela et al., 2012a; Cattani et al., 1986; Cooper et al., 2009; Rodríguez-Rodríguez et al., 2019c) and cultural diversity; it is very likely that different human behaviours are relevant for malaria transmission across the country (Bannister-Tyrrell et al., 2017; Bousema et al., 2012; Cotter et al., 2013). Social and cultural patterns, livelihoods, and the response of people to specific interventions, together with local political and economic realities, have been responsible for changes in malaria epidemiology in the past (Radford et al., 1976). For instance, travel and trade, clay collection for pottery, inter-area marriages, bird of paradise

hunting, gardening, sago harvesting, salt collection, and road and transport developments have been identified as social aspects influencing the malaria epidemiology in PNG (Attenborough and Alpers, 1992; Mueller et al., 2003; Radford et al., 1976).

This study aims to better understand the role of human behaviour in relation to malaria transmission and transmission heterogeneities by: i) identifying activities and livelihood aspects relevant for malaria transmission, ii) understanding which measures are currently being used to prevent or reduce mosquito biting in the study sites and iii) identifying behavioural differences between population groups.

6.3. METHODS

STUDY DESIGN

A mixed-method design was used to investigate human behavioural patterns with potential relevance for malaria transmission in two sites in PNG. Knowledge, perception and practices related to malaria transmission, prevention and sickness were also assessed. In-depth interviews (IDI), focus group discussions (FGD), a cross-sectional malaria indicator survey and a population census were implemented in parallel.

STUDY SITES

The study was conducted within the catchment area of two health facilities: 1) Mugil Health Centre, Sumkar District, Madang Province and 2) Lemakot Health Centre, Kavieng District, New Ireland Province (Figure. 6.1.). Villages with a high malaria burden at the health facility were selected for an exploratory visit. Following the visits, four villages were selected in each site based on accessibility and the explicit consent from the village leaders to participate. Two villages in each site included a scattered population distributed across a larger area while the two others included a population concentrated in a smaller area. The location of all selected villages can be observed in Figure 6.1.

Mugil area

Mugil area is located in the rainforest area on the North Coast of mainland PNG (Marfurt et al., 2010). Cash crops such as copra (the dried meat of the coconut), cocoa beans and betel nut (*Areca catechu)* are the main source of income. Selected villages in the Mugil area included two coastal villages (Megiar and Mirap) and two inland villages (Bulal and Wasab).

Lemakot area

Lemakot area is located on the main island of New Ireland Province, which is long (~200 km), narrow (~8 km) and mountainous with a wet tropical climate (Holdsworth et al., 1980). Oil

palm companies are well established in the area as a major provider of formal employment. The most important cash crops are oil palm fruit, copra and to a lesser degree sago (*Metroxylon sagu*) and betel-nut. The last two are grown for own consumption or to sell on a small scale within the community. Selected villages in Lemakot area included two West Coast villages (Lamusmus 1 and Lavolai) and two East Coast villages (Luburua and Lossuk).

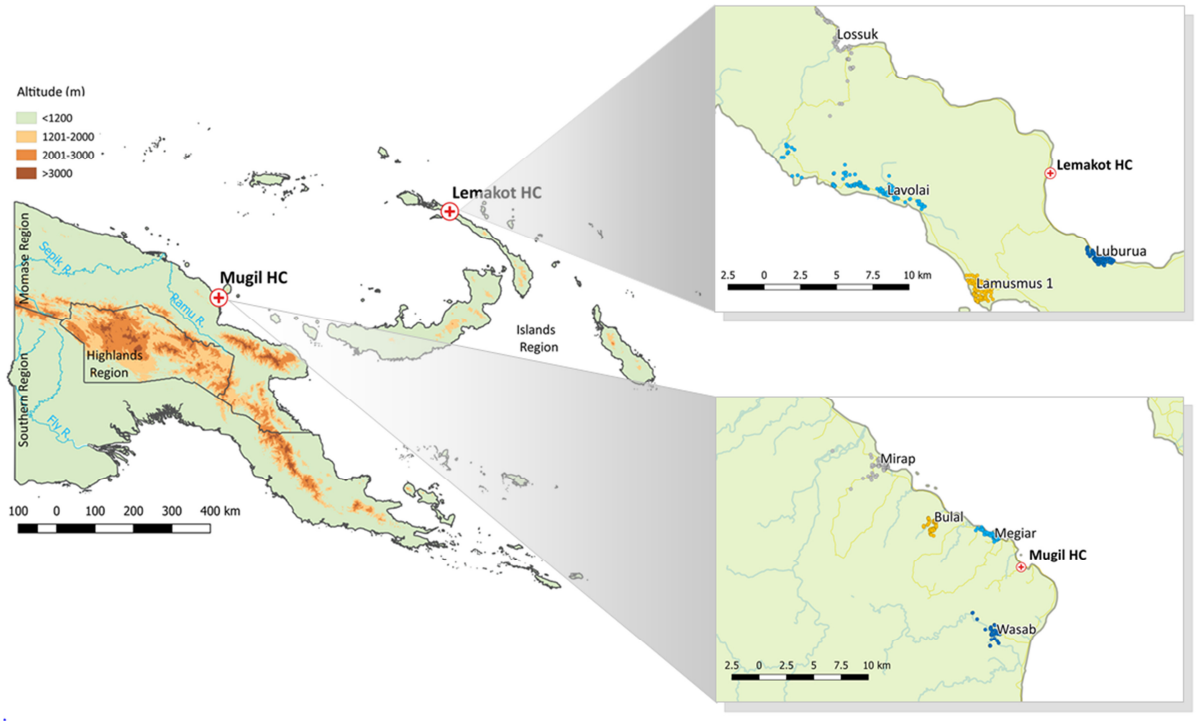


Figure 6.1. Location of the two study sites and the selected villages in each study site. Coloured dots represent all identified households in the selected villages.

Data Collection

Quantitative data collection

Quantitative data on key behaviours and livelihood factors such as sleeping habits, mosquito-net use, malaria prevention preferences, housing and household structures, recent malaria treatment and travel history were collected from participants and caregivers (for children) during a cross-sectional malaria indicator survey (MIS) between September 2016 and October 2017. After a census was concluded, a sample of 80% of the households was purposively selected to represent a spatially even distribution across all hamlets in the village. All eligible and available residents (age > 6 months) of the selected households were invited to participate in the MIS. All data were collected electronically on tablets using one questionnaire for each household (household questionnaire) and one for each individual participating in the survey (individual questionnaire). Variables collected for the household questionnaire included size of the household, kinds of livestock in the household, type of water source, type of house structure,

presence of window screening, type of toilet facility, education level of the household head, income source, best malaria prevention methods and LLINs in the household. Variables collected for the individual questionnaire included: demographic details (age and sex), use of LLIN the previous night, frequency of LLIN use, age of the used LLIN, sleeping times, sleeping place, history of antimalarial treatment in the last two months and travel history in the last month. All forms were developed using the open-source platform Open Data Kit (ODK). A population census in the eight selected villages preceded the survey. Household size, house coordinates, age and sex of all household members were collected electronically using ODK. A photo of the house and its members was taken if participants agreed. The census and the survey were conducted by a team of trained local field interviewers with previous research experience.

Qualitative data collection

Qualitative data on behaviour, livelihood and activities of different demographic groups with location and timing were collected using IDIs and FGDs. A local village representative was appointed by the village leaders to recruit study participants. Participants were selected to represent single and married men and women in the area. Inclusion criteria were age (age ≥ 15 years) and fluency in Tok Pisin. Participants across all age groups were selected to represent younger and older demographics.

Explorations during the IDIs applied a time line follow-back method (TLFB). TLFB gathers behavioural information during a pre-selected time period. A calendar is used to structure the interview assist the respondent's recall strategy (Sobell and Sobell, 1992). IDIs were guided by a calendar that covered everyday activities with particular focus on time spent indoors and outdoors during *Anopheles* biting times during the last 7 days. Two IDIs were conducted in each village; one with a man and one with a woman. IDIs were conducted in T*ok Pisin (*local *lingua franca)* by a trained interviewer. IDIs were recorded, then transcribed verbatim and finally translated to English for the analysis.

Two FGDs (one with men and one with women) took place in each selected village. FGDs were conducted in *Tok Pisin* by a trained interviewer. FGDs were recorded and notes were taken by a second team member who was not the interviewer. Since the environment is a crucial part of understanding behaviour, FGDs were accompanied by a hand drawn map on a flip chart. The map was based on a sketch (Additional file 1) of the village developed by the community leaders. The sketch delimited the village according to the local community. On occasions, it included sub-divisions of the village and or highlighted the landmarks (e.g. houses, schools, plantations, swamps, etc.) considered important by each community. During the FGDs, the maps (Additional file 2) added a sense of distance and time to the narrative. In addition, potential sites for transmission within or around the village were identified for each community. After data

collection the interviewer reviewed and adjusted the notes while listening to the recording. Notes were used for the analysis.

IDIs and FGDs were conducted in parallel in each village. After completing the first round of 8 IDIs and 8 FGDs in one study site (Mugil area) re-emerging topics were identified and the collected data reached saturation without new topics emerging. The sample size was then established for both study sites.

DATA ANALYSIS

Quantitative data analysis

Descriptive quantitative analysis was conducted using Stata/IC version 13.1 (Stata Corp LP., College Station, USA). Responses from the household questionnaire were stratified by site and percentages with 95% confidence intervals were calculated for each response. Three tables were generated: Table 6.1. enlists household characteristics by site, Table 6.2. enlists perceived 'best' malaria prevention methods by site and Table 6.3. describes LLIN ownership and use by site. Hourly cumulative percentage of people sleeping and people sleeping under a LLIN by behavioural group (demographic groups exhibiting similar behavioural patterns) was calculated for each site. The proportion of individuals being actually protected by behavioural group during a given percentage of time (100, 75, 50, 25 and 0%) between 6pm and 6am was calculated. The shape of the curve for sleeping and waking up times was assumed to be symmetrical.. Sleeping and waking up times recorded during FGDs supported the assumed symmetry. Responses from the individual questionnaire were stratified by behavioural group and percentages with 95% confidence intervals were calculated for each site (Table 6.4.).

Qualitative data analysis

English transcriptions of the IDIs and the notes of the FGDs were analysed independently by two researchers using a pre-established framework designed to identify activities and related information relevant to malaria transmission (time of the day, duration, location, sex and age of the person or people reported for each activity). Coding differences were discussed and harmonized by the researchers coding the data. Consensus on coding was agreed-on after discussions. The analysis prioritised activities occurring between 4 pm and 8 am, 2 hours either side of dusk and dawn. Seven demographic groups exhibiting similar behavioural patterns (behavioural group) subsequently emerged from the framework, including: preschool aged children (age ≤ 5 years), school boys (age>5 and ≤16 years), school girls (age>5 and ≤16 years), adult men in Mugil, adult women in Mugil, adult men in Lemakot and adult women in Lemakot (age for all adult groups >16 years). A composite profile for each behavioural group was then drafted, drawing on the respective IDI and FGD data for that grouping, to portray the most

common activities and livelihoods with a special focus on potential exposure to mosquito bites. A further thematic analysis of the IDI and FGD data was also conducted independently by two researchers focused on identifying knowledge, attitudes and practices towards malaria transmission and vectors, malaria prevention, and clinical episodes. Emerging themes were compared, discussed, harmonized and added to the composite profiles. Saturation was reached with most IDIs and FGDs discussing the same or similar topics.

6.4. RESULTS

The baseline census identified a total of 3,364 individuals in the four villages in the Mugil area and 5,470 in the four villages in the Lemakot area. Age and sex distribution of each study site are depicted in Figure 6.2. Women comprised 48% (1,620/3,364) of the population in Mugil and 47% (2,888/5,470) in Lemakot. The survey sample included 1927 participants and 398 households in Mugil and 1202 participants and 309 households in Lemakot. A total of 16 IDIs were conducted, age ranged from 21 to mid-fifties in Mugil and from 28 to 68 in Lemakot. A total of 16 FGDs were conducted with 61 people aged between 15 and 61 participated in Mugil and 68 in Lemakot aged between 19 and 76.

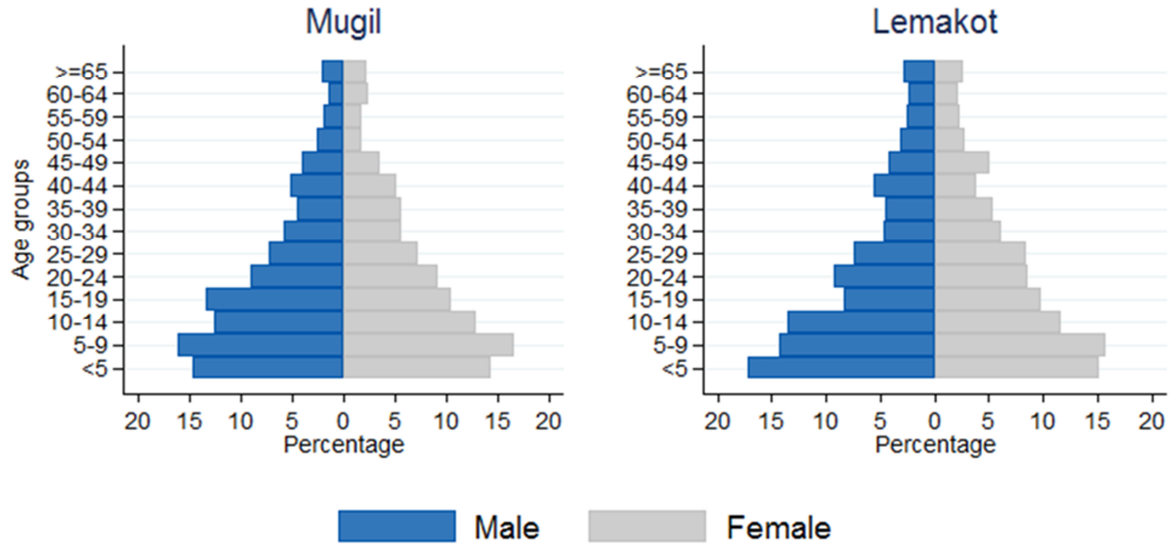


Figure 6.2. Age and sex distribution of the population in the study sites according to the baseline census.

QUANTITATIVE DATA

Household characteristics and livelihood

Table 6.1. includes key characteristics of households. In Mugil, more people lived in traditional houses than in Lemakot, where mixed construction was more common. Figure 6.3. exemplify the types of housing in both study sites. The study considered traditional houses those

constructed only with raw materials (roof, walls and floor). Modern houses were constructed only with improved materials and mixed houses with both, raw and improved materials. In Lemakot, the number of households with access to water at the dwelling (water tanks and piped) was considerably higher than in Mugil where most households used surface water. The number of self-sustained households in Mugil was considerably higher than in Lemakot where more wage employment was available.

Table 6.1. Household characteristics by site

Characteristic		Mugil (N=398)		Lemakot (N=309)	
		Mean	**95%CI**	**Mean**	**95% CI**
Number of household members		6.0	5.7 - 6.3	5.7	5.4 - 6.1
		Percentage	**95%CI**	**Percentage**	**95% CI**
Households keeping animals					
	Chicken	47	42 - 52	47	41 - 52
	Pigs	51	46 - 55	49	43 - 54
	Dogs	47	43 - 52	48	43 - 54
	Cats	30	26 - 35	30	25 - 35
Housing Type					
	Traditional	74	68 - 78	47	42 - 53
	Mixed	26	22 - 31	51	46 - 57
	Modern	0	0 - 2	2	1 - 4
Window screening					
	On all windows	25	21 - 29	19	15 -24
	On some	37	33 - 42	34	29 - 39
	On none	30	26 - 35	44	39 -50
	No windows	8	6 - 11	3	2 – 6
Water source					
	Surface water or well	79	75 - 83	34	28 - 39
	Water tank or piped into dwelling	21	17 - 25	66	61 - 72
Toilet facility					
	None	46	42 - 51	52	46-58
	Outdoors latrine	53	48 - 58	47	41 - 52
	Indoors toilet	1	0 - 2	1	0 - 3

Income source					
	Regular wage	6	4 - 9	21	17 - 26
	Self-employed	12	9 -15	9	7 - 13
	Self- sustained	81	77 - 84	67	62 - 72
	Other	1	1 - 3	2	1 - 5
Educated household head		92	89 - 94	97	94 – 98
Achieved education					
	≤6th grade	51	46 – 56	51	45 – 57
	≥7th and ≤12th grade	45	40 – 50	38	33 – 44
	Higher education	4	2 – 7	11	8 - 15

Figure 6.3. Examples of housing structures in Mugil (top): traditional structure (left), mixed (centre) and modern (right). Examples of housing structures in Lemakot (bottom): traditional structure (left), mixed (centre) and modern (right).

Malaria prevention

Malaria prevention methods were reported in similar frequency by respondents in both sites. The use of mosquito nets was the prevention method mentioned most frequently in both study sites. Interestingly, four of the mentioned prevention methods were linked or related to cleanliness of self and around the house. A complete list of the perceived "best prevention methods" is available in Table 6.2.

Table 6.2. Best malaria prevention methods according to the respondent by site

Methods	Mugil (N=398)		Lemakot (N=309)	
	Percentage	95%CI	Percentage	95% CI
Mosquito net	82	78 – 85	81	76 - 85
Remove rubbish	65	60 - 69	72	67 - 77
Clean the house	45	41 – 50	42	37 - 48
Clear grass around the house	45	41 - 50	39	33 - 44
Drain stagnant water	32	28 - 37	34	29 - 39
Burn leaves or husks	14	11 - 18	11	8 - 15
Stay in good health	12	9 - 15	13	10 - 18
Cleanliness	8	6 - 11	8	6 -12
Mosquito coil	4	2 - 6	3	2 - 6
Herbs	2	1 – 4	0	0 – 2
Avoid mosquito bites	1	0 - 2	0	0 - 2

LLIN ownership and use

Table 6.3. describes LLIN ownership and use by household. Compared to Mugil, people in Lemakot own fewer nets and use them less consistently. However, when sleeping under a LLIN the number of people sleeping per LLIN is similar in both sites. In both sites, the most common reason for not using a given LLIN was that it was considered a "spare" one. In some households, certain LLINs were considered "spare" despite not all occupants sleeping under one. In Lemakot, a considerably higher percentage of LLINs were not used because the weather was too hot.

Table 6.3. LLIN ownership and use by site

Indicator Number of households	Mugil (N=398)		Lemakot(N=309)	
	Percentage	95%CI	Percentage	95% CI
At least 1 LLIN	100	98 – 100	93	90 – 94
1 LLIN for every 2 people	72	68 – 76	61	57 – 65

Reported LLINs	**N=1,554**		**N=971**	
LLINs used the previous night	70	67 – 72	34	31 – 37
Number of people sharing a LLIN				
1	43	40 – 46	43	38 – 48
2	33	30 – 36	29	24 – 34
3	18	15 – 20	23	19 – 28
4	5	4 – 7	4	3 – 7
5	1	1 – 2	1	0 – 2
Not used LLINs	**N=471**		**N=637**	
Reasons for a particular LLIN not being used				
Spare LLIN	81	77 – 84	49	45 – 53
Too hot	6	4 – 9	26	22 – 30
Damaged LLIN	5	3 – 7	2	1 – 3
User away	4	3 – 7	4	2 – 6
Other	2	1 – 4	7	5 – 9
No mosquitoes	1	1 – 3	2	1 – 3
Don't know	0	--	10	8 – 13

Sleeping times and LLIN use the previous night

The percentage of people sleeping under a LLIN in all groups is considerably smaller in the Lemakot area compared to the Mugil area (Figure 6.4.). Additionally, individuals in both sites go to sleep at a later time the older they become, adult men going to sleep the latest. By 9pm, 90% of pre-school aged and school aged children (boys and girls) were asleep in both sites. In the Mugil area, almost all of them slept under a LLIN whereas in the Lemakot area less than half of them slept under a LLIN. By 11pm, 90% of the adult women were asleep in both sites, almost 90% of them under a LLIN in the Mugil area and less than half of them in the Lemakot area. By 12am, 90% of adult men were asleep in both sites. In the Mugil area almost 80% of them slept under a LLIN while in the Lemakot area, only a third of them slept under a LLIN. A difference in LLIN-use between men and women was visible especially in Lemakot where LLIN use is lower. LLIN-use as a percentage (Table 6.4.) might underestimate exposure to anophelines, especially

for adults (men and women). For instance, despite a high LLIN-use reported in the Mugil area (89%) by 9 pm, roughly 70% of adult men and 50% of women are still unprotected in the areas where anophelines start biting much earlier (Keven et al., 2019; Reimer et al., 2016; Robinson et al., 2018). Considering the anophelines biting times from 6pm to 6am and the reported sleeping times, many individuals using LLINs are unprotected a considerable amount of time within this period. Figure 6.4. (bar) depicts this relationship and reveals an important gap of protection especially in adults despite them using a LLIN. For instance, in the Mugil area it was estimated that 43% of the women and 50% of the men are protected under a LLIN only 25% of the time between 6pm and 6pm. Similarly, 34% of the school boys and girls are protected only 50% of the time between 6pm and 6am.

Less than 10% of respondents reported sleeping outdoors in both study sites. Overall, LLIN use in Lemakot was 37% (95% CI, 35 – 40), less than half than in Mugil (89%; 95% CI, 88 – 91). Preschool aged children were the group with the highest percentage sleeping under an LLIN in both sites with 96% in Mugil (95% CI, 93 – 98) and 52% (95% CI, 44- 59) in Lemakot. In contrast, adult men were the group using LLINs the least; 81% (95% CI, 77 – 84) in Mugil and 26% (95% CI; 21 – 31) in Lemakot. Individuals recently traveling or using antimalarials were few; however, the number of preschool aged children using antimalarials was considerably higher in Mugil compared to Lemakot. Adult men were the groups with the highest percentage reporting recent travels in both sites. Further details are described in Table 6.4.

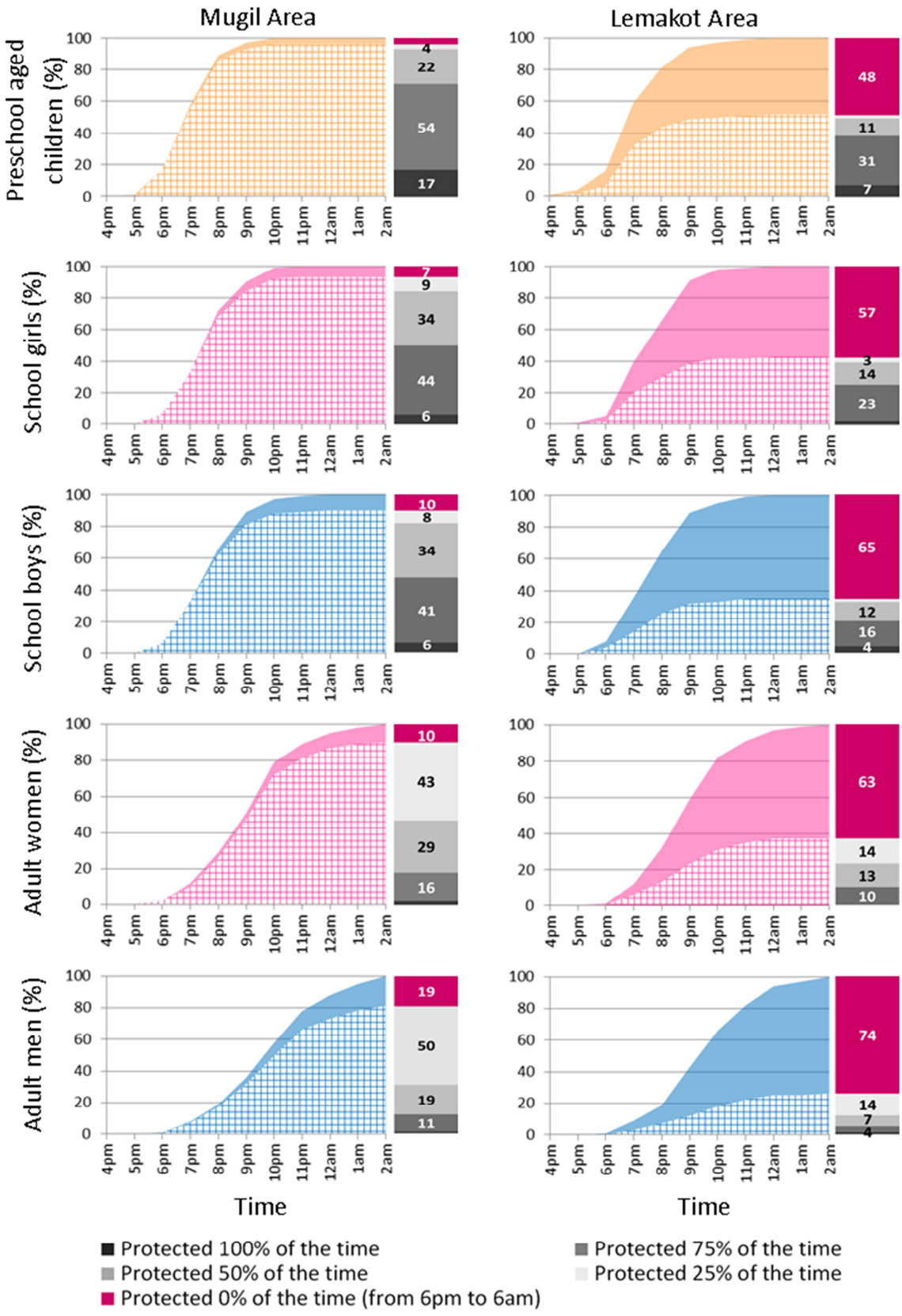


Figure 6.4. The graph represent sleeping times and net use for each behavioural group by site, sleeping under a LLIN (net pattern) and not sleeping under a LLIN (solid).The bar and colour code represent the percentage of individuals by behavioural group actually protected under a LLIN during a given percentage (100, 75, 50, 25 and 0%) of the period between 6pm and 6am.

Table 6.4. Self-reported sleeping habits and LLIN use, history of recent travelling and recent use of antimalarials by behavioural group and site

Group	Slept indoors % (95% CI)	Slept under LLIN % (95% CI)	LLIN every night % (95% CI)	Travel in previous 30 days % (95% CI)	Antimalarials in previous 60 days % (95% CI)
Mugil	96 (96 – 97)	89 (88 – 91)	77 (75 – 79)	9 (7 – 10)	8(7 – 9)
Preschool aged children	98 (96 – 99)	96 (93 – 98)	90 (86 – 93)	5 (3 – 9)	10 (7 – 14)
School girls	100 (100- 100)	93 (89 – 95)	83 (79 – 87)	5 (3 – 8)	11 (8 – 15)
School boys	99 (97 – 100)	90 (86 – 93)	78 (73 – 82)	6 (4 – 10)	9 (7 – 13)
Adult women	98 (96 – 99)	90 (87 – 92)	77 (73 – 80)	9 (7 – 12)	5 (3 – 7)
Adult men	90 (86 – 92)	81 (77 – 84)	63 (58 – 67)	14 (11 – 18)	6 (4 – 9)
Lemakot	92 (90 – 93)	37 (35 – 40)	24 (22 – 27)	10 (8 – 12)	4 (3 – 5)
Preschool aged children	97 (94 – 99)	52 (44 – 59)	36 (30 – 44)	7 (4 – 11)	3 (1 – 6)
School girls	98 (94 – 99)	43 (36 – 50)	29 (23 – 36)	5 (3 – 9)	5 (3 – 10)
School boys	94 (89 – 96)	35 (29 – 43)	21 (16 – 28)	7 (4 – 12)	4 (2 – 8)
Adult women	94 (92 – 96)	37 (32 – 42)	24 (20 – 29)	11 (8 – 15)	3 (2 – 6)
Adult men	80 (75 – 85)	26 (21 – 31)	15 (12 – 20)	15 (11 – 20)	5 (3 – 8)

QUALITATIVE DATA

The seven composite profiles (one for each behavioural group) constructed from the IDI and FGD data are presented in full in additional files 3-9. A summary of the seven profiles, highlighting common and distinct activities, malaria transmission risks and preventive actions by site is presented below. The following section initially describes general behaviours and livelihoods identified throughout all behavioural groups and time periods. Activities carried out by specific groups across five relevant time periods (pre-dinner, dinner, post-dinner, morning and weekends) by site follow the initial summary.

Outdoor activities between dusk and dawn, absence of outdoor prevention for mosquito biting, lack of protective clothing (e.g. long sleeves and long pants) and open structures accessible to mosquitoes for gatherings and sleeping were identified as factors conducive to malaria transmission in both study sites. Clothing and footwear were similar in both sites, during day and night and among all behavioural groups. Commonly worn garments, such as shorts and short sleeve t-shirts, left arms, legs, and feet exposed to mosquito biting, the torso of men and children were commonly exposed as well. The most common footwear were thongs (flip-flops) and in many occasions people walk barefoot (especially children). While at the water, women and girls are usually wrapped in a laplap (sarong) or a towel while boys and men are wearing only a pair of shorts. Babies are usually naked. Open areas accessible to mosquitoes were very common for private and public gatherings (e.g. dining area and church). Figures 6.5. to 6.7. depict clothing and open spaces in both study sites. Using smoke to scatter mosquitoes was the only reported method to repel mosquitoes outdoors in both study sites. The use of topical repellents was absent and mosquito coils were rarely used. Night time activities occurring outdoors and gathering static groups of people include: cooking, eating, chatting, selling, watching TV, watching live sports (at the field), drinking alcohol, smoking, chewing betel nut, doing homework, playing and praying. Most of the areas where people gather in the village are open or semi-open and mosquitoes freely enter and exit such structures. Specific activities carried out by different groups in each study site and their relevant aspects for potential exposure to mosquito biting are described in detail in the section below.

Figure 6.5. Garments commonly worn in Mugil area by children and adults (left and right). Outdoor gathering at nigh and betel nut chewing (left). Veranda space were people commonly spend evenings (right).

Figure 6.6. Garments commonly worn in Lemakot area by children and adults (left and right). Outdoors-space cleared from vegetation for the family to gather using mats to sit on the floor (right).

Figure 6.7. A Outdoor cooking in Mugil (2016). B Outdoor spaces in Mugil (2016). C Night gathering, extraordinary celebration in Lemakot (2017). D Outdoor sitting and cooking spaces in Lemakot (2017).

