Epidemiología y tratamiento de *Plasmodium vivax*

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TESIS DOCTORAL

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"Epidemiología y tratamiento de Plasmodium vivax"

The epidemiology and treatment of Plasmodium vivax

Tesis presentada por Inoni Betuela

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1. Glossary

ACPR	Adequate clinical and parasitological response
ACT	Artemisinin-based combination therapy
AL	Artemether-Lumefantrine
AQ	Amodiaquine
ART	Artesunate
BCS	Blantyre Coma Scale
CQ	Chloroquine
CQRPf	Cloroquine resistant *Plasmodium falciparum*
CQRPv	Cloroquine resistant *Plasmodium vivax*
DDT	Dichloro-diphenyl-trichloroethane
DHA-PQP	Dihydroartemisinin-Piperaquine
G6PD	Glucose 6 phosphate dehydrogenase
IPTi	Intermittent Preventive Treatment in Infants
IRB	Institutional Review Board
IRS	Indoor residual spraying
LDR-FMA	Ligase detection reaction-fluorescent microsphere assay
LLIN	Long-lasting insecticidal nets
LM	Light microscopy diagnosis
MalERA	Malaria eradication research agenda
MDA	Mass drug administration
MOI	Multiplicity of infection
NDoH	National department of health
nPCR	Nested polymerase chain reaction
PCR	Polymerase chain reaction
PCR-RFLP	Polymerase chain restriction fragment length polymorphism
P. falciparum	*Plasmodium falciparum*
P. malariae	*Plasmodium malariae*
P. ovale	*Plasmodium ovale*
P. vivax	*Plasmodium vivax*

Glossary (continuation)

PNG	Papua New Guinea
PQ	Primaquine
qPCR	Quantitative real time polymerase chain reaction
RDT	Rapid diagnostic test
SAO	Southeast Asian ovalocytosis
SP	Sulfadoxine-Pyrimethamine
WHO	World Health Organization

2. Resumen (Castellano)

La carga de enfermedad y las muertes relacionadas con la malaria han disminuido a nivel global, principalmente gracias a las mejoras en la implementación de las intervenciones para prevenir y tratar la malaria, y en particular al uso de las mosquiteras impregnadas con insecticidas de larga duración y al tratamiento de los episodios clínicos con terapias combinadas con artemisinina. El reciente aumento de los fondos mundiales destinados a financiar los esfuerzos de control de la malaria ha permitido que la mayoría de la población pobre que vive en países endémicos de malaria tenga acceso a medidas de prevención, a diagnóstico en caso de sospecha de malaria y a terapias combinadas con artemisinina. De acuerdo a los datos de 104 países recopilados por la OMS en el Informe Mundial sobre el Paludismo del 2012, se estima que se evitaron 274 millones de casos de malaria y 1.1 millones de muertes relacionadas con la malaria. El éxito de las medidas de control actuales ha reactivado el objetivo de la eliminación de la malaria y su erradicación del globo.

Sin embargo, la mayor parte de los logros globales se deben a la reducción de enfermos graves y muertes debidos a *Plasmodium falciparum*, principalmente en los países africanos. Fuera de África, la mayoría de las regiones endémicas de malaria cuentan con más de una especie del parásito *Plasmodium*. Medidas actuales como las mosquiteras impregnadas con insecticidas de larga duración y las terapias combinadas de artemisinina se han mostrado más eficaces contra *P. falciparum* que contra *Plasmodium vivax*. En la mayoría de los países endémicos donde *P. vivax* y *P. falciparum* coexisten, con programas eficaces de control de la malaria, parece haberse producido un incremento proporcional de la prevalencia de *P. vivax*, sustituyendo a *P. falciparum* como especie predominante. *P. vivax* es más difícil de controlar que *P. falciparum* a causa de la fase hepática de *P. vivax* llamada hipnozoito. Los hipnozoitos pueden permanecer inactivos en el hígado durante largos períodos de tiempo antes de volver a activarse para reinfectar la sangre y causar una recaída de malaria por *P. vivax* en el huésped humano. En las regiones tropicales los hipnozoitos se activan rápidamente, y son comunes las recaídas

múltiples a intervalos de cerca de 3 semanas, mientras que en las zonas subtropicales las recaídas pueden producirse con intervalos más largos (de 8 a 10 meses). Lograr la eliminación y erradicación de la malaria será difícil sin la erradicación de los hipnozoitos del hígado, especialmente en regiones endémicas con transmisión de la malaria perenne como Papúa Nueva Guinea.

Actualmente, la primaquina es el único fármaco autorizado en el mercado que tiene efecto sobre los hipnozoitos. Las terapias combinadas de artemisinina, aunque son muy eficaces contra la infección en fase sanguínea de ambos *Plasmodium*, no tienen ningún efecto sobre los hipnozoitos. Una de las estrategias para la eliminación de la malaria es la administración masiva de medicamentos. Sin embargo, para que esta estrategia sea eficaz en áreas endémicas de *P. vivax*, debe incluir la primaquina en el régimen de tratamiento para así erradicar los hipnozoitos en el hígado, pero el uso indiscriminado de este fármaco puede conllevar problemas de seguridad importantes. Los futuros posibles sustitutos de la primaquina deben ser fármacos con una fase de eliminación terminal larga con el fin de proporcionar una profilaxis post-tratamiento eficaz para detener la transmisión de *P. vivax*, previniendo las recaídas desde el hígado. La mayoría de las infecciones por *P. vivax* en niños mayores y adultos como resultado de las recaídas por activación del hipnozoito en el hígado son asintomáticas. Por otro lado, la gametogénesis (producción de gametos, los estadios infectivos responsables de la transmisión desde el humano al siguiente mosquito vector) de *P. vivax* ocurre de forma espontánea con el desarrollo de la infección en fase sanguínea, hecho que lleva a una gametocitemia temprana que incrementa la probabilidad de transmisión a mosquitos antes incluso de la aparición de síntomas y por tanto del tratamiento de los casos sintomáticos. Se ha demostrado que la primaquina también es el único fármaco eficaz contra los gametocitos de estadío V de *P. falciparum*, los verdaderos responsables de la transmisión en esta especie El uso de la primaquina para la administración masiva de medicamentos será, por lo tanto, una herramienta efectiva para la prevención de la transmisión de *P. vivax* y *P. falciparum*.

En la actualidad se desconoce la contribución de los hipnozoitos a la carga de infección y enfermedad por *P. vivax*, especialmente en los niños que viven

en zonas de alta endemicidad. La primaquina, el único fármaco efectivo contra los hipnozoitos de *P. vivax* y *P. ovale* conocido, existe desde hace más de 60 años pero, sin embargo, se sabe muy poco de su seguridad y tolerabilidad en los niños, la población que sufre la mayor carga de infección y enfermedad por *P. vivax*. Incluso en su uso en adultos, todavía no se sabe mucho acerca de la dosificación y modo de acción. Existen importantes limitaciones en el uso de la primaquina en regímenes de tratamiento de la malaria. En primer lugar, el riesgo letal de anemia hemolítica grave asociada al tratamiento con primaquina para las personas con las variantes graves de deficiencia de deshidrogenasa de glucosa-6-fosfato (G6PD). En segundo lugar, la falta de un test de diagnóstico rápido barato, fiable y accesible para detectar la deficiencia de G6PD durante la actividad clínica rutinaria, así como durante el ejercicio preventivo de administración masiva de medicamento.

En Papua Nueva Guinea, varios estudios de cohorte longitudinales han demostrado que *P. vivax* es la causa más común de infección y enfermedad por malaria en los niños menores de 3 años de edad, mientras que *P. falciparum* predomina como causa de morbilidad en niños de 3 a 10 años de edad. La enfermedad clínica secundaria a *P. vivax* tiene su punto culminante a los 3 años de edad. A pesar de que la prevalencia de la infección por *P. vivax* y *P. falciparum* son similares en el grupo de mayor edad, los niños con infección de *P. vivax* permanecen asintomáticos o presentan enfermedad clínica leve. No hay variación estacional en la prevalencia de *P. vivax* en comparación con la de *P. falciparum*. Esto puede ser debido a las recaídas por activación de los hipnozoitos en el hígado, que contribuyen a la reinfección de la fase sanguínea, sin requerir la transmisión mediante el vector anofelino.

Esta tesis presenta datos de dos estudios de cohortes longitudinalales en niños de 1 a 10 años de Papua Nueva Guinea con niveles normales de G6PD para evaluar el efecto de la primaquina sobre los hipnozoitos en el hígado. Los objetivos eran dos: El primero: determinar la contribución de las recaídas de la activación desde los hipnozoitos a la carga de infección y enfermedad por *P. vivax* en los niños que viven en una zona de alta transmisión de malaria, después de un pre-tratamiento con primaquina. El segundo: evaluar la seguridad y tolerabilidad del uso de la primaquina en los niños como pre-

tratamiento en el terreno. Tras los estudios de cohortes, se estudiaron las propiedades farmacocinéticas de una dosis única de primaquina en niños de Papua Nueva Guinea para evaluar la viabilidad de un eventual tratamiento corto con dosis altas de primaquina para la cura radical de la malaria por *P. vivax*. Estos estudios constituyen la base de esta tesis.

En el primer estudio, niños de entre 1 y 5 años con G6PD normal fueron seleccionados y distribuidos aleatoriamente en tres grupos: grupo control sin medicación pre-tratamiento; grupo pre-tratado con artesunato + primaquina, y aquellos solamente tratados con artesunato. Las dosis de tratamiento se administraron bajo observación directa, y siempre junto con comida. Los niños fueron seguidos activamente cada dos semanas durante los primeros tres meses, luego mensualmente hasta nueve meses haciendo detección de casos con infección de malaria asintomática y de casos clínicos. La detección pasiva de casos se hizo mediante las enfermeras establecidas en las clínicas locales durante la duración total del estudio. Los resultados de este estudio de cohorte muestran que el pre-tratamiento con artesunato más primaquina (14d, 0.5 mg) redujo la incidencia de malaria por *P. vivax* en un 49% durante los 3 primeros meses ($p = 0.031$) y un 19% para los meses 4 a 9 ($p = 0,25$). También redujo el tiempo de la primera detección por microscopía óptica y por PCR de los casos positivos por infección en un 57% y 48%, respectivamente ($p < 0,001$), en comparación con el grupo que sólo tomó artesunato. El efecto preventivo del pre-tratamiento en el grupo de primaquina se limitó a los primeros 3 meses de seguimiento y el 30% de los niños sufrieron una reinfección a las 2 semanas de seguimiento.

En el segundo estudio, se realizó un ensayo aleatorizado, doble ciego, controlado con placebo para estudiar el efecto de la primaquina en niños de 5 a 10 años de edad con niveles normales de G6PD. Todos los niños del estudio recibieron cloroquina en los días 1 a 3, y arteméter lumefantrina en los días 15 a 17 días de la fase de pre-tratamiento. Los niños fueron asignados aleatoriamente en dos grupos para recibir primaquina o placebo, juntamente con cloroquina. Todas las dosis pre-tratamiento se administraron bajo observación directa y junto con comida utilizando el mismo programa de seguimiento del primer estudio. Los resultados del segundo estudio muestran

que el pre-tratamiento con primaquina redujo el riesgo de adquirir una infección en fase sanguínea nueva en un 78% ($p<0.0001$), y de enfermedad clínica en un 68% ($p<0.0035$). La edad se asoció con un riesgo menor de presentar episodios clínicos causados por *P. vivax* de cualquier densidad ($p<0.0167$) y de alta densidad ($p<0.016$). La mayoría de reinfecciones ocurrieron durante las 12 semanas de pre-tratamiento.

El tercer estudio, diseñado para monitorear la seguridad y la tolerabilidad del tratamiento con primaquina en los niños del estudio, se llevó a cabo durante la fase de seguimiento de los dos estudios de pre-tratamiento de cohortes. Se utilizó un cuestionario semi-estructurado de todos los posibles eventos adversos a medicamentos antes de la toma de la dosis de primaquina. Todas las dosis de primaquina se administraron con alimentos excepto en el grupo de edad más joven, en el que los niños fueron amamentados/alimentados por las madres. Los resultados de este estudio mostraron que se necesitaban 14 dosis diarias de 0.5mg/kg de primaquina para garantizar la seguridad y la tolerabilidad en los niños de ambos estudios de cohortes. En ninguno de ellos hubo eventos adversos graves y/o abandonos asociados con la toma de primaquina. En los niños que reportaron náuseas o vómitos debido a la ingestión de primaquina, la recuperación fue casi inmediata a la ingesta de alimentos.

A raíz de los estudios de cohortes, se estudiaron las propiedades farmacocinéticas de dos dosis únicas de primaquina en niños de Papua Nueva Guinea de entre 5 y 10 años de edad, para evaluar la viabilidad de un tratamiento corto, con regímenes de tratamiento con primaquina en dosis altas para la cura radical de malaria por *P. vivax*. Se evaluaron los perfiles de las dosis de 0.5 mg/kg y 1.0 mg/kg. Durante el reclutamiento, los niños del estudio fueron ingresados en el Centro de Salud durante 2 días para poder hacer un seguimiento exhaustivo y permitir la toma de muestras de sangre frecuentes. Durante todo este tiempo los participantes llevaron una cánula intravenosa permanente para tomar muestras de sangre. Las dosis de primaquina se co-administraron con alimentos.