MUGIL – ACTIVITIES, POTENTIAL MALARIA TRANSMISSION RISK & PREVENTIVE ACTIONS

Pre-dinner period (4 to 6pm)

At 4pm on a typical working day preschool aged children in the village play outdoors while waiting for the school children to come back from school and adults to come back from the market or the "blocks" (cash-crop plantation). The school children arrive at the village at 4 or 5 pm depending on how far they have to walk from school (usually between 30 min and 2 hours). They follow the main road and once at the village they follow the walking paths. Once at home, school girls look after their younger siblings and help with chores mostly outdoors close to the house. After school, boys help in the garden or the blocks at the outskirts of the village; they collect betel nut or bananas and carry them to the house. Men and women return to the village at 4 or 5pm. Women return from the food garden or the market, men from the block. They all go to the river and bathe. Most people bathe or wash twice a day; once early in the morning and once before dinner. They walk to a stream or the river bank 5 to 10 min away. The girls go with their mothers and younger siblings to bathe and fetch water. Women and girls combine bathing times with other water related chores like doing the laundry or washing the dishes. Such additional chores prolong up to 90 min the time women spend by the water. Once at the river, the boys swim and play before going back to the house. Men and boys spend more recreational time at the water, not only bathing but also swimming or fishing. Areas for males and females at the river banks or streams are separated. Women, girls and small children are back at the house

at 5pm or 6pm. Then, women cook with the help of their children. The kitchen is usually outdoors or partially-open (Figure 6.7.).

All activities between 4pm and 6pm occur outdoors and many of these activities occur by the water. The timing and place of these activities seems conducive for malaria transmission since *Anopheles* are known to bite between dusk and dawn. Such an open environment easily exposes the community to mosquito bites and especially to anophelines when the duration of the activities extends until dark. Bathing, doing the laundry, cleaning the dishes, fetching water and swimming are activities likely to convey a high risk of exposure. In addition, the walking paths commonly followed to and from the garden, school or water bodies are likely to represent a risk since those paths are transited daily and connect the scrubland to the village resulting in ideal place to encounter mosquitoes (Keven et al., 2019).

Dinner period (6 to 8pm)

Dinner gathers the whole family and it takes place between 6 and 8pm. The family sits outdoors, either on the veranda or at an open space next to the kitchen (Figures 6.5. and 6.7.). Dinner is usually followed by tea, chatting and betel nut chewing and is accompanied by a smoking fire. The smoke of a fire with coconut husks or leafs is the most common method people use to repel mosquitoes. Dinner conveys risk of exposure since it occurs outdoors and it gathers the family in a static activity in an unprotected space with arms, legs and feet exposed to mosquito bites for a considerable amount of time. In addition, the youngest of the children and babies usually fall asleep outdoors and stay exposed up to two hours before they are taken to their sleeping place.

Post dinner period (8pm –till bed time)

Different groups (men, women, and youth) gather on different days of the week for praying fellowship. Young men gather after dinner. Depending on the season they go spear fishing (Quinn, 2009) or hunting at night or they watch a match or movies on the available screen in the village. They also chat, listen to music on a mobile phone, chew betel nut and smoke at the market-stands by the road. When the battery of the phone runs out, they look for places where they can charge it and then wait (usually outdoors) while the phone charges.

Most people sleep indoors in shared rooms and mostly on the floor on a mat or a thin mattress. Small children go to sleep by 8pm, school boys and school girls by 9pm, women by 11pm and men are the last going to sleep by midnight or even later (Figure 6.4.). Women usually share the net with one young child. School girls usually share the net with one other girl and school boys with one other boy. When boys reach puberty they start sleeping alone, if nets are available they

sleep under one otherwise they do not. Generally, adult males also sleep alone under their own net.

Evening activities, like the praying fellowship, convey potential exposure risk since the activities gather people in open or semi-open spaces; in addition, people walk to and from the venue during *Anopheles* biting times without any protection. Boys and men seem to be at higher risk of exposure since they are awake longer than their female counterparts. Activities like chewing, smoking, and "watching TV" after dinner are a potential risk of exposure. Once asleep the main potential risk is sleeping without a LLIN.

Morning period (waking time to 8am)

During the week days the women are the first up (4 to 5am) to prepare breakfast. School boys and girls wake up at 6 am to go to school. School starts at 8am. This means that children need to start walking before 7am. Before going to school, some boys collect firewood or cut the grass around the house. Girls fetch water and wash the dishes from the previous night. Men and preschool aged children are the ones waking up the latest (7am and later). Potential risk during the morning period could be associated to waking up times, the earlier someone awakes the longer the exposure to biting anophelines. Women tend to rise earlier than men. For school children, the potential exposure could be associated to the distance to school; the greater the distance the earlier they rise, shower and walk, therefore the longer the exposure time.

Toilet facilities are scarce in the area with 46% of the households having no access to a toilet facility (Table 6.1.). People in the area mainly use the ocean, the bush or an outdoors latrine (when they have access to it),. Walking distance to the closest toilet area varies between 50 to 800m. The use of the toilet occurs across all time periods but mostly before washing in the morning and afternoon. Stagnant water is commonly found close to the toilets, potentially exposing people using the toilet to mosquito bites.

Weekend & seasonal variation

During the weekend, everybody in the house wakes up later (7 am) than during the weekdays. The family has breakfast at around 8am. The afternoon is similar to the week days. The family has dinner together between 6 and 8pm. In general, the family stays awake until later. Sometimes, the whole family joins a communal movie screening in the village. Screening areas are often open. Visiting or getting visitors is more common during the weekend. The extended family gathers and moves around within the village and the neighbouring villages. Saturday is the common day to go get supplies at the closest town (Madang). The first bus passes by at around 8 am; everyone going to town needs to wait by the main road shortly before 8am. On Sunday, people prepare for church service which usually starts at 9 am; therefore, people wake

up and bathe between 7 and 8 am. At 6 or 7pm, the praying fellowship starts. Most of the family members join the prayer. The prayer finishes at 9pm. The families then walk home and go to sleep at about 10pm. During the weekend, potential exposure during the morning period is likely to be reduced since people wake up later than during the week. Conversely, they also stay awake until later potentially increasing exposure at night.

When the family plans to grow a new garden or block, school boys help to clear out the area after school. They work a couple of hours in the afternoon and walk back home during or shortly after sunset. Households grow more than one kind of crop having multiple planting and harvesting seasons a year. During harvest season for coconut and cocoa beans, boys and young men take overnight turns to supervise the drier for a small fee. People are more active in the gardens and blocks during planting and harvesting season making it more likely that they work until dark increasing potential exposure to mosquito bites

Volleyball and soccer teams (young men and women), assemble and train for the local tournaments a few times a year. They usually train between 4 and 6pm at the school or the church grounds. Sports convey potential exposure, especially for boys, since they gather and chat close to the fields after practice, the timing coincides with anophelines biting times. In contrast, girls go directly home after training. School holiday could also pose potential risk especially for boys since they stay awake and outdoors for longer periods at night.

LEMAKOT –ACTIVITIES, POTENTIAL MALARIA TRANSMISSION RISK & PREVENTIVE ACTIONS

Pre-dinner period (4 to 6pm)

A typical afternoon in Lemakot is very similar to Mugil. In general, school children return home from school and adults return home from work. At home, people engage in chores and bathing. Girls and women perform household chores including laundry, dishes and cooking. Boys and men work at the garden, cut the grass or collect food, betel nut or firewood. Young men play rugby. People wash themselves twice a day in the morning and in the late afternoon or evening. People commonly wash at home, next to the water tank or water drum. The availability of water tanks, public taps and wells is higher in Lemakot than in Mugil area, therefore people walk shorter distances to a water source. During rainy season families with a water tank share with their neighbours, resulting in people fetching and storing water closer to home. During this period all activities occur outdoors and many of them close to the water. Activities like bathing, doing the laundry, doing the dishes and swimming are likely to convey potential exposure for malaria especially when they extend in duration until dark. However greater access to protected water sources might reduce contact with mosquito breeding sites and potentially reduce exposure to mosquito bites.

Dinner period (6 to 8pm)

Dinner period takes place between 6 and 8 pm like in Mugil and is accompanied by a smoking fire to repel mosquitoes. The families sit outdoors and eat. After dinner people have tea and chew betel nut or smoke. During dinner exposure is associated with the extended period of time families spend gathered and static outdoors.

Post dinner period (8pm –till bed time)

Recreational activities like watching movies or games on a screen, chatting and chewing betel nut or religious events (fellowship prayer) are common after dinner. However, the dynamics slightly change every two weeks with fortnightly wage payment. Over 20% of the households in the Lemakot area receive their main income from wage employment (Table 6.1.). Firstly, a larger number of road stands are set up and opened for longer hours. They commonly sell snacks, food, soft drinks and other recreational commodities like alcohol, betel nut and cigarettes. A great number of people especially men gather outdoors by the selling stands or the roads to drink, chew and chat during the night. Alcohol consumption is a common practice that could continue until the early hours of the morning. After dinner, the potential risk is mainly related to recreational activities. In Lemakot, a constant influx of cash in the community could potentially increase risk of exposure during the fortnight weekends starting on Friday night. Alcohol consumption is also likely to increase exposure since it extends exposure time.

People sleep mostly indoors in a shared room and on the floor using a mat or mattress. Most women are sleeping by 11pm and men by 12pm. The number of people sleeping under a mosquito net is significantly lower in Lemakot compared to Mugil area (Figure 6.4.). Hot nights seem to increase the risk of exposure since people are more likely to sleep outdoors and more reluctant to sleep under a LLIN. Locals reported perceiving the air under the net hotter and damper.

Morning period (waking time to 8am)

People in Lemakot generally wake up earlier than in Mugil with most of them awake by 6am. Women and men seem to rise at the same time in contrast to Mugil where men sleep longer. Plantation employees work shifts from 6am to 2pm. Therefore, transport circulates before 6am for the plantation workers. People walk towards the main road as early as 5am, meaning many of them wake at 4 or 5am and spend a considerable amount of time outdoors and unprotected while anophelines are still active.

Most of the toilet facilities available are outdoors or non-existent. 52% of the households in the area reported not having access to a toilet (Table 6.1.). People walk between 50 and 800 m to a latrine or a toilet area in the bush or at the beach posing a potential exposure to mosquito bites.

Weekend & seasonal variation

The weekend dynamics are similar to those in Mugil area; people tend to wake up and go to sleep later. Entire families work at the garden or the block during the day. Visits to town (Kavieng) are usual during the weekend. People wait for the bus at the main road earlier than 8am. Fishing is a time consuming activity. Mostly men go to the sea for hours returning in the afternoon and cleaning the fish at the beach during sunset. Night fishing (Quinn, 2009) is also a common practice in the area, especially in times when the fish are scarce during daytime. The harvesting of sago is more common in Lemakot than in Mugil. Men and women go to the sago swamp for a whole day potentially increasing exposure to bites when times in the swamp start before 8am or finish after dark. Recreational activities like family gatherings in the late afternoon and night are more common than over the week. Other common night activities include religious gatherings and movie screenings. Recreational and religious activities at night are likely to convey risk of exposure since people gather in open and/or semi-open spaces..

People in the area grow different kinds of crops having multiple planting and harvesting seasons throughout the year. Clearing of wild vegetation, planting and harvesting are likely to increase the risk of exposure since these activities prolong the time people spend in the planting areas and postpone walking times back to the village until the early evening. Once a month with the new moon, people living in the villages between the swamp and the beach (especially at the East Coast) collect the mud crab. The harvest takes place while the crab moves from the swamp to the beach (Quinn, 2009). Collecting the mud crabs is likely to increase exposure to mosquito biting during the new moon since it occurs outdoors, close to the mangroves and at dusk.

Once a year, a big festival known as Malangan happens for three days and three nights and the whole community gathers to celebrate. The preparations for the feast take months with people gathering regularly in the afternoons. Feast preparations include “mumu”, a cooking tradition that occurs overnight and requires hours of outdoor preparations (dig the hole, heat up the stones in a fire, prepare the meat and vegetables, burying the food for a long cooking time and unearthing it once cooked). Events like funerals gather people outdoors at night. The community (Lemakot and Mugil) usually meets at the house of the deceased and pay respects to the family. The mourning usually continues day and night until the burial. Depending on the circumstance, time to the burial could take up to a week. Extraordinary activities, such as the Malangan and funerals, are likely to involve higher risk of exposure to mosquito biting since they extend until night and take place outdoors.

6.5. DISCUSSION

This study highlights the substantial amount of time spent outdoors or in structures that offer little protection against mosquitoes when "indoors", during peak anopheline mosquito biting times in the study sites. Between 4pm and 8am, all age groups in both sites are likely to be exposed to mosquito bites across all types of activities; an exception being sleeping under a LLIN. LLINs are the only reliable prevention method available in both study sites. In the Mugil area 89% of the people reported sleeping under a LLIN, in comparison, only 37% of the people in Lemakot reported sleeping under a LLIN. The study findings underline the potential of exposure to outdoor biting constantly occurring in both study sites while people are not sleeping under a LLIN.

Activities like bathing, washing laundry and dishes, swimming, fishing, hunting, harvesting sago and collecting mud crabs pose a potential major risk of malaria transmission since these activities occur outdoors and close to or by the water during anophelines biting times. Except for harvesting sago, hunting and fishing, which occur in the swamp, the bush and the ocean respectively, these activities occur within the village. Transmission outside the village in the gardens or blocks seems less likely since most people consistently return to the village before dark. Hence, transmission is more likely to happen on the way to or from the plantations and gardens. In addition, people in the study sites do not sleep at the gardens. In contrast to some practices in Asia and Africa (Gryseels et al., 2015; Hetzel et al., 2008), people reporting a second house to sleep at the garden or plantation were the exception. Gatherings outdoors at night are likely to be conducive for malaria transmission as well. Most reported outdoor activities have been previously identified as potential risk for malaria transmission in other settings (Dlamini et al., 2017; Dunn et al., 2011; Monroe et al., 2014; Moshi et al., 2017).

Potential seasonal exposure was identified and linked to farming, and sociocultural practices. However, planting and harvesting times occur a few times a year and are determined by the kind of crops grown by each household. Extraordinary events entailing substantial night time activity that might increase exposure to mosquito bites include funerals, religious activities (e.g. retreats, annual festivities) and festivals (e.g. Malangan). Similar socio-cultural outdoor activities have been previously describe elsewhere as potential risk for transmission (Dunn et al., 2011; Makungu et al., 2017; Monroe et al., 2014).

The study found little evidence of reliable forms of outdoor mosquito biting prevention between 4pm and 8am. When outdoors, the only preventive measure consistently reported across quantitative and qualitative data was producing smoke to repel mosquitoes. Keeping the house and its surroundings clear of vegetation, water and rubbish were considered good measures to

prevent malaria in both study sites, according to the quantitative data. When indoors, sleeping under an LLIN was the only method consistently reported to prevent mosquito bites and malaria. Behavioural and livelihood elements like: little skin coverage by clothing, nil use of mosquito repellent, minimal use of mosquito coils and open housing structures could exacerbate and maintain malaria transmission despite the use of LLINs. The potential of LLINs to significantly reduce malaria-related morbidity is well known (Lengeler, 1998; Pryce et al., 2018). However, inconsistent or low use (e.g. Lemakot) limits their effectiveness, and varying levels of ownership and use (e.g. Lemakot area vs. Mugil area) are likely to vary the effectiveness of LLINs in different sites (Rodríguez-Rodríguez et al., 2019c). Whereas there is room for improvement of LLIN-use in both sites, the gap in Lemakot seems bigger since people are less committed and less accustomed to use LLINs. In the Lemakot area, less than 30% of school children (boys and girls) and adults (men and women) reported sleeping under a LLIN every night. In comparison over 60% of all age groups reported sleeping under a LLIN every night in the Mugil area. A common reason for not using LLINs in the Lemakot area was the notion that it is too hot to sleep under them. Despite similar temperature range in both sites, this notion is rarely described within the Mugil area. A more consistent use of the LLINs seems to diminish the "too hot" perception suggesting that LLIN users need to adapt their perception before consistently using the LLIN. The ownership and use of LLINs found in this study coincide with previous regional findings of net use in PNG. Higher ownership and use have been consistently reported in Madang Province (Mugil area) compared to New Ireland Province (Lemakot area) (Hetzel et al., 2014a; Pulford et al., 2012, 2011). The study also highlights the limits on the protection offered by LLINs, especially for adults. For instance, despite a high LLIN-use in the Mugil area (90% for women and 81% for men) between 6pm and 6am 69% of the men and 72% of the women were under the protection of the LLIN only 50% or less of the time within this period. This finding highlights the need for complementary interventions over more intensive LLIN distributions.

Interestingly, recent prevalence data from both study sites identified a 3.7-fold higher malaria prevalence in the Mugil area compared to the Lemakot area (Rodríguez-Rodríguez et al., 2019d). Such prevalence suggests that in places with a high LLIN-use like the Mugil area transmission beyond the protection of the LLINs still occurs. The considerable amount of time spent outdoors translates to a great risk of exposure to mosquito bites. Since LLINs prevent indoor biting, complementary methods to LLINs are needed to prevent outdoor biting in the evenings and the morning. Some potential options include vector control measures like larval source management, topical and spatial repellents and attractive toxic sugar baits (Williams et al., 2018; World Health Organization, 2017a). However, an essential feature of understanding

human behaviours is the potential to target places, groups and activities. In the study sites, places where people regularly gather at night, like churches or movie screening areas, could be targeted for group prevention, either treating surfaces with insecticide or using spatial repellents. In contrast, when people roam widely at night or early in the morning, like men and plantation workers do, then personal protection may be more important. Personal repellent or more protective clothing might be useful in this case. Specific groups (e.g. boys, men and plantation workers) could be targeted with behavioural change communication to encourage more protective clothing choices at night and early in the morning. However, given the current lack of outdoor prevention and concern of mosquito biting, ensuring effective treatment is readily available at the health facilities is a crucial control measure.

Age and sex were important factors determining behavioural patterns. The main event drastically changing a child's behaviour is starting school. Once in school, behavioural differences between boys and girl become evident. Girls chores are more house oriented (e.g. fetching water, cooking, doing the laundry or washing the dishes) while boys have less house-oriented chores (e.g. chopping wood, cutting the grass) and more freedom to spend their time with peers and away from the house. Once out of school the range of activities carried out by men and women are affected by their environment. In this case the kind of cash crop and availability of wage employment of each site considerably change livelihoods in the communities. In Lemakot, the regular influx of cash prolongs exposure to anophelines bites for informal vendors and customers during fortnight weekend. The study identified seven groups based on the mentioned patterns of activities: preschool aged children, school boys, school girls, male adults in Mugil, female adults in Mugil, male adults in Lemakot and female adults in Lemakot (Figure 6.4., Table 6.4. and Additional files 3-9). Previous studies have associated some activities to specific groups, household chores like fetching water and laundry have been associated with women while drinking and socializing to men and sports to adolescent boys (Dlamini et al., 2017; Dunn et al., 2011).

Adult men were the group with the smallest proportions using LLINs in both sites. In addition, women in both sites were more likely to use the LLINs than men. This could be due to the fact that women are more likely to share the LLIN than men. Adult women in Mugil were sleeping by twos sharing the net with one child (mainly a daughter) while women in Lemakot were sleeping mainly by threes sharing with two children.

As it is, subnational variations in prevalence might not be attributable to behavioural differences since both communities presented behaviours that increased risk of exposure and livelihoods that are likely to be conducive for malaria in both settings. However, it is difficult to

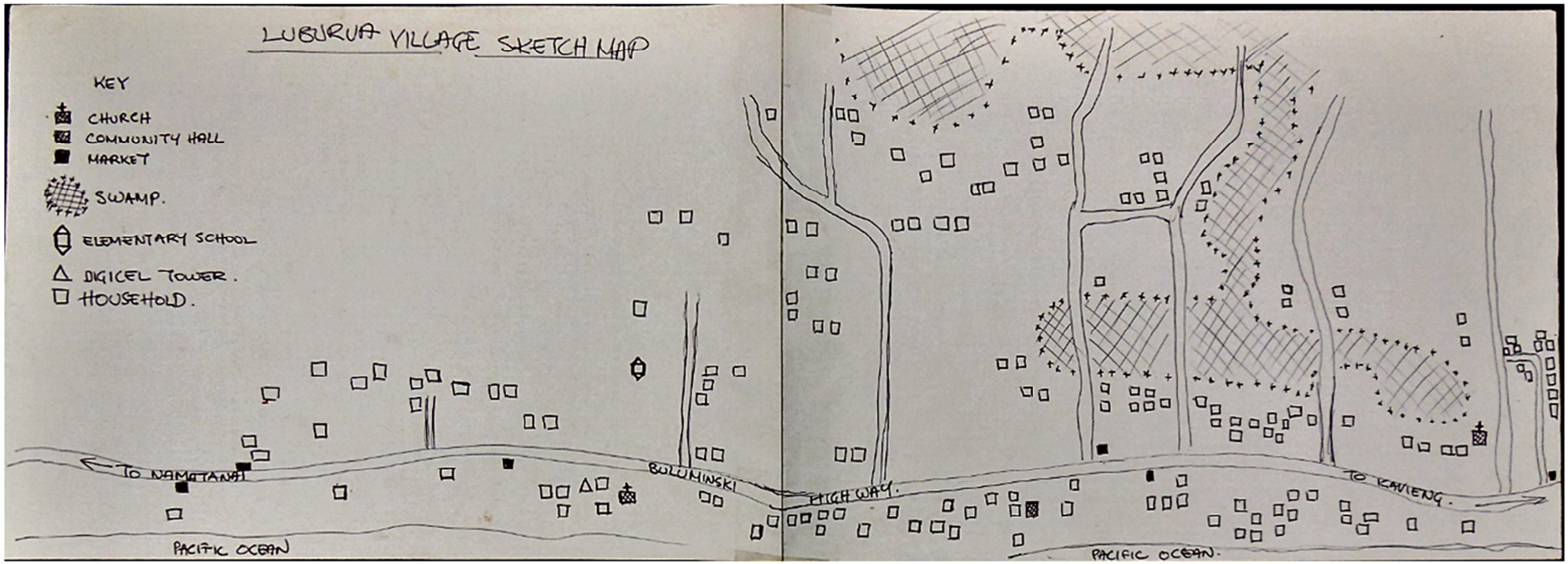
quantify differences in behavioural risk of exposure between communities and their significance to transmission using solely behavioural data. Complementing our findings with entomological and prevalence data from the region is necessary to quantify behavioural risk of exposure and understand local transmission. Therefore, a second step to this study is to triangulate our main findings with entomological and malaria infection prevalence data collected simultaneously in both study sites.

Our study was not free of limitations. Recall bias may have affected the data collected, especially with IDIs. It is possible participants could not recall all relevant activities carried out the previous week, in the exact time, order and place they occurred. An alternative is to conduct two separate interviews asking to recall activities for the previous three or four days rather than seven. When discussing annual events and seasonality, some participants struggled recalling annual activities and the exact month when they occur. FGDs were conducted with men or women of different ages, since the topic was not considered to be very sensitive. However it is possible that during the discussions young participants were intimidated by the senior members of the group limiting younger people inputs in the discussion. In order to prevent this, the interviewer actively integrated every participant to the discussions. The qualitative analysis was framed around activities and did not allow for a deeper thematic analysis that could raise other unforeseen relevant issues (e.g. gender or cultural dynamics). Finally, the study did not include any prevalence of malaria in the analysis; therefore it assesses potential exposure to mosquito bites rather than risk of malaria infection. An in-depth analysis of the prevalence distribution in the study sites is detailed elsewhere (Rodríguez-Rodríguez et al., 2019d).

Our findings highlight the potential of 'outdoor biting' to hamper malaria control and elimination efforts if not addressed appropriately since people spend a remarkable amount of time outdoors without protection from mosquito biting. Complementary interventions to LLINs targeting groups, places and activities in order to prevent outdoor biting in the evening and the morning based on local knowledge of community behaviours seem crucial in order to advance towards elimination.

6.6. ADDITIONAL FILES

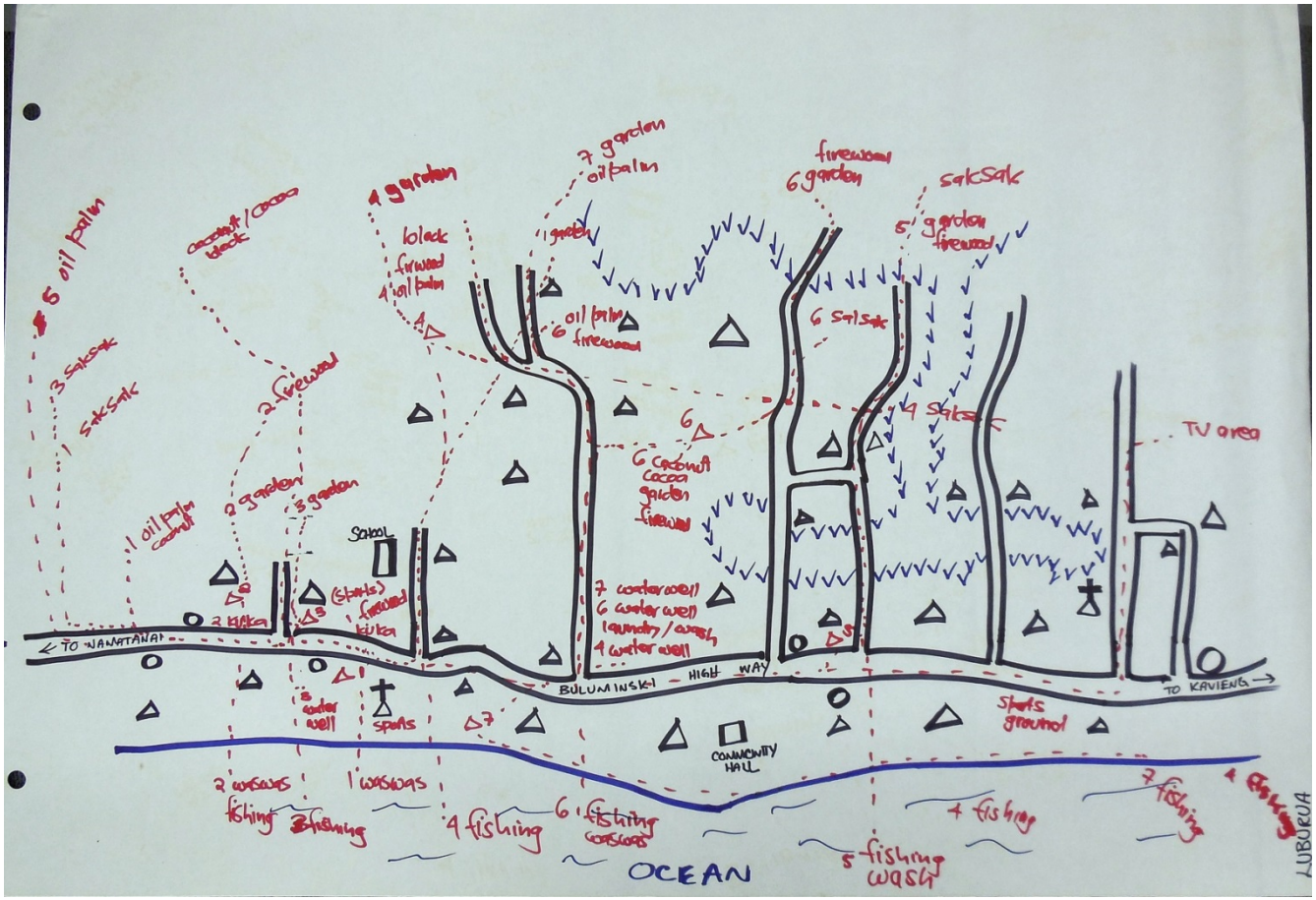
ADDITIONAL FILE 1



Sketch Example

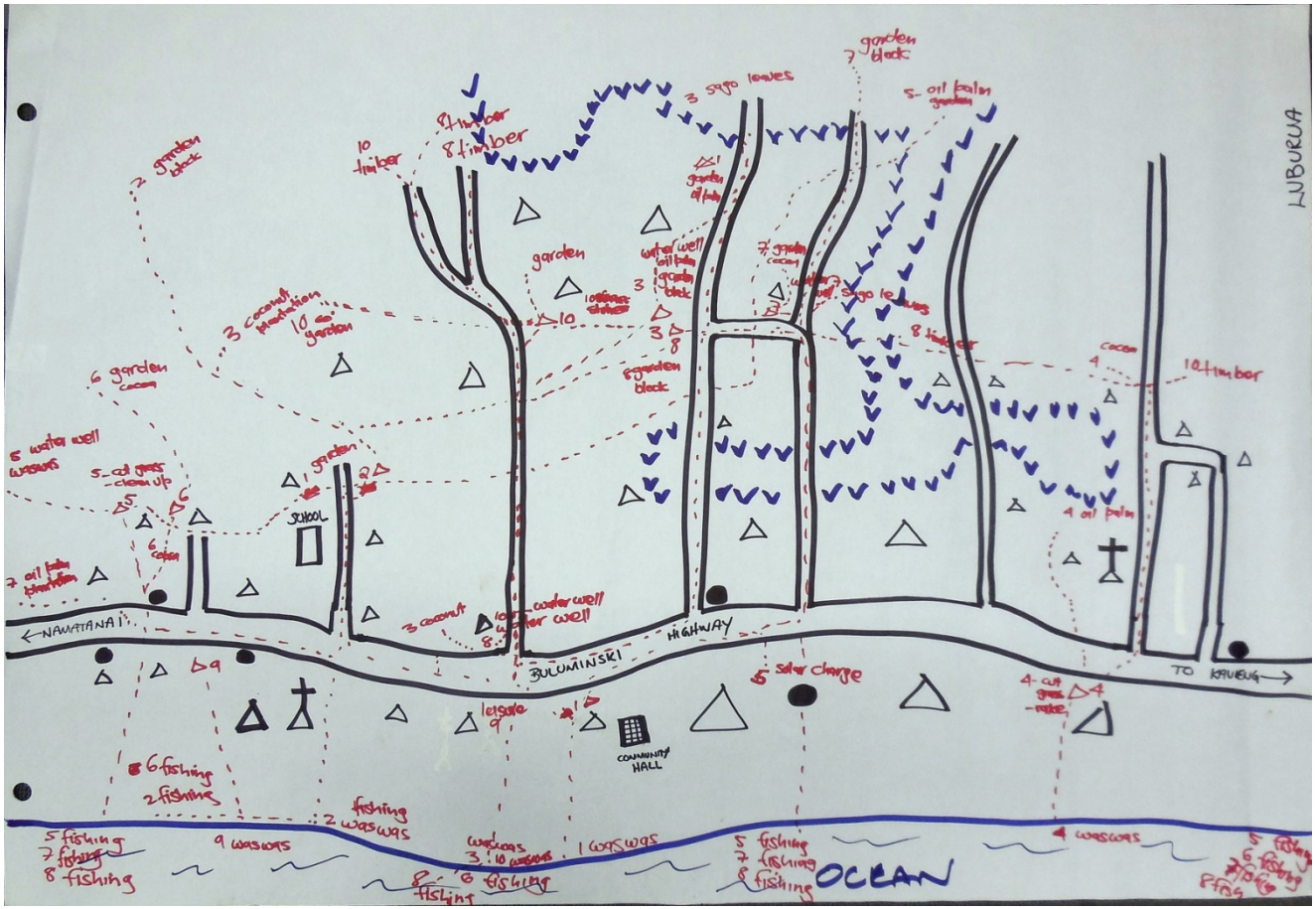
Sketch of Luburua (Lemakot area) as provided by the village leaders.

ADDITIONAL FILE 2



Example 1 of map used during the FGDs

Map of Luburua used to accompany the FGD with women. In black the village characteristics, in blue the swamp and ocean delineation and in red the location of the reported activities.



Example 2 of map used during the FGDs

Map of Luburua used to accompany the FGD with men. In black the village characteristics, in blue the swamp and ocean delineation and in red the location of the reported activities.

ADDITIONAL FILE 3: CHILD- SERAH, 4 YEARS OLD

During the week Serah is the last waking up in the house. Her parents and older siblings wake up earlier to get ready for school but as a young child Serah sleeps until 7am. She usually shares the sleeping space and mosquito net with her mother and her baby brother. During the hot nights she sleeps wearing only underpants. She wakes up calls for her mother or older sister and they will take her to use the outdoor latrine. They usually walk 200m to get there, and it is a quick visit that usually takes 10 minutes. When they are back at the house her sister dresses Serah and her little brother. Serah wears a skirt or a pair of short. She only wears a shirt when it is rainy and cold. Her little brother is usually only wearing a cloth nappy. Both of them are always barefoot. Once dressed Serah and her brother sit outside close to the kitchen and play until breakfast is ready at about 8am. She eats breakfast with her mother and her younger brother who like her is not in school age yet. They spend most of the day together following their mother around, playing and learning the house chores. Serah as a girl imitates her older sisters and wants to help cooking, sweeping and fetching water while her baby brother collects pieces of wood and helps raking around the house. After breakfast they play around the house until their mother finishes cleaning up after breakfast. Then they follow her to the river or the beach where they bathe and accompany their mother while she does laundry and dishes. This usually takes one or two hours. After the river they return to the house if Serah feels tired she takes a nap in the kitchen on a mat her mother unrolls for her and her baby brother. During the day they do not sleep under a net. Sometimes they sleep in the room; in any case the longest they nap is about an hour. After her nap she eats a little something and plays around with the kids next door. Sometimes when her mother needs to do some gardening Serah accompanies her to the garden. If she is tired and her older siblings are around she stays with them or her grandmother at the house. During the late afternoon she awaits for her siblings in school they come back at 4pm and spend time with her. They take Serah and their baby brother to the river and bathe them before dinner. They leave at around 5pm and come back at 6 or 6:30pm. Her family usually eats dinner at 6 or 7pm during the week. They eat together and they all sit outside. After the food they drink tea and chat, around 8pm Serah gets sleepy. Sometimes she falls asleep, for half an hour, on the veranda. Her big sister would take her in the room when she goes inside. Other times, when her mother is also tired she would take the two youngest children into the house and put them to sleep. Her baby brother and she usually share the bed and mosquito net with their mom.

The weekends are different since her older siblings do not go to school. They all wake up later and spend more time together. They go fishing or swimming to the beach or the river. They go at around 10am and come back in the afternoon. Sometimes the whole family goes to the garden

and harvest food for the week. They go early so they can work before the sun reaches the hottest point. At noon they rest and continue working in the afternoon when it cools down. They usually have a long dinner on Saturday. Serah stays up longer and her arms and legs are exposed to mosquito bites since her clothing does not cover them. Her family cannot afford mosquito repellent to apply on the skin but they usually make a fire with the intention of dispersing the mosquitoes around the house. On Sunday they all go to church service together. It usually starts at 9 am and lasts till noon. They have to bathe at 7am to be on time. After church they all go home have lunch and rest. At 6 or 7pm the praying fellowship starts. On Sunday most of the family including Serah join the prayer they walk to the church area and back. It takes about 20 minutes since they walk slowly and chat. They wave away the mosquitoes with their hands if they feel them on the way to church. The church is a semi-open with screened windows but during the prayer the door remains open. They usually finish at 9pm. The family walks home and go to sleep shortly before 10pm.

Additional file 4: Male School Child – Tom, 15 years old

Tom wakes up at 6am. He is the oldest of his siblings therefore he stopped sharing a bed net and mattress with his younger siblings. He usually sleeps on a mat in a shared bedroom. He does not use a net. He wakes up at 6am. Since his family does not have a toilet facility, he goes to the close by bush first thing in the morning. He leaves the house wearing only the shorts he slept in, his arms and legs quite exposed to mosquito bites. Sometimes he collects fire wood on his way back; it takes him some extra 10 minutes to collect the wood. Once a week he cuts the grass around the house it takes him about 15 minutes. Afterwards he goes to the river bank and bathes. He walks 10 minutes to the water and bathes in less than 10 min. His body is shortly submerged under the water. He is mostly standing with the water up to his waist. He comes back home shortly before 7am and wears his school uniform (a pair of shorts and a button short sleeve shirt). He prepares his lunch and eats breakfast before leaving the house. At about 7am he starts walking to school it takes him 45 to 60 min to get there. School day starts at 8 am and ends at 3 pm. The school year runs from January to December and it has four terms. Tom has school break in April, June, September and December). Tom does not travel, not even during holidays therefore he spends them in his village just like during the weekends. Tom and his classmates hang around at school after class. They chat and make plans for the weekend. Then he walks back home. He is back at 4:30. At home he changes his uniform to his working shorts and sometimes a t-shirt. Depending on the day of the week he might need to go to the family garden and collect betel nut to sell or bananas for dinner and breakfast. During dry season and if the family plans to grow a new garden or block Tom joins his brothers and clear out the area. They work a couple of hours in the afternoon and walk back home during or shortly after

sunset. The way to the garden follows parts of the main road. The main road is unpaved and the tire tracks they pass are good *Anopheles* breeding sites. At home he grabs his towel and goes to river to bathe and swim. Often he meets his friends at the river and they play together in and out of the water, it is usually dark by the time he goes home. Once at home he eats dinner with his family at around 7pm. They all sit outside and usually make a fire. They add the coconut husks to the fire to produce smoke and repel the mosquitoes. After dinner Tom takes some time to do his homework on the veranda under the only light available in the house. When he does not have school work he stays around the fire talking to the rest of the family and chewing betel nut. He goes to bed at 10 or 11 pm. Some nights if there is an important Rugby match on TV; Tom meets with his friends to watch. The viewing area is covered from the rain but it has no walls. On Friday; they watch movies until roughly midnight. If there is nothing to watch the boys chat and listen to music at the selling-stand by the road. Some of his friends have phones and they gather together to listen music and find a place where they can charge the phone. Whenever a generator is on in the village they ask if they can use the plugs. They sit around the fire and wait until the battery is charged. Tom walks back home and sleep at 1 am or so. He does not own a mosquito-net since his younger siblings use the available ones and he feels sleeping under the net is too hot in any case.

During the weekend Tom wakes up at 9 or 10 in the morning. He eats breakfast and right after he meets with his friends at the beach. Sometimes they play rugby or volleyball games. Other times if any of the boys needs help with a big task they work together and get bush material for building a house. When they need sago palm leaves for the roofing they go to the swamp to get them for two or three hours after breakfast. When he wakes up before 9am he goes to the garden with his family and helps her mother carry the food back to the village. In the afternoon, he would bathe at the river and have dinner at home. He then meets his friends. He knows the village very well and he has a lot of freedom to move around and meet with the other boys. They watch a movie if any is screening in the village if not the boys gather at the road and buy betel nut. They spend the night talking, chewing betel nut and listening to music. He gets back home passed midnight and goes to sleep. On Sundays if he wakes up early he goes to church service at 9 am with his family. After service, the family eats together at 4 or 5pm. After the meal they have tea and sit around the fire. If Tom is tired he goes to sleep at about 10 pm otherwise he stays up and chats with the others or he finishes his school work before going to sleep at 11pm or midnight. His family never uses mosquito repellent since its use is unknown to them, it is not available in the local store and in town it is too expensive for his family to purchase it regularly.

ADDITIONAL FILE 5: FEMALE SCHOOL CHILD – KATI, 10 YEARS OLD

Kati sleeps with her older sister; they share a net and a thin sponge mattress on the floor. She sleeps on a t-shirt and a pair of shorts. Day and night her clothes exposed her arms and legs, she never wears socks and her shoes are a pair of flip-flops. She wakes up at 6 am she uses the outdoor latrine first thing in the morning. The latrine is about 100 meters away from the house it takes her less than 10 minutes to go there and back. Immediately after she goes to the river and bathes. A little stream runs downhill from her house, she usually goes there when she has pots to wash from the previous night. When there is water left in the drum she bathes by the drum and gets dress in her school uniform at the house. She wears a mid-leg skirt and a short sleeve shirt to school. Once ready she helps her mother prepare breakfast. Shortly before seven in the morning she starts walking to school. She walks together with her siblings it usually takes an hour to get there. They follow the main road but when they are lucky they get a ride from a passing car that they know and they get early to school. School day starts at 8 am and continues until 3 pm. When school day is over; she walks back home with some of her friends. She is back home at 4 pm but some of her friends; the ones living further from school; walk an extra hour to get home. Once at home she changes from her school uniform (to either a skirt or shorts and a t-shirt) and helps with the house chores. First she goes to fetch water mostly at the river but one neighbour has a big tank that is closer than the river. During rainy season they can get water from the tank more often than when rain is scarce. In any case the river is a 10 min walk away. When she is back her mother starts cooking. Kati helps to peel the vegetables or scrape the coconut. They seat on two little stools in the outdoors kitchen. When she is done with the cooking she grabs her towel and goes to the river. She washes her own laundry twice a week. She sits on a rock by the river bank with her feet in the water when she does her laundry. When she finish she bathes her little siblings and herself. They come back home after 30 to 40 min at the river. They walk back home between 6 and 7 pm. Then the whole family has dinner together. They sit outside next to the fire. They have an outdoor space were they are protected from the rain but there are no walls. Those days with a lot of mosquitoes her family throws coconut husks or tree leaves into the fire to repel the mosquitoes with the smoke. After dinner depending on the day of the week she accompanies her mother to the evening prayer at the church grounds. They usually go at 8 pm. They walk 20 min to the church. They have a small flashlight to light the way there and back. If she has school work she stays home and finishes her homework on the veranda. In very rare occasions a mosquito coil is available to repel the mosquitoes. Insecticide commonly referred to as “Mortein” is available on the local store. Once or twice a year, when too many mosquitoes disturb their sleep her father sprays the rooms to kill the mosquitoes in the sleeping areas. Unlike her brothers she spent most of her free time at

home helping her mother with house chores and babysitting her youngest siblings. She usually goes to sleep before 10pm.

On the weekends she wakes up at 7 or 7:30 in the morning. She uses the latrine, eats her breakfast and goes to the river. She bathes and does the laundry with her mother and sister. The three of them spend over an hour at the river bank. They come back home hang up the laundry and she helps her mother with the house chores. Sometimes if there is need for planting or harvesting they go to the garden where they grow the family food or to the block where they grow the cash crop. The block is further away. They usually walk for an hour or an hour and a half. The leave the house at about 10am. Once in the garden they work for three or four hours with a break in between. When they finish they walk back to the village. Some weekends they go fishing for most part of the day. They usually get back at 4 or 5pm. Back at home they rest for an hour before preparing dinner. Kati helps with the cooking as usual and at 6 pm or 7pm they have dinner. Some nights she visits her aunts and stays for an hour or so. At their place sit outside chat and chew betel nut. Her aunts live close by just a few minutes walk down the road. Other times she brings food to her grandfather, he lives alone so her family looks after him. In rare occasions her whole family joins a movie screening at the neighbours. She only stays until 9 pm. At this time her mother takes Kati and her little siblings back home. Her dad and her big brother stay until late. On Sunday she wakes up at 7am she goes to the river, bathes and washes the dishes then she prepare for Sunday school and church service. Sunday school starts at 8 am, one hour before the mass. After the mass the family goes back home and she helps prepare dinner. As usual all the cooking and eating takes place outdoors. They all eat together at 4 pm. They drink tea and they chat, they chew betel nut and rest. Kati prepare her school work for next week before going to sleep.

Additional file 6: Adult male (Madang) – John, 23 years old

John is not married and he lives with his two older brothers. Their house is close to their parent's house, they live independently but they share the latrine. John and his brothers share a sleeping room and each of them has a mosquito net. Most nights they sleep under the nets and they sleep wearing a pair of shorts and no shirt. John wakes up at 7am and uses the outdoor latrine 100 m away from the house. Right after, he collects some wood in the garden behind the house and starts a fire in the outdoors kitchen. At about 8am he cooks some bananas directly on the fire and makes tea. Afterwards, the three brothers go to the river and bathe then they dress in shorts and a t-shirt. Their legs and arms are exposed to mosquito bites day and night. They never wear socks and their shoes are a pair of flip-flops. They walk to the coconut and cocoa block were they work. It takes them 30 to 40 minutes to get there. They follow a narrow walking path and they have to cross a pair of small streams on the way. Depending on the amount of

work that needs to be done and the season the time they spend at the block varies. They either clear the block, plant new trees or harvest. On harvest season they collect coconuts and dry them under the sun. Once they are dried they compact them in sacks and arrange transport to pick them up. There is no big plantation or company in the area, therefore they have to arrange the sell within the community or go to Madang town and sell there. They also harvest and sell the betel nut. Buyers from the highlands regularly come with big trucks to buy sacks of betel nut and transport them to the highlands. When they harvest the cocoa beans they sell them green within the village since they do not have a drier to dry and store the beans. These are the three main cash crops they grow in the area. Close to the block they have a garden where they grow their own food. After working in the blocks or the garden for two or three hours they go to the beach close by at noon. They take shelter from the strong sun for a couple of hours. They swim and take a nap or rest at the beach before going back to work or fishing. In the afternoon if they don't have betel nut to chew they collect it from the block before returning to the village. During a couple of months of the year John trains for the volleyball or soccer tournament. Every afternoon from 4 to 6 pm he goes to the field and train with his team mates. The training area is outdoors in the church grounds 150m away from a stream downhill. After training he goes back to the house grabs his towel and goes bathe at the river. He stays about 30 min at the river bank with his feet in the water until he gets hungry and goes home. John cooks something if there is nothing ready at the house. Otherwise he eats dinner and then leaves to meet his friends. During the right season he and his brothers dive in river at night and spear-fish (Quinn, 2009). They spend most of the time submerge or with their bodies under the water but on the way to the river and back their torso, arms and legs are exposed to mosquito bites. Most nights when John is not diving and someone is playing something on a screen he watches. Screens to watch are usually in open areas like verandas. When there is nothing to watch he gathers with his friends at the road to chew betel nut, chat and listen to music. He walks back to the house after 11 pm and goes to sleep. John knows malaria is transmitted by a specific kind of mosquito. That is one of the reasons he sleeps under the mosquito-net. However when he is outdoors he is not very concern of getting bitten by mosquitoes but he is annoyed by them.

During the weekends he wakes up at 9 or 10 am he uses the outdoor latrine, lights a fire and drinks tea sitting outside. Sometimes when he has business in town he walks to the road gets in a bus and goes to town (Madang). He spends a few hours there buying supplies. He gets back to the village in the afternoon using the same bus. In the afternoon he rests a bit and naps at the beach. Other times he goes to the garden to plant and harvest. He also checks on the block and collects betel nut. Some Saturdays he goes fishing to the sea with one of his brothers, they go for some hours in the afternoon; they get back at around 4pm and clean the fish at the beach before

walking back to the house at around 5pm. At night he walks around the village with his peers. Sometimes they watch a rugby match on TV, sometimes they chew betel nut, smoke brus (dry tobacco leaves) and - if they have money –they buy beer. They gather outside for hours sometime around a fire sometimes just under the light of a selling stand at the road. They stay up till passed midnight. On Sunday morning he gets ready for church. He wakes up, uses the outdoor latrine, bathes at the river and has breakfast before 9am. Then he joins the church service at 9 am. In the afternoon he joins his family, they usually have a gathering at his parent's house where they all eat, have tea and chat for hours sitting outside around the fire. Like most families in his village the only measure they take to repel mosquitoes is the smoke from the fire. When John feels tired he walks to his house. If he is hungry he has another cup of tea and biscuits or chews betel nut again while sitting on the veranda. He listens to music in his phone before going to sleep. If his phone runs out of battery he walks around and finds a place to charge it; sometimes at a house with a generator or a solar panel power source. He walks back home after his phone is charged and sleeps passed midnight.

ADDITIONAL FILE 7: ADULT FEMALE (MADANG) – JENNY, 48 YEARS OLD

Jenny is a mother of five. She sleeps with her youngest daughter and they both share a LLIN. She wakes up between 5 and 6 am every day. Her family does not have a toilet therefore she walks 800m to the beach area commonly used as a "toilet" by women in the village. Twenty minutes later she is back in the house. She cooks breakfast for the school children in the semi-open kitchen. Once the older children leave to school she sweeps around the house and cleans up the kitchen area. She collects all dirty dishes and pots and goes to the river with the youngest children. She walks to the river at 9 or 10 in the morning and she spends one hour there with the children. They stay by the edge of the river sitting on a big rock. She washes the dishes and pots first. Then she bathes herself and the children. Some days of the week instead of dishes she takes the laundry to the river. In any case she walks back home and gets dressed before noon. She usually wears a skirt or a laplap (wrap) with a shirt or a meri-blouse (long loose blouse worn by women in PNG) but when she goes to the garden she wears garden-duty clothes (shorts and T-shirt). Her feet are always exposed since she do not wear socks and her shoes are a pair of flip-flops . Once dressed, she prepares to go to the garden or the market depending on the day of the week. When no one is around to babysit the little children she takes them with her otherwise they stay home with their older siblings or their grandmother. Early in the week she would go to the market with the food the family harvest during the weekend. To go to the market she follows a small road that eventually connects to the main road it takes her 45 to 60 min to walk there. If a car passes by and she knows the owner they give her a ride on the open back of the car and drop her at the main road. Once at the main road she walks further or gets

on the bus depending on which market she goes to: the village market is close by but Kubugam market the big market in the area is further away so she takes the bus. Later in the week she goes to the garden and harvests food for dinner and breakfast. She walks 30 min to the garden and crosses a few small creeks on her way there specially during rainy season. Some areas get easily flooded. The family diet changes according to the season and the availability of food at the food-garden. Jenny also helps maintain the blocks of cocoa and coconuts twice a week. The blocks belong to her family and are far from the village. To get there she walks 90 minutes to 2 hours, she follows a narrow walking path between blocks to get there. In the afternoon she returns back home between 3 and 5 pm. When she gets home she rest a little and waits for the school children to get back from school, they are usually back at 4:30pm. They all go together to the river and fetch water and bathe; the trip tales up to 45 min. When they are back Jenny cooks dinner. When dinner is ready at about 6pm Jenny calls out the family and she serves the food. They all eat together, drink tea and chew betel nut until dark. They sit outdoors and eat in an open area next to the fire. They use the fire smoke to try to repel mosquitoes. Twice a week she goes to the prayer fellowship at 8 pm if she is not too tired. She walks to the church area followed by some of her children. They walk 20 minutes to get there. If they feel mosquitoes around they use a small towel and wave it around their legs and arms to try to prevent mosquito bites. The church is a semi-open space with mosquitoes freely flying in and out. When the prayer is over, the family walks back. The latest Jenny goes to sleep during the week is 11 pm.

During the weekend she wakes up a bit later since the children do not have school. On Saturday morning she wakes up, prepares breakfast and then gets ready to go to the garden with the children, they all go together to carry the food back from the garden. Some Saturdays they go fishing they go to the beach with line and hook and fish there. Jenny stands on a submerge rock close to a reef and fishes for hours with the water up to her waist. If Jenny still has food or betel nut to sell from the week she goes to the market. She gets back in the afternoon at 4 or 5pm and rests before cooking dinner. On Saturday her eldest daughter visits and brings her grandchildren. She looks after them while cooking and when dinner is ready they all eat together nest to the fire. Whenever she has someone visiting they stay up till late, chatting, chewing betel nut and drinking tea. She goes to sleep passed midnight until all visitors leave. On Sunday she wakes up at 7am. She send all the children to the river early and only after they are all back and having breakfast she goes herself and bathes. The family walks to the church area for service at 9 am. They stay in church until midday when they walk back home. She starts cooking right away since her extended family usually gathers on Sunday. Sometimes they gather at her house some other times they gather at another house but they all contribute with the

meal, either they cook together or they bring a prepared dish. When the meal is ready they sit outside, eat and talk for hours. When it gets dark the family disperses and walk back to their home. Jenny makes sure the school children get ready for the week and she has tea before going to sleep at 10 pm. Jenny is afraid of malaria, especially of her children getting sick. When they get a fever she treats them at home but if they do not improve she know she need to go to the health facility and that expense could be catastrophic for the family.

Additional file 8: Adult male (New Ireland Province) – Andrew, 51 years old

Andrew shares the sleeping area with his wife and the children. But unlike them he does not have a LLIN. He sleeps wearing a pair of shorts and no shirt. During the week he wakes up at 6 or 7 am. First thing in the morning he goes to the beach, to the area men usually use as "toilet". It takes him 20 minutes to walk there and back. He grabs his towel and go bathe next to the shared well behind his house. He usually wears shorts and a T-shirt and his arms, legs and feet are exposed to mosquito bites. If needed, he cuts the grass around the house or chops wood for the fire; it takes him 30 minutes to complete each task. Once finished, he has breakfast and goes to the work in the block where he is growing coconuts and oil palms. The oil palm block is far from the house he walks 60 minutes to get there. He follows the main road, most of the blocks around the area are surrounded by an ample road that allows the trucks to drive around and collect the oil palm harvest. The road is full of tire tracks and puddles that are very suitable *anopheles* breeding sites. He and his family harvest the oil palm block every two weeks during harvest season. The rest of the year he clears the block area, works in his food garden or collects and dries the coconuts. When he has enough dry coconuts to sell he goes to the nearby big plantation where once a week a local buyer weights the sacks and buys them from the farmers in the area. He spends most of his day in the blocks but he takes a break at noon when the heat is the strongest. In the afternoon he walks back to the village and goes to the river. After his bath he has dinner with the family, they all sit outside and dine at 6 or 7pm. When his wife is not around he starts the fire and cooks some bananas. Once a week there is a prayer fellowship for men, he joins one or two times a month, the meeting starts at 8pm and finishes at 9pm. He walks 20 min to the church area. Other nights he goes for a walk at 9pm before going to sleep. He smokes and chews betel nut with the neighbour or other peers that he meets during his walk. If there is a rugby game on TV he goes watch the match. A venue in the village shows all the big matches. The venue is semi open and mosquitoes fly around. Andrew is the last person going to sleep in the house. He goes to bed at 11 pm or midnight.

During the weekends he wakes up at 8 or 9 am. When the wind and the sea are calm he goes fishing. He spends three or four hours at sea. He mainly fishes using a line and hook but other men in the area also go diving and collect sea cucumbers or spear fish. When fish is scarce

during the day they go fishing at night. Saturday is also the day he goes to Kavieng to buy supplies if they are needed. The days he goes to town he wakes up earlier since he has to walk 30 min to the main road to catch the bus. He takes the bus at 8 am gets to town at 10 am purchases the goods and returns to the village with the afternoon bus. Saturday afternoon he stays home and rest. He makes a fire and sits next to it. He smokes and chews betelnut. He waits for dinner, once ready he eats with the family and then, he walks to the road junction. Men gather at the road, chew betel nut, smoke and talk. Every two weeks on pay day the bottle shops are full. The bottle shops are enclosed and sell beer through a window. There is no space to sit down and drink. Men usually buy the beer and walk to a convenient space to sit and drink (usually a rock at the side of the road, a bench close to a selling stand or at the beach). Andrew does not stay too long since the later it gets the more drunken men around. He goes back home and sleeps before midnight. On Sunday he wakes up and gets ready for church service. Service starts at 9 am. He and his family have tea in the morning and immediately walk to the church area. After the service they all walk back. Sometimes he stops at the garden and collects betel nut to chew during the week. The family has dinner together at the usual outdoors area. They get visitors on Sundays that come by to share food, betel nut or a smoke. He stays awake until the visitors leave; Andrew drinks tea with them and they talk for hours. They sit outside next to the fire with only the smoke protecting them from mosquito bites. When the visitors leave he goes to sleep usually after midnight. Andrew is not sure how does malaria gets transmitted, he thinks it could be transmitted by a mosquito but other things could also give malaria to a child. In any case malaria is part of life and there is not much he could do about it.

Additional file 9: Adult female (New Ireland Province) – Francisca, 22 years old

Francisca sleeps with her youngest sister and her youngest brother. They share the sleeping area and a LLIN but they do not use it every day. Francisca sleeps wearing a shirt and a laplap. During the week Francisca wakes up at 4 am. Immediately after waking up, she uses the outdoor latrine situated 200m away from the house. Then, she starts the fire and prepares breakfast. She bathes at home with water from the drum. She usually wears a pair of shorts and a t-shirt, her usual work-clothes. Her shoes are flip-flops. Her arms, legs and feet are usually exposed to mosquito bites. She walks 10 minutes to the main road at 5:30 am and waits for the bus or a company car to pass by and take her to work. She works as a loose-fruit-collector at the big oil palm plantation. Her shift starts at 6 am and continues until 2 pm. The plantation blocks are located in a swampy area are treated with insecticide to protect the palms from pests. Once at the plantation she can change the flip-flops to rubber boots if the picking-areas are flooded. After work Francisca gets home at 3 pm. She changes her work clothes to clean ones if she is not

going to the garden. She cleans the kitchen and if there is no food for dinner she goes to the garden. Her garden is 15 minutes away. She follows a narrow walking path behind her house to get there. She gets banana or taro and greens. Sometimes she also gets some betel nut to sell outside her house. She has a table next to the road where she sells the betel nut and sometimes home-made doughnuts. Fortnight-Fridays she prepares doughnuts or flour balls to sell. She prepares the dough after work. It needs time to grow before the frying so she let it grow while she goes to the garden. Before cooking dinner with her sisters she fries the dough. Then she sets the table outside the house to start selling as early as possible and until it gets dark. At about 6 pm the family has dinner. They all sit outside nest to the fire. Francisca keeps an eye on the table while she dines. When the selling stand is open she takes turns with her sisters so she can go bathe at the river. She walks towards the river at 7 or 8 pm. She bathes and does her laundry with the help of a flashlight. It takes her about one hour. Right after, she walks back home and attends the selling again. She chats and chews betel nut with people passing by. Francisca never wears repellent, no one in the village does. She closes her little stand at 10 pm or earlier if she sold all the goods. Twice a week, when she is not selling she joins the praying fellowship at 8 or 9 pm. The church area is 20 minutes away from her house. She walks back home at 10 pm and goes to sleep right away.

During the weekend Francisca wakes up at six or seven in the morning. She uses the outdoor latrine and goes to the river. She takes the dirty dishes and cleans them. She goes with her sisters and they also do the laundry. It takes them a little bit longer than one hour. They stay at the river bank with their feet in the water while doing the laundry. If they need to go to the garden they go in the morning after their bath. Some Saturdays they go fishing at sea or they go to the swamp and work the sago. Working the sago takes the whole day at the swamp starting 9 or 10 am till 3 or 4pm. When the tide is low and the swamp is dry they collect kina shells (clams) but they go a few times a year. On fortnight weekend she goes to town (Kavieng) and buys supplies. She wakes up early, walks to the main road and catches the bus at 8 in the morning. She gets to town at 10 am and buys the flour and sugar for the doughnuts among other items. She gets back to the village in the afternoon and rest for a while in the house. She sits on a patch of grass on a mat and chats with her family. Then, she goes to the river and bathes. Francisca and her sisters cook dinner together and the whole family eats and drinks tea while sitting outside next to the fire. On Sundays she wakes up at 7 and she gets ready for church, she usually wears a meri-blouse and laplap to Church. Service starts at 9 am. She goes to the church grounds and stay they until noon. Her family walks back together and gather. She cooks with her sisters and the family eats an early dinner at 4 or 5 pm. After food they all chat and chew betel nut for hours until she gets tired. They spend all this time outside while adding coconut

husks to the fire to repel the mosquitoes. Before going to sleep she goes to the well bathes and fetches water for Monday morning. She goes to sleep at 10 or 11pm and she is usually too tired to care about putting up the net and sleeping under it Once a year Francisca helps with the preparations for the Malangan; a big celebration that takes place once a year for three days and three nights. Three months before the festival regular meeting in the afternoon take place in order to arrange all Malangan preparations . Francisca is scared of malaria and thinks mosquitoes are a bad thing. However there are so many the only thing she can do is to light a fire and put lots of coconut husks on it to repel mosquitoes.

7. PREVALENCE AND RISK FACTORS OF MALARIA INFECTION IN TWO SITES OF PAPUA NEW GUINEA

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7.1. ABSTRACT

BACKGROUND

Papua New Guinea (PNG) exhibits a particularly complex malaria epidemiology owing to the country's diverse environments and the variety in parasite and vector species composition. Since 2004, much progress has been made in reducing malaria burden in PNG. The National Malaria Control Programme has led to a rapidly increasing coverage with LLINs and a significant overall reduction in prevalence of malaria by 2014. Despite this success, malaria resurged by 2016/2017 with an estimated 8.6-fold increase in prevalence compared to 2013/2014. Identifying areas or groups at higher risk in a local scale is crucial to fine tune the current control strategies and potentially target interventions. This study aims to investigate the distribution of malaria prevalence across spatial clusters and population sub-groups in order to assess the extent of residual malaria at the time of study and identify possible drivers of residual transmission and risk factor for malaria infection in two distinct settings in PNG.

METHODS

A cross-sectional malaria indicator survey covering a sample of households was used to assess malaria prevalence in two distinct sites in Papua New Guinea. A census preceded and complemented the survey with demographic (population size, age and sex distribution) and geospatial (household GPS location) information. Household prevalence was mapped for each village and behavioural and demographic variables were used to identify possible drivers of malaria transmission in the selected sites.