El estudio de monitorización de la seguridad y tolerabilidad mostró que

ambas dosis de primaquina fueron bien toleradas, sin efectos adversos graves. No hubo cambios observados en los síntomas y la gravedad de las náuseas o dolores abdominales en ninguno de los grupos de dosificación de primaquina. No hubo diferencias entre los grupos en la concentración de hemoglobina y niveles de metahemoglobina. Los regímenes cortos de dosis altas simulados mostraron que las concentraciones de primaquina y carboxyprimaquina en plasma predecidas y obtenidas no fueron significativamente mayores que las observadas en los estudios farmacocinéticos anteriores en adultos. Esto sugiere que tanto los regímenes de dosis de 0.5 mg/kg como de 1.0mg/kg de primaquina podrían ser evaluados con más detalle en estudios de seguridad y eficacia sobre terreno.

Varios estudios hechos en el pasado sobre la primaquina reportaron un aumento de la eficacia de la primaquina cuando asociada a una droga con una fase de eliminación terminal larga. La primaquina es más eficaz cuando se administra conjuntamente con cloroquina debido a su efecto profiláctico post-tratamiento más largo, tal y como se muestra en el segundo estudio de cohortes. Los estudios también han demostrado que la dosis terapéutica de primaquina se basa en el efecto de la dosis total, y no en los intervalos de dosificación, ya que una mayor dosis diaria durante 7 días parece ser tan eficaz como el régimen de 14 días con eventos adversos mínimos cuando se co-administraron con comida. La actual dosis diaria de primaquina durante 14 días es más eficaz cuando se administra en las condiciones de investigación con la observación directa de la ingesta del fármaco, mientras que en la vida real, debido a la falta de cumplimiento, el tratamiento con primaquina tiene un efecto más reducido. Un tratamiento corto con altas dosis de primaquina compatible con el régimen de 3 días de aplicación de las terapias combinadas con artemisinina sería óptimo para mejorar el cumplimiento durante el tratamiento de rutina y facilitar la administración como administración masiva de medicamentos para la eliminación de la malaria. Por tanto, el último artículo de esta tesis revisa el posible uso de la primaquina como herramienta no sólo de control sino que también para la eliminación de la malaria en el contexto de una zona como Papúa Nueva Guinea.

Los resultados de estas cohortes longitudinales y los estudios de dosis de

primaquina contribuirán al conocimiento científico sobre la epidemiología y el manejo de *P. vivax*, la seguridad del uso de primaquina en niños y a la mejora del cumplimiento de las tomas preventivas del fármaco. Esta será una herramienta útil para el control y eliminación del paludismo para combatir los hipnozoitos en el hígado del huésped humano. La estrategia de la administración masiva de medicamentos tendría potencialmente capacidad para detener la transmisión de la malaria no sólo eliminando hipnozoitos de *P. vivax* en el hígado, sino también los gametocitos en fase V de *P. falciparum*.

3. Summary (English)

Globally, the burden of malaria related disease and deaths has decreased in the last 10 years, mainly due to interventions for malaria prevention and treatment with long lasting insecticide treated bed nets and the artemisinin combination therapies, respectively. The recent increase in Global funding for malaria control efforts has enabled majority of impoverished populations living in malaria endemic countries to access preventative measures, diagnosis of presumptive malaria cases and artemisinin combination therapies. According to data summaries from 104 countries compiled by WHO in the 2012 malaria report, an estimated 274 million malaria cases were averted and 1.1 million lives saved from dying, from malaria related illness. The success of current control measures has once again, renewed the goal of malaria elimination and its eradication from the globe.

However, most of the global achievements are due to reductions in severe disease and deaths from *Plasmodium falciparum* malaria mostly in African countries. Outside of Africa, most malaria endemic regions have more than one *Plasmodium* parasite species present. The current measures, using long lasting insecticide treated nets and artemisinin combination therapies are more effective against *Plasmodium falciparum* compared to *P. vivax* malaria. In most *P. vivax* endemic countries with effective malaria control programs, there has been a relative increase in the prevalence of *P. vivax,* replacing *Plasmodium falciparum* as the predominant species. *P. vivax* is more difficult to control than *Plasmodium falciparum.* This is due to the liver stages of *P. vivax* called hypnozoites. The hypnozoites remain dormant in the liver for prolonged periods of time before activation, to re-infect the blood leading to *P. vivax* relapse infection and malaria in the human host. In Tropical regions, the hypnozoites activate quickly, and multiple relapses commonly occur, at about 3 weekly intervals, while in the sub-tropics, relapses occur at about 8-10 month intervals. Achieving the goal of malaria elimination and eradication will be difficult without eradication of the hypnozoites from the liver, especially in endemic regions with perennial malaria transmission such as Papua New Guinea.

Currently, primaquine is the only licensed drug in the market that has an

effect upon the hypnozoites. The artemisinin combination therapies while highly effective against the blood stage infection of both *Plasmodium falciparum* and *P. vivax*, have no effect upon the hypnozoites. One of the strategies for malaria elimination is mass drug administration. However, for this strategy to be effective in *P. vivax* endemic areas, primaquine must be included in the treatment regimen to eradicate the hypnozoites from the liver. The partner drug of primaquine must have a long terminal elimination phase in order to provide effective post-treatment prophylaxis to prevent relapses from the liver to stop *P. vivax* transmission. Most *P. vivax* infections in older children and adults as a result of relapses from hypnozoite activation in the liver are asymptomatic. Moreover, the gametocytogenesis of *P. vivax* occur spontaneously with the development of blood stage infection leading to early gametocytemia with high probability of transmission to mosquitoes before treatment of symptomatic cases. Primaquine has also been shown to be the only drug effective against the gametocyte stage five of *Plasmodium falciparum*. Primaquine use for mass drug administration will therefore be an effective tool for preventing *P.vivax* and *P. falciparum* transmission.

Currently, the contribution of the hypnozoites to the burden of *P. vivax* infection and disease especially in children living in highly endemic areas is notwell understood. Primaquine, the only licensed drug known to be effective against the hypnozoites of *P. vivax (and P. ovale)* existed for over 60 years, yet very little is known of its safety and tolerability in children; the population with the highest burden of *P. vivax* infection and disease. Even in adult use, much is still not known about dosing and mode of action. There are major limitations to primaquine use in malaria treatment regimens. Firstly, the risk of severe life threatening haemolytic anaemia associated with primaquine treatment in persons with the severe variants of glucose-6-phosphate dehydrogenase deficiency. Secondly, the lack of cheap and reliable, point of care rapid diagnostic test for glucose-6-phosphate dehydrogenase deficiency for routine use and during mass drug administrations.

In Papua New Guinea, several longitudinal cohort studies have shown *P. vivax* to be the commonest cause of malarial infection and disease in children less than 3 years old; while *Plasmodium falciparum* accounts for the majority of

malarial disease burden in children over 3-10 years old. The clinical disease of *P. vivax* peaks at 3 years of age. Even though the prevalence of infection with *P. vivax* and *P. falciparum* are similar in the older age group, children with *P. vivax* infection remain asymptomatic with less clinical illness. There is much less seasonal variation in *P. vivax* prevalence compared to that of *P. falciparum.* This may be due to relapses from hypnozoite activation in the liver contributing to blood stage re-infection.

Two longitudinal cohort studies in G6PD normal Papua New Guinean children aged 1 to 10 years were carried out to assess the effect of primaquine on the hypnozoites in the liver. There were two aims for the cohort studies. Firstly, to determine the contribution of relapses from hypnozoite activation to the burden of *P. vivax* infection and disease in children living in an area of high malaria transmission, following treatment with primaquine. Secondly, to assess the safety and tolerability of primaquine use in the children as part of the cohort studies, drug treatment phase in the field. Following the cohort studies, pharmacokinetic profiles of two single high-dose primaquine in Papua New Guinean children weredetermined to assess the feasibility of short course, high-dose primaquine treatment regimens for radical cure of *P. vivax* malaria. These studies form the basis of this thesis.

In the first study, G6PD normal children aged 1-5 years old were screened and randomised into three groups: control group with no pre-treatment drugs; primaquine plus artesunate group; and those with artesunate only, as pre-treatment. The treatment doses were administered by direct observed therapy with food. Children were followed actively every two weeks for the initial three months, then monthly up to nine months for asymptomatic malarial infection and clinical case detection. Passive case detection was done by nurses based at the local clinics throughout the study duration. The result from this cohort study show, pre-treatment with artesunate plus primaquine (14d, 0.5mg) reduced incidence of *P. vivax* malaria by 49% for the initial 3 months (p = 0.031) and 19% for months 4-9 (p = 0.25); and reduced time to first light microscopy and PCR-positive infections by 57% and 48%, respectively (p < 0.001), when compared to the artesunate only group. The effect of pre-treatment in the primaquine group was limited to the first 3 months of follow-up and 30% of

children had re-infection by 2 weeks of follow-up.

In the second study, a randomised, double-blind, placebo controlled trial of primaquine effect was performed in G6PD normal children aged 5 to 10 years old. All study children received chloroquine at days 1-3 and artemether lumefantrine at days 15-17 of the pre-treatment phase. The children were randomised to receive either primaquine or placebo, to be administered with chloroquine. All pre-treatment doses were administered by direct observed therapy with food using the same follow up schedule as study one. The results from this study show, pre-treatment with primaquine reduced the risk of acquiring a new blood stage infection by 78%, (p <0.0001), and clinical disease by 68%, (p <0.0035). Age was associated with a reduced risk of *P. vivax* clinical episodes of any density (p <0.0167) and high density (p <0.016). Most re-infections occurred within 12 weeks of pre-treatment.

The third study, monitoring of safety and tolerability of primaquine treatment in the study children was performed during the pre-treatment phase of follow up in the two cohort studies. A semi-structured questionnaire of all possible drug adverse events was performed prior to primaquine dose ingestion. All primaquine doses were administered with food, however in the younger age group, children were fed by the mothers. The results from this field-based study show, 14 daily doses of 0.5mg/kg primaquine to be safe and well tolerated in children in both cohort studies. There were no serious adverse events and/or withdrawals of children associated with primaquine ingestion in the two cohorts studies. In children with reported nausea or vomiting due to primaquine ingestion, recovery was almost immediate upon food intake.

Following on from the cohort studies, pharmacokinetic properties of two single-dose primaquine in Papua New Guinean children aged 5-10 years old was performed; to assess the feasibility of short course, high-dose primaquine treatment regimens for radical cure of *P. vivax* malaria.The profiles of single doses of primaquine 0.5mg/kg and 1.0mg/kg were assessed in the children. At recruitment, study children were admitted to the Health Centre for 2 days for close monitoring, and, to allow for frequent blood sampling. During all this time participants had indwelling intravenous cannula in place for blood sampling. The

primaquine doses were co-administered with food.

The safety and tolerability monitoring showed both primaquine doses were well tolerated, with no severe adverse events. There were no observed changes in symptoms and severity of nausea or abdominal pains in the two primaquine dosage groups. There was no between-group difference in haemoglobin concentration and methaemoglobin levels. The simulated short course high dose regimens showed predicted plasma primaquine and carboxyprimaquine concentrations achieved were not significantly greater than those seen in previous pharmacokinetic studies of adults. This suggests both 0.5mg/kg and 1.0mg/kg primaquine dose regimens could be further assessed in safety and efficacy field studies.

Several primaquine studies in the past observed the efficacy of primaquine enhanced by a partner drug with a long terminal elimination phase. Primaquine is more effective when co-administered with chloroquine due to its longer post-treatment prophylactic effect as shown in the second cohort study. Studies have also shown that the therapeutic dose of primaquine is based on the total dose effect, and not the dosing intervals. Indeed, a higher daily dose for 7 days was as effective as the 14 day regimen with minimal adverse events when co-administered with food. The current 14 day, daily dosing of primaquine is most effective when given in research conditions with direct observation of drug treatment; whereas in real life situations, due to poor compliance, primaquine treatment has a reduced effect. A shorter course of high dose primaquine treatment compatible with the 3 day dosing regimens of artemisinin combination therapies would be ideal; to improve compliance during routine treatment and easier to administer as mass drug administration for malaria elimination. Thus, the last manuscript of this thesis reviews the potential use of primaquine as a tool for control and eventually elimination of malaria in the PNG context.

The results from these longitudinal cohorts and the primaquine dose studies will contribute to the scientific knowledge on *P. vivax* epidemiology and the safety of primaquine use in children and an attempt to improve compliance. This will be a useful tool for malaria control and elimination to tackle the hypnozoites in the liver of the human host. The mass drug administration strategy would

have an effective arsenal to potentially, not only eliminate hypnozoites of *P. vivax* from the liver, but stage five gametocytes of *Plasmodium falciparum* as well, to stop malaria transmission.

4.0 General Introduction

4.1 The Global burden of *P. vivax* malaria: implications for malaria eradication

Of the five *Plasmodium spp*. known to infect mankind, *Plasmodium vivax (P. vivax)* is the most widely distributed globally, with an estimated 2.5 billion people at risk [1]. According to Gething et al., the Americas contribute 22% in global area at risk of *P. vivax* transmission but the endemic areas in this region account for only 6% of the population at risk globally. The other regions of Central Asia, Duffy negativity parts of Africa, and South East Asia contribute 82%, 3.5%, and 9%, respectively. Papua New Guinea (PNG) and Indonesia have the highest endemicity for *P. vivax* in the South East Asia region. Globally, these two countries and areas in the Amazon have the highest *P. vivax* endemicity.

The World Health Organisation (WHO) world malaria report 2012, had an estimated 154-289 million reported malaria cases in 2010 with over 660 000 deaths [2]. Of these WHO global estimates, 80% of malaria cases and deaths come from 17 and 14 countries, respectively mostly from Africa. Malaria is strongly associated with poverty; countries with majority of the population living in poverty also have the highest malaria burden and mortality [2]. The estimated annual global *P. vivax* clinical cases by Mendis et al., and Price et al., range from 80 to 390 million [3, 4]. *P. vivax* malaria is no longer regarded as a benign disease. It is now widely recognised as cause of severe malaria and mortality. *P. vivax* severe malaria cases and deaths have been reported from different countries including PNG [5, 6]; Indonesia Papua [7, 8]; India [9, 10]; Turkey [11] and Brazil [12]. *P.vivax* also contributes to early pregnancy loss and low birth-weight, and the overall all cause infant mortality rates (4-8).