RESULTS

This study illustrates malaria heterogeneity at different scales; between sites and between villages within the sites. Malaria prevalence and LLIN-use significantly differed between Mugil and Lemakot area. Behavioural groups (or demographic groups exhibiting similar behavioural patterns), housing and village of residence were relevant risks identified for malaria infection in Mugil area. In comparison behavioural groups and LLIN ownership were relevant identified risks in Lemakot area.

CONCLUSIONS

This study reveals spatial heterogeneity in the prevalence distribution of malaria in the study sites. The identification of site specific risk factors provides evidence to potentially inform complementary intervention in a sub-national scale that target specific groups or areas.

KEYWORDS

Malaria, Residual transmission, Risk factors, Papua New Guinea, Malaria Prevalence

7.2. INTRODUCTION

Renewed intensification of global malaria control efforts since 2000 met with significant success in reducing the global burden of malaria, yet data from 2015 to 2017 suggest the progress in reducing malaria cases has stalled (Bhatt et al., 2015; World Health Organization, 2018a). Papua New Guinea (PNG) exhibits a particularly complex malaria epidemiology owing to the country's diverse environments and the variety in parasite and vector species composition (Betuela et al., 2012a; Mueller et al., 2003). *Plasmodium falciparum*, *P. vivax*, *P. malariae* and *P. ovale* have been identified in the country, several *Anopheles* species have been incriminated as vectors, and heterogeneities in transmission and disease burden have been described at various scales (Cattani et al., 1986; Cooper et al., 2009; Hetzel et al., 2017a; Rodríguez-Rodríguez et al., 2019c). Due to this complexity, malaria elimination has long been considered challenging in PNG. Since 2004, PNG has been financially supported by The Global Fund to Fight AIDS, Tuberculosis and Malaria in the implementation of its national malaria control programme (NMCP). The NMCP has focused on: i) the free mass distribution of long-lasting insecticide-treated nets (LLIN), ii) the improvement of diagnostic capacity (microscopy and rapid diagnostic tests; RDTs) and the introduction of artemisinin-based combination therapy (ACT) in health facilities nationwide (Hetzel et al., 2014c), and iii)behavioural change communications. The program has led to a rapidly increasing coverage with LLINs (Hetzel et al., 2014a) and a significant overall reduction in prevalence of malaria across PNG by 2014 (Hetzel et al., 2017a). However, a resurgence in the prevalence of malaria was observed in 2017 (Hetzel et al., 2018). As control efforts develop towards elimination, it becomes more important to understand the factors influencing the persistence of malaria transmission at finer spatial scales (Bannister-Tyrrell et al., 2018). The efficacy of interventions, whether individual or combined, varies by setting and is dependent on many local factors including vector ecology, human behaviour, environmental conditions, health systems, and coverage levels (Bhatt et al., 2015). Therefore, understanding local transmission dynamics is essential to maintain the accomplished progress and eventually eliminate malaria in a given setting (Bannister-Tyrrell et al., 2018). Identifying areas or groups at higher risk on a local scale is crucial to fine tune the current control strategies and potentially target interventions. This study aims to investigate the distribution of malaria prevalence across spatial clusters and population sub-groups in order to assess the extent of residual malaria at the time of study and identify possible drivers of residual transmission and risk factor for malaria infection in PNG.

7.3. METHODS

7.3.1. STUDY DESIGN

A cross-sectional malaria indicator survey covering a sample of households was used to assess malaria prevalence in two distinct sites in Papua New Guinea. A census preceded and complemented the survey with demographic (population size, age and sex distribution) and geospatial (household GPS location) information. Household prevalence was mapped for each village and behavioural and demographic variables were used to identify possible drivers of malaria transmission in the selected sites.

7.3.2. STUDY SITES

The study was conducted within the catchment area of two health facilities: 1) Mugil Health Centre, Sumkar District, Madang Province and 2) Lemakot Health Centre, Kavieng District, New Ireland Province (Figure 7.1.). Villages with a high malaria burden at the health facility were selected for an exploratory visit. Following the visits, four villages were selected in each site based on the accessibility and the explicit consent from the village leaders to participate. In addition, two villages in each site represented a scattered population distributed in a larger area while the two others included a concentrated population over a smaller area. The location of all selected villages is mapped in Figure 7.1.

Mugil area

Mugil is located in the rainforest area at the North Coast of Madang (Marfurt et al., 2010). Cash crops in the Madang community include dry coconut, cocoa beans and betel nut (*Areca catechu)*. Villages in Mugil were selected to represent the two typical settings in the area: coastal villages (Megiar and Mirap) and inland villages (Bulal and Wasab).

Lemakot area

Lemakot is located on the main island of New Ireland Province, which is long (~200 km), narrow (~8 km) and mountainous with a wet tropical climate (Holdsworth et al., 1980). Oil Palm Companies are well established in the area. The most important cash crops in the area are oil palm fruit, dry coconut and to a lesser degree sago (*Metroxylon sagu*) and betel-nut. Selected villages in the Lemakot area included the two main settings in the island: West Coast villages (Lamusmus 1 and Lavolai) and East Coast villages (Luburua and Lossuk). The main difference between the two coasts is the access to a well maintained paved highway on the East Coast.

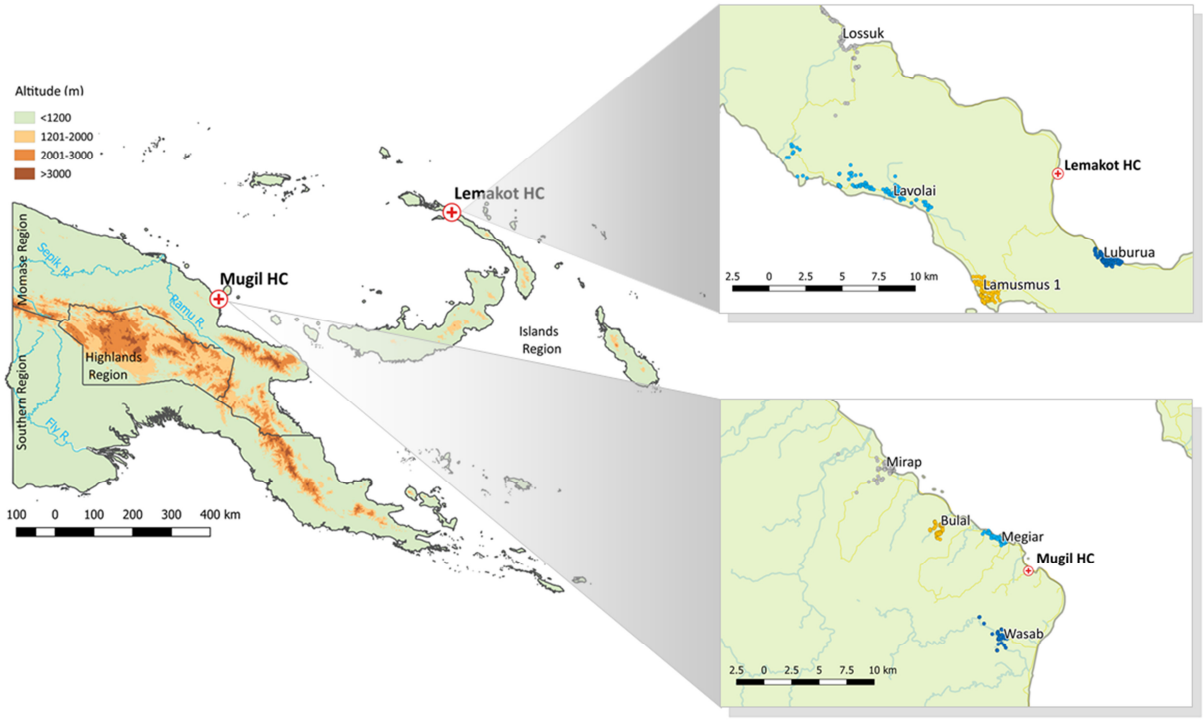

Figure 7.1. Location of the two study sites in Papua New Guinea and location of the selected villages in each study site.

7.3.3. Data Collection

Prior to the survey, a census was conducted in the selected villages. Household size, house coordinates, age and sex of all household members were collected electronically with a census form. The survey was conducted between October 2016 and February 2017 in the Mugil area and between September and October 2017 in the Lemakot area. Data was collected electronically using two questionnaires: one for the household information (household questionnaire) and one for each member of the household participating in the survey (prevalence questionnaire). The questionnaires followed the same structure as the instruments previously used for the National Malaria Indicator Survey (Hetzel et al., 2018). All forms were developed using the open-source platform Open Data Kit (ODK). After the census was concluded, a sample of 80% of the households was purposively selected to represent a spatially even distribution across all hamlets in the village. All eligible and available residents (age > 6 months) of the selected households were invited to participate and following written informed consent, household heads provided details of each household member's demographic characteristics and coverage by malaria interventions. Individual history of febrile illness and finger prick blood samples were collected alongside information on LLIN use and treatment seeking for recent febrile illness episodes. Trained study nurses prepared in duplicates thick and thin blood films for light microscopy. Haemoglobin levels were measured with a portable HemoCue Hb 201+ photometric analyser (HemoCue AB, Ängelholm, Sweden) and axillary

temperature with an electronic thermometer. Symptomatic household members were diagnosed using a malaria rapid diagnostic test (ICT Combo, ICT Diagnostics, Cape Town, South Africa), and positive cases were treated or referred to the nearest health-facility when appropriate.

Malaria diagnosis by light microscopy was performed at the Papua New Guinean Institute of Medical Research following established procedures (Hetzel et al., 2017a, 2015; Robinson et al., 2015). Each slide was examined independently by two microscopists, each viewing a minimum of 200 thick film fields. Slides with discordant results were examined by a third microscopist who was certified at World Health Organization (WHO) level 1 or 2. A slide was considered positive for malaria if judged positive by at least two microscopists. The study was approved by the Papua New Guinea Medical Research Advisory Committee (MRAC no. 16.08).

7.3.4. DATA ANALYSIS

Mapping of malaria prevalence

The proportion of infected individuals per household was calculated for each screened household and mapped by village. The *Plasmodium* species-composition of each infected household and village was calculated and is displayed on the village-specific maps.

Risk factors for malaria infection

Measures of prevalence were calculated by site and by village including species-specific prevalence and reported LLIN-use the night prior to the survey. Age specific prevalence was also explored for *P. falciparum, P. vivax,* and mixed infections (*P. falciparum* and another species) by site.

A series of univariable models of risk factors of malaria infection were explored to identify relevant variables for the final models. Individual, household and village level variables were then introduced into two multilevel logistic regression models, one for each site. The outcome was a binary variable for the presence or absence of any species of *Plasmodium* identified by light microscopy. A multilevel analysis was used since the nested structure of the data required simultaneous examination of individual and household level variables (Diez-Roux, 2000; Messina et al., 2011). The multilevel approach improved standard errors and parameter estimates for correlated variables of individuals within a group. To avoid multicollinearity, correlations between similar or derived variables were tested prior to inclusion into the model. All models were computed using Stata/IC v.13.1 (Stata Corp LP., College Station, USA).

The final model for Mugil area included variables of intervention use (LLIN-use the night prior to the survey, one LLIN for every two people), behavioural variables, (sleeping time and

demographic groups exhibiting similar behavioural patterns referred to as "behavioural groups"), housing variables (housing type and window structure), socio-economic status variables (education level of the households head) and spatial/environmental variables (village of residence). Behavioural groups were categorized according to a previously defined classification of demographic groups exhibiting similar behaviours within the study sites (Rodríguez-Rodríguez et al., 2019a).

Since very few malaria cases were detected in Lamusmus 1 and Luburua, these two villages were omitted from the model for the Lemakot area. In general, the low prevalence in Lemakot reduced the predicting ability of some variables and restricted the model flexibility. The final model included the following variables: LLIN use the previous night, sleeping time, behavioural groups, one LLIN per two people and village of residence. In both final models, age groups were omitted due to collinearity with the behavioural groups.

7.4. RESULTS

According to the baseline census, a total of 3,364 individuals were identified in the four villages in the Mugil area and 5,470 in the four villages in the Lemakot area. Age and sex distribution of each study population are depicted in Figure 7.2. Women comprised 48% (1,620/3,364) of the population in Mugil and 47% (2,888/5,470) in Lemakot.

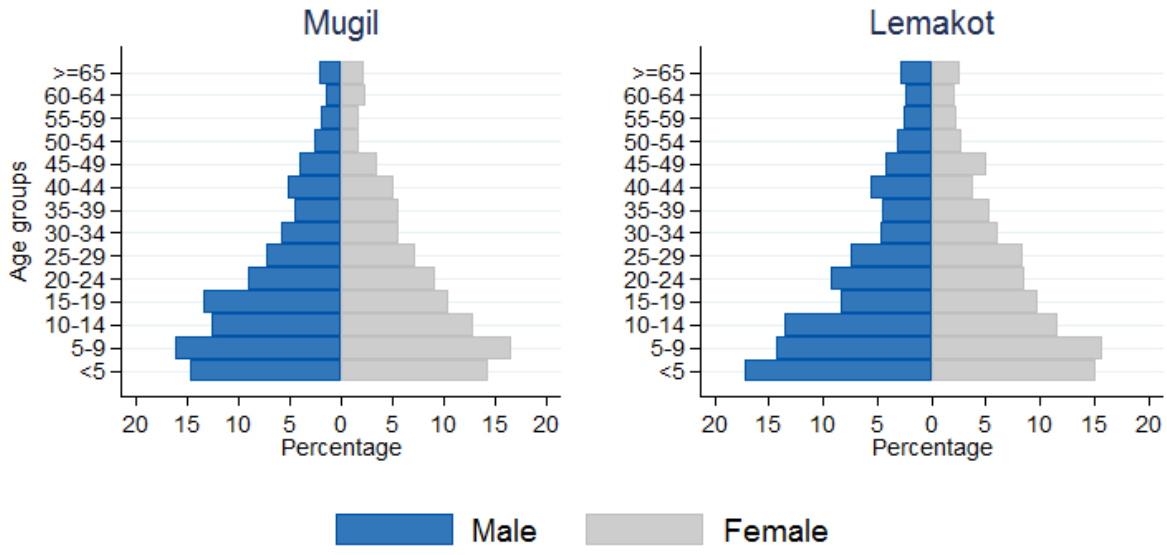

Figure 7.2. Age and sex distribution of the population of the four selected villages in 2017 for Mugil area (N= 3,364) and Lemakot area (N= 5,470).

The total number of surveyed households was 398 in the Mugil area and 309 in the Lemakot area. The number of survey participants was 1,927 in the Mugil area and 1,202 in the Lemakot area. Blood slides were collected from 3,120 individuals in both sites. The age distribution of study participants by site is shown in Table 7.1. The overall median age was 18 (interquartile

range, IQR: 7–36); 15% (478/3,120) were aged under 5 years and 52% (1,615/3,093) were female.

Table 7.1. Number of participants and proportion by age group in both Study sites

	Mugil area		**Lemakot area**	
Age group	N	(%)	N	(%)
<5	296	(15)	182	(15)
5-9	337	(18)	214	(18)
10-14	237	(12)	128	(11)
15-19	160	(8)	95	(8)
20-39	517	(27)	304	(25)
>40	379	(20)	271	(23)
Total	**1,926**	**(100)**	**1,194**	**(100)**

Pooled malaria prevalence of both sites was 9.8 % (95% CI 8.8, 10.9), 13.7% (95% CI 12.2, 15.3) in the Mugil area and 3.7% (95% CI 2.8, 4.9) in the Lemakot area. Prevalence values for each selected village are presented in Table 7.2. Luburua and Lamusmus 1 had the lowest prevalence and both are located in Lemakot area while Mirap and Megiar had the highest and both are located in the Mugil area. *P. vivax* was the more abundant species in Bulal and Lavolai. Luburua was the only village with one single *Plasmodium* species (*P. falciparum*). Self-reported LLIN use the previous night differs between sites, with percentages above 81% in Mugil and below 45% in Lemakot.

Table 7.2. Malaria incidence determined by light microscopy for each village and reported LLIN use the night previous to the survey.

Site		**Prevalence (%)***				**LLIN use (%)**
	N	All	*Pf*	*Pv*	*Pm*	Mix
Mugil area	1,902	13.7	6.8	6.0	0.2	0.7	89.3
Bulal	382	10.5	3.7	5.5	0.5	0.8	93.5
Megiar	589	13.5	7.0	5.1	0.0	1.4	81.2
Mirap	669	19.5	10.0	9.0	0.2	0.3	93.9
Wasab	262	4.3	3.1	1.2	0.0	0.0	89.9

Lemakot area	1,190	3.7	1.5	1.8	0.3	0.1	37.4
Lamusmus 1	308	1.0	0.7	0.3	0.0	0.0	29.0
Lavolai	273	6.3	1.5	4.4	0.4	0.0	39.1
Lossuk	249	8.4	3.6	3.2	1.2	0.4	44.4
Luburua	361	0.8	0.8	0.0	0.0	0.0	38.5

**Po was not detected by light microscopy in any of the study sites.*

Disaggregation of malaria infection by age in both study sites is displayed in Figure 7.3. including species-specific distribution. "All species" include *P. falciparum*, *P. vivax* and *P. malariae*. *Plasmodium ovale* was not detected in any of the samples and only seven cases of *P. malaria* were identified (three in Mugil area and four in Lemakot area) therefore, both species were omitted from the Figure 7.3. In both sites, the overall age-peak is in the 5-9 age group and *P. vivax peak* is in younger age groups than *P. falciparum*. Compared to Mugil area, age peaks of infection are less pronounced in Lemakot area.

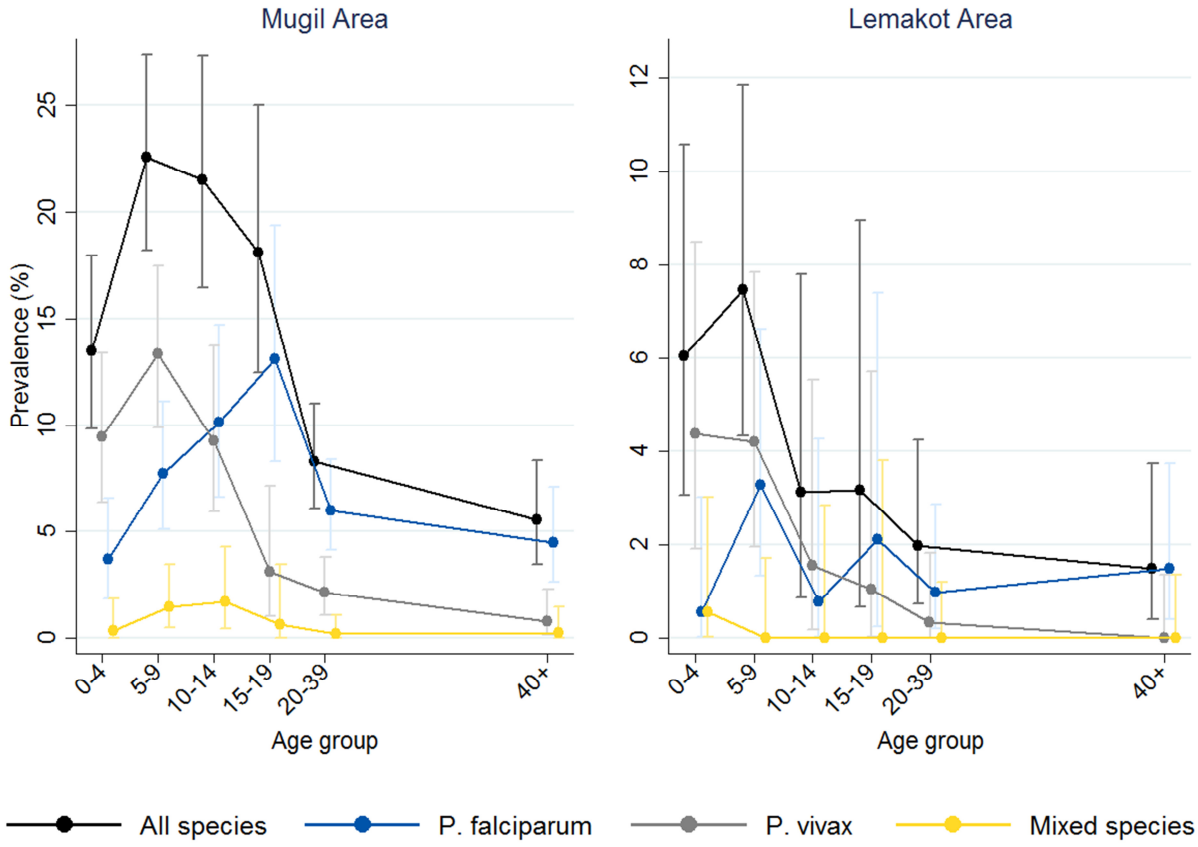

Figure 7.3. Malaria prevalence of all Plasmodium species, P. falciparum, P. vivax and mixed species (Pf and another species) by age group and site.

7.4.1. MAPPING OF MALARIA PREVALENCE

The total number of households with at least one malaria infection was 173 in the Mugil area and 30 in the Lemakot area. The highest household prevalence was 67% in the Mugil area and 40% in the Lemakot area. The highest number of infected individuals per household was seven in the Mugil area and four in the Lemakot area. Small households with one or two members did not have any infected individuals detected by microscopy in both sites except for two houses in Megiar with two members and one infected individual detected in each. Detailed figures depicting the number of infections per household by household size are provided in the Additional File 1. Village maps including the spatial distribution of malaria prevalence at a household level are included in Figures 7.4. to 7.7. The following paragraphs described the patterns of household prevalence that were visually identified.

In **Wasab** (Figure 7.4., left), most detected malaria infections were attributed to *P. falciparum*. *Plasmodium vivax* infections were identified in only two households. Coincidentally, *P. vivax* distribution was in nearby houses on the same side of the main river (Yamab River) and in between two bodies of water.

In **Bulal** (Figure 7.4., right), *P. falciparum, P vivax, P. malariae* and mixed infections were identified and *P. vivax* was the most dominant species. *Plasmodium falciparum* and *vivax* infections seem evenly distributed across the village except for a cluster of houses towards the South of the village and away from the river (River Wagem). *Plasmodium malariae* was identified in two neighbouring houses.

In **Megiar** (Figure 7.5., left), a coastal village with houses clustered together, malaria prevalence was higher than in the inland villages and *P. falciparum* was the dominant species closely followed by *P. vivax*. *Plasmodium vivax* infections were more dominant towards the northern part of the village whereas *P. falciparum* was more dominant towards the south of the village. *P. malariae* was not identified and individuals with mixed infections were identified throughout the village.

In **Mirap** (Figure 7.5., right), a setting similar to Megiar, infections due to *P. falciparum, P. vivax, P. malaria* and mixed species were identified. *Plasmodium falciparum* was the most dominant followed closely by *P. vivax*. *Plasmodium malariae* and mixed infection were very few. Infections were distributed across the village and even in some remote inland houses.

In **Lamusmus 1** (Figure 7.6., right), only three infections were identified, two due to *P. falciparum* towards the north and one due to *P. vivax* towards the south.

In **Lavolai** (Figure 7.6., right), most infections were attributed to *P. vivax*. Most *P. vivax* infections were identified in two clusters in the mid-section of the village.

Lossuk (Figure 7.7., left), is a village on the Easts-Coast with a very scattered population. *P. falciparum, P. vivax, P. malariae* and mixed infections were identified in individuals from this village mostly residing on the coastal side of the road. The most prevalent species was *P. falciparum* closely followed by *P. vivax.* Infections due to *P. vivax* were cluster towards the mid-section of the village*. Plasmodium falciparum* was more prevalent towards the north of the village. *Plasmodium malariae* and mixed infections were few. Clusters of uninfected households are visible towards the north and the south ends of the village.

In **Luburua** (Figure 7.7., right), only three infections were identified all due to *P. falciparum*; two in neighbouring inland houses and one in a further house on the coastal side of the road.

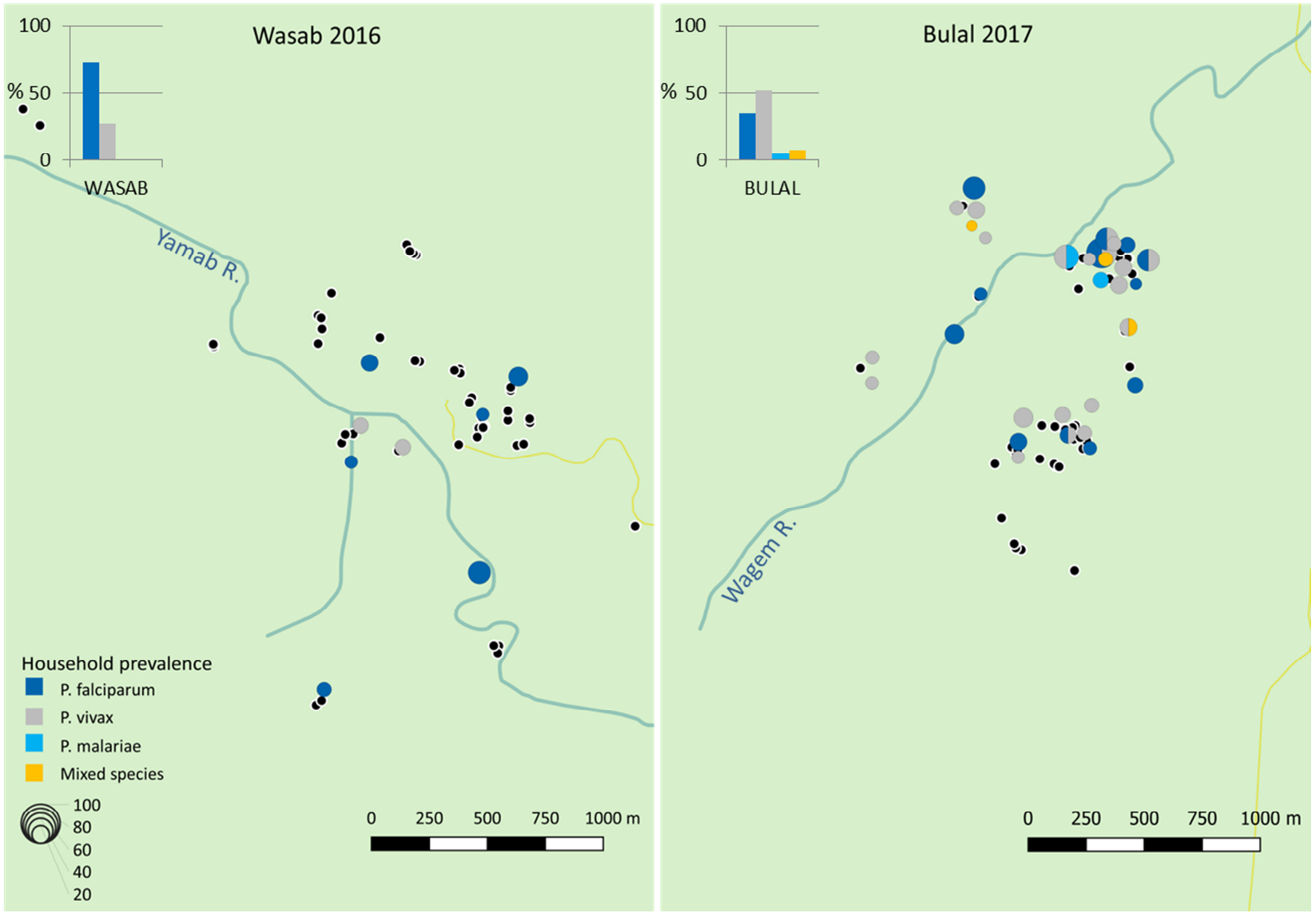

Figure 7.4. Spatial distribution of malaria prevalence in Bulal and Wasab (Mugil area, inland villages). Each dot represents a surveyed household. Black dots represent households without infected individuals. The other colours indicate the Plasmodium species. The size of the bubble represents the percentage of people with malaria infection in each household. Bar graphs on the top corner display the overall Plasmodium species composition for each village. Mixed species include P. falciparum and another species.

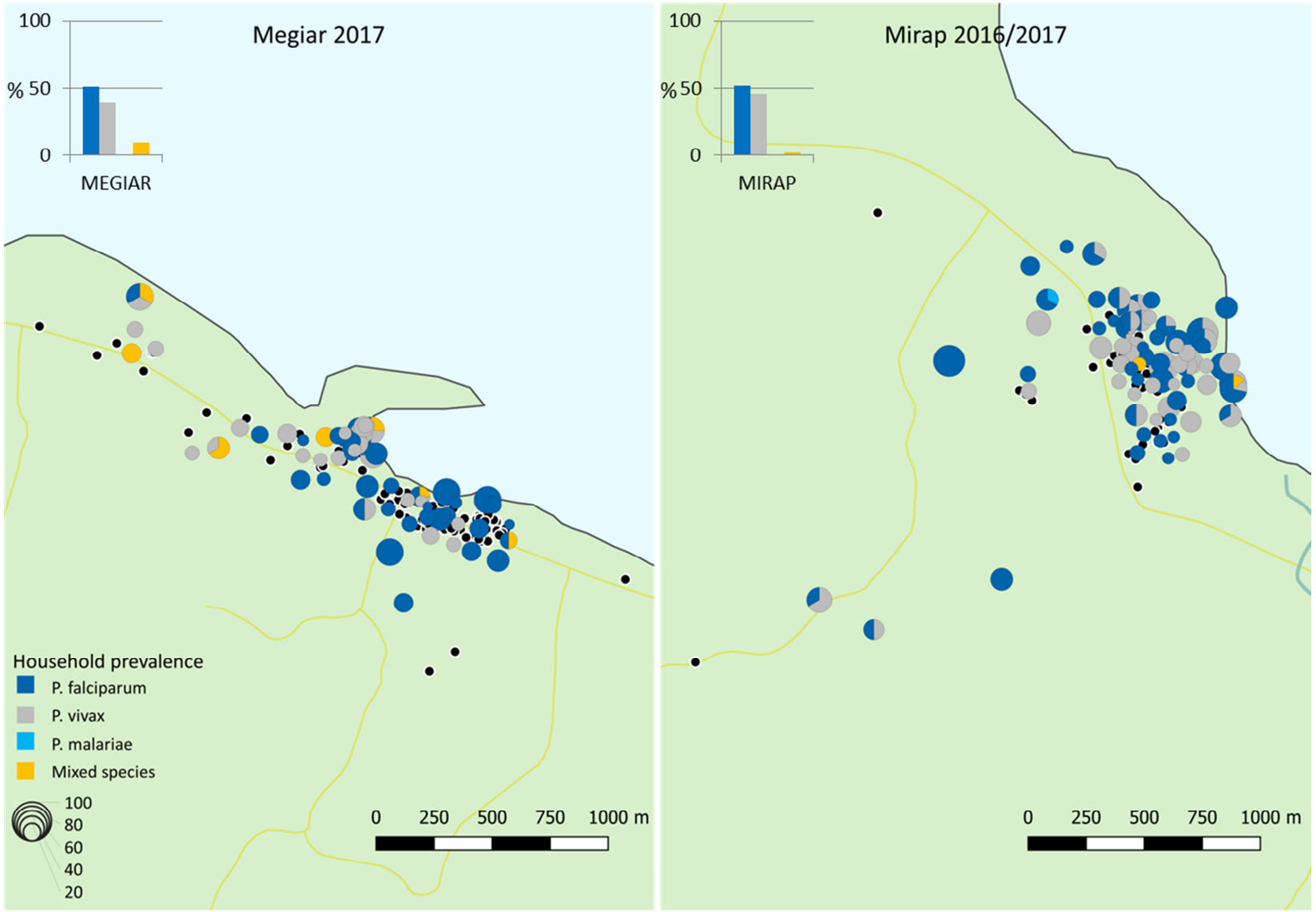

Figure 7.5. Spatial distribution of malaria prevalence in Megiar and Mirap (Mugil area, coastal villages). Each dot represents a surveyed household. Black dots represent households without infected individuals. The other colours indicate the Plasmodium species. The size of the bubble represents the percentage of people with malaria infection in each household. Bar graphs on the top corner display the overall Plasmodium species composition for each village. Mixed species include P. falciparum and another species.

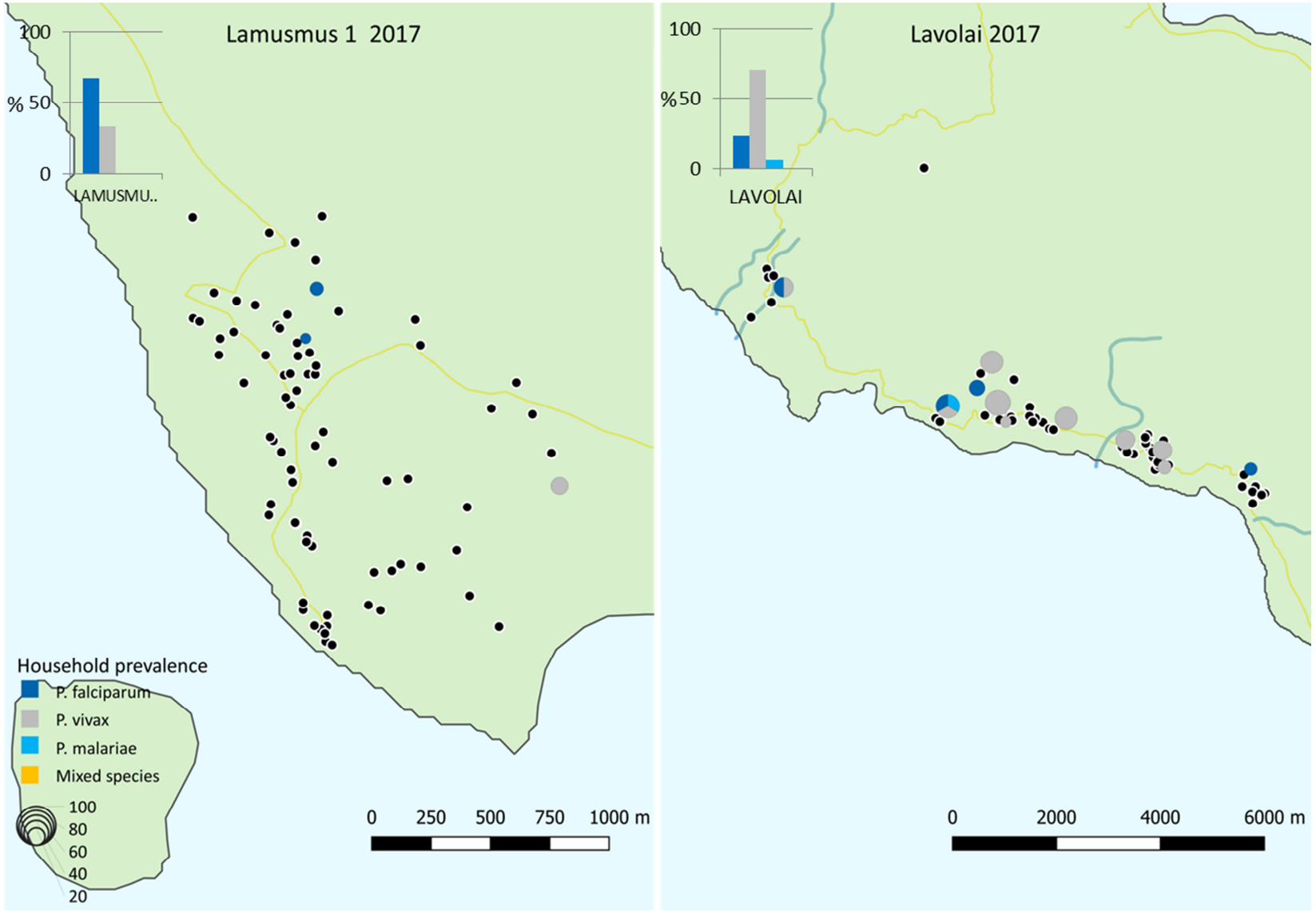

Figure 7.6. Spatial distribution of malaria prevalence in Lamusmus 1 and Lavolai (Lemakot area, west-coast villages). Each dot represents a surveyed household. Black dots represent households without infected individuals. The other colours indicate the Plasmodium species. The size of the bubble represents the percentage of people with malaria infection in each household. Bar graphs on the top corner display the overall Plasmodium species composition for each village. Mixed species include P. falciparum and another species.

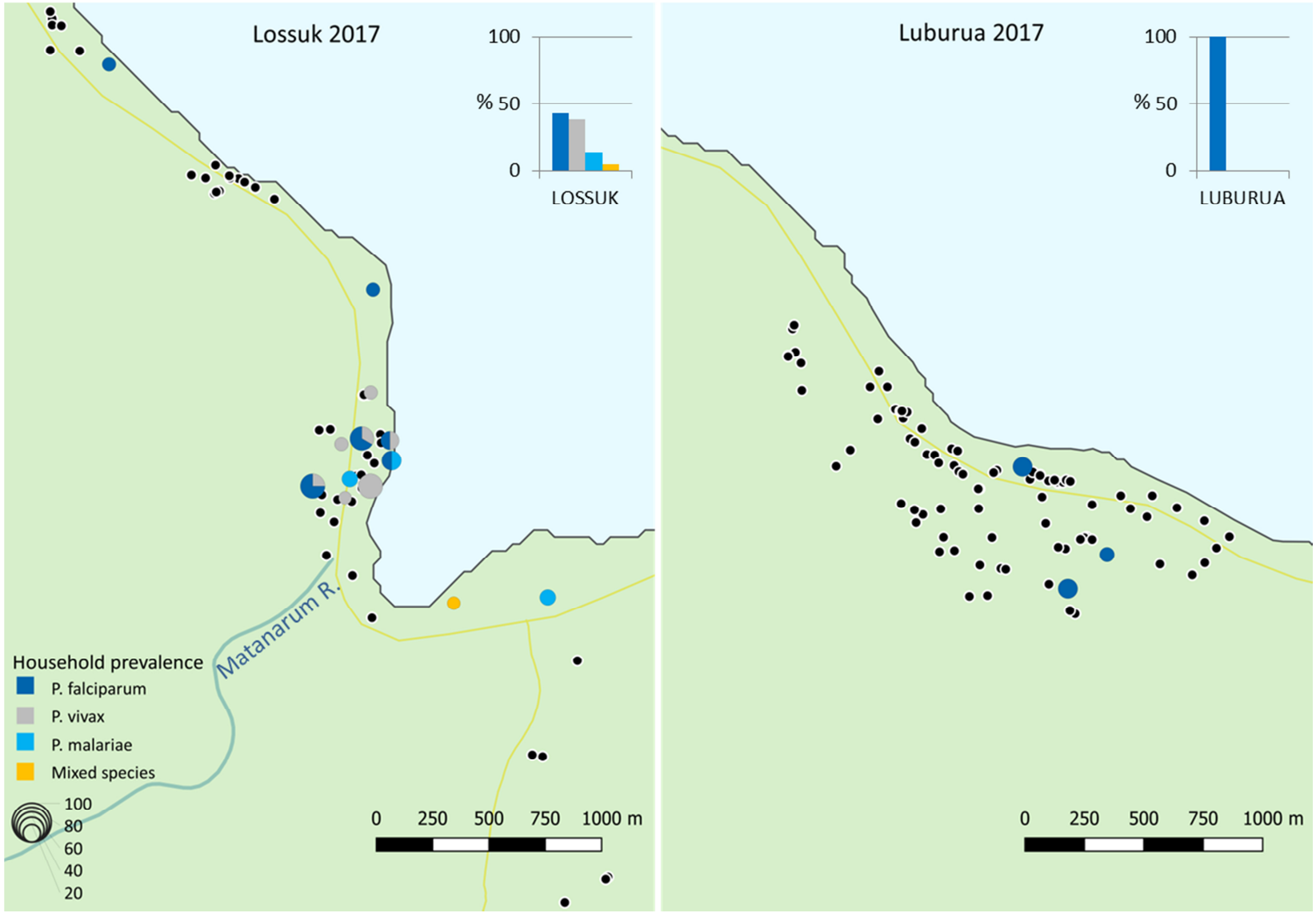

Figure 7.7. Spatial distribution of malaria prevalence in Lossuk and Luburua (Lemakot area, east-coast villages). Each dot represents a surveyed household. Black dots represent households without infected individuals. The other colours indicate the Plasmodium species. The size of the bubble represents the percentage of people with malaria infection in each household. Bar graphs on the top corner display the overall Plasmodium species composition for each village. Mixed species include P. falciparum and other species.

7.4.2. *RISK FACTORS FOR MALARIA INFECTION*

The model estimates for risk factors of malaria infection in the Mugil area are shown in Table 7.3. Compared to preschool aged children, school boys and girls are at higher risk of infection; 1.9-fold (95% CI, 1.19-2.98) for school girls and more than two fold (95% CI, 1.37-3.42) for school boys; and female adults are at a lower risk of malaria infection (51%; 95% CI, 16-71%). These results support the age-specific prevalence data shown in Figure 7.3., which shows peak prevalence in children aged 5 to 9 that sharply decreases after 19 years of age. In addition, housing seems relevant to malaria transmission since individuals living in houses built using a mix of improved and traditional material were at higher risk of infection (1.80; 95% CI, 1.25-2.6) compared to those living in traditional houses. Modern houses (roofs, floors and walls built with improved materials) were very few and no malaria cases were identified in these houses; therefore the model omitted an estimation for this category. Having some screening on the windows seems to be protective compared to not having any screening on windows. This variable reflects housing standards and potential access for mosquitoes to the household members but it could also reflect socio-economic status. Poor households are less likely to screen windows since the screening requires an additional expense. As observed in the maps, prevalence differs between villages. Accordingly compared to Bulal residents, residents in Mirap are at higher risk of malaria (2.18; 95% CI, 1.40-3.39) while Wasab residents are at lower risk (0.32; 95% CI, 0.15-0.65).

The model estimates for risk factors of malaria infection in the Lemakot area are shown in Table 7.4. The model suggests a risk difference for behavioural groups. Compared to preschool aged children, adult women are at 80% (95% CI, 33- 95%) less likely to acquire a malaria infection. The model also suggests that individuals in households with at least one LLIN for every two people are at considerable reduced risk of malaria infection (65%; 95% CI, 13- 86%) than those in households with more than two people per LLIN. The relationship between the number of people and the number of LLINs per households and malaria infection is visible in the univariable analysis, for instance, the risk of malaria infection increased 19% (95% CI, 5- 36%) with each extra member in the household and decreased 85% (95% CI, 38- 96%) with every additional LLIN in the household. When the relationship between 'households size and owning at least one LLIN for every two people in the household' was explored, the likelihood to have at least one LLIN for every two people was reduced 37% (95% CI, 31- 43%; P-value <0.01) with every additional household member.

Table 7.3. Relevant results for the univariable and multivariable models explored for Mugil area. Significant odds ratios are display in bold.

Mugil area		**Univariable**				**Multivariable**			
Variables	**Values**	**OR**	**95% CI**		**P-value**	**OR**	**95% CI**		**P-value**
Individual level variables									
Age groups (years)	0-4	1.00				-			
	5-9	**1.86**	**1.22**	**2.84**	**<0.01**	-			
	10-14	**1.75**	**1.11**	**2.77**	**0.02**	-			
	15-19	1.44	0.85	2.43	0.17	-			
	20-39	**0.60**	**0.38**	**0.95**	**0.03**	-			
	40+	**0.38**	**0.22**	**0.66**	**<0.01**	-			
Sex	Female	1.00				-			
	Male	1.22	0.94	1.59	0.14	-			
LLIN-use last night	No	1.00				1.00			
	Yes	1.50	0.92	2.42	0.10	1.25	0.73	2.13	0.42
Frequency of use	On and off	1.00				-			
	Every night	0.74	0.51	1.07	0.11	-			
	Never	**0.55**	**0.31**	**0.96**	**0.04**	-			
Slept indoors or outdoors	Indoors	1.00				-			
	Outdoors	0.56	0.22	1.40	0.21	-			
Sleeping times	Before 9pm	1.00				1.00			
	9 to 11pm	**0.74**	**0.56**	**0.97**	**0.03**	1.07	0.76	1.51	0.70
	After 11pm	0.63	0.37	1.08	0.09	1.11	0.58	2.13	0.75

Behavioural groups	Preschool aged children	1.00				1.00			
	School girl	**1.67**	**1.09**	**2.57**	**0.02**	**1.88**	**1.19**	**2.98**	**0.01**
	School boy	**1.91**	**1.25**	**2.92**	**<0.01**	**2.17**	**1.37**	**3.42**	**<0.01**
	Adult female	**0.52**	**0.33**	**0.83**	**0.01**	**0.49**	**0.29**	**0.84**	**0.01**
	Adult male	0.67	0.42	1.05	0.08	0.64	0.37	1.11	0.11
Antimalarials in the last 2 months	No	1.00				-			
	Yes	1.26	0.80	2.00	0.32	-			
Household level variables									
No. of household members		1.02	0.98	1.07	0.34	-			
Housing type	Traditional	1.00				1.00			
	Mixed	**1.48**	**1.12**	**1.95**	**0.01**	**1.80**	**1.25**	**2.60**	**<0.01**
	Modern	NA				NA			
Windows	Not screened	1.00				1.00			
	No windows	1.10	0.68	1.77	0.71	1.51	0.87	2.64	0.14
	Some screened	**0.69**	**0.51**	**0.95**	**0.02**	**0.69**	**0.48**	**1.00**	**0.05**
	All screened	**0.70**	**0.49**	**1.00**	**0.05**	0.74	0.48	1.15	0.18
House elevated (stilts)	No	1.00				-			
	Yes	2.18	0.88	5.40	0.60	-			
Household head	No education	1.00				1.00			

education level									
	Less that 6th grade	1.43	0.81	2.52	0.21	0.89	0.46	1.70	0.72
	7-12th grade	1.48	0.84	2.63	0.18	0.88	0.45	1.70	0.70
	Higher education	1.37	0.55	3.43	0.50	0.89	0.31	2.54	0.82
Number of LLINs owned		0.99	0.92	1.06	0.78	-			
No. of LLINs per household member		0.94	0.62	1.41	0.75	-			
One LLIN per 2 people	No	1.00				1.00			
	Yes	0.95	0.73	1.25	0.73	1.35	0.97	1.88	0.08
Village level variables									
Village	Bulal	1.00				1.00			
	Megiar	1.32	0.88	1.98	0.17	1.20	0.74	1.94	0.46
	Mirap	**2.06**	**1.41**	**3.01**	**<0.01**	**2.18**	**1.40**	**3.39**	**<0.01**
	Wasab	**0.37**	**0.19**	**0.74**	**0.01**	**0.32**	**0.15**	**0.65**	**<0.01**
Village location	Inland	1.00				-			
	Coastal	**2.32**	**1.68**	**3.20**	**<0.01**	-			

- *Not included in the multivariable model*

NA Result omitted by the model

Table 7.4. Relevant results of the univariable and multivariable models explored for Lemakot area. Significant odd ratios are display in bold.

Lemakot area		**Univariable**				**Multivariable**			
Variables	**Values**	**OR**	**95% CI**		**P-value**	**OR**	**95% CI**		**P-value**
Individual level variables									
Age groups (years)	0-4	1.00				-			
	5-9	1.35	0.58	3.11	0.49	-			
	10-14	0.79	0.23	2.64	0.70	-			
	15-19	0.19	0.02	1.54	0.12	-			
	20-39	**0.27**	**0.09**	**.82**	**0.02**	-			
	40+	**0.11**	**0.02**	**.52**	**0.01**	-			
Sex	Female	1.00				-			
	Male	1.04	0.54	2.03	0.89	-			
LLIN last night	No	1.00				1.00			
	Yes	1.79	0.92	3.49	0.09	1.37	0.62	3.03	0.44
Sleeping times	Before 9pm	1				1.00			
	9 to 11pm	**0.39**	**0.19**	**0.83**	**0.02**	0.89	0.35	2.25	0.81
	After 11pm	0.26	0.03	1.98	0.19	0.70	0.07	6.65	0.76
Frequency of use	On and off	1.00				-			
	Every night	0.63	0.24	1.64	0.34	-			
	Never	**0.40**	**0.16**	**1.01**	**0.05**	-			
Behaviour groups	Children	1.00				1.00			
	School girl	1.16	0.47	2.86	0.74	1.17	0.43	3.14	0.76
	School boy	0.95	0.36	2.50	0.91	0.92	0.32	2.66	0.88

	Adult female	**0.18**	**0.06**	**0.60**	**0.01**	**0.20**	**0.05**	**0.77**	**0.02**
	Adult male	**0.23**	**0.07**	**0.75**	**0.01**	0.31	0.07	1.24	0.10
Antimalarials in the last 2 months	No	1.00				-			
	Yes	1.17	0.34	4.00	0.81	-			
Household level variables									
No. of household members		**1.19**	**1.05**	**1.36**	**0.01**	-			
Housing type	Traditional	1.00				-			
	Mixed	0.98	0.50	1.92	0.95	-			
	Modern	1.00	NA			-			
Number of LLINs owned		0.92	0.75	1.14	0.46	-			
No. of LLINs per household member		**0.15**	**0.04**	**0.62**	**0.01**	-			
One LLIN per 2 people	No	1.00				1.00			
	Yes	**0.29**	**0.13**	**0.66**	**<0.01**	**0.35**	**0.14**	**0.87**	**0.02**
Village level variables									
Village	Lavolai	1.00				1.00			
	Lossuk	1.39	0.72	2.71	0.33	1.62	0.74	3.56	0.23

- *Not included in the multivariable model*

NA Result omitted in the model

7.5. DISCUSSION

This study contributed to the understanding local transmission dynamics in two sites in PNG which is crucial to accelerate elimination of malaria. The study investigated the spatial distribution of malaria prevalence at different levels (site, village and household) at the time of study and identified risk factor for malaria infection on a local scale.

Malaria prevalence in the Mugil area was 3.7-fold higher than in the Lemakot area. LLIN-use was 2.4 times higher in the Mugil area compared to the Lemakot area. Malaria prevalence in the study sites confirms some of the finding of the 2016/2017 Malaria Indicator Survey (MIS) in PNG. Overall, prevalence in Mugil area (13.7%) was similar to the estimate for Madang Province in the 2016/2017 MIS (16.0%) and higher than the estimate for 2014 (6.3%), confirming the increase of prevalence in Madang Province since 2014. In contrast, prevalence in Lemakot (3.7%) was lower than the MIS estimate for the province (8.7%) and similar to the prevalence levels in 2014 (3.2%) (Hetzel et al., 2018, 2014b). This illustrates the heterogeneous spatial distribution of malaria prevalence at different scales; between sites and between villages within the same area.

Prevalence differences between sites are intriguing especially since the settings are comparable in temperature, altitude and rainfall (Attenborough and Alpers, 1992). Moreover, LLIN-use has been consistently higher in Mugil area and Madang province than in Lemakot area and New Ireland Province (Hetzel et al., 2018; Robinson et al., 2018; Rodríguez-Rodríguez et al., 2019b) yet prevalence was higher in the Mugil area. The percentage of people reporting sleeping under a LLIN the previous night was 89.3% in the Mugil area and 37.4% in the Lemakot area which roughly corresponds with the latest MIS that estimated 77.1% of people use LLINs in Madang and 34.0% in New Ireland Province (Hetzel et al., 2018). Our models suggest that at the time of the study LLIN-ownership seems protective in Lemakot and not so in Mugil. Since the identified risks drastically differed between sites a broader range of elements, such as vector ecologies; might be driving transmission differently in the study sites. A quick up-take of LLINs and prolonged use has been documented in Madang Province and has been linked to shifts in peak biting times of *Anopheles* to earlier than 9pm (Thomsen et al., 2017). Such a shift has been identified elsewhere and has been linked to a reduction in the effectiveness of LLINs due to a relative increase of outdoor biting (Sherrard-Smith et al., 2019). That, in turn might amplify the effect of other aspects of transmission such as human behavioural patterns and housing as observed in Mugil. In contrast, an area like Lemakot with a much slower LLIN up-take and lower mosquito density (Robinson et al., 2018) might still benefit from increasing LLIN ownership and use. Further detailed investigations of the local vectors might contribute to clarify some of the

key heterogeneities in transmission between these two sites. A future step in this study is to complement the prevalence results with mosquito data obtained from entomological surveys implemented together with the prevalence survey.

The spatial distribution of malaria infections at a village level might become more relevant as control efforts move towards elimination (World Health Organization, 2018b, 2015). At least in two of our survey villages (Lamusmus 1 and Luburua) prevalence was low enough to treat all identified infections and possibly use reactive case detection (Chitnis et al., 2019). In Mugil, the number of infections in inland villages was considerably lower than in coastal villages. In Lossuk, the majority of infections occurred on the coastal side of the road. The mapping of infections at a household level seems useful to identify areas at increased or reduced risk therefore it could be used to direct targeted control efforts. A recent study suggests that targeting households rather than 'hotspots' might be a better intervention targets for malaria elimination (Bannister-Tyrrell et al., 2019). Prevalence surveys similar to the one in this study could be used to inform interventions such as reactive case detection in the villages with the lowest prevalence (World Health Organization, 2018b, 2015).

As outdoor biting is becoming more common in settings with high coverage and use of LLINs, the importance of human behaviour for malaria transmission is likely to increase (Monroe et al., 2019b; Sherrard-Smith et al., 2019). Categorization of demographic groups in the population using behavioural patterns proved relevant in both settings. A better understanding of such behavioural groups in a local scale could prove useful for targeting interventions. For instance, our study suggests that school children (boys and girls) in Mugil area were at a higher risk of malaria infection. Such risk could be linked to earlier wake-up times and later sleeping times paired with a lower LLIN-use (specially for school boys) compared to children in preschool aged (Rodríguez-Rodríguez et al., 2019a). In addition, most roads to school in the study site are unpaved and covered with tire tracks that provide ideal breeding sites for anophelines known to be active early in the morning (Charlwood et al., 1986) along roads transited by school children in the morning and on a daily basis. This information could translate to targeted interventions distributed at or around schools to target this specific population or interventions along school roads (e.g. larval source management) could target specific areas. Malaria control and health promotion in school have been recommended as viable interventions by the WHO including promotion of LLINs, IRS, chemoprevention, case management & treatment and larval and environmental control (Brooker, 2009; World Health Organization, 2010c). The risk of adult women to acquire malaria infection in both sites was lower compared to other behavioural groups. This could be explained by their acquired immunity as adults, when compared to children; and by their habit of sleeping earlier and using a LLIN; when compared to

adult men who sleep later and spend a considerably larger amount of time outdoors and unprotected at night (Rodríguez-Rodríguez et al., 2019a). Behavioural change campaigns targeting men could aim to improve LLIN-use and promote protective clothing (long pants and long sleeves) especially at night.

Similar studies in other settings have identified specific population groups at higher risk (Gryseels et al., 2015; Monroe et al., 2019a) but evidence of the effect of targeting such groups is yet to be produced. Most currently available evidence on the effect of targeted intervention focuses on targeted IRS in areas at higher risk or so called hotspots (Bousema et al., 2016, 2012; Hast et al., 2019) or IPTp (Cates et al., 2018; Senn et al., 2012; Unger et al., 2015). The effect of targeting hotspots in reducing the local malaria burden has been underwhelming (Bousema et al., 2016; Hast et al., 2019) and the effect of IPTp has been mainly related to health outcomes at birth (e.g. low birth weight) rather than the effect on the burden of malaria. Therefore, further research could focus on addressing this gap and assessing the effect of targeted interventions based on locally identified risks for specific groups.

The completion of this survey was not without challenges and limitations. The study aimed to include 80% of the residents of each selected village. However, saturation of the sample (all households willing to participate were enrolled) was reached before our goal was achieved in most villages in Mugil. In Lemakot, time was the major constraint since in such a remote site the survey needed to be completed in less than 12 weeks. As a result, the sample size differs between sites and was below 80% in all villages but two (Bulal and Luburua). In addition, the survey was conducted from Monday to Friday which coincides with the time children are at school. In the most remote sites (Lavolai and Bulal), the proportion of school children is lower since sample collection happened while children were at school and concluded before they were back in the village. One major challenge for computing the model for Lemakot was the very low prevalence limited the power of some variables (Education level of the household head, windows type and house elevated on stilts).

This study reveals spatial heterogeneity in the prevalence distribution of malaria and LLIN use between study sites. Malaria prevalence in the Mugil area was 3.7 fold higher than in the Lemakot area. LLIN-use was 2.4 times higher in the Mugil area compared to the Lemakot area. Spatial heterogeneity of malaria was also observed at a village and households level. Prevalence between villages ranged from 0.8% to 19.5% and between households from 0% to 66.6. In the Mugil area identified risk factor related to behavioural groups and housing while in the Lemakot area LLIN ownership was a predictor for infection. The identification of site-specific risk factors

provides evidence to potentially inform complementary interventions in a local scale that target specific groups or areas.

ADDITIONAL FILES

ADDITIONAL FILE 1

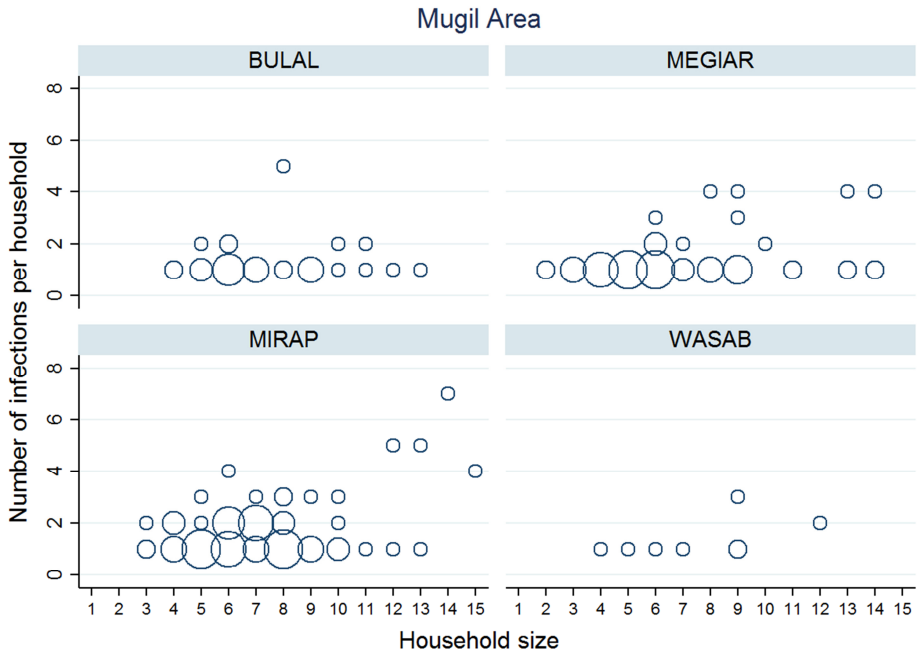

Figure 7.8. Number of infections per household by household size in the Mugil area (N=173). Size of the bubble represents occurrence frequency (range 1-8).

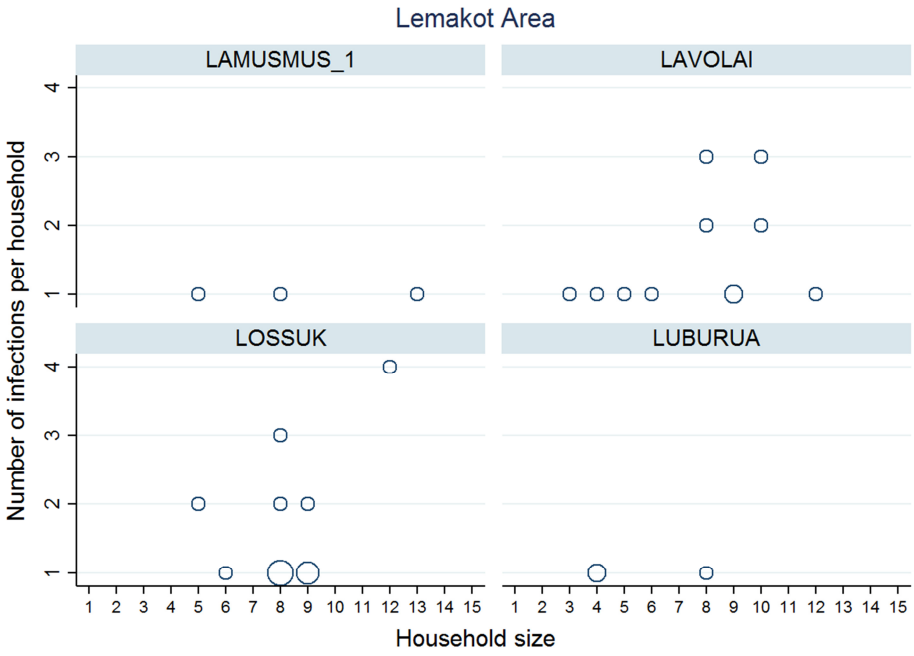

Figure 7.9. Number of infections per household by household size in the Lemakot area (N=30). Size of the bubble represents occurrence frequency (range 1-4).

8. DISCUSSION

The present work investigated the malaria trends, relevance of interventions and human behaviour for residual transmission and the role of surveillance in the context of increasing heterogeneity. The aim was to provide a better understanding of the dynamics of the heterogeneous malaria transmission and interventions rolled-out by the PNG NMCP.