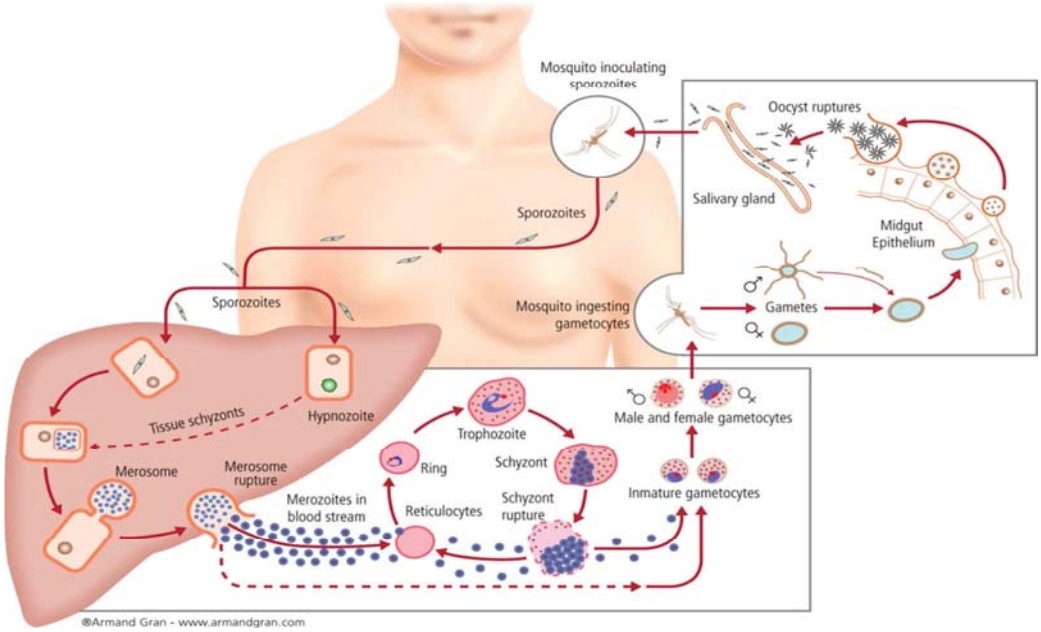
P. vivax is more difficult to control than *Plasmodium falciparum* (*P. falciparum*) as reported in countries like Thailand [13] and Brazil [14]. The use of effective treatment and control programs in these countries has resulted in a proportional increase in the prevalence of *P. vivax*, replacing *P. falciparum* as the predominant species. This may be due differences in the biology of these Plasmodia species at the liver stage of their life-cycle. *P. vivax* has a latent liver

stage called hypnozoites which remain dormant for prolonged periods of time before activation to re-infect the blood and cause relapse infection and malarial disease. The hypnozoites in the liver are therefore an important reservoir for *P. vivax* blood stage infection and transmission. Currently, primaquine (PQ), (an 8-aminoquinoline) is the only licensed drug that has an effect against the hypnozoites of *P. vivax* in the liver. PQ has also been shown to be the only drug effective against the stage V gametocytes of *P. falciparum*. The gametocytes of both *P. falciparum* and *P. vivax,* and asymptomatic blood stage infections play a crucial role in transmission of malaria to the human host and to potentially faster spread of drug resistance [15]. The asymptomatic infections of all human Plasmodia species contribute to transmission and the development of antimalarial drug resistance [16]. According to the WHO world malaria report 2012 [2], resistance to artemisinin combination therapies (ACTs) and long lasting insecticide bed nets (LLIN) has been reported in several countries in Asia and Africa, respectively. The ACTs and LLIN use in most malaria endemic countries has dramatically reduced clinical cases and mortality due to severe malaria. The emergence of resistance to these highly effective interventions for malaria will have implication on the success of the malaria eradication agenda globally. The use of antimalarial drugs has been recognised by the MalERA (Malaria Eradication Research Agenda) Consultative group as an essential tool for all stages of elimination including early malaria control phase to lower transmission and its sustainability toward eradication [17]. One of the proposed strategies for malaria elimination is mass drug administration (MDA). However, for this strategy to be effective against the hypnozoites of *P. vivax* in the liver, PQ must be included in the MDA regimens [18-20].

The eradication of hypnozoites in the liver remains a major challenge for malaria elimination programs as the current ACTs, while highly effective against blood stage malarial infections have no effect upon the hypnozoites. The hypnozoites allow *P. vivax* to adapt and survive in different geographic regions, in varying climatic and temperature conditions. Therefore, strategies for malaria elimination and its eventual eradication globally, will need to overcome the challenges of treatment and control of *P. vivax*, especially for the impoverished populations living in highly endemic countries such as PNG.

4.2 The Life cycle of *P. vivax* malaria (hypnozoite)

Figure 1: Life Cycle of *P. vivax* in Human and Mosquito gut



P. vivax has the ability to produce hypnozoites that can remain dormant in the parenchymal cells of the host liver following an acute infection. After a period of time, which varies in duration depending on the geographical area [21], hypnozoites can spontaneously reactivate, causing the release of new merozoites into the blood stream triggering a new reproduction cycle. Clinically, relapses may present as a new malaria episode indistinguishable from a new infection, with the potential to further transmit through the development of gametocytes, the infection to a new mosquito and eventually to a new human host. The gametocytogenesis in *P. vivax* occur simultaneously with the asexual blood stage and gametocytes mature in 48-72 hours thus leads to early gametocytemia[15]. *P. vivax* also has greater transmission ability at low parasitaemia levels. The mosquito vectors for *P. vivax* are early biting mosquitoes [22]. *P. vivax* is able to adapt and is more resilient toward altitude and temperature differences. In Tropical regions the hypnozoites have a shorter time to relapse (≈3 weeks) and multiple relapse episodes occur, whereas in temperate regions relapses occur at around 8-10 months after the initial *P. vivax* infection [21].

4. 3 *Plasmodium vivax* in Papua New Guinea

Several reviews of previous work in PNG have been done by Mueller, et al.[23] and Kazura, et al.[24]; on the epidemiology of malaria and research challenges and gaps in malaria knowledge, respectively. The work presented as the initial manuscript of this thesis is a review of the clinical and molecular epidemiology of *P. vivax* malaria in PNG and previous cohort studies which lay the foundation of this thesis.

In PNG, *P. vivax* is a major cause of morbidity especially in children, and in recent times, a recognised cause of severe disease in *P. vivax* endemic regions of Asia, Oceania, Central and South America and the horn of Africa [4]. The disease burden in PNG children is seen mostly in children less than 3 years of age, the older children remaining asymptomatic carriers of the parasite up to adulthood [25, 26]. *P. vivax* infection also contributes to early pregnancy loss and low birth-weight and the overall, all cause infant mortality rates as shown in studies from other countries [4, 27-30]. *P. vivax* malaria is now no longer seen as a 'benign' illness, but a cause of severe disease [4], and has a larger at risk population globally [1].

4.3.1 Impact of Earlier Malaria Control and Eradication efforts in PNG

P. vivax was the dominant *Plasmodium spp.* in most parts of PNG before the malaria eradication program commenced in the 1950's [31]. A combination of dichlorodiphenyltrichloroethane (DDT) indoor residual spraying of houses [32], and mass administration of chloroquine (CQ) [33], was used for malaria control and the attempt at its eradication. After the cessation of DDT spraying and MDA as control measures in the1970's; the composition of *Plasmodium spp*. changed from *P. vivax* to *P. falciparum* predominance [34-36]. Prior to the launch of the global eradication efforts, cases of DDT and chloroquine resistance reports had existed from other parts of the world [37]. An oversight of these reports may have contributed to the failure of the eradication program and changes in malaria epidemiology, mosquito behaviour and enhanced the development of antimalarial drug resistance in PNG. The first reported cases of CQ-resistant *P. falciparum* (CQRPf) in PNG was documented in 1976 [38-40], a few years after cessation of MDA, followed by *P. vivax* in 1989 [41]. Active drug resistance

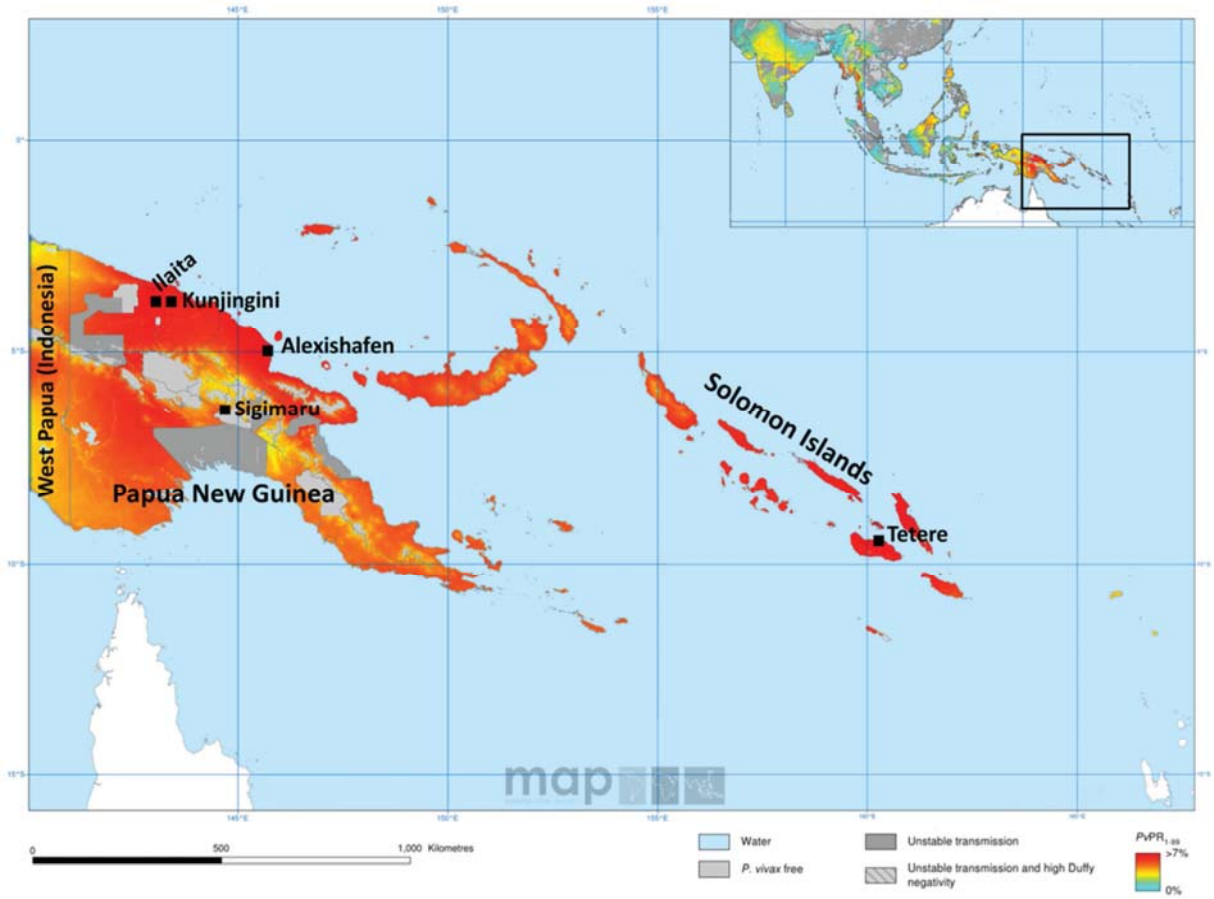
monitoring and surveillance system during the malaria elimination program may have detected the cases earlier. The emergence, spread and increase in prevalence of CQ resistance [42] has forced the PNG National Department of Health (NDoH) to change the treatment regimen for uncomplicated malaria illness for both *P. vivax* and *P. falciparum* to ACTs [43]. The new treatment regimen is more expensive and less effective against *P. vivax* malaria.

4.3.2 Endemicity of *P. vivax* (and *P. falciparum*) in PNG

Four of the five human *Plasmodium spp*., *P. falciparum*, *P. vivax*, *Plasmodium ovale* (*P. ovale*) and *Plasmodium malariae* (*P. malariae*) co-exist in PNG, a country diverse in geography and ecology, with a population of about 7million people (detailed description in [23, 24]). In PNG, the malaria transmission intensity is highly variable, with no malaria in some parts of the highland to hyper-endemic coastal areas and islands. Along the coastal and inland low-lying areas especially the north coast provinces of Madang and East Sepik, there is high intensity perennial transmission. On the island of Lihir, a very low transmission area surrounding the gold mine has being observed due to intensive malaria control activities while the rest of the island remains a hyper-endemic area [44]. Malaria transmission is less intense along the south coast of the mainland PNG, outlying island provinces such as New Britain, New Ireland and Bougainville and at higher altitudes in central highlands [45-47]. There is also smaller scale spatial heterogeneity of malaria endemicity at the village or even household cluster level within these geographic regions [48-50]. This heterogeneity may be explained by the variability in use of antimalarial drugs, availability of bed nets, and the differences in vector abundance and ecology [49, 51, 52].

The spatial distribution of *P. vivax* endemicity in PNG and parts of the Solomon Islands has been made possible by recent advances in molecular analysis of samples [53]. The endemicity map of PNG as shown in figure 2 (reproduced from Gething et al [1], courtesy of the MAP project) is based on light microscopy data.