This chapter starts with some clarification on the terminology used throughout this work followed by a description of Papua New Ginea's current National Malaria Strategic Plan 2014-2018 (NMSP) and the Global Technical Strategy 2015-2030 (GTS) with special focus in the role of surveillance . An assessment of the current NMSP based on the results of this work and the GTS framework follows. The chapter continues with a discussion on outputs for each specific objective (described in Chapter 2) and how the results support or challenge the NMSP expected targets. A discussion of challenges and opportunities found at different stages of this work follows. The chapter ends with the overall conclusion.

As our understanding of malaria expands, new concepts emerge and consequently the malaria terminology evolves. In some cases, concepts are not clearly defined leading to conflicting and even contradicting understanding of the same term by different groups or stakeholders. Such is the case of some concepts discussed in this work. "Malaria heterogeneity" is the term used throughout this work. However, the term is not defined by the WHO (Global Malaria Programme and World Health Organization, 2016). Variation of malaria (trends) is also referred to as 'micro-epidemiology' or emergence of 'hot spots' or 'pockets of transmission´. A recent concept that also relates to this variation is the 'transmission continuum', introduced in WHO documentation in the latest malaria surveillance manual (World Health Organization, 2018b) and described in detail later on in this section (section 8.1). Since no official term has been coined, the term 'heterogeneity' was used consistently throughout this work to refer to variations in malaria prevalence, incidence or transmission at subnational level and distinctions between the terms previously listed were not assumed. Similarly, 'residual malaria' has been a controversial term. Despite an official definition by WHO, the term has been misinterpreted and used inconsistently. Other terms commonly used interchangeable with 'residual' are 'persistent', 'refractory' and 'ongoing'. Over the course of this work, the term 'residual malaria' has been used as defined by the WHO:

“Persistence of transmission after good coverage has been achieved with high-quality vector control interventions to which local vectors are fully susceptible” (Global Malaria Programme and World Health Organization, 2016)

8.1. The National Malaria Strategic Plan 2014-2018, the Global Technical Strategy for Malaria 2016-2030 and the Role of Surveillance

A revision of the latest NMSP linked to the key findings and aligned with the GTS could provide a basis for future strategies and contribute to the understanding of the current situation of malaria in PNG. Achievements identified during the implementation of the latest NMSP should be considered when setting new targets in the future and possible solutions for identified challenges should be incorporated in upcoming strategies (Andrada et al., 2019). The following section describes the framework of the current NMSP in PNG. It continues describing the GTS focusing on the role of surveillance followed by the description of the some dynamics between the NMCP and surveillance in PNG. The section ends with an assessment of the NMSP based on key finding of this work.

The National Malaria Strategic Plan 2014-2018

The NMSP 2014-2018 was drafted in March 2014 with the long-term goal of malaria elimination from PNG by 2030 (Papua New Guinea Department of Health, 2014).The plan used five thematic areas to summarize key issues, yet this work mainly relate to the first three:

- Prevention: vector control, intermittent preventive treatment in pregnancy and infants, epidemics and emergency preparedness and response
- Diagnosis and Case Management
- Epidemiology, surveillance, monitoring, evaluation and operational research

The NMSP includes 7 objectives (Table 8.1.). Objectives 1 to 4 specially relate to this work therefore the discussion will mainly focus on these objectives (Papua New Guinea Department of Health, 2014).

Table 8.1. PNG NMSP goal and main objectives

Goal. To achieve a substantial and sustained reduction in the malaria burden in PNG (reduce Annual Parasite Incidence to 84/1,000 by 2015 and to 72/1,000 by 2018). **Objective 1.** Maintain high coverage of LLINs and increase the utilization of appropriate malaria prevention measures. **Objective 2.** Maximize access to and utilization of early diagnosis and appropriate treatment for malaria. **Objective 3.** Strengthen malaria epidemic preparedness and response capacities at all levels. **Objective 4.** Maintain malaria monitoring and evaluation systems and continue to develop the national capacity for surveillance. **Objective 5.** Expand Public Private Partnerships for key malaria interventions. **Objective 6.** Strengthen malaria advocacy, communication and social mobilization (ACSM). **Objective 7.** Further strengthen malaria program management at all levels with district level as the priority.

THE GLOBAL TECHNICAL STRATEGY FOR MALARIA 2015-2030 AND SURVEILLANCE

After the release of PNG NMSP 2014-2018 in March 2014 the GTS 2016-2030 was released. The GTS provides a framework to develop custom-made programmes to accelerate progress towards malaria elimination (World Health Organization, 2015). It aids malaria-endemic countries and their partners to define a path for malaria control and elimination until 2030. The GTS emphasizes the need for universal coverage of core malaria interventions for populations at risk (Objective 1- NMSP), and highlights the importance of employing high-quality surveillance data for decision-making in order to drive local responses consistent with national goals (Objective 3 and 4 - NMSP). The strategy also identifies areas where innovative solutions will be essential for attaining its goals (World Health Organization, 2015). In Addition, this strategy contributes to implement the sustainable development framework. A scale-up of malaria responses will help countries reach the health-related targets for 2030, and contribute to poverty reduction and other development goals. However, PNG's Millennium Development Goals (MGDs) agenda is unfinished and remains crucial in the transition from MGDs to Sustainable Development Goals (SGDs). Ideally, the GTS should be the foundation for national strategies therefore alignment between the GTS and the NMSP should be essential in PNG.

The GTS has recently transformed the role of malaria surveillance in health systems from an 'optional' tool to a core intervention (World Health Organization, 2015). Its relevance and perceived value towards elimination have been elevated. In addition, the most recent WHO guidelines for malaria surveillance introduced the concept of "malaria surveillance on the

transmission continuum" (Figure 8.1.). This notion considers the progress towards malaria elimination in a given country as a continuous process with heterogeneous levels of transmission rather than a set of independent stages with homogeneous transmission (World Health Organization, 2018b).

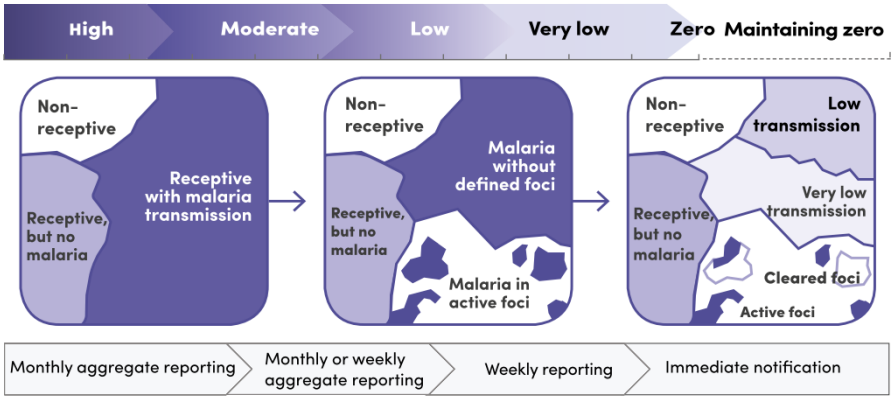

Figure 8.1. Surveillance systems and malaria heterogeneity across the transmission continuum, from World Health Organization, 2018.

The heterogeneous transmission of malaria within a given country often results in progress at different speeds at a subnational level. As transmission decreases, distribution of infection becomes more focal. In response, the intensity and frequency of surveillance reporting should increase to identify emerging areas with focal transmission. Surveillance systems should be flexible enough to migrate from reporting aggregate case data (e.g. monthly cases/district) to reporting almost real-time individual case data (World Health Organization, 2018b). A functional malaria surveillance system should: i) identify areas or groups at risk to deliver the necessary interventions, ii) assess the impact of interventions in order to adjust interventions when needed, iii) detect and respond to epidemics, iv) provide information for certification of elimination , and v) monitor re-establishment of transmission (World Health Organization, 2015).

Surveillance and the PNG National Malaria Control Programme

The achievement of the PNG national malaria control program reducing malaria prevalence to levels below 1% in 2013/14 (Hetzel et al., 2018) was unprecedented and steered the national control agenda towards elimination (Papua New Guinea Department of Health, 2014; Rosewell et al., 2017). The WHO recognized the remarkable success attained during the previous decade and stated that malaria was not a strategic priority but that efforts to strengthen a malaria surveillance system should be put in place (WHO and Government of Papua New Guinea, 2016). In July 2016 a mid-term review of the NMCP even recommended commencing a push towards elimination in some areas of the country (Rosewell et al., 2017). Unfortunately, the control efforts suffered a major setback. Results from the 2016/17 Malaria Indicator Survey revealed a

8.6-fold increase in prevalence (Hetzel et al., 2018). This thesis work progressed during the "tide change" of malaria trends in PNG and compiled data from 2010 until 2017. Therefore, the results not only documented malaria trends but also captured glimpses of the unfolding setback. Beyond the outcomes of the research, this work presents some lessons learned from the success and failures of PNG NMCP.

A sentinel surveillance system was established in PNG to evaluate and monitor the progress of the PNG NMCP (Hetzel et al., 2014c). Sentinel surveillance was chosen as a viable alternative to a fragmented, delayed and unreliable National Health Information System (NHIS) that could take up to 3 months to report summary data and up to 3 years to enter data and report individual malaria cases (Rosewell et al., 2017). In many settings, sentinel surveillance has been considered sub-optimal. However, considering the limited resources in the given setting, sentinel surveillance became a reliable source of malaria health facility data during the surveillance period (2010-2014). In addition, the health facility data was complemented with regular nation-wide prevalence surveys, catchment area censuses and entomology collections (Hetzel et al., 2014c). Chapters 4 and 5 relied mainly on the data collected at the SHFs and complemented it with the census data to add a spatial dimension to the analysis (Chapter 5).

Results in Chapter 4 and Chapter 5 depict the continuum of transmission in malaria as described by the WHO. However, the currently established surveillance systems (NHIS and SHFs) lack the flexibility to easily adapt to fluctuating trends and different levels of transmission. In an attempt to improve malaria surveillance in PNG a pilot project to strengthen the National Health Information System (NHIS) was deployed in 184 health facilities. The so called eNHIS uses a mobile interface to electronically register malaria cases at the health facility in almost real time (Rosewell et al., 2017). Despite the potential of the proposed system to improve surveillance, to date the status of the pilot is unclear and plans to scale it up are unknown.

An important set back of malaria surveillance in PNG was the halt of data collection in the SHFs between 2015 and 2017. In a setting with limited resources, it is understandable to direct resources into relief, rather than surveillance. Such was the case in PNG after losing external support from one of its major donors in 2015 (Hetzel et al., 2018). As consequence, surveillance at the SHFs stopped and the data gap coincided with the malaria resurgence in the country. The opportunity to have a better understanding of the malaria resurgence in sites with consistent surveillance was lost. The familiar "cautionary tale" that emphasizes the appalling effects of relaxing malaria control efforts before elimination proved right once more (Cohen et al., 2012) and justification for transitioning surveillance into a core intervention became clearer.

Fortunately, thanks to recent efforts to strengthen surveillance in PNG, the main sentinel sites (East Cape, Sausi, Karimui and Lemakot) have been re-established (Australian High Commissions Papua New Guinea, 2019) and the malaria trends in known SHFs are being collected once more. Continuing the efforts to strengthen surveillance in PNG and expanding a functional surveillance system nationally in the near future could better direct control efforts, identify setbacks early on and inform response.

PNG NMSP TARGET EVALUATION

In order to evaluate the progress of the NMSP a program indicator framework with 26 indicators was developed (Papua New Guinea Department of Health, 2014). More than 10 entities were responsible for the data collection, including the PNGIMR, NHIS, NMCP and Rotarians against malaria (RAM) among other institutions (Papua New Guinea Department of Health, 2014). Clear targets were included for 21 of the indicators and seven of them (Table 8.2.) were relevant for this work and assessed by this work or other publicly available data (e.g. World Malaria Report).

Table 8.2. Relevant indicators as defined by the programme indicator framework of PNG NMSP (Papua New Guinea Department of Health, 2014)

Indicator	Definition	Source	Addressed objective	Baseline	Targets
Parasite prevalence	Percentage of children aged 6-59 months with malaria infection detected by microscopy	Malaria Indicator Survey	All	13% (2010-2011)	9% (2014) 5% (2017)
Annual parasite incidence	Number of malaria cases confirmed by microscopy or RDT per 1,000 population in one year	NHIS and sentinel sites	All	205 per 1,000 (2010-2011)	84 per 1,000 (2015) 72 per 1,000 (2018)
Mass LLINs	Number of LLINs distributed through mass campaigns	LLIN distribution records	Objective 1	NA	1,312,718 (2014) 1,023,228 (2015) 1,914,830 (2016)
Household LLIN rate	Percentage of households with at least one LLIN	Malaria Indicator Survey	Objective 1	87% (2011)	>90% (2014) >90% (2017)
LLIN utilization rate	Percentage of people who slept under an LLIN the previous night.	Malaria Indicator Survey	Objective 1	49%-59% (2011)	>80% (2017)
Case confirmation rate	Percentage of reported cases confirmed either by microscopy or RDTs	NHIS	Objective 2	NA	80% (2016)
Appropriate treatment rate	Percentage of confirmed cases receiving treatment as per national treatment guidelines	Malaria Indicator Survey	Objective 2	63% (2013)	70% (2014) 80% (2015) 85% (2016)

Parasite prevalence in Chapter 7 revealed a pooled prevalence of 9.8% in 2017. In contrast, site-specific prevalence was 13.7% in the Mugil area and 3.7% in the Lemakot area. Collectively, the target (5%, 2017) was not achieved but when disaggregating by site the target was achieved in the Lemakot area. When looking at village prevalence three out of eight villages in both sites (Lamusmus 1, Luburua and Wasab) achieved the target. This finding highlights heterogeneity's role adding complexity to the control efforts. Since targets are achieved in some sites and at some levels, assessing prevalence seems as relevant as understanding the underlying drivers of heterogeneity. Reported **annual parasite incidence (API)** in PNG was 73 per 1,000 in 2015, 90 per 1,000 in 2016, and 106 per 1,000 in 2017 (World Health Organization, 2018a, 2017b, 2016). The target for 2015 (84 per 1,000) was achieved but the target for 2018 (72 per 1,000) was not and between 2015 and 2017 API increased rather than the expected reduction. Chapter 4 reported malaria incidence in four SHFs from 2010-2014. By 2014, incidence ranged from 1 to 79 per 1,000 and three of the SHFs achieved incidence levels below the target established for 2018 four years earlier than expected. Nevertheless, an increase in the malaria burden has been observed since 2014 (Hetzel et al., 2018) hampering further progress of malaria control in PNG.

According to the World Malaria Report, the **number of LLINs distributed** in PNG during 2017 was 1,694,315 (World Health Organization, 2018a), 220,515 less than the expected target for 2016. LLIN ownership and use were described in detail in Chapter 6. The target for percentage of **households with at least one LLIN** (>90%) was easily achieved in both sites (Mugil area – 100% and Lemakot area – 93%). In contrast, **LLIN utilization** differ substantially between sites and the target (>80%) was only achieved in the Mugil area were 89% of the respondents reported sleeping under a LLIN the previous night. When disaggregating by behavioural groups (or demographic groups presenting similar behavioural patterns) the target was achieved in all groups as well. But looking further into LLIN use, the percentage of respondents sleeping under a LLIN consistently **every night** was lower in all groups and only preschool aged children and school girls reached the >80% target. Moreover, only 30% of the adult men reported being in bed by 9pm despite 81% of the reporting using a LLIN. When considering LLIN use and sleeping times the indicator appear to be misleading since it reports a high but suboptimal use of LLINs especially in adults. For instance, despite a high LLIN-use in the Mugil area (90% for women and 81% for men) between 6pm and 6am 69% of the men and 72% of the women were under the protection of the LLIN half or less of this time period.

According to the World Malaria Report percentage of **confirmed cases** was 66% in 2016 and 54% in 2017 far below the established target of 80% (World Health Organization, 2018a, 2017b). The reduction in the number of confirmed cases in 2017 reflects to some extent the

reported stock-outs during the time of the study. RDT and ACT shortages are discussed in detail in section 8.2 and have recently presented a challenge controlling malaria in PNG.

Chapter 4 explored adherence to appropriate treatment at the SHFs in Additional files 8 and 9. In 2014, five out of seven SHFs reported the highest percentage of ACTs prescribed appropriately in the period between 2010 and 2014. The percentage reported was above 80% in the same five SHFs. The most recent MIS reported the percentage of malaria cases **treated appropriately** was 85% in 2016-2017 achieving the established target of 85% by 2016 (Hetzel et al., 2018). Besides ACTs, Chapter 4 also explored the adherence to Primaquine for non-P. f. and mixed or P. f. infections. In 2014, only one SHF reached 80% of such infections treated with Primaquine. In general, adequate diagnosis and treatment of malaria with ACT appears to be high in symptomatic infections with fever reaching the health facility. The prescription of Primaquine to clear liver- stage parasites appeared to be less common. According to this finding when provided with the appropriate diagnosis and treatment tools health care personnel adhere to the treatment guidelines. Therefore, it seems of utmost importance to prevent RDT and ACTs stock outs since when available they are being used as required.

Using these targets alone seems insufficient to evaluate the performance of the NMSP. However, some relevant information can be extracted from this short evaluation. Up to 2014 the malaria situation in PNG was progressing towards elimination and targets appeared achievable. In general, after 2014 malaria trends increased and simultaneously quality of care indicators revealed setbacks in delivery of care at the health facilities. Some indicators disclosed heterogeneous trends in a smaller scale. The variation within one single indicator highlights the inadequacy of some national estimates and calls for metrics in a finer scale and across groups. A similar assessment of NMSP in 22 countries in the African setting (Andrada et al., 2019) recently revealed a disconnect between newly developed NMSP and their predecessors. In many cases challenges were identified and solutions proposed but the solutions were not considered in new strategies or were only partially implemented. Furthermore, many of the targets were unachievable since targets adhered to global goals (in order to secure funds) rather than considering country specifics. The current malaria situation in PNG calls for an improved NMSP that considers the success and failures of its predecessors and set achievable goals that weights the global goals against PNG specific needs.

Despite limitations of the indicators the results could be used to guide a future strategy specially to set realistic targets that in combination with the GTS could improve the future NMSP and accelerate progress towards malaria elimination in PNG.

8.2. SPECIFIC OBJECTIVES AND RESEARCH OUTPUTS

Malaria is a complex matter with many aspects of the disease transmission yet to be understood, hence each specific objective focused on different aspects of malaria transmission and several data collection tools and analysis methods were used to address each specific objective. Specific objectives 2.1. and 2.2.; addressed by Chapters 4 and 5 respectively; are closely related since the analysis used data extracted from the same source (SHFs). Therefore, they will be assessed jointly in this section of the discussion.

Specific objective 2.1. *- To use health facility surveillance data to assess changes in malaria case incidence since the roll-out of interventions and compare the malaria burden between sites from 2010 to 2014*

Specific objective 2.2. *- To use health facility surveillance data and investigate the usefulness of the spatial disaggregation of routine data for informing targeted interventions*

Chapters 4 and 5 describe malaria trends estimated from health facility data and compare trends from 2010 to 2014 between and within SHFs. The key findings of these chapters describe to a certain extent a success story of malaria control efforts in the country. By the last year of surveillance (2014), the number of malaria cases had significantly declined in five out of seven SHFs. Distribution of LLINs were associated with reductions in the number of malaria cases at the SHFs. Furthermore, each distribution seemed to further reduce the number of malaria cases. For instance, in Karimui only five cases were reported in 2014.

Nevertheless, the same year, a resurgence was first visible in Sausi (Chapter 4 and 5) and later confirmed in other places of PNG (Hetzel et al., 2018). In addition, other events hampered malaria control progress. Firstly, a reduction in the Global Fund support to the PNG malaria control programme after 2013; next, a simultaneous decline in PNG public expenditure in the health sector (Hetzel et al., 2018); and a decrease in the availability of ACT and RDTs across PNG (Kurumop et al., 2016; World Health Organization and Government of Papua New Guinea, 2016). Lengthy stock-outs were reported in many places including the study sites (Mugil and Lemakot). Mugil HC reported stock outs to the project in May, June and July 2017 and January 2018. Lemakot health Centre did not report directly to the project at the time, but stock outs were reported informally between March and October 2017. Interestingly, the regression model in Chapter 4 did not find associations between ACT roll-out and a decrease in the number of malaria cases suggesting the lack of ACTs should not dramatically increase the number of cases at the health facilities. Yet, the roll-out of ACT was the replacement of one treatment for another implying a modest change in availability of treatment. In comparison, stock-outs imply a drastic

change in the availability of treatment. In any case, only a small proportion of all potential malaria cases (symptomatic and asymptomatic) are tested and treated in PNG (Hetzel et al., 2018). Therefore, it is unlikely that stock-outs alone are to blame for the malaria resurgence.

Outdoor and earlier biting of *Anopheles* species has been identified as a threat to LLINs effectiveness in PNG and other settings (Sherrard-Smith et al., 2019; Thomsen et al., 2017). Studies in Sausi have described a shift in mosquito biting to earlier hours following the first LLIN distribution. The peak exposure time to infective bites shifted from later than 9pm in 2008 to between 6 and 7 pm in 2011 (Reimer et al., 2016; Thomsen et al., 2017). Results in Chapter 4 and Chapter 5 identified an increase in the number of cases in Sausi by 2014 despite consistently high LLIN ownership and use in the area (Hetzel et al., 2012). Furthermore, Chapter 6 identified potential exposure to mosquito bites due to the amount of time spent outdoors (when not asleep) or in unprotected structures. Between 4pm and 8am all age groups were exposed to mosquitoes across all types of outdoors activities and when not in bed. Therefore, it is possible that the reduced efficacy of LLINs in synergy with human behaviour and ACT stock-outs led to the observed increase especially in places with historically high mosquito densities such as the Sausi and the Mugil area (Keven et al., 2019; Reimer et al., 2016).

The threatened effectiveness of LLINs should be carefully considered since LLINs remain a core intervention not to be substituted but complemented with other interventions. LLIN distributions in PNG have been hugely successful despite the vectors biting outdoors more than in the African setting (Charlwood et al., 1986; Keven et al., 2019; Sinka et al., 2011). Results in Chapter 4 suggest that removing LLIN distributions is not advisable, since their effect on reducing malaria cases is considerable and likely to increase with additional distributions. Instead, complementing the LLINs with other targeted interventions specific for outdoors activities (Chapter 6) and earlier biting is more likely to interrupt transmission.

Chapter 7 showed prevalence in Mugil was 3.7 times higher than in the Lemakot area. A more accentuated malaria heterogeneity with greater variation at a subnational level could translate to a greater challenge for malaria control and surveillance & response. Some places in the country such as Lemakot seem ready to move towards pre-elimination. In contrast, places like Mugil are back to the prevalence levels observed in 2010. Such differences suggest the need for a flexible surveillance & response system that can transition to individual case reporting in the Lemakot area while reporting aggregated data on a monthly basis in the Mugil area. In addition, an increase such as the one recently observed should be prevented in the future and response to such increase should be deployed at early stages of any potential outbreak. The absence of a

response to the recent resurgence highlights the need for an improved surveillance & response system in PNG.

A functional surveillance & response system with clear epidemic warning protocols should be considered a priority in PNG (Objective 3 – NMSP) since malaria response has not been clearly established to target interventions or respond to epidemics (Rosewell et al., 2017). A method similar to the one presented in Chapter 5 could offer an initial step to inform malaria control but a simple tool for calculating and mapping malaria case incidence at district or sub-district level is required to operationalize the approach. Recent efforts to strengthen surveillance in PNG could enable an initial warning system. The WHO cooperation strategy with PNG established interest in consolidating a malaria surveillance system (World Health Organization and Government of Papua New Guinea, 2016). In addition, a malaria registry that records the village of residence of test-confirmed malaria cases has already been introduced in all health facilities in PNG. A opportunity to validate this approach at larger scale could be the eNHIS pilot (Rosewell et al., 2017) since it includes features to map malaria cases at a village level and identify malaria outbreaks that, if updated timely, could constitute an adequate surveillance system that could inform a response.

Specific objective 2.3. *- To investigate the distribution of malaria infection across spatial clusters and population sub-groups in order to identify the extent of residual malaria at the time of study*

Results from Chapter 7 explored the heterogeneity of malaria trends between sites, villages, and households. For instance, it is likely that the transmission level is lower in Lemakot than in Mugil area. The age distribution of malaria cases in Lemakot is more homogeneous than in Mugil area (Chapter 7 –Figure 7.3.). The resulting flat age curve is expected in areas with lower, unstable transmission, where a small number of individuals are infected and the risk of symptomatic infection is comparable in all age groups since none have acquired immunity (Fowkes et al., 2016). In contrast, the age distribution of malaria cases in Mugil area is more heterogeneous with a peak in the youngest age groups. In places with higher transmission, young children are the most affected since they have not yet acquired immunity and are susceptible to develop high density infections (Fowkes et al., 2016). Observing the spatial distribution of household prevalence, a statement made in 1937 by Hackett comes to mind: "[Malaria is] so moulded and altered by local conditions that it becomes a thousand different puzzles (Hackett, 1937)". Each would have to be solved on its own terms. Some spatial patterns were identified in the villages. However, the fewer number of cases, the more difficult it

becomes to use conventional statistical methods (e.g. regression) to identify clusters and risk factors.

Risk factors were additional 'puzzle pieces' explaining variations and commonalities between settings. In both sites, adult women were at lower risk of malaria infection compared to preschool aged children. In contrast, school girls and boys were at higher risk of malaria infection than preschool aged children only in the Mugil area. LLIN-ownership (one LLIN per two people) had a protective effect at the time of the study in the Lemakot area; a site with lower ownership and use of LLINs than the Mugil area. It seemed like in areas with consistently high LLIN ownership and use, other factors – like behaviour – could drive transmission. Whereas in areas with lower ownership and use, LLINs still play a major role driving transmission. Housing was a relevant aspect of transmission in the Mugil area. Traditional housing and screened windows were associated with a fewer infections in the household. These findings highlight the potential for improved housing to reduced residual transmission. Window screening and protective walls could be used as an intervention if the setting allows for it (Kaindoa et al., 2018; Tusting et al., 2017). In general, the housing structures were better in the Lemakot area. Materials were improved, less impervious to mosquitoes, and in better condition than in the Mugil area. Interestingly, the use of improved material in the Mugil area seemed counterproductive since those materials were more difficult to acquire compared to raw materials and more expensive to replace. Damaged walls and roofs with big entry points for mosquitoes were often observed. In comparison, households built only with raw materials were easier to maintain resulting in wall and roofs with fewer holes for mosquitoes to enter. This could explain the identified risk for malaria infection in residents of houses built by mixing raw and improved materials compared to residents of traditional houses in the Mugil area. In the Lemakot area, the better condition of the road, the constant access to cash and transport provided by the surrounding oil palm plantations are factors likely to improve housing, which in turn could potentially reduce risk to malaria infection and partially explain the low prevalence in the area despite the lower use of LLINs.

Chapter 7 has been crafted as a working paper since it is part of a broader project that is likely to combine 'puzzle pieces' of different aspects of malaria to provide a more comprehensive understanding of transmission in the study sites. Entomology data and sub-microscopic prevalence will complement the behavioural data and light microscopy prevalence. Some preliminary results have assembled 'pieces' of entomological data with the results of Chapter 6 and 7. A surprising finding in one of the study villages was a high number of *Anopheles* in the bathing area (river bank) designated to females while none were found in the area where men

bathe. A more in-depth analysis of all the findings combined is expected to offer a profound understanding of local residual transmission in our study sites.

Specific objective 2.4. *- To better understand the role of human behaviour in relation to malaria transmission and transmission heterogeneities in selected sites*

Understanding of human behaviour is crucial to identify when and where malaria transmission occurs (Lindblade, 2013; Monroe et al., 2019b). Identification of activities occurring during biting times of malaria vectors is essential for targeting existing interventions and developing complementary interventions (Monroe et al., 2019b). Chapter 6 unveiled the potential exposure to mosquito bites for all age groups while not asleep and raises concern since standard control interventions do not offer protection beyond secure sleeping areas. The considerable amount of time spent outdoors while malaria vectors are active and the suspected shift in peak biting times (Sherrard-Smith et al., 2019; Thomsen et al., 2017) translates to potentially great exposure to outdoor mosquito bites. Behavioural patterns within demographic groups were identified (Preschool aged children, school girls, school boys, adult women in Mugil, adult women in Lemakot, adult men in Mugil, and adult men in Lemakot) in Chapter 6. Women in Lemakot and Mugil were at lower risk of infection than preschool aged children. In Mugil school children (boys and girls) were at increased risk of infection than preschool aged children. Interestingly, the behavioural pattern identified in men (e.g. later sleeping time and lowest used of LLINs) would be expected to expose them to a greater risk of infection but such risk was not identified in Chapter 7. Compared to children, adult men are more likely to have acquired immunity resulting in lower parasitaemia less likely to be detected by light microscopy. When moving toward elimination, identifying and treating asymptomatic carriers becomes a pressing issue along with the need for improved diagnosis beyond light microscopy. Appendix 1 discusses the importance of suitable diagnostic tools for malaria elimination. In order to identify most if not all infections with potential to transmit the malaria parasite qPCR was the recommended diagnostics method. However, implementation challenges need to be considered. Perhaps an alternative to qPCR are ultra-sensitive RDTs but further evidence is needed in order to justify their use and assess their effectiveness in the PNG setting.

A very relevant aspect of behavioural studies is the possibility to identify groups, places or activities that are likely to expose people to the vectors. For instance, spatial repellents could be used in areas where people are likely to gather at night like screening areas, churches or training fields. Roads and schools could also be targeted in sites where school children are at higher risk. Further studies in different settings within the country could help generalize

behavioural groups in sites not explored by this work. Similar studies in other settings have identified specific population groups at higher risk (Gryseels et al., 2015; Monroe et al., 2019a) but evidence of the effect of targeting such groups is yet to be produced. Currently available evidence on the effect of targeted intervention mainly focuses on targeted IRS in areas at higher risk or so called hotspots (Bousema et al., 2016, 2012; Hast et al., 2019) or IPTp targeting vulnerable groups like pregnant women (Cates et al., 2018; Senn et al., 2012; Unger et al., 2015). The effect of targeting hotspots in reducing the local malaria burden has been underwhelming (Bousema et al., 2016; Hast et al., 2019).The effect of IPTp has been mainly associated to health outcomes at birth (e.g. low birth weight) rather than to the effect on the burden of malaria. Therefore, further research could focus on addressing this gap and assessing the effect of targeted interventions based on locally identified risks for specific groups. In addition, a standardized method (additional key questions in the MIS referring to sleeping and waking times, and outdoor activities after 6pm) to assess behaviour and compare results between different setting could accelerate identification of possible targets (activities, places, or groups) for complementary interventions.