Figure 2: Spatial distribution of *P. vivax* endemicity



The epidemiological studies described by Mueller, et al. [23], including the PNG highlands malaria surveys [54-59], were done prior to the use of polymerase chain reaction (PCR) based diagnostic assays in PNG for analysis of large sample sets. Light microscopy diagnosis (LM) was the primary method used for plasmodium infection and species identification in these reports.

A summary report [47] of the highlands malaria surveys [54-59] (carried out between December 2000 and July 2005 of 112 villages situated in the central highlands of PNG) show overall that prevalence varies with altitude for parasite species and epidemics. For non-epidemic surveys, the prevalence ranged from 0.0% to 41.1% (median 4.3%, IOR, 0.1, 5.3) and in epidemic outbreaks at altitude 1250-1960m IQR (1520-16400), the prevalence ranged from 6.6% to 63.2% (median 21.2% IOR 10.3, 35.2, p = <0.001) [47].

The prevalence rates were negatively correlated with increasing altitude in non-epidemic surveys and for epidemics (p = <0.001), more so for *P. falciparum*

than *P. vivax*. The proportion of *P. vivax* infection increased with altitude and was predominant above 1600m (p = 0.002). Analysis of correlations between altitude, population mean haemoglobin and anaemia prevalence [60] showed, an increase in mean haemoglobin with altitude; below 500m 10.5g/dl, to 12.8g/dl at altitudes >1500m (p = 0.001). The correlations of altitude with parasite and spleen rates, population mean haemoglobin and anaemia prevalence in children 2-10 years old were high, (r(2): -0.77, -0.68, 0.73 and -0.81; p = <0.001), respectively [60].

4.4 Sensitivity of Microscopy versus PCR-based test of Malaria

Prior to the introduction of PCR based diagnostic methods, LM was the primary method of *Plasmodium* parasite detection and species identification for describing PNG malaria epidemiology. LM has a significantly lower sensitivity compared to PCR based methods for detecting malarial infection [61, 62]. This low sensitivity has been a major limitation of malaria diagnosis in highly endemic countries such as PNG, with four of the 5 human plasmodium parasite species (*P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*) present. Mixed infection among the 4 parasites species is very common in PNG [23]. Several observations in the PNG setting have noted LM to be poor in identifying the less common *P. malariae* and *P. ovale* [63] and in diagnosing mixed infections [64]. The development of a semi quantitative PCR- ligase detection reaction-fluorescent microsphere assay (LDR-FMA) [65], made the analysis of large field and intervention sample sets in PNG feasible. There are now several quantitative PCR methods [66-68] that allow for estimation of parasite levels as well as positivity. The duplex quantitative real time PCR (qPCR) is a new robust, sensitive and species specific assay when compared to LDR-FMA, nested PCR (nPCR) and light microscopy [69]. The epidemiological studies in PNG now include use of qPCR for molecular diagnosis and species identification of *Plasmodium* parasites.

4.5 LDR-FMA versus Microscopy of *P. vivax* Epidemiology

The LDR-FMA was first used by Kasehagen et al. for molecular analysis and diagnosis of a subset of 1, 182 out of 16, 209 samples from four cross-sectional surveys in the Wosera district, East Sepik Province, PNG, between August

2001 and June 2003 [70]. A direct comparison with light microscopy was performed. The results showed an increase in the capacity of detection for all parasite species when compared to LM as shown in the table 1. The overall prevalence of mixed infections increased from 2.4% to 16.8% ($p < 0.001$). *P. vivax* infections were commonly found in the older children, 7- 9 years old. For *P. falciparum*, *P. malariae* and *P. ovale* the prevalence of infection peaked at 10-19 years old.

Table 1. Direct Comparision of Prevalence rates for LM and LDR-FMA

Prevalence %	*P. falciparum*	*P. vivax*	*P. malariae*	*P. ovale*
LM	20.6	12.7	4.0	0.25
LDR-FMA	32.9	27.1	12.4	5.5
LDR-FMA vs, LM *P*- value	0.001	0.001	0.001	0.001

A treatment re-infection study carried out by Michon, et al., in school age children in Madang, PNG [26], to assess the risk of re-infection and symptomatic malaria caused by different *Plasmodium spp*. used PCR based assay for diagnosis. Results from this study show different trends in incidence of clinical episodes among children infected with *P. falciparum* and *P.vivax*. Although the number of detectable infection by PCR of both *P. falciparum* and *P. vivax* was similar (incidence of 5.00 new infections with *P. falciparum* and 5.28 with *P. vivax*/child/year; ($p < 0.2$), symptomatic disease was more common in children infected with *P. falciparum* (1.17/year) compared to *P. vivax* (0.06/year). Children older than 9 years had reduced risk of acquiring *P. vivax* infection of low to moderate parasite density. Symptomatic infections with *P. malariae* and *P. ovale* were rare. By 9 years, children had acquired almost complete immunity to symptomatic *P.vivax* infections with tight control of parasite densities while *P. falciparum* immunity remained incomplete. There was no geographic variation observed with *P. vivax* infections.

The use of highly sensitive PCR based diagnostic assay for *Plasmodium spp*. infection has improved our understanding of species interaction, shift in age distribution and reduced local variation.

In 15 cross-sectional surveys of villages in the Wosera and Maprik districts of East Sepik Province, PNG, performed by Mueller and others [50], significantly higher rates of infection by *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale,* were observed by LDR-FMA compared to those diagnosed by LM, ($p < 0.001$). There was marked increase for *P. malariae* (LM: 3.9% vs. LDR-FMA: 13.4%) and *P. ovale* (LM: 0.0% vs. LDR-FMA: 4.8%). A significant excess of mixed infections over expectation was detected by use of LDR-FMA for diagnosis ($p < 0.001$). The age of peak prevalence shifted for *P. falciparum* and *P. vivax* to the older age groups; *P. falciparum* (LM: 7-9yrs 47.5%, LDR-FMA: 10-19yrs 74.2%) and *P. vivax* (LM: 4-6yrs 24.2%, LDR-FMA: 7-9yrs 50.9%). There was significant and higher geographic variation in prevalence rates observed in LDR-FMA for all *Plasmodium spp*. compared to LM diagnosed infections (overall, 84.4% vs. 37.6%, respectively). The variation was to a large extent explained by the coverage and use of insecticide treated bed nets.

Following on from observations in these previous studies, a longitudinal cohort study of children aged 1-3yrs was carried out in the Ilahita area of Maprik District by Lin and others [25]; including some children from the same villages in the cross-sectional surveys [50]. Out of overall 199 infections diagnosed infections among the children at enrolment by LDR-FMA and LM, *P. vivax* was the most common parasite as shown in the table 2.

Table 2. Higher *P. vivax* Prevalence rates by LM and LDR-FMA in 1-3yr old children

Prevalence %	***P. falciparum***	***P. vivax***	***P. malariae***	***P. ovale***
LM	32.6	44.3	4.2	0.0
LDR-FMA	49.6	53.0	9.9	2.7

Mixed species infection was detected in 85/199 (42.7%) by LDR-FMA and 45/168 (26.8%) by LM among the children.

The prevalence of P. vivax infections increased significantly with age for LDR-FMA as shown in table 3, but this did not occur for LM diagnosed infections ($p < 0.30$).

For *P. falciparum* infections the prevalence increased significantly for both LDR-FMA and LM, ($p < 0.001$) from children <18 months (LDR-FMA: 27.3%, LM: 12.3%) to children 18-23 months (LDR-FMA: 38.1%, LM: 19.3%) and children 24-29 months (LDR-FMA: 54.3%, LM: 32.2%) with no further increases in older children ($p = 0.24$).

Table 3. Prevalence rates for *P. vivax* increase with age by LDR-FMA diagnosis

Age group	**<18 months**	**18-23 months**	**24-29 months**
***P. vivax* Prevalence % by LDR-FMA**	57.6	67.3	73.5

In children with *P. vivax* infection, the prevalence of febrile episodes decreased significantly with age (OR (per year increase in age = 0.57, $p = 0.001$). This trend was not observed in *P. falciparum* and mixed infections (OR = 0.97, $p = 0.77$). The analysis of age trends in burden of *Plasmodium* infections and disease showed, pronounced rise of *P. falciparum* and *P. vivax* infections with increasing age. However, while the overall episodes of clinical disease was comparable (*P. falciparum*: 2.56, *P. vivax*: 2.46 episodes/child/yr), the infectious episodes of both *Plasmodium spp.* showed strong but opposing trends. The incidence of *P. vivax* clinical episodes decreased significantly with age throughout the age range (any density ($p = 0.002$); *P. vivax* density >500: ($p < 0.001$). Incidence rate ratios for *P. vivax* decreased from 3.07 (any density) and 2.13 (for *P. vivax* density >500) episodes/child/year in children <18 months; to 1.5 (any density) in children 36-41 months; and 0.59 (for *P. vivax* density >500) in children ≥42 months, respectively. *P. vivax* incidence decreased in dry season, while the prevalence remained the same. Compared to *P. vivax*, for *P. falciparum,* incidence and prevalence showed more marked seasonal variation. These differences in seasonal variation between the two *Plasmodium spp*. may be due to relapses from activation of hypnozoites of *P. vivax* in the liver, which lead to blood stage re-infection and disease during the dry seasons when mosquito populations are low, hence effect on low transmission.

4.6 Molecular Epidemiology of *P. vivax*

4.6.1 Plasmodium parasite species Interaction

The molecular detection methods allow assessment of species interaction in areas such as PNG where mixed infections commonly occur [25, 50, 64, 70], allowing the assessment of whether one species modifies the course or the risk of infection in relation to the other. A study in Vanuatu using microscopy detection of parasite species suggested epidemiological evidence of cross-species immunity between *P. falciparum* and *P. vivax* [71]. In PNG, an analysis of mixed-species infection in semi-immune children, showed that, there may be density-dependent regulation of all malaria parasites that coexist in the blood [72]. A high parasitaemia of one clone may reduce the effective replication of another parasite either from the same or different species. However the detection of mixed species was done using non-quantitative PCR, with estimation of parasite density using LM which has a very low sensitivity and specificity compared to PCR based tests [36, 50, 69, 70].

Analysis of complexity of infection by Mehlotra and others showed, the coexistence and distribution of each of the 4 *Plasmodium spp*. in a population and in age groups to be random, irrespective of having been diagnosed by LM or PCR [36]. The establishment of mixed infection in the blood is common, and is independent of each *Plasmodium spp*. genotype infection.

4.6.2 *Plasmodium vivax* genetic diversity

In 1999, Bruce and others used a combined PCR- restriction fragment length polymorphism (PCR/RFLP) protocol for the first time in PNG to investigate the allelic diversity of *P. vivax* at the merozoite surface protein-3alpha (*msp*3α) locus [73]. The study showed preliminary evidence of multiple genotypes in samples of single infections indicating extensive polymorphism of *P. vivax* parasites. Since then different PCR protocols made the discrimination of parasite species and genotype with ease in large number of blood samples possible. The same authors also genotyped *P. vivax* at the *msp*3α locus of samples from a highly endemic area of PNG in 2000, showing frequent changes in the genotypes of parasites detected in asymptomatic individuals [74, 75]. *P. vivax* genotypes found in an individual and amongst participants were highly

diverse and complex. This genotypic diversity was not correlated with age. The asymptomatic infection episode was longer in the 4 year olds compared to older children [74], suggesting faster acquisition of immunity to *P. vivax* [75].

Genotyping also allows for mapping of small area variations and identifying the origins of parasites during surveillance of transmission in malaria elimination and in epidemics. Analysis of blood samples by PCR from an epidemic of malaria in the Eastern highlands province, PNG, in 2002 by Mueller and others showed for the first time in PNG, *P. falciparum* infections originating from a single genotype, whereas *P. vivax* infections were genetically diverse [76]. The results suggest local endemicity for *P. vivax* with low-level transmission, and introduction of *P. falciparum* from outside as the most likely cause of the malaria epidemic.

4.6.3 Complexity, Multiplicity of *P. vivax* infections (MOI) and Molecular Force of Blood-stage Infection ($_{Mol}$FOB)

Molecular parameters are useful for monitoring the dynamics of transmission and incidence of infection or disease using high precision genotyping. Multiplicity of infection (MOI) is defined as the number of concurrent parasite clones per parasite positive host. The molecular force of blood-stage infection ($_{Mol}$FOB) on the other hand is defined by the appearance of new parasite clones in the bloodstream per unit time interval. The $_{Mol}$FOB is useful for monitoring of efficacy of antimalarial drugs and malaria vaccine effects during field trials[77]. As countries move from malaria control to elimination, $_{Mol}$FOB, may also be used as a surveillance tool for monitoring changes in malaria transmission patterns.

A report in 2005 by Cole-Tobian et al. assessed complexity of infection in a subset of 4 children aged 4-12 years and a 31 year old adult *P. vivax* positive by nPCR following a cross-sectional survey [78]. Over a 4 month period of repeated sampling, the authors observed in each individual between 5 and 13 unique *P. vivax dbpII* genotypes. There were more clones observed for *msp3α,* ranging from 7 to 38 (median= 21 clones). The median number of unique parasite genotype observed with *msp3α* in an individual at a single time point was 12 (range: 2-24). Compared to children, the adult participant had only 2

unique genotypes. Occasionally, a similar clone was observed in the same individual or multiple children. The highest complexity of genotypes was seen in a participant who was LM negative at the time of sampling. This re-emphasizes the observation that at very low parasitaemia levels PCR based assay is superior to LM. Also, it highlights that *P. vivax* has the potential for transmission of different clones even at very low densities. The clones will potentially undergo sexual recombination in the mosquito gut when transmission occurs, leading to more genetic diversity of *P. vivax.*

In search for better and less labour-intensive PCR based assays for evaluation of *P. vivax* genotyping markers, Koeplfi and others, assessed a high-throughput system based PCR followed by capillary electrophoresis [79]. This could be a very useful tool for genotyping samples from drug resistance monitoring and large intervention studies. The authors selected microsatellite markers: Pv3.27; MS16; Pv1.501; and Pv3.502 for assessment. Results show MS16 being the most diverse (p = 0.023, H_E = 0.988), followed by Pv3.27 (p = 0.07; H_E = 0.94). Using the two combinations together (MS16 & Pv3.27) reduced further the probability of two samples having the same *P. vivax* genotype by chance (Overall probability, πP_i = 0.0016). The probability of two independent *P. vivax* infections carrying the same genotype when the two microsatellite markers are combined for analysis was 0.16%. Adding more microsatellite markers to the analysis reduces the probability further, by a factor of almost 10 for each extra microsatellite marker. *P. vivax* parasite clones with the MS16 marker, also had the higher multiplicity of infection (MOI = 2.37) in the study samples.

Following on from this study, the same authors used the same PCR based analysis in a larger sample set from a cohort of 268 PNG children aged 1 to 4.5 years, (the Lin et al. study, [25]); using MS16 and *msp1*F3 molecular markers [80]. The authors observed high diversity of *P. vivax* genotypes, (MS16: = H_E = 97.8%); (*msp1*F3: H_E = 88.1%); and multiplicity of infection (mean MOI = 2.7, [IQR = 1-3]) of *P. vivax* infections. The *P. vivax* positive samples had multi-clonal infections in 74% of them (n = 1162). There was minimal increase in multiplicity of *P. vivax* infection with age (p = 0.02). The highest rise in multiplicity of infection was observed in the younger age group (MOI = 2.4). This

corresponds to the observation from Michon et al. and Lin et al., [25, 26] of clinical illness more commonly seen in the younger children as compared to the older age group. A proportional increase was seen with the number of clonal infections and age. In 18 children aged 300 to 400 days, MOI of 1.67 was observed, and only two of these children harboured more than 2 clones. There was no seasonal variation in the multiplicity of *P. vivax* infection (p = 0.50). In comparison to *P. falciparum* infections in the same cohort, the authors observed a lower MOI of 1.5 with the *Pf msp2* microsatellite marker and, in only 35.2% of the samples; multiple clone infections were observed [80].

Furthermore, Koepfli and others have recently genotyped 14 microsatellites markers from 295 *P. vivax* positive samples collected from 4 different geographic areas of PNG and a single area from Solomon Islands [53]. The authors observed a high percentage (63-88%) of people in the study with multiple clone infections.

Similar studies by Arnott and others, observed high *P. vivax* genetic diversity in samples from 2 areas of North coast PNG, using MS16 and *msp1*F3 as molecular markers [81]. The prevalence of *P. vivax* infection in the sample set from the areas in Maprik and Madang was 15% and 27-35%, respectively. Even though the observed prevalence were markedly different, one area twice the other; the genetic diversity was similar H_E = 0.77 - 0.98.

The difference in the *P. vivax* prevalence was most likely related to LLIN use in Maprik which was not the case for Madang study areas. Relapses from hypnozoites in the liver may account for maintaining the clonal diversity in the Maprik area, despite the use of LLIN. *P. vivax* has the ability to maintain an effective clonal population greater than *P. falciparum* by reducing inbreeding, continual genetic mixing from sexual recombination of different genotypes, and because it has co-evolved with the human host over millions of years as an evolutionary force.

Genotyping of parasites from the aforementioned studies [25] [82] has also shown that, on average, children had 14.0 new *P. vivax* blood-stage clones/child/years-at-risk. The authors observed the incidence of clinical *P. vivax* illness to be strongly associated with molecular force of blood-stage

infection ($_{Mol}$FOB) (IRR = 1.99, 95% CI, [1.80, 2.19]), with no change of $_{Mol}$FOB with age. Furthermore, the incidence showed a faster decrease with age for children with a high exposure (IRR = 0.49, 95% CI, [0.38, 0.64]), $P < 0.001$) compared to that with low exposure (IRR = 0.63, 95% CI, [0.43, 0.93]), $P < 0.02$). The $_{Mol}$FOB *P. vivax* of 14.0 was found to be much higher compared to the $_{Mol}$FOB of 5 new *P. falciparum* blood-stage clones/child/years-at-risk. The rapid acquisition of immunity against clinical *P. vivax* malaria may be due to the high number of *P. vivax* clones that infect children in early childhood.

4.6.4 Genetic Polymorphisms

Several human genetic polymorphisms have been suggested to play an important role in the natural selection process due to malaria in populations of Melanesian origin such as PNG and Solomon Islands. These polymorphisms are thought to offer protection against blood stage infection and severe malaria. Among these polymorphisms is G6PD deficiency, an X-linked hereditary genetic defect due to mutations in the G6PD gene [83], which is prevalent in areas of malaria high endemicity. Studies in other parts of the world have observed Mediterranean type deficiency to offer some protection against *P. vivax* infection [84]. To-date, this effect has not been seen in the PNG studies. The overall prevalence for G6PD deficiency in PNG is yet to be determined, although some studies estimate the prevalence of the severe form of G6PD to be around 6.6 percent [24]. The use of PQ for *P. vivax* treatment in PNG has being limited due to G6PD deficiency associated risk of haemolytic anaemia. Other polymorphisms include: α-thalassemia, Southeast Asian ovalocytosis (SAO); a promoter mutation that silences expression of the Duffy Antigen Receptor for Chemokines on the red blood cells; Melanesian Gerbich blood group negativity and expression of red blood cell Complement Receptor 1 [24]. Some of the polymorphisms found in PNG have a high prevalence among the population.

The α-thalassemia alleles, -α/αα and –α/-α, are observed in 36.9, and 56.0 percent of the population, respectively [24]. However, no association was found from analysis of large sample set from cohort of children aged 3-21 months [85] between α(+)-thalassemia and the risk of *P. vivax* and *P. falciparum* infection and disease [86]. The severe malaria study [5], observed at least a single α-

thalassemia deletion found in 83.6 percent of the 320 children with severe malaria.

Along the north coast of Madang, SAO is present in up to 10-15% of people in some populations [24]. In the severe malaria study [5], seven children with SAO had coma/impaired consciousness. In seventeen (5.3%) children with severe disease, SAO was present (1 child with *P. vivax* mono-infection; 2 with mixed (*P. vivax & P. falciparum)* infection; and 14 had *P. falciparum* mono-infection).

Analysis of pooled sample sets from independent studies; 2 cohorts and 1 case-control in PNG children showed, a reduced risk of *P. vivax* malaria observed in children with SAO [87]. Genotyping of 1975 children from the 3 studies showed a reduction by 46% of the incidence of *P. vivax* clinical illness in infants 3-21 month with SAO, ([IRR] = 0.54, p <0.0001). There was also a significant reduction by 52% in *P. vivax* (blood-stage) re-infection diagnosed by PCR (p = 0.003) and 55% by light microscopy (p = 0.014), respectively, in children aged 5-14 years old from the pooled data set. *P. vivax* parasitaemia was significantly reduced, (p = 0.011) in the 3-21 month old children.

In Inland regions of East Sepik Province, exon 3 deletion of the red blood cell glycophorin C (Gerbich blood group) is found in 46.0% of the population [24].

4.7 Naturally Acquired Immunity

Our present day knowledge and understanding of immunity was suggested by Robert Koch almost a century ago [88]. Since Koch's visit to PNG, the mechanism of *P. vivax* protective immunity has not been as yet fully understood, especially in children. From longitudinal cohort studies, in PNG, naturally acquired immunity to *P. vivax* appears to develop very early in life and quickly, with the burden of disease seen mostly in children under 3 years of age [25, 26, 50]. While in populations moving from areas with no malaria or low transmission such as the trans-migrants in Indonesia New Guinea, both children and adults have equal risk of infection and of disease following the first malarial infection with *P. falciparum* and *P. vivax* [89]. However, the pattern of acquiring immunity seems to be different for the native populations and trans-migrants for

the two parasite species. [90]. After several clinical episodes of malaria, a year or two later, the pattern changes to acquiring age-dependent immunity to high density parasitaemias and fever. The relative benefit of life-long exposure to *Plasmodium* parasite infections in the native populations is observed for *P. vivax* but not for *P. falciparum* [90]. Several cohort studies in PNG [25, 26], have observed development of clinical immunity to *P. vivax* to be almost complete by the time a child reaches 9 years old, whereas immunity to symptomatic *P. falciparum* malaria remains incomplete. These observations suggest that clinical immunity to *P. vivax* malaria starts very early in life, in the second to third year after birth [25]. It is characterized by increasing ability to control parasite densities below the pyrogenic threshold and nearly complete immunity after 5 years of exposure to high perennial transmission. As such the rate of acquisition of immunity to *P. vivax* is similar to that observed against *P. falciparum* in African children. The rate of acquisition of immunity to *P. falciparum* is however substantially slower in PNG compared to African children

4.8 Vectors

The main vectors for malaria transmission in PNG by morphologic *spp*. are members of the *Anopheles punctulatus* complex: *An. farauti; An. koliensis;* and *An. punctulatus* [51]**.** These vectors are also seen in Indonesia Papua, Solomon Island and Vanuatu. Other vectors such as *An. bancrofti*, *An. kawari* and *An. longirostris* have a minor role as vectors for malaria [52]. The use of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), identified more *spp*. of *An. farauti* and *An. koliensis* which are morphologically indistinguishable as *An. farauti s.s, An. farauti No.2* (also called *An. hinesorum*) and *An. farauti No. 4* [91].

The location and biting cycles are different for each spp. *An. farauti s.s* is found in the coastal areas; *An. farauti Nos* 2, 3, and 4 are found in coastal and inland areas and *An. farauti No*s 5 and 6 are found in the highlands areas. *An. farauti No7* was first identified in the Solomon Islands [92]. The biting hours of *An. farauti s.s,* has a more uniform biting activity estimated between 6-6am with a peak around 9-10pm, whereas 2 and 4 are early biters around 6-10pm and *An. punctulatus* during early hours of the morning 2-6am [91].

In 2009, Coopers et al [93] described *An. hinesorum* (new name for *Farauti No 2*) as a malaria vector in PNG for the first time using PCR and the other *Anopheles spp*.: *punctulatus*, *koliensis*, *farauti*, *farauti* 4, confirmed as the main vectors for malaria; and *An. farauti* 6 the main vector in the highlands river valleys (>1500m above sea level). A new taxon within the *Punctulatus* group was also identified as *An. farauti* 8. According to the authors, *An. bancrofti and An. longirostris* show high incidence of infection in some areas.

Malaria transmission studies in 1988 by Burkot et al [51], described *An. punctulatus* and *An. kolensis* as more anthropophilic than *An. farauti,* and the geometric mean *P. vivax* sporozoite density was higher in *An. punctulatus* than in either, *An. kolensis* or *An. farauti.* The sporozoite and inoculation rates varied greatly within villages and over time. As far as it is know, these species transmit both *P. falciparum* and *P. vivax.*

4.9 Clinical Disease and Severe *P. vivax* malaria

In PNG, *P. vivax* malaria is a major cause of morbidity especially in the younger children less than 3 years old [25, 26]. Globally, *P. vivax* malaria is a recently recognised caused of severe disease, and recent evidence [94] and studies from different areas of PNG and Indonesia Papua [5-8] have documented severe manifestations of malarial illness and deaths from *P. vivax* infection.

4.9.1 Severe *P. vivax* malaria - Community-based observational study

A prospective cohort study [6] over an 8 year period of presumptive malaria cases presenting to 2 Health Centres by Genton et al., documented for the first time an association between *P. vivax* monoinfection and mixed (*P. vivax* and *P. falciparum)* infections with severe malaria. Malaria cases were confirmed using LM with severe malaria classified according to WHO 2000 [95] case definition with several modifications. The authors used *P. vivax* case definition of severe malaria as that of *P. falciparum*, but parasite densities > 250, 000/µl were disregarded, as not applicable to *P. vivax* infections. The results showed *P. vivax* infections being common in children aged two to less than five years old, with up to 20% parasitaemia. Overall, *P. vivax* accounted for twenty-one

percent (21%) of severe malaria cases. *P. falciparum* mono-infections and mixed (*P. vivax* and *P. falciparum)* infections accounted for seventy-one (71%) and five percent (5%), respectively. In the severe malaria case presentations, respiratory distress was more common in cases due to *P. vivax* infection than *P. falciparum,* (60.5% vs. 41%, $p = 0.002$). The mixed (*P. vivax* and *P. falciparum)* infections also more often presented with respiratory distress than did *P. falciparum*, (66.7% to 41%, $p = 0.015$). In *P. vivax* severe malaria cases, up to 25.6% had neurological symptoms. Anaemia was present in 18.6% of the severe *P. vivax* malaria cases. The mixed (*P. vivax* and *P. falciparum*) infection cases of severe malaria had neurological symptoms and anaemia in 8.3% and 29.2%, respectively.

4.9.2. Severe *P. vivax* malaria - Hospital based severe malaria study

A hospital based study of severe malaria cases in PNG children [5] by Manning et al. showed, in children with confirmed severe malaria, that *P. vivax* infections were responsible for up to 7.9% of the cases, while *P. falciparum* mono-infection accounted for 71% of the cases, and mixed (*P. vivax & P. falciparum*) infections caused up to 14.7% of the cases. The WHO 2000 guidelines [95] for severe malaria were adopted for this study.