A relevant aspect of livelihood in our study sites was housing. A distinction conventionally made between indoors and outdoors spaces does not seem to apply to many households in the study sites. Usually, housing structures with walls ease the use of LLINs (e.g. provide a place to hang LLINs) but additional protection from mosquito exposure is likely to be as limited as it is outdoors since many structures are open. Appendix 2 offers visual examples of how the indoors and outdoors definition might not always apply to this setting. In addition, the only consistently reported method to prevent mosquito bites while outdoors was smoke from a fire. Outdoor prevention methods such as protective or treated clothing, mosquito coil or mosquito repellent were very rarely used. This finding suggests that such prevention methods are not suitable for this setting. The cost and local unavailability of these products drastically limit their use. Innovative tools adapted to the setting are needed for mosquito bite prevention, perhaps the use of insecticide treated bilums or attractive toxic sugar baits to reduce mosquito density and potential exposure could be explored. In addition, results in Chapter 6 highlight the limits on the protection offered by LLINs, especially for adults. Despite a high LLIN-use in the Mugil area (90% for women and 81% for men) 69% of the men and 72% of the women were under the protection of the LLIN only half of the time between 6pm and 6am. These findings highlight the need for complementary interventions that address outdoor biting rather than more intensive LLIN distributions.

Physical, biological and social environments play a crucial role in the epidemiology of malaria transmission (Heggenhougen et al., 2003). Under certain circumstances, physical environments

provide favourable conditions for the *Anopheles* to thrive, biological environments provide beneficial relationships between vector and host or other species; and social environments such as the ones explored in Chapter 6 could increase exposure risk. Interestingly, physical and biological environments modify social environments as well. A clear example is the collection of the mud crab in the Lemakot area. The physical and biological characteristics of the local environment allow the mud crab to thrive in Lemakot area, thus becoming an important source of food and income (Frijlink et al., 2016) which in turn is relevant to malaria transmission since collection of the mud crabs occurs between 6pm and 8pm in mangrove areas and could potentially maintain malaria transmission in the area. Better understanding of such environmentally driven behaviours paired with local entomology surveys could provide evidence to target complementary intervention and possibly interrupt transmission in areas with a small number of malaria cases, such as the Lemakot area.

Challenges and Limitations

One limiting factor while assessing the impact of interventions (Chapter 4) was the scarcity of contextual data. Reliable nationwide demographic data has not been updated since 2000. The census that year was the latest to release a complete database for PNG population. Most of the currently available data has been aggregated in national, regional or provincial estimates that do not allow for analysis at a finer scale (e.g. site or village level). Since incidence cannot be estimated without a denominator, the lack of denominators for the catchment population of three of the SHFs forced the analysis to focus on number of cases for most part (Chapter 4) or exclude these three facilities from the analysis (Chapter 5).

Weather variables were another crucial but scarce piece of data. Previous to the use of remote sensing data (Chapter 4) an effort to measure weather variables on site was made. Weather stations (Davis Instruments, n.d.) were set up in the sentinel sites but receivers deteriorated rapidly due to frequent voltage spikes in the power line and receivers had limited space in memory that required regular visits to extract the data. Lack of internet access in the SHFs obstructed the possibility to send the data digitally. Efforts to collect weather data on site were abandoned after the attempts to fix the receivers failed. In addition, it appeared not much of these data is routinely collected and or made available to the public by any governmental agency in PNG. Rainfall, vegetation and temperature estimates were extremely difficult to obtain at a SHF level. Ultimately, satellite data was used for the analysis but satellite data has its limitations especially in Melanesia, a regions with extensive (in area) and extended (over time) cloud cover (Hoekman et al., 2010; McAlpine et al., 1983). Cloud cover considerably reduces optical remote sensing monitoring accuracy. Moreover, extraction & management of satellite

data requires special software and satellite data files use up a considerable amount of space in memory restricting accessibility to this data in a setting with limited infrastructure.

The weather data in Chapter 4 disclosed a chaotic pattern that suggested a lack of seasonal variations in the observed variables (rainfall and EVI). Specific periods that correspond to dry and rainy season in the study sites were not identified. Interestingly, data collected qualitatively confirms this notion. Seasonality was one of the topics explored by IDIs and FGDs. When asked about wet and dry season participants were unfamiliar with the concept. Most of the responses referred to rainy and sunny days rather than periods of weeks or months. And when explicitly asked to delimit months of the year with higher amounts of rain respondents could not identify a specific period during the year. The few identified periods often did not coincide between respondents in the same site. Some respondents referred to their traditional knowledge to signal seasons, yet they described them as imprecise or out-dated to identify seasons. Allusions to a changing weather increasingly difficult to predict also emerged during data collection. This allusion to a changing weather could refer to visible signs of climate change. Nevertheless, the effects of El Niño/ La Niña phenomena are known to drastically alter weather patterns in the area (Carlowicz and Schollaert Uz, 2017).

Systematic collection of weather data is relevant for the health sector to monitor malaria and other environmentally driven diseases (e.g. dengue) but it is also relevant to other sectors (e.g. agriculture, fisheries). Collaboration with the Papua New Guinea Weather Services in future projects would be recommended to maximize resources and promote cooperation between different sectors. Such collaboration has been previously reported in a similar setting (Smith et al., 2017) highlighting the viability for intersectoral collaborations. Inter-disease programme and inter-sectorial collaboration could also be explored for vector control and such approaches are in line with the GTS and the Global vector control response 2017–2030 (World Health Organization, 2017a, 2015).

Data collection at the SHFs required substantial coordinated efforts since numerous logistical difficulties complicated data collection and microscopy readings. For instance, in order to standardize microscopy readings in all SHFs, one central microscopy laboratory was established for the whole study. Therefore, all blood slides were fixed and stained at the SHFs and then air shipped regularly for microscopy reading. In addition, all data was collected on paper forms which resulted in air shipping the forms to a central data entering office on a regular basis. Aside from the logistics, appropriate training for the nursing personnel before the surveillance and regular supervision to ensure quality of the data was essential. Efforts and resources were put in place to overcome challenges and data collection in the SHFs was a successful

surveillance system from 2010 to 2014. Maintaining surveillance in the sentinel health facilities as an initial surveillance system is advisable since these health facilities have by now a known history of malaria trends. The precedent of successful health facility surveillance despite the challenges of the setting eases the possibility of expanding surveillance nationally in a longer term towards elimination.

The location and time period for data collection restricted the sample size in Lemakot area. The Mugil community is located a 50km drive away from PNGIMR Madang branch. Such proximity allowed frequent visits and extended periods of time to carry out data collection. In contrast, Lemakot is an Island Province only accessible by air. The remoteness of the site posed a significant challenge to overcome during data collection. A fully equipped field team of 10 people was deployed for 11 weeks to complete data collection in Lemakot resulting in a smaller sample size.

OPPORTUNITIES

This work was completed through a close collaboration of mainly two institutions: The Papua New Guinea Institute of Medical Research and The Swiss Tropical and Public Health Institute. Both institutions are deeply committed to improve health and wellbeing of populations through scientific research and action guided upon knowledge ("PNGIMR - About PNG Institute of Medical Research," n.d., "Swiss TPH - About Swiss TPH," n.d.; Reeder, 2003). Aligned with such motivation and vision the ultimate goal of this work is to improve health of Papua New Guineans by generating evidence and informing health services and communities. Malaria is a scourge that devastates populations hence efforts to alleviate the disease improves peoples' health and wellbeing.

Since the ultimate aim of this work is to inform health authorities, stake holders, and communities, efforts have been made to achieve this. The initial formats used in the field to inform communities were informal meetings at the community, brochures and information in the health facility through the research nurse. Brochures (Appendix 3) were developed in a lay accessible way with two aims: to inform about the study beforehand and to disseminate preliminary findings. The development of an updated version with final results in Chapter 6 and 7 is in the making and should be distributed within the participating communities during upcoming meetings in the near future. The meetings will include a general discussion aiming to answer all questions raised by the community and their leaders. An important message to convey to all communities is the importance of getting a diagnosis before taking any treatment and of completing the course of treatment when prescribed by a health professional. Promotion of LLIN-use especially for men as way to prevent them to get infected but also as a way to

protect others will be addressed. The last key message would be to raise awareness on the potential of outdoors biting to fuel transmission. Some recommendations include wearing protective clothing (long sleeves and long pants) at night when outdoors and a shift to earlier sleeping times.

Spatial disaggregation of data over time is challenging to share in a conventional paper and most academic papers are inaccessible to non-academic partners and stakeholders. Therefore, Chapter 5 used a video as an innovative format for a scientific publication to communicate complex spatial relationships in an engaging and accessible way for project managers, health authorities, funding organizations and project partners. This video was produce following the WHO strategic communication framework that recommends making information visual and easy to understand (World Health Organization, 2017c).

PNGIMR offered a range of opportunities to successfully complete this work. Over the past decade, PNGIMR has established a number of malaria research sites for which data on malaria burden and intervention coverage are available. These sites have developed as ideal locations for in depth investigations of residual malaria transmission after the roll-out of standard interventions. PNGIMR has established enduring international research partnerships, close links to all NMCP stakeholders and crucial relationships with disease-affected communities in the study sites. The institute is in a unique position to contribute to further progress towards malaria elimination in PNG. The field teams are a group of committed people from local communities that understand local context and are truly engaged with improving people's health. Despite the human resources crisis in PNG health workforce (The World Bank, 2011) our local team included nursing personnel trained in research and with ample experience.

Finally, a great advantage that eased and hastened data collection & management for the study was the transition from paper based to electronic based data collection The availability of an open source platform such as ODK (Anokwa et al., 2009; Hartung et al., 2010; Rajput et al., 2012) lessened the financial burden that often comes with technological improvements. The already available mobile phone technology in the country facilitated the transition. The data collection devices (tablets and phones) were purchased locally and telecom infrastructure was regularly available with few network failures. Field staff was familiar with the technology and enthusiastic to migrate data collection to electronic instruments.

8.3. CONCLUSION

During the course of this work, malaria elimination from PNG by 2030 became less likely than when it was originally envisioned in the National Malaria Strategic Plan 2014-2018. The resurgence in malaria is likely to worsen unless malaria control is re-intensified and maintained. Structuring programmes in response to evidence of the local malaria burden together with an analysis of transmission will enable the adapting the strategy to the local context and optimize the use of resources. However a strong and functional surveillance & response system is needed to monitor the local burden and inform control efforts. Evidence in this study documented reasonable high LLIN ownership across study sites; however LLINs use can be improved in some areas. RDTs and ACTs were not always available in the health facilities therefore efforts need to be made to assure availability especially in areas with higher transmission. Since outdoor biting was consistently identified as an exposure risk and specific groups and areas at higher risk were also identified targeted complementary interventions could be explored and piloted in PNG. Further studies could address the current evidence gap on the effectiveness of targeted interventions.

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APPENDIX 1

Articles

Assessment of ultra-sensitive malaria diagnosis versus standard molecular diagnostics for malaria elimination: an in-depth molecular community cross-sectional study

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Summary

Background Submicroscopic malaria infections contribute to transmission in exposed populations but their extent is underestimated even by standard molecular diagnostics. Sophisticated sampling and ultra-sensitive molecular methods can maximise test sensitivity but are not feasible in routine surveillance. Here we investigate the gains achievable by using increasingly sensitive methods with the aim to understand what diagnostic sensitivity is necessary to guide malaria interventions.

Methods Venous blood samples were collected from participants in a cross-sectional survey in two coastal medium-endemic villages in Madang province, Papua New Guinea. Using ultra-sensitive quantitative PCR (us-qPCR) on concentrated high-volume blood samples (2 mL) as reference, we quantified the proportion of *Plasmodium falciparum* and *Plasmodium vivax* infections and gametocyte carriers detectable in fingerprick blood volumes (200 µL) by standard 18S rRNA qPCR, us-qPCR, rapid diagnostic test (RDT), and ultra-sensitive *P falciparum* RDT. We further compared the epidemiological patterns observed with each diagnostic approach in the study population.

Findings Venous blood samples were collected from 300 participants between Dec 5, 2016, and Feb 24, 2017 (ie, during peak rainy season). Standard qPCR identified 87 (54%) of 161 *P falciparum* infections and 73 (52%) of 141 *P vivax* infections detected by the reference method. us-qPCR identified an additional 11 (7%) *P falciparum* infections and 14 (10%) *P vivax* infections. 80 (86%) of 93 *P falciparum* gametocyte carriers and 75 (91%) of 82 *P vivax* gametocyte carriers were found among infections detectable by us-qPCR. Ultra-sensitive RDT missed half of *P falciparum* infections detected by standard qPCR, including high gametocytaemic infections. Epidemiological patterns corresponded well between standard qPCR and the reference method. As the prevalence of *P vivax* decreased with increasing age, the proportion of *P vivax* infections undetectable by standard qPCR increased.

Interpretation Almost all potentially transmitting parasite carriers were identified with us-qPCR on fingerprick blood volumes. Analysing larger blood volumes revealed a large pool of ultra-low-density *P falciparum* and *P vivax* infections, which are unlikely to be transmitted. Therefore, current RDTs cannot replace molecular diagnostics for identifying potential *P falciparum* transmitters.

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Introduction

During the past decade, malaria epidemiological studies have increasingly applied molecular methods for diagnosis of infections. This approach has revealed that a large proportion of malaria infections in naturally exposed populations is characterised by low parasite densities that are undetectable by light microscopy or a rapid diagnostic test (RDT).[1,2] Although chronic low-density infections are associated with negative clinical consequences in the long term,[3] they have no acute pathological impact and might even confer protection against severe malaria episodes.[4] In the context of malaria control, the main relevance of chronic low-density infections is their contribution to maintaining malaria transmission.[5,6]

Therefore, maximal detection of low-density malaria infections is often considered important for countries aiming to eliminate malaria; however, this goal is challenging in the context of routine surveillance strategies. The detection of low-density infections requires active surveillance of entire populations with use of molecular diagnostic tests, which are most commonly based on amplification of the plasmodium 18S rRNA gene from fingerprick blood samples.[7] In 2017, the first ultra-sensitive *Plasmodium falciparum* RDT (us-RDT) was launched for simplified detection of low-density malaria infections in surveillance screens.[8]

In the past decade, improved nucleic acid amplification techniques have set increasingly high standards in test sensitivity by use of multi-copy target genes[9] or increasing the blood volumes processed.[10] In Tanzania and southeast Asia, these approaches have revealed low-density infections that would not be detected by standard

Research in context

Evidence before this study

We searched PubMed for publications, without any language restriction, until March 1, 2018, using the search terms "plasmodium" AND ("falciparum" OR "vivax") AND ("sub-microscopic" OR "submicroscopic" OR "ultra-sensitive" OR "ultrasensitive") AND ("pcr" OR "polymerase chain reaction"). We retrieved 135 studies, which were screened for the sample type (venous blood *vs* fingerprick), sample volume, and type of (molecular) analysis method used for detection of malaria infection. At the Thailand–Myanmar border and in Vietnam, a few studies investigating ultra-low parasitaemias in asymptomatic carriers applied a detection method by Imwong and colleagues that uses venous blood combined with standard quantitative PCR (qPCR). However, this method does not allow species determination of the lowest plasmodium parasitaemias, and no direct comparisons were made to standard sampling and molecular detection methods used by the vast majority of malaria epidemiological studies. One study by Das and colleagues assessed the performance of a new ultra-sensitive lateral flow *Plasmodium falciparum* rapid diagnostic test (us-RDT) in Myanmar and Uganda. We found no studies investigating the presence of gametocytes among ultra-low-density malaria infections, which serves as a surrogate marker of their potential to contribute to malaria transmission.

Added value of this study

In many endemic areas, the aim of antimalarial interventions has shifted from just treating clinical cases to also reducing or eliminating malaria transmission. This goal entails the identification and treatment of asymptomatic parasite carriers who are characterised by low parasite densities, but still can maintain malaria transmission. Improved diagnostic techniques have revealed a large reservoir of such infections below the microscopic detection threshold, and even below the limit of detection of standard molecular techniques. However, the venous sampling required for detection of the lowest parasitaemias is not feasible in routine surveillance and intervention monitoring. Our study therefore addresses the question of how many *P falciparum* and *Plasmodium vivax* infections are missed in population-based studies using standard molecular malaria diagnostics or a new *P falciparum* us-RDT. Our study evaluated the relevance of these missed infections in the context of malaria interventions by detecting gametocytes (transmission stages) in high-volume samples.

Implications of all the available evidence

Our findings show that a large proportion (up to 50%) of *P vivax* and *P falciparum* infections are undetected by standard molecular diagnostics using finger-prick blood volumes in cross-sectional studies. Despite this large number of missed detections, standard molecular malaria diagnostics suffice to investigate the epidemiological patterns in the population and to identify virtually all parasite carriers with gametocyte densities that are meaningful for onwards transmission. By contrast, us-RDT missed a large number of *P falciparum* infections with high gametocyte densities. Our findings thus reduce the pressure to apply venous blood sampling for ultra-sensitive molecular diagnostics, while casting doubt on the effectiveness of implementing the us-RDT in interventions aiming at reducing malaria transmission.

molecular malaria diagnosis (ie, 18S rRNA quantitative PCR [qPCR] on fingerprick samples).[9,11] More awareness of the extent, epidemiology, and relevance of these missed ultra-low-density *P falciparum* and *Plasmodium vivax* infections is required in the context of efforts towards malaria elimination and for discovery of remaining pockets of transmission.

Venous blood sampling and sophisticated sample processing are required for the most sensitive molecular diagnostic tests, which are feasible in research studies but not in large-scale surveillance. Therefore, in this study, we aimed to address the question of whether the use of highly sophisticated molecular detection methods provides more useful information for design and monitoring of malaria interventions compared with standard molecular detection. To this end, we systematically validated the proportion of *P falciparum* and *P vivax* infections as well as gametocyte carriers detected in samples from a community survey using different blood volumes, different molecular diagnostics, standard RDT (st-RDT), and a novel us-RDT.[8] We compared the epidemiological patterns observed with each diagnostic approach to investigate whether certain subgroups of the human host population are of greater importance than others for harbouring of low-density malaria infections. The knowledge gained could be used as a benchmark for the design of surveillance strategies, in which maximisation of test sensitivity has to be balanced against the feasibility of venous bleeding.

Methods

Study design and participants

Venous blood samples were collected from participants in a cross-sectional survey in two coastal medium-endemic villages in Madang province, Papua New Guinea.[12] Sample collection was embedded in a larger census-based cross-sectional survey, during which participants aged 5 years and older (excluding pregnant women) could volunteer for venous sampling. After written informed consent was obtained, a health status assessment was undertaken and a standard electronic prevalence questionnaire was completed, followed by a brief interview. 5 mL of venous blood were collected in sodium-heparin-coated vacutainers (BD Biosciences, Franklin Lakes, NJ, USA). 800 µL of blood were immediately stabilised in RNAprotect Cell Reagent (Qiagen, Hilden, Germany).

Participants presenting with signs and symptoms of malaria infection (>37·5°C axillary or reported fever

For more on the **electronic prevalence questionnaire (Malaria Indicator Survey Toolkit)** see http://malariasurveys.org/toolkit.cfm

in the previous 2 days) were tested using the CareStart HRP2/pLDH (Pf/PAN) Combo RDT (AccessBio, Somerset, NJ, USA). Test-positive participants were treated according to national guidelines.

Ethical approval for the study was obtained from the Papua New Guinea Institute of Medical Research Institutional Review Board (PNGIMR IRB number 1516) and the Medical Research Advisory Committee of the Papua New Guinea Ministry of Health (MRAC number 16.01).

Sample processing and nucleic acid extraction

See Online for appendix

Whole-blood aliquots of 200 µL (chosen to mimic fingerprick blood samples) and 2 mL were separated into red blood cell (RBC) pellet and plasma. RBC pellets from the 2 mL blood aliquots were depleted of white blood cells by Ficoll Paque Plus (GE Healthcare, Chicago, IL, USA) gradient centrifugation. RBC pellets, RNAprotect samples, and whole-blood aliquots of samples with sufficient volume were stored at −20°C.

DNA was extracted from the RBC pellets within 3 months using the QIAamp 96 DNA Blood Kit (Qiagen) for small RBC volumes and QIAamp DNA Blood Midi Kit (Qiagen) for large RBC volumes according to the manufacturer's instruction. DNA obtained from small RBC volumes was eluted in 100 µL, yielding twofold template concentration with respect to the original blood sample. DNA from large RBC volumes was eluted in 400 µL, yielding fivefold template concentration. For samples that were qPCR-negative for *P falciparum* or for *P vivax* when analysing DNA from small and large blood volumes, a 200 µL aliquot of DNA from the large blood volume was further concentrated tenfold by sodium acetate and ethanol precipitation, yielding a final 50-fold concentrated template. RNA was extracted from the pelleted RNAprotect samples within 6 months using the RNEasy Mini Kit (Qiagen) according to the manufacturer's protocol, including an on-column DNAse digest.[13] RNA was eluted in 80 µL, yielding a tenfold template concentration compared with the original blood sample.

	Plasmodium falciparum			*Plasmodium vivax*		
	$n_{positive}$	Prevalence (95% CI)	Proportion detected*	$n_{positive}$	Prevalence (95% CI)	Proportion detected*
Standard qPCR						
All positive	87	29% (24–35)	54%	73	24% (20–30)	52%
Gametocyte carriers†	71	24% (19–29)	76%	67	22% (18–28)	82%
Ultra-sensitive qPCR						
All positive	98	33% (27–38)	61%	87	29% (24–35)	62%
Gametocyte carriers†	80	27% (22–32)	86%	75	25% (20–30)	91%
High-volume ultra-sensitive qPCR						
All positive	159	53% (48–59)	99%	135	45% (39–51)	96%
Gametocyte carriers†	93	31% (26–37)	100%	81	27% (22–32)	99%
Any PCR (reference)*						
All positive	161	54% (48–59)	NA	141	48% (42–53)	NA
Gametocyte carriers†	93	31% (26–37)	NA	82	27% (22–33)	NA

$n_{positive}$=number of positive samples. qPCR=quantitative PCR. NA=non-applicable. *The proportion of parasite carriers detected by each method was calculated against the combined detections by any qPCR (defined as reference). The overlap between diagnostic methods, as well as an assessment of diagnostic performance of each assay (sensitivity and specificity), is shown in the appendix (p11–12). †Gametocyte carriers among all positives detected by the respective qPCR method.

Table: **Comparison of molecular methods with increasing sensitivity for the detection of *Plasmodium falciparum* and *Plasmodium vivax* infections and gametocyte carriers**

Detection of malaria infections

Standard qPCR for detection of *P falciparum* and *P vivax* used previously published 18S rRNA assays[13,14] with a modified *P falciparum* reverse primer (PFS18S_revMAO 5′–TATTCCATGCTGTAGTATTCAAACACAA–3′).[15] Ultra-sensitive qPCRs with increased limit of detection compared with standard qPCR[9] (appendix pp 1–2) targeted the *P falciparum var* gene acidic terminal sequence (*Pf*-varATS)[9] or the *P vivax* mitochondrial *cox1* gene (*Pv*-mtCox1).[16] Presence of gametocytes was investigated in all *P falciparum*-positive or *P vivax*-positive samples using previously published *pfs25* and *pvs25* qRT-PCR assays.[13]

All molecular assays used 4 µL of template material; hence, the blood volume equivalent per reaction ranged between 8 µL and 200 µL whole blood (appendix p 3). Parasitaemia or gametocytaemia was quantified in relation to a standard row of target-specific plasmid[13] and adjusted according to the concentration factor of DNA template with respect to whole blood.

All small blood volume DNA samples were tested using *P falciparum* and *P vivax* 18S rRNA, *Pf*-varATS, and *Pv*-mtCox1 qPCRs. Throughout this Article, 18S rRNA qPCRs on small blood volume DNA samples are referred to as standard qPCR (st-qPCR) and *Pf*-varATS and *Pv*-mtCox1 qPCRs on small blood volume DNA samples as ultra-sensitive qPCR (us-qPCR).

Eluted high-volume DNA samples were tested using *Pf*-varATS and *Pv*-mtCox1 qPCRs. Samples negative for *P falciparum* or *P vivax* on eluted DNA from both small and large blood volumes were further tested in *Pf*-varATS and *Pv*-mtCox1 qPCRs using concentrated large-volume DNA. Results obtained by *Pf*-varATS or *Pv*-mtCox1 qPCRs on eluted and on concentrated high-volume DNA were combined and are further referred to as high-volume us-qPCR (hv-us-qPCR).

Parasite densities correlated well between different molecular detection methods, with stronger correlations observed for *P falciparum* than *P vivax* (Spearman's r=0·86–0·92 for *P falciparum*; r=0·80–0·86 for *P vivax*; appendix p 4).

Samples for which frozen whole blood was available were tested with a *P falciparum*/*P vivax* st-RDT (Malaria Ag P.f/P.v, Standard Diagnostics, Yongin-si, South Korea) and a *P falciparum* us-RDT (Malaria Ag Pf Ultra-Sensitive, Standard Diagnostics) using 5 µL of thawed whole blood. Mean *P falciparum* and *P vivax* parasite densities in samples tested by RDT were not significantly different from the full set of samples or samples not tested by RDT.

Statistical analysis

We aimed to evaluate whether certain population subgroups harbour more ultra-low-density infections than others and to compare the epidemiological patterns observed with the different diagnostics. To this end, we modelled the effect of covariates on the odds of detecting a *P falciparum* or *P vivax* infection or gametocytaemia using multivariable logistic linear regression. We selected covariates a priori on the basis of previous knowledge. We calculated univariate factors for RDT-diagnosed *P falciparum* infections resulting from the low number of positive detections. We used R version 3.4.1 for all analyses. Packages plyr and reshape2 were used for structuring of data; packages limma, gplots, beeswarm, and forestplot for production of graphics; package *zoo* was used to calculate a rolling mean of diagnostic sensitivity.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Venous blood samples were collected from 300 participants between Dec 5, 2016, and Feb 24, 2017 (ie, during peak rainy season). Whole blood was available for 247 of 300 samples after RNAprotect samples had been prepared and DNA extractions from small and large blood volumes had been done. Demographics of the study population were comparable between the two study villages. In Megiar (n=163), mean participant age was 30 years (SD 16·7; median 31 years [IQR 14–43]), and in Mirap (n=137) it was 28 years (16·1; median 24 years [14–40]). 78 (48%) participants in Megiar and 76 (55%) participants in Mirap were male. 124 (76%) participants in Megiar and 123 (90%) in Mirap reported having slept under a bednet in the preceding night. 20 (7%) participants presented with fever or reported fever within the 2 preceding days, and 24 (8%) participants reported antimalarial treatment within the past month.

We detected *P falciparum* in 159 (53%; 95% CI 48–59) and *P vivax* in 135 (45%; 39–51) participants using large blood volumes and hv-us-qPCR (table). Using st-qPCR (on small volumes), we identified 87 *P falciparum* infections and 73 *P vivax* infections, which corresponded to 54% of 161 *P falciparum* infections detected by any qPCR method, and 52% of 141 *P vivax* infections detected by any qPCR method. Parasite prevalence in the population was thus two times lower by st-qPCR (29% [95% CI 24–35] for *P falciparum* and 24% [20–30] for *P vivax*) than by hv-us-qPCR (table).

us-qPCR on small blood volumes identified an additional 11 (7%) of 161 *P falciparum* infections and an additional 14 (10%) of 141 *P vivax* infections. Use of us-qPCR therefore increased parasite prevalence estimates in the population slightly (33% [95% CI 27–38] for *P falciparum*; 29% [24–35] for *P vivax*; table) compared with st-qPCR. Parasite densities in these additionally positive infections were similar to the lowest parasite densities detected by st-qPCR (figure 1B,C), with a median of 1·01 (IQR 0·86–1·76) estimated *P falciparum* parasites

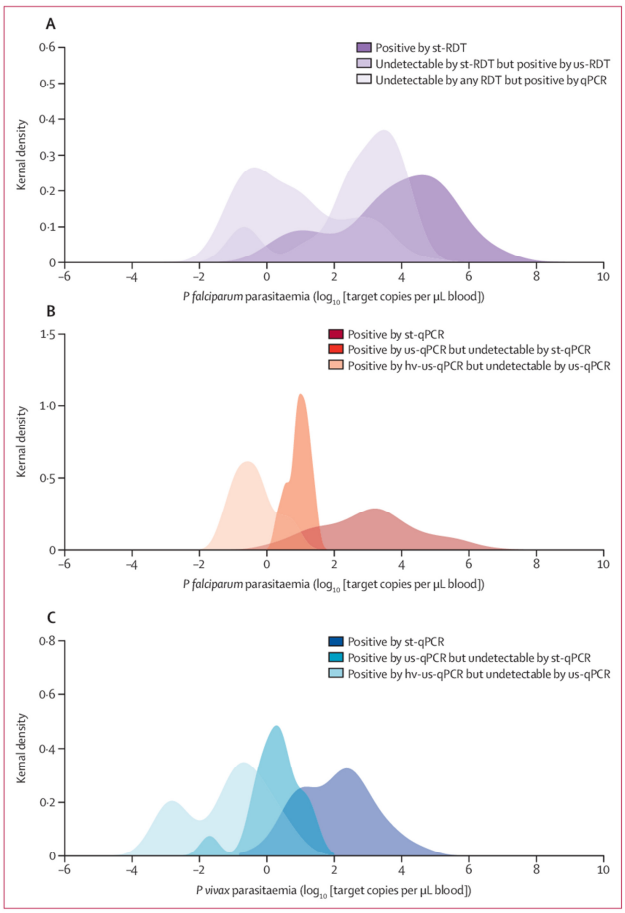

Figure 1: **Parasite density distributions in *Plasmodium falciparum* (A,B) and *Plasmodium vivax* (C) infections detected by RDTs and molecular methods with different sensitivity**

Parasite density by hv-us-qPCR is plotted (see underlying histograms in the appendix p 5), so only samples positive in hv-us-qPCR are shown. Samples were categorised according to their positivity by the specific detection methods. An unknown number of target sequences is amplified in *P falciparum* and *P vivax* us-qPCR, so parasite densities cannot be directly compared between the two species (see discussion on quantifying parasitaemia by molecular methods in the appendix pp 6–10). hv-us-qPCR=high-volume ultra-sensitive qPCR. qPCR=quantitative PCR. RDT=rapid diagnostic test. st-qPCR=standard qPCR. st-RDT=standard RDT. us-qPCR=ultra-sensitive qPCR. us-RDT=ultra-sensitive *P falciparum* RDT.