The commonest clinical presentations of severe *P. vivax* malaria included impaired level of consciousness or deep coma in 29.6% of the cases; severe anaemia in 11.1% and metabolic acidosis or hyperlactatemia in18.5% of the cases. Respiratory distress was five times more common in severe *P. vivax* malaria compared to severe *P. falciparum* cases (OR = 5.04, $p = 0.001$), after adjusting for hyperlactatemia and severe anaemia. The other presenting features were similar in both severe *P. vivax* malaria and *P. falciparum* cases. Children with severe malaria from mixed (*P. vivax* and *P. falciparum*) infections were more likely to present with higher median respiratory rate than *P. falciparum* mono-infections, ($p = 0.014$). Also, mixed infections with severe malaria also had a lower Blantyre Coma Score (BCS), ($p = 0.018$) and were more likely to show deep coma, ($p = 0.030$) compared to *P. falciparum* mono-infection cases. Children with severe malaria due to mixed infections also had lower mean haemoglobin and serum sodium concentrations, and a higher median plasma bilirubin levels compared to those from *P. vivax* mono-infection.

The mortality rate from severe malaria due to *P. vivax* was 3.7%, raising up to 8.0% in mixed (*P. vivax* and *P. falciparum*) infections. Surprisingly, *P. falciparum* severe malaria cases had a mortality of rate of 0.4% in this study.

In the longitudinal cohort by Lin et al. [25], the incidence of severe *P. vivax* and mixed (*P. vivax* and *P. falciparum*) infection was 16.1 and 20.2 per 1000/person/yr at risk, respectively [5]. The risk of severe compared with an uncomplicated malaria from mixed (*P. vivax* and *P. falciparum*) infections was similar to *P. falciparum* alone; 3.4% vs. 4.2%, (p = 0.81) but was greater than *P. vivax* mono-infections 3.4 and 0.9 percent, (p = 0.035).

4.9.3 Comparing PNG and Indonesia Papua - Severe *P. vivax* disease

Observations from studies in Indonesia Papua [7, 8, 28, 30], the western half of the Island of New Guinea confirm the finding from PNG [5, 6], with some differences in the clinical presentation of severe *P. vivax* malaria. A review by Price et al., documented results from studies done in PNG, Indonesia Papua, Thailand and India showed that, in those countries, up to 21-27% of the patients with severe malaria occur after *P. vivax* mono-infections [94]. In Indonesia Papua, severe anaemia is seen in 67-80% of the cases with an overall mortality rate ranging from 0.8 – 1.6% [7, 8]. The mortality rose considerably (10-39%) when severe anaemia and other manifestation of severe disease were concurrently present [8]. In the majority of severe *P. vivax* cases, cough was a frequent complaint [96]. Respiratory distress, the major clinical feature in PNG [5, 6] seems less common elsewhere. Jaundice and renal failure has been described in a study from Indonesia Papua [7]. In a study of infants from southern Indonesia Papua [30], more infants were hospitalised with *P. vivax* malaria even though *P. falciparum* was the most prevalent species in the area. There was an increase risk associated with *P. vivax* infection of severe anaemia (OR=2.4) and thrombocytopenia (OR=3.3) compared to *P. falciparum* infection in infants from this study [94]. In the studies from PNG and Indonesia Papua [6, 8], mixed (*P. vivax* and *P. falciparum)* infection was associated with an increase risk of severe anaemia.

Several factors associated with *P. vivax* severe anaemia have been suggested [94] such as: recurrent infection from *P. vivax* leading to removal of

infected and uninfected red blood cells, dyserythropoiesis and retention of RBC in the spleen (25, 26), and increased RBC fragility and haemolysis (24).

4.9.4 Drug Treatment and Drug-resistant

The unique biological differences between *P. falciparum* and *P. vivax* arise from their complex life-cycles. *P. vivax* and *P. ovale* have a liver stage called hypnozoites, which remain dormant and can re-activate weeks, months or in some cases years after the primary infection to cause a new relapsing malarial infection and thus clinical disease [21]. Current treatment regimens are based on the parasite-stage-specific and the functional grouping of the drug [97]. Drug treatment for malaria can be classified according to their activity against the different stages of the malaria life cycle as: sporontocidal; tissue schizonticidal and hypnozoitocidal; blood schizontocidal and gametocytocidal [97]. Complete cure of *P. vivax* malaria infections require drugs effective against active tissue schizonts, quiescent hypnozoites, asexual blood stages and gametocytes. Currently, primaquine (PQ) is the only licensed drug that was shown to be effective in eradicating the *P. vivax* liver stages and gametocytes. It has however only limited efficacy against blood-stages and is thus not recommended as a cure for *P. vivax* malaria infection and disease.

4.9.5 Chloroquine Resistant *P. vivax*

For a long time the treatment of choice for *P. vivax* malaria infection and disease has been CQ. Of the combination regimens used for *P. vivax* malaria treatment, the co-administration of CQ plus PQ has been the most effective regimen against relapse malaria. However, the emergence and spread of chloroquine-resistant *P. vivax* (CQRPv) in countries such as PNG, Indonesia, Burma, Vietnam, South Korea, Turkey, some countries within the horn of Africa, Madagascar, South America and Eastern India [94], has led to changes in treatment regimens in some of these countries.

In PNG, CQRPv was first documented in 1989 [41], 13 years after reports of CQRPf in 1976 [38, 40] and 9 years after report of sulfadoxine-pyrimethamine (SP) resistant *P. falciparum* in 1980 [98]. CQ resistance was first observed only a few years after CQ MDA for malaria eradication [33] ceased in the early 1970's. A single regimen for *P. vivax* and *P. falciparum* using CQ with a dose of

PQ 0.75mg/kg as a gametocytocidal regimen was recommended for use prior to 2000. The treatment regimen was changed to CQ or amodiaquine (AQ) plus SP as first line for both *P. vivax* and *P. falciparum,* and artesunate (ART) plus SP as second line treatment. Quinine plus SP was reserved for severe malaria and as rescue treatment. Following reports of low efficacy and spread of CQRPv and CQRPf to most regions of PNG [42, 99], a standard treatment trial of antimalarials was carried out; to compare CQ regimens against (ACTs) [100]. The four different comparative trial arms consisted of CQ+SP; ART+SP, arthemether/lumefantrine (AL) and dihydroartemisinn/piperaquine (DHAPQ). The observed, *P. vivax*, 28 day (28d) and 42 day (42d) adequate clinical and parasitological responses (ACPR) for the different study arms were: CQ+SP (28d: 51.0%, 42d: 13%); ART+SP (28d: 51.3%, 42d: 33.3%); DHA-PQ (28d: 84.2%, 42d: 69.4%) and AL (28d: 48.5%, 42d: 30.3%). Genotyping of the *P. vivax* parasites from the study samples showed most of the infections in the AL group were of different genotypes, classified as new *P. vivax* infections and not recrudescence of the initial infection from the same genotype observed at baseline [101]. The revised PCR-corrected ACPR for AL was, 79.3% and 77.8% for 28d and 42d, respectively. Contrary to *P. falciparum* infections (which do not relapse), molecular diagnosis using microsatellite markers to detect parasites in patients with new *P. vivax* infections may not adequately discern between a new infection (theoretically with a different genotype from that of the baseline infection), a recrudescent infection (arising from the incomplete or inadequate treatment of the original infection, and thus with an identical genotype) and a relapsing one (deriving from hepatic newly formed or previously existing hypnozoites which can be genetically identical or different from the initial clinical infection, particularly if older heterologous hypnozoites are the ones reactivated [102, 103].

4.9.6 PNG National Standard Treatment for uncomplicated malaria

The recommendation for AL as first line treatment for both *P. vivax* and *P. falciparum* was based on the ACPR (28d: 97.3% and 42d: 95.2%) for *P. falciparum* malaria among the study participants. DHA-PQ was chosen as the second line drug. A review of the treatment of *P. vivax* infections with AL concluded that this drug can be effectively be used but that treatment regimens

should be administered in combination with PQ [18]. Analysis of effectiveness of AL use as treatment of uncomplicated *P. vivax* and *P. falciparum* clinical illness in young PNG children [104], from a study of intermittent preventative treatment in infants (IPTi) [85], found AL effective against *P. vivax* and *P. falciparum* infections. However, a higher rate of *P. vivax* recurrent clinical episodes between 28d and 42d was observed among participants. The authors also recommended use of PQ in combination with AL.

The current 2008 PNG [43] recommended standard treatment for uncomplicated malaria from *P. vivax* and *P. falciparum* infections includes use of PQ for confirmed *P. vivax* cases. However, due to insufficient information on the prevalence of severe variants of G6PD deficiency in PNG at the time of policy formulation, and, no cheap and easy to use, point of care rapid diagnostic test (RDT) for G6PD deficiency testing, a 14 day, low dose PQ 0.25mg/kg was recommended for use nationwide. The efficacy of such a lower dose remains uncertain.

4.9.7 Primaquine and G6PD deficiency

The radical cure of *P. vivax and P.ovale* infections requires the treatment of both blood and liver stages of the parasite (hypnozoites). For over 60 years, the only drug known to have any effect in eradicating the liver stages of both *P. vivax* and *P.ov*ale has been primaquine (PQ). However, to-date questions about PQ remain as shown in the following panel.

• How does PQ work?
• What is the best PQ regimen to balance efficacy, compliance and side effects?
• Are the current WHO dose recommendations valid for every malaria endemic setting?
• What is the real problem of PQ tolerance and/or resistance?
• Should PQ radical cure be used in highly endemic areas?
• Is it safe?

• Can it be used in children?
• Should the use of PQ as a transmission blocking weapon be promoted in all malaria-endemic areas?

There are several important major drawbacks of the (low dose) PQ regimen and its implementation into routine clinical practice for the treatment of hypnozoites. Firstly, studies and reviews [105-107] have indicated the Chesson strain found in PNG requires a higher dose of PQ (0.5mg/kg or higher) to be effective. Secondly, PQ has a high associated risk of side effects, particularly among people with G6PD deficiency. This enzymatic deficiency, of which ~140 different variants exist, is an absolute contraindication for the use of PQ when the enzyme's activity is below the threshold of 5%, but the drug can be used in milder cases with the provision of spreading the treatment on a weekly basis during a two month-long 0.75 mg/kg schedule. G6PD deficiency is frequent in malaria endemic areas such as PNG. To-date, several molecular studies have been done in PNG [108-110]. However, overall prevalence and epidemiological screening in any given population is imprecise [83], as it does not quantify the clinical phenotypes (allelic mutations associated with haemolysis) at the individual level. Due to the risks associated with PQ treatment, the National Health Department has recommended G6PD testing in all health facilities as part of its strategy for malaria control in the country [111].

4.9.8 G6PD testing

The available methods of testing for G6PD deficiency, such as the Motulsky dye decolouration test [112], NADPH fluorescent spot test [113, 114] and variations of the MTT formazan methods [115-118] require specialized equipment and have therefore not been successfully implemented in clinical settings in malaria-endemic area [115, 119]. More recently, the FDA approved BinaxNOW G6PD test (Inverness Medical, Switzerland) and the Dojindo G6PD WST-8 Assay Kit (Dojindo Molecular Technologies, Japan) [115, 120, 121], which have provided a more suitable alternative but challenges to their implementation in malaria-endemic areas remain. Both tests are temperature/light sensitive, expensive (approximately US$8 and US$5 per test, respectively), are not stand-alone kits (requiring additional equipment) and

require a higher level of training to perform and interpret than the commonly utilised malaria RDTs. Recently, a new G6PD test kit called CareStart™ (Access Bio, New Jersey, USA), currently undergoing research and development was tested for the first time in field conditions to assess its performance [122]. The CareStart™ is an RDT-format test, which could be used together with current RDT testing for malaria diagnosis once fully developed and approved, as point of care, easy to use diagnostic tool for G6PD deficiency testing.

Until such challenges are overcome and routine G6PD screening is implemented at outpatient health services in PNG, *P. vivax* malaria and relapses from the dormant stages in the liver will remain a challenge for PNG national malaria control programs as the low dose PQ treatment recommended in the absence of G6PD testing is unlikely to be effective against the circulating *P. vivax* strains present in PNG [107].

4.9.9 Hypnozoitocidal and Gametocytocidal Drugs

The hypnozoites in the liver represent an important source of re-infection, disease and transmission of *P. vivax*. In order for malaria prevention and control programs to be effective, treatment options for eradication of the liver stages of *P. vivax* must be evaluated and implemented together with other control measures such as ACTs and LLIN. All confirmed cases of malaria, including *P. falciparum* monoinfections will need to be treated with PQ as high proportion (over 50%) become *P. vivax* positive post-treatment and remain asymptomatic [100]. Asymptomatic carriers of all *Plasmodium spp*. parasites contribute to disease, transmission and development of resistance to antimalarial drugs [123].

Besides its activity against hypnozoites, PQ is highly effective in killing the sexual forms of all *Plasmodium spp*. parasites (i.e. the gametocytes) [20]. This is particularly important in the treatment of *P. falciparum*, whose stage V gametocytes are relatively resistant to treatment with most other blood-stage antimalarials [124, 125]. Consequently, *P. falciparum* gametocytes are commonly seen for up to 4 weeks after successful treatment of asexual forms and can contribute both to transmission and to potentially faster spread of drug

resistance [15]. For these reasons, a single dose of 0.75mg/kg PQ was included in the PNG national treatment guidelines until 2000 when the PQ single dose was dropped in conjunction with the switch from CQ/AQ mono-therapy to CQ/AQ plus SP combination therapy. Following a large consultative process WHO has recently issued a recommendation for the inclusion of a single dose of 0.25mg of PQ for treatment of *P. falciparum* malaria irrespective of G6PD status in places in which there is a threat of artemisinin resistance or where elimination programs are in place [126]. The WHO recommendation also indicated that countries already using the previous recommended dose of 0.75mg to continue until additional data on the efficacy on the lower dose becomes available. PNG should therefore consider re-introducing such a single PQ dose in its national treatment guidelines. Although the improved use of PQ could result in a significant improvement of both current treatment of *P. vivax* malaria and reduce transmission of any *Plasmodium* spp., the need for G6PD testing as well as problems with adherence to the long treatment schedule are considerable obstacles to a large-scale roll-out of PQ therapy. Consequently, there is a great need to develop alternative anti-hypnozoite drugs. One such novel drug is tafenoquine [127-129]. As another 8-aminoquinoline, it shares the problem of potential haemolysis in G6PD deficient individuals with PQ, however, due to its long half-life, it can be given as a single dose or 3-day long treatment and can thus potentially be combined with standard 3-day blood-stage regimens. Tafenoquine is currently undergoing phase II/III testing and has shown preliminary promising results[130]. Even though Tafenoquine will address the problem of poor compliance with current PQ regimens, additional anti-hypnozoite drugs that can be safely given in G6PD deficient individuals are a high priority in the malaria elimination research agenda [17].

5. Specific introduction to this thesis

This thesis is based on work undertaken through a partnership between Barcelona's Centre for International Health Research (CRESIB) and the Papua New Guinea Institute of Medical Research (PNGIMR). The first longitudinal cohort study in children 1 to 5 years old was funded by Cellex Foundation (Barcelona, Spain) through the *P. vivax* Vaccine Consortium as part of the baseline immune-epidemiological studies for setting up a field trial site for testing candidate *P. vivax* vaccines in Papua New Guinea. This was followed by the second cohort of children aged 5 to 10 years old, funded by the PNGIMR. The study assessing the safety and tolerability of PQ in children was done during the pre-treatment phase of the two cohort studies. The pharmacokinetic profiling of single high doses of PQ prior to doing a safety and tolerability assessment of high dose, short course, pilot efficacy studies in children 5 to 10 years old was funded through a component of the Australian Aid to PNG from the NDoH. The overall goal of the studies presented in this thesis was to determine the contribution of *P. vivax* relapses from the hypnozoites in the liver to infection and burden of *P. vivax* malarial disease; and to assess effective PQ treatment regimens to partner ACTs to prevent relapse malaria and reduce the disease burden of *P. vivax* in children.

5.1 First paper

The first paper is a report on the reanalysis of combined data set from individual published surveys by Mueller and others of 153 households in 112 central PNG highland villages. The surveys were conducted between 2000 and 2005 to document the epidemiology of malaria in the PNG highlands and to provide data for evidence-based planning and monitoring of malaria control activities. Infections were diagnosed by LM and risk factors assessed by a structured questionnaire. The prevalence of malaria infections in these surveys ranged from 0.0% to 41.8% (median 4.3%) for non-epidemic surveys and 6.6% to 63.2% (median 21.2%, $P < 0.001$) during malaria epidemics. *P. vivax* was predominant at altitudes >1600 m while in lower altitudes <1400 m, *P. falciparum* was the dominant parasite species. Below 1400 m, malaria was the primordial source of febrile illness. At higher altitudes asymptomatic infections

are common and malaria only becomes a significant source of febrile illness during epidemic outbreaks. Bed net use was associated with reduced malaria prevalence (OR= 0.8, $P < 0.001$). Sustained malaria control efforts have led to substantial reductions in malaria transmission and may lead to local elimination in some central PNG highland areas.

5.2 Second paper

The second paper reports the effect of PQ against *P. vivax* relapses from the hypnozoites in the liver against infection and disease burden in children from the first longitudinal cohort study. It involved a cohort of 433 G6PD normal PNG children aged 1 to 5 years old given pre-treatment of either, PQ plus ART, ART alone or no treatment (control) and followed up for recurrent *Plasmodium* infection for 40 weeks. The use of 0.5mg/kg PQ plus ART significantly reduced the risk of *P. vivax* episodes in the first 3 months of follow up compared to the artesunate only and control groups. *P. vivax* re-infection was observed in 30% of the children at two weeks of follow-up.

5.3 Third paper

The third paper documents the safety and tolerability of PQ in 247 G6PD normal children aged 1 to 10 years old from the two longitudinal cohort studies. The children were given 14d daily dose of 0.5mg/kg PQ pre-treatment as direct observed therapy (DOT), and clinical data were collected trough daily semi-structured questionnaire assessment of side effects by the attending field Nurse. To-date the WHO recommended treatment guidelines for malaria [131] documents PQ use as contraindicated in children due to little or no available data. The use of 0.5mg/kg PQ in PNG children aged 1 to 10 years was found to be safe and well tolerated. However, an intensive health facility based safety and tolerability with haematological, methaemoglobin, biochemistry and cardiovascular profiling of high dose PQ use in children is still needed.

5.4 Fourth paper

The fourth paper reports on the pharmacokinetic profiling of single, high doses of PQ (0.5mg/kg and 1.0mg/kg) prior to doing the safety and tolerability

of short course, high dose PQ in children. This study involved 28 healthy G6PD normal children. The single high doses of PQ were well tolerated when co-administered food. Model simulations, using pharmacokinetic parameters for both dose groups, showed the two proposed short course high dose PQ treatment regimens (1 mg/kg/day for 7 days and 1mg/kg bd for 3.5 days) would not exceed safe drug concentrations for administration to children.

5.5 Fifth paper

The fifth paper is an Opinion paper on the use of PQ as an essential tool for malaria elimination in the PNG setting. It highlights several key challenges for treatment, control and elimination of *P. vivax*. These include the biology of the *P. vivax* hypnozoites, the difficulties of malaria elimination without the eradication for the hypnozoites from the liver with PQ and the lack of cheap, point-of-care test for G6PD deficiency. The implementation of public health programs for routine use of PQ including MDA for malaria control and elimination are hindered by the PQ associated haemolytic anaemia in persons with the severe variants of G6PD deficiency. In PNG and other Melanesian countries the higher dose of 0.5mg/kg PQ should be administered to *P. vivax* confirmed cases as the Chesson strain present in these countries are relatively resistant to PQ.

5.6 The way forward

The challenge for *P. vivax* endemic countries such as PNG moving toward malaria elimination is the eradication of hypnozoites from the liver and the PQ associated side effect of severe haemolysis in persons with the severe variants of G6PD deficiency. The current drugs for MDA such as ACTs have no effect upon the hypnozoites therefore, in order for MDA regimens to be successful in the elimination of *P. vivax*, PQ will need to be included in the treatment regimen. A point-of-care, easy to use RDTs for diagnosis of G6PD deficiency such as the CareStart™ [122], currently under research and development could be used concurrently with a malaria RDT for routine treatment with PQ. However, RDT population screening for G6PD deficiency during MDA will be expensive. Therefore, data on the distribution of the severe forms of G6PD deficiency in *P. vivax* endemic areas are needed to minimise cost of mass screening during

MDA with PQ. The PQ treatment regimen can be tailored to reduce the risk of severe haemolysis in persons with the severe variants of G6PD deficiency.

The 14 day course of PQ to achieve the total dose effect is very efficacious in research settings when administered as DOT. In real life settings compliance to long treatment regimens is a major obstacle, as the total dose effect may not be realised in order to eradicate the hypnozoites from the person's liver. Therefore it is important to explore the option of using a short course, high dose PQ treatment regimen, especially in children to reduce the disease burden and seek other treatment options such as Tafenoquine. A short course, high dose may improve compliance and ease of use with ACTs, however the PQ associated haemolytic anaemia will remain an obstacle until an alternative safer hypnozoitocidal drug comes into routine practice.

6. Hypotheses and objectives

6.1 Hypotheses

Hypothesis 1:

- Pre-Treatment with PQ will significantly reduce the burden of *P. vivax* infection and disease during (early) follow up period in children 1-10 years of age

Hypothesis 2:

- PQ is well tolerated and safe in G6PD normal children less than 10 years old and effective in preventing *P. vivax* relapse malarial infection and clinical disease

6.2 General objectives

1. To determine the effects of (pre-)treatment with blood stage and blood plus liver stage drugs on the incidence of *P. vivax* infections and disease in children 1-5 years of age

2. To determine the contribution of long-lasting liver stages to the risk of infection with *P. vivax* in children 5-10 years old

3. To determine the safety, tolerability and efficacy of the current PQ regimen in children 1-10 years.

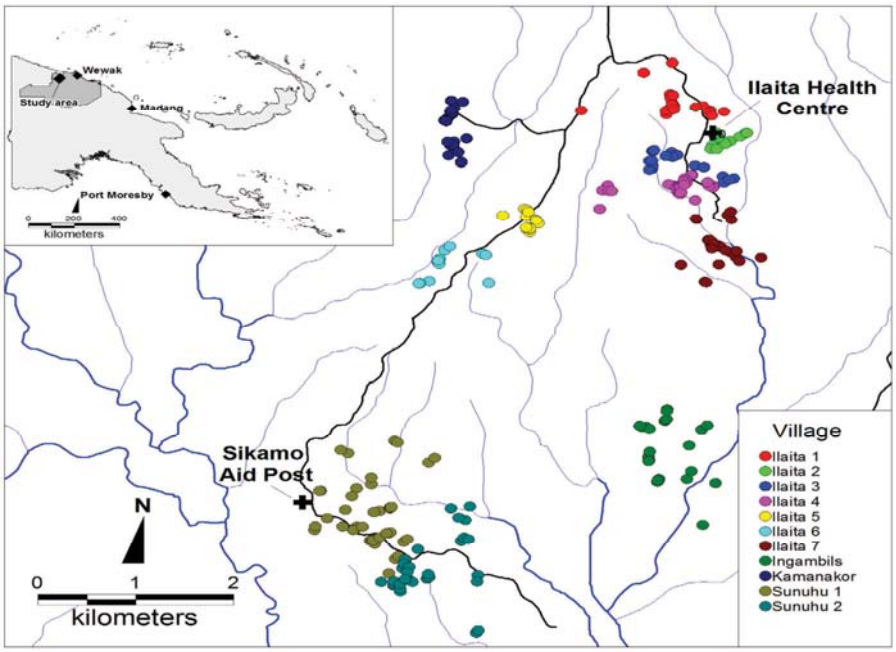
4. To design PQ treatment regimens for *P. vivax* relapse malaria in order to assist the PNG National Department of Health implement its treatment and control strategies for *P. vivax* malaria.

7. Materials and methods

7.1 Study outline for cohort 1 (Cellex)

A) Cohort 1(Cellex)

Ilahita Study Area – Location of study villages



In this study, 450 children aged 1-5 years were screened, enrolled, and, randomized to 3 arms of a longitudinal cohort study, after written informed consent by the parent or guardian. The randomized arms comprised:

- 150 Children pre-treated (days 8-14) with 7 days artesunate (ART)
- 150 Children pre-treated with 7 days artesunate (days 0-7) plus 14 days primaquine (days 0-14) (ART+PQ)
- 150 Children with no pre-treatment (CONTROL)

At enrolment, all eligible children aged 1-5 years were screened for Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency and the presence of severe anaemia or malnutrition. G6PD deficient children were not randomized, but added to the control arm of the study (no pre-treatment). All enrolled children were issued with a LLIN at enrolment.

Following baseline measurement and G6PD screening, children were started on their respective treatment, i.e. ART (7d), ART (7) + PQ (14) and without treatment

Subsequently, all enrolled children were followed actively and passively at a local health centre over the duration of 9 months. Active follow-up consisted of two-weekly morbidity visits to villages to monitor clinical malaria illness not reported to a health facility. At each visit, 250µl of blood and two blood slides were taken through finger prick sampling for the detection of asymptomatic malaria at 2 weekly intervals for the first 12 weeks then 4 weekly thereafter. The morbidity surveillance visits included the collection of a medical history of the preceding 2 weeks to determine incidence of malarial infections, bed net usage, an axillary temperature recording, a physical examination and 250µl finger prick blood sampling in asymptomatic children. All blood samples were assessed for the presence, type, and number of malarial infections (by light microscopy and PCR). DNA from the finger prick samples was also used for molecular parasitological studies. Hb levels and spleen sizes were only assessed at enrolment (as part of the initial assessment of inclusion criteria) and at the in-depth health check during the final follow-up time point.

During the entire study period, a passive case detection system was maintained at the local Health Centre. Every participating child presenting at the health centre with a febrile illness was clinically assessed and had a finger prick blood sample taken for determination of malaria infections by a Rapid Diagnostic Test (RDT), two blood slides for microscopy confirmation and HemoCue® for Hb concentration. Only the children with a positive RDT did receive artemether-lumefantrine (COARTEM®) from the attending field Nurse. The negative RDT cases were asked to wait for the microscopy result before appropriate treatment was administered by the attending clinician. All the data collected were recorded in the morbidity forms and the health book of each participating child, reviewed afterwards by the study clinician.

Over the duration of 9 months, each participant had therefore 21 study contacts (1 enrolment, 1 baseline, 18 active morbidity surveillance, and 1 final

visit). Additional finger prick blood samples may have been collected at the time of febrile illness during morbidity follow-ups and passive case detection.

This design allows assessment of the incidence of symptomatic malaria in an estimated maximum of 2700 (i.e. 150 x 18) 2-week intervals per arm following pre-treatment with ART or ART+PQ and the Control (without treatment).