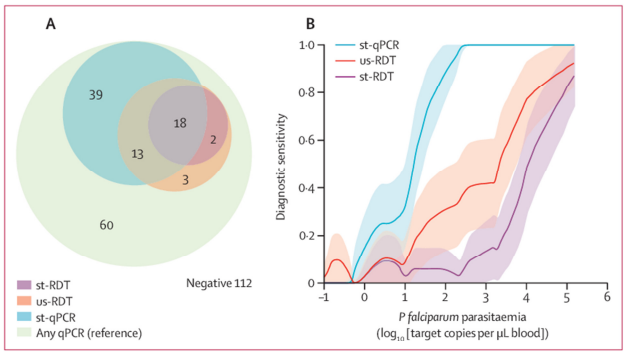

Figure 2: **Diagnostic performance of *Plasmodium falciparum* RDTs compared with qPCR methods in a subset of 247 samples**

Frozen whole-blood for RDT analysis was only available for 247 of 300 samples. (A) Venn diagram of *P falciparum* positivity by st-RDT, us-RDT, and molecular detection methods. Five samples were positive with us-RDT, or us-RDT and st-RDT, but negative with st-qPCR, and would thus have been considered false positive by RDT. However, *P falciparum* parasites were detected in all RDT-positive samples with hv-us-qPCR. (B) Diagnostic sensitivity of st-qPCR, us-RDT, and st-RDT in relation to parasite density (by hv-us-qPCR). Diagnostic sensitivity was calculated as a rolling mean of ten observations using combined detections by any qPCR as reference, and is shown with 95% CI (shaded areas). Curves were smoothed using locally weighted scatterplot smoothing function (span=0·16). An assessment of overall RDT diagnostic performance (sensitivity and specificity) is shown in the appendix (p 12). hv-us-qPCR=high-volume ultra-sensitive qPCR. qPCR=quantitative PCR. RDT=rapid diagnostic test. st-qPCR=standard qPCR. st-RDT=standard RDT. us-RDT=ultra-sensitive *P falciparum* RDT.

per µL of blood and 0·08 (0·03–0·16) estimated *P vivax* parasites per µL of blood (based on a conversion formula in the appendix pp 6–10). In other words, detection of infections with few or a single parasite in the small blood volume is more reliable with us-qPCR than st-qPCR, since the higher number of DNA sequences targeted in us-qPCR reduces the effect of chance.

Detection of the lowest parasitaemias was only achieved by hv-us-qPCR, in which a larger blood volume equivalent is examined (figure 1B,C). However even at such maximised sensitivity, a chance effect remained in detecting low-density infections, which was apparent from an imperfect overlap of positivity between the molecular detection methods (appendix p 11).

In the 247 samples that were tested using RDT, st-RDT detected 20 (15%) of all 135 *P falciparum* infections detected by any qPCR method in this subset of samples (figure 2A). us-RDT detected 36 (27%) *P falciparum* infections, corresponding to 51% of the 70 st-qPCR-detectable *P falciparum* infections in this sample subset (figure 2A). us-RDT detected *P falciparum* infections with reduced parasitaemia compared with st-RDT (figure 1A) and showed improved diagnostic performance over the whole range of *P falciparum* densities (figure 2B).

One *P vivax* infection was identified by st-RDT, and 118 *P vivax* infections were detected by qPCR methods in the subset of samples that were tested with RDT.

Gametocytes were detected in 19 (95% [95% CI 73–100]) of 20 *P falciparum* infections identified by st-RDT and in 12 (75% [47–92]) of 16 infections additionally identified by us-RDT (figure 3A). Gametocytes were also detected in 44 (44% [95% CI 35–55]) of 99 us-RDT-negative and qPCR-positive *P falciparum* infections (figure 3A). Of all 75 *P falciparum* gametocyte carriers, 44 (59%) were not detected by us-RDT. The range of gametocyte densities in us-RDT-negative gametocyte carriers was similar to that in us-RDT-positive or st-RDT-positive gametocyte carriers (figure 3D).

Parasite and gametocyte densities correlated better for *P vivax* (r=0·69) than for *P falciparum* (r=0·42; appendix p 13). For both species, parasite density was the single most important predictor for gametocyte carriage (appendix p 14). Therefore, when using molecular diagnosis, gametocytes were most common in st-qPCR-detectable *P falciparum* and *P vivax* infections. 71 (82%; 95% CI 72–89) of 87 st-qPCR-detectable *P falciparum* infections were gametocyte positive, and 67 (92%; 82–97) of 73 *P vivax* infections were gametocyte positive (figure 3B,C). More than half of infections additionally detected by us-qPCR also carried gametocytes (ten [67%; 95% CI 39–87] of 15 *P falciparum* infections; 11 [58%; 34–79] of 19 *P vivax* infections; figure 3B,C). The proportion of gametocyte carriers was considerably lower in infections only detectable in hv-us-qPCR (12 [20%; 95% CI 11–33] of 59 *P falciparum* infections; four [8%; 3–20] of 49 *P vivax* infections). As a result, diagnosis of infections using st-qPCR identified 71 (76%) of all 93 *P falciparum* and 67 (82%) of all 82 *P vivax* gametocyte carriers in the population (table). Using us-qPCR, 80 (86%) of *P falciparum* gametocyte carriers and 75 (91%) of *P vivax* gametocyte carriers were identified.

Mean *P falciparum* and *P vivax* gametocyte densities were significantly lower in infections that were not detected by st-qPCR than in those that were (geometric mean 1·0 *vs* 31·6 *pfs25* transcripts per µL of blood, p<0·001 for *P falciparum*; 0·3 *vs* 5·6 *pvs25* transcripts per µL blood, p<0·001 for *P vivax*). In infections that were only detected by hv-us-qPCR, estimated gametocyte densities did not exceed one gametocyte per µL of blood (based on previously published conversion formulas;[13,17] figure 3E,F; appendix pp 6–10). Similarly, in infections that were detected by us-qPCR but not by st-qPCR, estimated gametocyte densities were lower than one gametocyte per µL of blood in all but one infection (figure 3E,F).

The same main risk factors for malaria infection were identified by st-qPCR and hv-us-qPCR (figure 4; appendix p 15). Age was the only significant predictor for the odds of a *P vivax* infection. The odds of a *P falciparum* infection was significantly associated with village of residence and haemoglobin level. Patterns in the odds of RDT-diagnosed *P falciparum* infections were similar to those of molecular *P falciparum* diagnosis; however, the power of risk analysis was low because of the low number of RDT-positive detections (appendix pp 16–17).

The proportion of ultra-low-density infections among all infections was up to two times higher in population

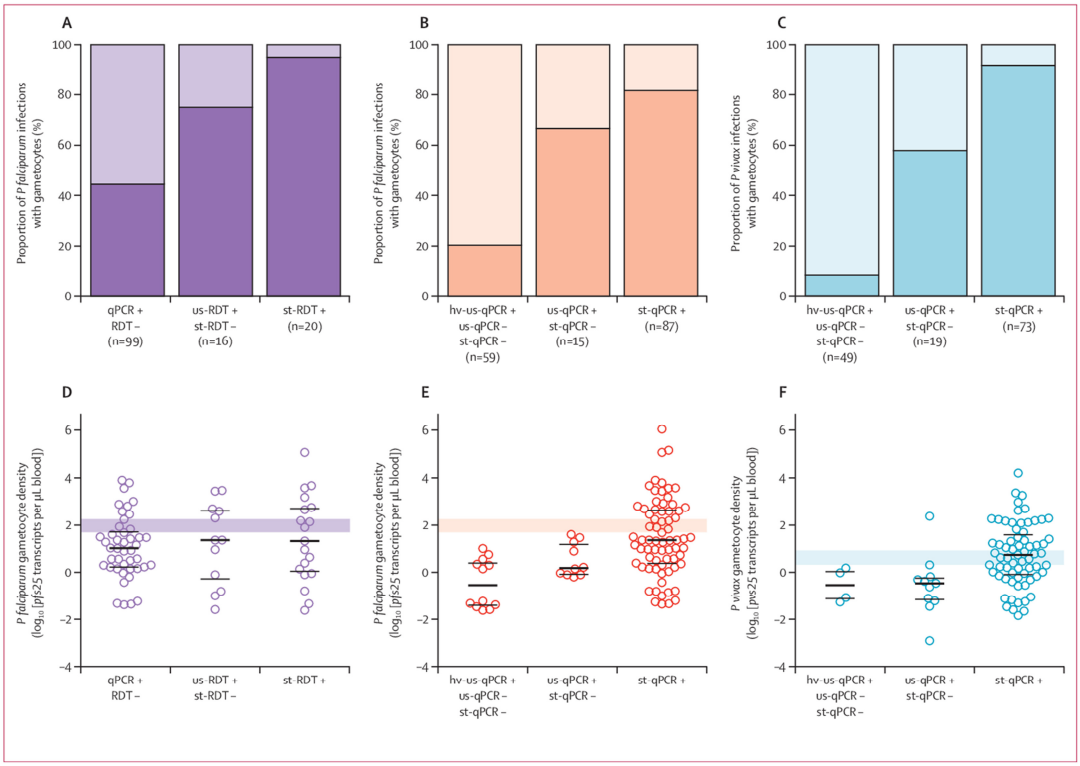

Figure 3: **Proportion of gametocyte-positive infections (A–C) and gametocyte density (D–F) in infections detected by RDTs (A,D) and molecular methods with different sensitivity (B,C,E,F)**
Samples were categorised according to their positivity by the different diagnostic methods as specified under each bar (corresponding to figure 1). (A–C) The proportion of gametocyte positive samples in each category is shown. (D–F) For each category, the concentration of gametocyte-specific transcripts in the corresponding samples is displayed, with each circle representing one sample. For each category, summary lines are displayed: thick black lines indicate the median, and thin black lines indicate the IQR. A different number of *pfs25* and *pvs25* transcripts is amplified per *Plasmodium falciparum* and *Plasmodium vivax* gametocyte; hence, gametocyte densities cannot be directly compared between the two species (see discussion on quantifying gametocytes by molecular methods in the appendix pp 6–10). *pfs25* and *pvs25* copy numbers corresponding to one gametocyte (within the confidence range, based on previously published correlations[13,17]) are delineated with a horizontal coloured line. hv-us-qPCR=high-volume ultra-sensitive qPCR. qPCR=quantitative PCR. RDT=rapid diagnostic test. st-qPCR=standard qPCR. st-RDT=standard RDT. us-qPCR=ultra-sensitive qPCR. us-RDT=ultra-sensitive *P falciparum* RDT.

subgroups with low parasite prevalence than in subgroups with high prevalence. For example, as *P vivax* prevalence decreased from 63% (30 of 48 [95% CI 47–76) in 11–15-year-old children to 31% (14 of 45 [19–47]) in adults older than 50 years (figure 5B), the proportion of ultra-low-density *P vivax* infections rose from 30% (nine of 30 [15–50]) in the 11–15-year-old children to 64% (nine of 14 [36–86]; figure 5B) in the oldest age group. Overall, *P vivax* density decreased with increasing age (figure 5D; Anova $p<0·001$), whereas no clear trends with age were observed for *P falciparum* (figure 5A). For *P falciparum*, parasite prevalence differed between villages and was inversely related to the proportion of ultra-low-density infections per village (appendix p 18). However, these differences between villages were not statistically significant.

Discussion

In this study, we applied multiple molecular diagnostic methods with maximised sensitivity to explore the true prevalence of *P falciparum* and *P vivax* in an endemic population in Papua New Guinea. This approach revealed an unexpectedly large reservoir of infections below the limit of detection of standard molecular diagnosis. Main limiting factors were the blood volume sampled and the blood equivalent added to the detection assay. However, complex laboratory procedures are necessary when using large blood volumes, which are not feasible for routine malaria surveillance or intervention monitoring. This issue raises the question of whether malaria interventions aimed at reducing transmission can benefit from detecting these ultra-low-density residual infections.

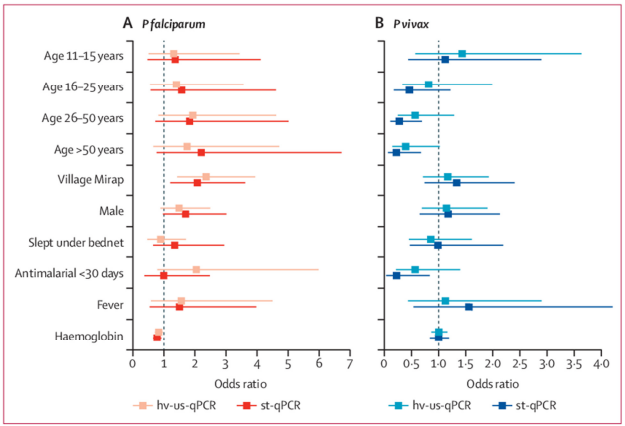

Figure 4: **Forest plot comparing the epidemiological patterns in *Plasmodium falciparum* (A) and *Plasmodium vivax* (B) infections detected using molecular methods with different sensitivity**

Odds ratios were modelled using logistic regression for infections detected using st-qPCR or using hv-us-qPCR. Error bars indicate 95% CIs. Detailed numeric model results for qPCR diagnosis (as well as for RDT diagnosis) are shown in the appendix pp 15–17. hv-us-qPCR=high-volume ultra-sensitive qPCR. qPCR=quantitative PCR. RDT=rapid diagnostic test. st-qPCR=standard qPCR.

In cross-sectional surveys, the density of gametocytes in the host's blood is often used as a surrogate marker for the transmission potential to mosquitoes. Directly measuring infectivity in cross-sectional surveys is challenging because it would require feeding of colony mosquitoes by direct exposure of the infected individual or by membrane blood feeding. Although gametocyte density is positively associated with infection success in membrane feeding experiments,[18–20] measuring gametocyte densities in the hosts's blood could provide only a restricted picture of the true probability of onward transmission. This onward transmission might instead depend on the density of mature gametocytes in the subcutaneous tissue, where gametocytes might aggregate to facilitate transmission to mosquitoes.[21]

In our study, gametocyte densities were estimated from the number of *pfs25* or *pvs25* transcripts, which are highly expressed in mature female gametocytes. High-volume RNA sampling maximised the limit of gametocyte detection to below one *P falciparum* or 11 *P vivax* gametocytes per 800 µL of blood (for a detailed discussion of molecular quantification, see appendix pp 6–10). Estimated gametocyte densities in our study were often below one gametocyte per 1 µL of blood, a threshold below which mosquito infection is rare in membrane feeding experiments.[18–20] In fact, with one exception, estimated gametocyte densities were below one gametocyte per µL of blood in all infections undetected by st-qPCR, suggesting that those densities are unlikely to be infective to mosquitoes. However, if parasitaemia in infections undetectable by st-qPCR at the time of sampling increased at a later timepoint, the likelihood of transmission would increase. Studies[22,23] on the longitudinal dynamics of chronic *P falciparum* infections revealed fluctuations in clonal densities by transient absence and later reappearance of clones. Large fluctuations in plasmodium densities over time have been described in Vietnam;[24] however, in the absence of parasite genotyping, it cannot be evaluated whether the observed density peaks represent new infections. In a cohort of children in Papua New Guinea, 70% of febrile malaria episodes showed a new genotype.[25] Low-density clones persisting around the levels of qPCR detection thus seem to be under density control (with fluctuations) and, in the absence of superinfection, asymptomatic individuals are unlikely to become highly effective transmitters.

Although molecular methods are required to detect very low gametocyte densities, the associated asexual parasite densities are approximately ten to 100 times higher and are thus detectable with less sensitive methods. In a multi-country trial,[26] high-quality research-grade microscopy identified more than 90% of infectious *P falciparum* carriers in high-transmission settings and two of three infectious carriers in a low-transmission setting. In the same study, all infectious carriers were detectable by standard molecular methods and fingerprick blood volumes. These results support our finding that little can be gained by laborious sampling and processing of larger blood volumes when diagnosis aims at identifying infectious individuals.

The relevance of maximising molecular diagnostic sensitivity in malaria surveillance surveys was further investigated by analysing the predictors of infection in cross-sectional data. If ultra-low-density infections accumulate in certain demographic pockets, these population subgroups would require specific targeting with improved detection methods. The same epidemiological patterns were observed with st-qPCR and hv-us-qPCR, supporting the view that standard molecular methods are adequate for investigating the relative distribution of malaria infections in populations. By contrast, the extent of undetected ultra-low-density infections should be considered when absolute parameters such as parasite prevalence are to be measured.

In a previous comparative diagnostic study,[8] the us-RDT missed 16% of PCR-detectable *P falciparum* infections in a high-endemic (Uganda) setting and 56% in a low-endemic (Myanmar) setting. In Papua New Guinea, us-RDT missed 50% of *P falciparum* infections that were detectable using st-qPCR, including samples with high gametocyte densities. Although the effect on us-RDT sensitivity of using frozen-thawed venous blood rather than fresh fingerprick blood in both studies is unknown, us-RDT seems to be a suboptimal substitute for molecular diagnosis in antimalarial interventions such as screen-and-treat interventions for reducing or eliminating malaria transmission in Papua New Guinea.

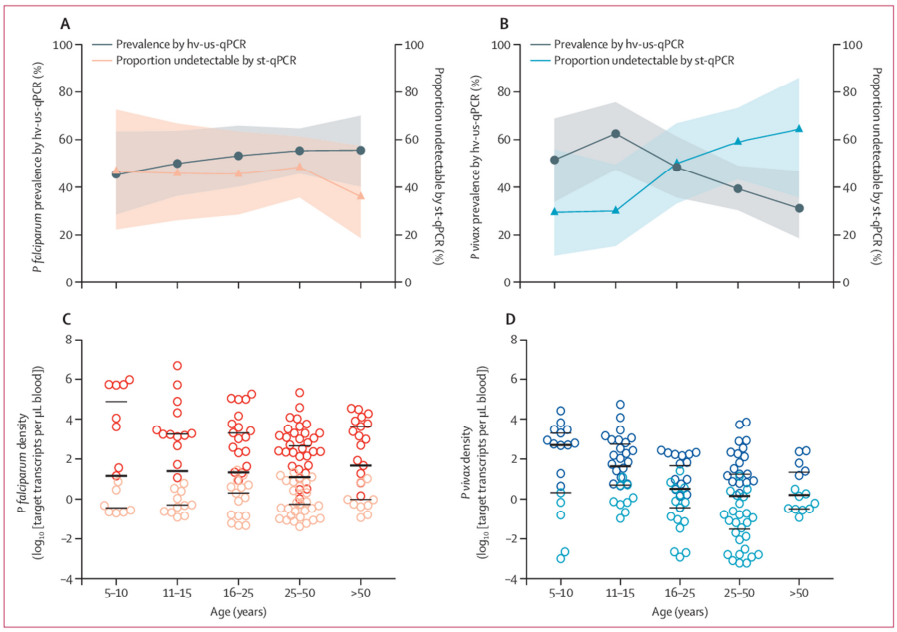

Figure 5: **Age patterns in *Plasmodium falciparum* (A,C) and *Plasmodium vivax* (B,D) infections**
(A,B) Age patterns in parasite prevalence (by hv-us-qPCR) and in the proportion of infections undetectable by st-qPCR. Shaded areas represent 95% CIs. (C,D) Age patterns in parasite density (by hv-us-qPCR). Each circle represents one sample in the respective age group, and summary lines are displayed (thick lines indicate the median; thin lines indicate the IQR). An unknown number of target sequences is amplified in *P falciparum* and *P vivax* ultra-sensitive qPCRs; hence, parasite densities cannot be directly compared between the two species (see discussion on quantifying parasitaemia by molecular methods in the appendix pp 6–10). hv-us-qPCR=high-volume ultra-sensitive qPCR. qPCR=quantitative PCR. st-qPCR=standard qPCR.

Although Papua New Guinea currently does not represent a low-endemic or pre-elimination setting, where detecting very-low-density infections is considered particularly relevant, its unique local epidemiology resembles that of other *P falciparum*-endemic and *P vivax*-endemic settings with declining transmission. Corresponding to global trends of an increasing proportion of submicroscopic infections with decreasing parasite prevalence,[1] parasite densities in Papua New Guinea declined over the past decade alongside a decline in clinical incidence and prevalence of malaria.[12] Furthermore, malaria transmission in Papua New Guinea is highly heterogeneous over small spatial scales,[25] which is considered a hallmark of declining transmission and has been described in various settings, such as western Kenya,[27] Thailand,[28] and the Peruvian Amazon.[29]

A main limitation of our study was the exclusion of children younger than 5 years for ethical reasons. Young children carry the main burden of malaria infection and disease; however, their contribution to mosquito infections is thought to be smaller than that of adolescents and adults.[30] Because parasite densities are higher in young children than adolescents and adults in Papua New Guinea,[17] ultra-low-density infections might be less common in young children, and therefore little would be gained applying ultra-sensitive diagnostics.

A technical limitation that applies to molecular malaria diagnostics, as well as microscopy, is the effect of chance in capturing a scarce parasite, which depends on the volume of blood or DNA solution investigated. In our study, some low-density infections were not detected by a supposedly more sensitive method but were positive by a supposedly less sensitive molecular method. The chance effect that is intrinsic to all malaria diagnostics can thus be lowered, but not abolished, by sampling of larger blood volumes and targeting of high-copy DNA sequences.

In conclusion, we have shown that the extent of both *P falciparum* and *P vivax* infections below the limit of detection of standard molecular malaria diagnostics is substantial. However, gametocyte densities in infections undetected by standard molecular diagnostics were very low and potentially not infective. The us-RDT did not achieve this level of sensitivity and missed infections with high gametocyte densities. Our findings reduce the

pressure to identify the very last parasite and advocate against the need for venous sampling in malaria control and elimination interventions.

Contributors

NEH was involved in data collection, data curation, data analysis, data interpretation, methodology, and writing of the original draft of the article. MG was involved in data collection, data curation, data analysis, methodology, and reviewing of the manuscript. EN, AU, DR-R, and MS were invoved in patient recruitment, data collection, data curation, and reviewing of the manuscript. IM and TAS were responsible for data analysis, and reviewing and editing of the manuscript. ML was involved in patient recruitment, project administration, and reviewing of the manuscript. LJR was involved in conceptualisation, ethical clearance, project administration, supervision, data interpretation, and reviewing and editing of the manuscript. IF was involved in conceptualisation, project administration, supervision, funding acquisition, data interpretation,and reviewing and editing of the manuscript.

Declaration of interests

We declare no competing interests.

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APPENDIX 2

Examples of dwellings in Mugil (top) and Lemakot (bottom) with open areas that do not offer protection despite being considered "indoors".

APPENDIX 3

Wokpainimaut long sik malaria PNGIMR mekim nau long Lemakot

Mipela bai wokim wokpainimaut bilong sik malaria long ples bilong yu. Long wokim dispela wok, mipela i laik askim long halipim bilong yu na comuniti bilong yu. Yupela bai halipim mipela long traim painimaut wanem as sik malaria em i kamap bikpela long sampela ples, tasol em i no save kamap bikpela long ol narapela ples. Dispela em i bikpela samting long wanem ol wok bilong daunim sik malaria i daunim dispela sik i go tamblo tru pinis. Olsem na em bai impoten tru long miplea long wokim dispela wokpainimaut long halipim mipela long save long wei bilong daunim sik malaria.

Displa wok mipla i mekim kain olsem sekim blut, kisim natnat na askim sompla askim long manmeri long wanem wok ol save mekim olgeta dei.

BAI MIPELA WOKIM DISPELA WOKPAINIMAUT OLSEM WANEM?
Iga planti hap bilong dispela wokpainimaut: i) manmeri, ii) natnat, na iii) malaria binatang.

i) MANAERI
Dispela wokpainimaut bai mipela wokim long 1500 manmeri na pikinini. Mipela bai kisim stori long papa bilong haus na ol stori wea mipela bai kisim em kain olsem hamas krismas bilong yu, wanem hap yu save stap (demographic); wanem kain wok yu save wokim na hamas moni yu save kisim (socioeconomic); na pasin bilong yu kain olsem yu save stap we long dei na nait taim (behavioural).

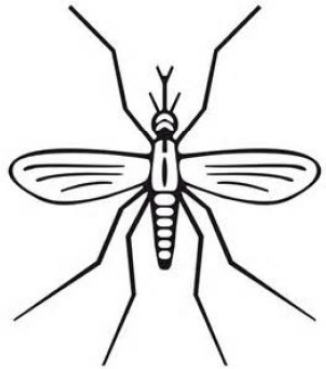
ii) NATNAT
Planti ol natnat wea i save karim binatang bilong malaria i save kaikai manmeri long autsait long haus bilong ol long avinun igo tulait.. Mipela bai katsim ol dispela natnat long stadim ol pasin bilong ol long karim sik malaria.

iii) MALARIA BINATANG
Bai mipela sekim liklik blut long pinga bilong yu na sapos yu luk olsem yu gat sik malaria na bai mipela testim long painim sik malaria. Blut long glas (blood slide) wea mipela kisim long yu bai mipela sekim long haus bilong wokpainimaut long Yagaum long sekim binatang bilong malaria.

SAPOS YU LAIKIM SAVE MOA LONG DISPLA WOKPAINIMAUT,

Ol sampela wei bilong banisim yumi long natnat

- Yumi mas silip long taunam igat marasin olgeta nait
- Yumi mas lukautim ol peles yumi stap long em. Sampela wei em long tromoi ol tin na pipia igo long hul na karamapim wantaim graun. Ol liklik ol peles we natnat iken putim kiau, karamapim wantaim graun o dikim baret long rausim tais, dikim na opim maus wara sapos em i pas.

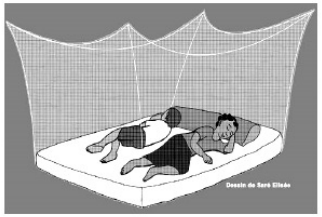


Wantaim displa mipela (PNGIMR) laik tok TENK YU TRU long komuniti long sapot yuplea isoim long stap insait long ol dispela wokpainimaut.

PNG-IMR
Save Tambulim Sik

Papua New Guinea
Institute of Medical Research

WOKPAINMAUT MIPELA I MEKIM NAU LONG LEMAKOT



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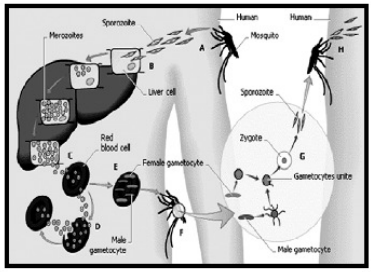
visit our website for more info www.pngimr.org.pg

Brochure developed to inform the community about the study beforehand (front).

Sik Malaria Em Wanem Samting?



A. Malaria em i wanpela sik natnat i save givim long taim em dringim blut blo yumi.
B. Taim natnat I kalkal mipla binatang I save go Insait long liver. Taim em stap insait long liver em i multiply.
C. Sampla taim bihain binatang bai bruk lusim liver na pain-im red blood cell insait long blut blo yumi.
D. Taim binatang stap insait long red blood cell em kaikai disla cell na multiply. Taim binatang multiply planti tru disla red blood cell bai bruk. Nau planti binatang cam aut na painim narpla red blood cell gen.
E. Sompla taim ol binatang bai kamap long narapla stage (gametocyte). Displa stage nau em i gutpla long natnat.
F. Taim natnat i kam kaikai mipla em bai kisim malaria binatang.
G. Nau binatang bai gro insait long natnat. Sompla dei bihain displa binatang em bai redi.
H. Taim em redi natnat bai go kaikai narapla manemeri na givim malaria binatang long em.

Binatang long malaria ol i kolim *Plasmodium* i save kamapim dispela sik na igat 4 pela kain i save kamapim sik lo ol manmeri.

- *P. falciparum* } Tupela i save kamapim sik planti taim
- *P. vivax* }
- *P. malariae* } Ino save kamapim sik tumas
- *P. ovale* }

Binatang *P.vivax* i ken istap insait long liver blong manmeri longpla taim na behain kamapim sik malaria behain long planti wik, mun o yia. Igat marasin lo daunim sik malaria olsem Mala I na Primaquine .

Malaria na PNG IMR

Sampla year igo pinis, PNGIMR i bin mekim sampla wokpain-imaut long sik malaria, we ol Komuniti long PNG bin hamamas na supportim na tu sampla i bin stap insait long em. Sampla blo ol displa wokpainimaut em bilong ol piknini igat sik malaria na ol manmeri. Olgeta displa wokpainimaut i bin kamap gut na tu i bin halivim planti ol manmeri na pikinini husait i bin stap insait long en. Sampla kaikai blong displa ol wokpainimaut em olsem:

Wokpainimaut bilong sekim sik malaria, we bilong kisim halivim na sekim sapos pikinini i sik ken behain lo kisim marasin

Tupla wokpainimaut i soim olsem:

- Givim marasin blong malaria long ol nupela bon pikinini long taim ol i RDT positive i seif na strong moa
- **Mala I em gutpla marasin** blong ol piknini i kisim malaria long PNG, yu mas kisim Mala I 3pla dei.
- Sampla taim marasin bai no inap wok sapos yu gat bi-natang *P.vivax* long blut o sapos yu no kisim marasin gut olsem dokta i tok.

- Sapos yu igat binating ***P.vivax*** lo blut, em i gutpla moa **yu MAS kisim** dispela **marasin primaquine** long 14pla dei olsem dokta i makim long rausim malaria binatang long bodi blong yu.

Wokpainimaut Igo Insait long save long strong blong PNG's National Malaria Kontrol Program

Mipla I bin sekim blut long ol manmeri stap insait long displa wokpainimaut na sekim sapos binatang blong sik malaria I stap. Long dispela mipela I luksave olsem

- Long 2006 igo long 2014, namba bilong *P.falciparum* I go daun na namba bilong *P.vivax* igo daun tasol long 2014 em I bin go antap gen olsem 20%..
- Namba blong manmeri I gat sik wantaim wanpela blong ol binatang blong malaria I go daun long year 2010 na 2014.
- Planti manmeri I bin gat binatang blong malaria tasol ol ino bin luk sik.

Dispela is soim olsem binatang blo malaria I ken kalap long wanpela man husait igat binatang blong malaria pinis tasol ino luk sik lo dispela taim igo long narapela man, meri or piknini.

Long dispela as tingting tasol na em I gutpla moa long **ol femili na kominiti long silip aninit long taunam igat marasin** olgeta taim.

Brochure developed to inform the community about the study beforehand (back).