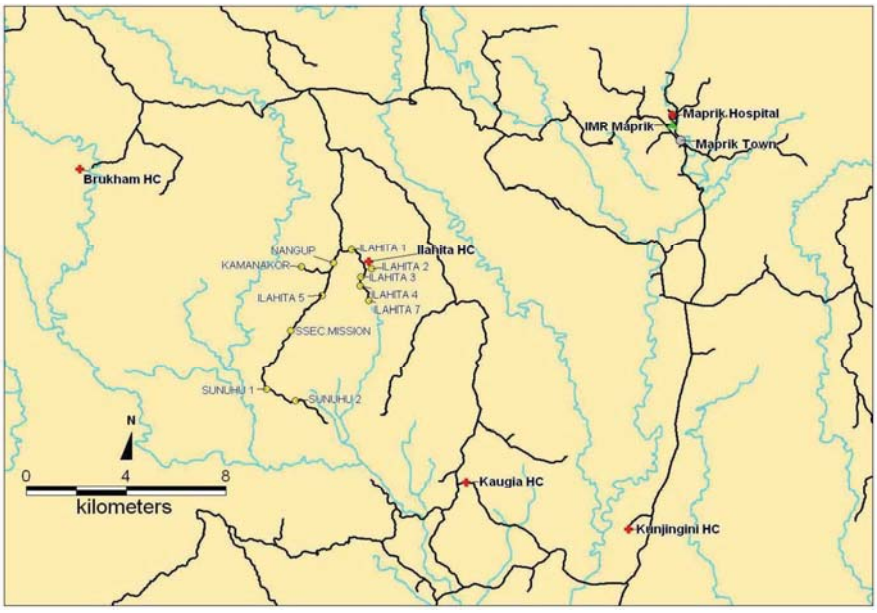
Main outcome variables:

- incidence of *P. vivax* malaria infection by light microscopy and PCR
- time to first (or only) *P. vivax* clinical episode during follow-up period
- time to first (or only) *P. vivax* infection

7.2 Study outline cohort 2 (Albinama)

B) Cohort 2 (Albinama):

Albinama study site involved neighbouring villages situated 4 kilometres north of the Ilaita area, linked by the same road network leading to Ilahita health centre and the study villages for Cohort 1 as shown in the Map.



In this study, 524 children aged 5-10 years were screened, enrolled, and randomized to 2 arms of a longitudinal cohort study after written informed consent by the parent or guardian. The randomized arms included:

- 262 children pre-treated with 3 days Chloroquine (CQ), 20 days Primaquine (PQ 0.5mg/kg) and 3 days Coartem (AL)
- 262 children pre-treated with 3 days Chloroquine (CQ), 20 days Placebo (PL) and 3 days Coartem (AL)

At enrolment all eligible children aged 5-10 years were screened for Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency and the presence of severe anaemia or malnutrition. Following the baseline measurement and G6PD screening, children were given the randomized treatment allocation from the two treatment study arms.

All children received CQ on days 1-3. Children did also receive either PQ (0.5 mg/kg) or placebo on days 1-5, 8-12, 15-19 and 22-26 (i.e. Monday to Friday for 4 weeks). During the third week of drug treatment all children did receive AL on days 15-17 (according to national treatment guidelines). Children were actively monitored for side effects including measurement of haemoglobin levels at days 1, 8, 15 and 22.

Following completion of treatment, a clinical examination was performed for each child and a finger prick blood sample collected on day 29 (i.e. 26 days after their last dose of CQ, 12 days after their last dose of AL and 3 days after their last dose of PQ or placebo.

Subsequently, all enrolled children were followed using 2-weekly active and continuous passive case detection at a local health centre for 32 weeks. Active follow-up consisted of morbidity surveillance visits to monitor clinical malaria illness not reported to a health facility, as well as finger prick blood sampling for the detection of asymptomatic malaria. These visits included the collection of a medical history of the preceding 2 weeks to determine incidence of malarial infections and bed net usage, a physical examination, measurement of axillary temperature. A 250µl finger prick blood sample was collected every 2 weeks for

the first 12 weeks and 4 weekly thereafter, i.e. weeks 2, 4, 6, 8, 10, 12, 16, 20, 24, 28 and 32.

All blood samples were assessed for the presence, type, and number of malarial infections (LM and PCR). DNA extracted from the finger-prick blood samples collected during follow-up was assessed as part of the molecular parasitological studies. Hb levels were only assessed at enrolment and as part of safety monitoring during the treatment period.

During the entire study period, a passive case detection system was maintained at the local Health Centre. Every participating child presenting at the health centre with a febrile illness was clinically assessed and had a finger prick blood sample taken for determination of malaria infections by a Rapid Diagnostic Test (RDT), two blood slides for microscopy confirmation and HemoCue® for Hb concentration. In accordance with the new national treatment guidelines, only children with a positive RDT did receive AL treatment from the attending field Nurse. All the data collected was recorded in the morbidity forms and the health book of each participating child to be reviewed by the study clinician.

Over the 32 week study duration, each participant had at least 32 contacts with the study team (1 for enrolment/screening, 20 for supervision of drug administration, 11 active follow-up visits). Additional finger prick blood samples may have been collected at the time of febrile illness during morbidity follow-ups and passive case detection.

Main Outcome variables:

- Time to first *P. vivax* infection by light microscopy and PCR

- Incidence of first and only *P vivax* infection during follow-up period.

- Time to first or only clinical *P. vivax* episode

7.3 Tolerability and (safety) of PQ in children 1-10 years old

C) Tolerability and (safety) of PQ in children 1-10 years old

In this study, the G6PD normal children of the longitudinal cohort studies above (1 & 2), which had been randomised to receive pre-treatment as direct observed therapy (DOT) were monitored closely for tolerability and safety of treatment drugs. Field nurses were trained to assess basic symptoms and signs which were recorded using a semi-structured side effects questionnaire, pre-defined with all most common possible side effects of the DOT drugs.

Main Outcome variable:

- Rates of adverse events in the PQ treated group compared to the Placebo.

7.4 PQ pharmacokinetic study in children 5-10 years old

In this study, the pharmacokinetic profiles of two single, high doses of PQ (0.5mg/kg and 1.0mg/kg) were assessed prior to commencing a safety and tolerability study of short course, high dose PQ treatment in children aged 5 to 10 years old. A total of 28 healthy, G6PD normal children, were recruited into the study. All participants were randomized into one of two treatment groups; Group A received single dose 0.5 mg/kg PQ and Group B received single dose 1.0 mg/kg PQ. After treatment children were admitted to the Alexishafen Health Centre to allow observation of safety parameters and for collection of frequent blood samples for drug assay (Baseline, 1, 2, 3, 4, 6, 8, 12, 18, 24, 36, 48, 72, 120 and 168 hours). Safety monitoring was performed throughout the follow-up period focussing on haemoglobin, methaemoglobin, liver and renal biochemistry tests.

The plasma concentrations of PQ and its principal metabolite carboxyprimaquine (CPQ) were determined simultaneously using a validated liquid chromatography-mass spectrometry (LC-MS) in Perth, Australia. Pharmacokinetic modelling and model evaluation and simulations were performed by collaborators from School of Medicine and Pharmacology, University of Western Australia.

7.5 Data management

7.5.1 Data management responsibilities

The primary data of the different studies included in this thesis were collected by field and laboratory staff at the different sites.

Upon receipt of Case Report forms (CRF) and source documents from the field team, the study coordinator (or his/her designee) crosschecked all CRFs for completeness, validity, and legibility. Upon crosscheck, all CRFs and laboratory worksheets were photocopied. Originals were maintained in the PNGIMR's field research office in Maprik (for studies done at the Maprik site) until completion of the field work, copies were sent to designated PNGIMR data entry unit in Madang.

All data from CRFs and laboratory worksheets in PNG were entered into a custom-made study database using DMSys (SigmaSoft) clinical trial data management software. This software meets all requirements of ICH GCP E6 and regulatory agency for data management in clinical research studies.

All data were double entered, with first entry done immediately after receipt of CRF and worksheet copies from the PNGIMR field station in Maprik. Second entry was done by a different data entry clerk once a batch of first entry was completed. All data entry was done by specifically trained data entry personnel. Queries for discrepancies, missing values, out-of-range and internal consistency were done by the data base manager on a weekly basis.

Access to the study database is limited to the data entry personnel, data managers of the PNGIMR and collaborating institutions.

Detailed data management procedures were described in the Data Entry and Data Management standard operating procedures. Regular data audits were conducted to assure quality of data and adherence to SOPs.

7.5.2 Data Capture Methods

Source documents in these studies consisted of the following:

- The Field study logbook relating to study enrolment and continued participation in cohort;
- Case report forms (CRFs) completed by the field study team during study visits (whether scheduled or non-scheduled) with each participant;
- Health Center logbooks;
- Health Center Surveillance form abstracts documenting illness and treatment histories for participants who seek care from participating health centers;
- Children's personal health books
- Microscopy laboratory worksheets documenting the evaluation of blood smears;
- Laboratory notebooks documenting DNA extraction and laboratory assays

The study coordinator and site investigator (for laboratory data) checked all CRFs prior to passing the forms on to data entry. If corrections were required, the incorrect entry was crossed out with a single line and the correct information will be printed adjacent to it. The correction was initialled and dated by designated, qualified study staff. Any requested information that was not obtained as specified in the protocol had an explanation noted on the CRF as to why the required information was not obtained.

7.6 Statistical Analysis Plan

7.6.1 Incidence of clinical *P. vivax* episodes during follow-up

This was the primary objective of the study and was evaluated by assessing differences in the risk of clinical malaria with *P. vivax* in treatment and placebo arms.

The analysis strategy for these outcomes was centered on different variations of Poisson regression. The outcome variables were total number of *P. vivax* episodes (over 32-36 weeks of follow-up) and incidence of 1st or only episode. Exposure was defined as the total risk time per child as outlined above. Analyses were adjusted for possible covariates, including infection status at baseline (i.e. before start of treatment), sex, age, host genetic traits, and nutritional state of child.

The analyses were done both for episodes defined as axillary temperature ≥37.5ºC plus any parasitemia or axillary temperature ≥37.5ºC plus *P. vivax* >500/µL.

7.6.2 Time-to-first *P. vivax* infection

Time to first infection was analysed using standard survival analysis, including log rank test for the comparison of survival curves and cox regression for modelling survival times. Time at risk was defined as outlined above. Cox regression analyses was adjusted for possible covariates including infection status at baseline (i.e. before start of treatment), sex, age, host genetic traits, and nutritional state of child. Analysis included time to first *P. vivax* only infection detectable by PCR or light microscopy as well as infections exceeding different density cut-offs.

7.6.3 Incidence of first or only *P. vivax* episode

The analysis approach to this objective was identical to the one for 1st infection and investigated both the time to first *P. vivax* episode of any density and *P. vivax* >500/µL.

7.6.4 Data analysis for PQ pharmacokinetic study

Data were summarized as mean and standard deviation (SD) or median and inter-quartile range (IQR). General linear modelling for repeated measures was used to determine whether variables differed significantly over time or by treatment group, and whether there was a treatment group time interaction

7.7 Ethical considerations

The protocols have been submitted for Ethical clearance/approval by both the Institutional Review Board (IRB) and the Medical Research Advisory Committee (MRAC) of Papua New Guinea:

Approval Numbers:

IRB #: 0720; MRAC #: 07.34 (Cohort 1)

IRB #: 0909; MRAC #: 09.11 (Cohort 2)

IRB #: 0931; MRAC #: 10.14 (PQ pharmacokinetic study)

All participants were only included in the study if their parents or guardians had signed a written informed consent document.

8. Articles

8.1 Study 1

Epidemiology of malaria in the Papua New Guinean highlands.

Betuela I, Maraga S, Hetzel MW, Tandrapah T, Sie A, Yala S, Kundi J, Siba P, Reeder JC, Mueller I.

Trop Med Int Health. 2012 Aug 28. doi: 10.1111/j.1365-3156.2012.03062.x. [Epub ahead of print] PMID: 22925472

Tropical Medicine and International Health doi:10.1111/j.1365-3156.2012.03062.x

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Epidemiology of malaria in the Papua New Guinean highlands

Inoni Betuela[1,2], Seri Maraga[1], Manuel W. Hetzel[1,3], Tony Tandrapah[1], Albert Sie[1], Simon Yala[4], Julius Kundi[4], Peter Siba[1], John C. Reeder[5] and Ivo Mueller[1,2,6]

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2 *Barcelona Centre for International Health Research (CRESIB, Hospital Clínic-Universitat de Barcelona), Barcelona, Spain*
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6 *Walter and Eliza Hall Institute, Parkville, Vic., Australia*

Abstract

OBJECTIVES To conduct an in-depth investigation of the epidemiology of malaria in the Papua New Guinea (PNG) highlands and provide a basis for evidence-based planning and monitoring of intensified malaria control activities.

METHODS Between December 2000 and July 2005, 153 household-based, rapid malaria population surveys were conducted in 112 villages throughout the central PNG highlands. The presence of malaria infections was determined by light microscopy and risk factors assessed using a structured questionnaire. The combined dataset from all individually published surveys was reanalysed.

RESULTS The prevalence of malaria infections in the different surveys ranged from 0.0% to 41.8% (median 4.3%) in non-epidemic surveys and 6.6% to 63.2% (median 21.2%, $P < 0.001$) during epidemics. *Plasmodium falciparum* was the predominant infection below 1400 m and during epidemics, *Plasmodium vivax* at altitudes >1600 m. Outside epidemics, prevalence decreased significantly with altitude, was reduced in people using bed nets [odds ratio (OR) = 0.8, $P < 0.001$] but increased in those sleeping in garden houses (OR = 1.34, $P < 0.001$) and travelling to highly endemic lowlands (OR = 1.80, $P < 0.001$). Below 1400 m, malaria was a significant source of febrile illness. At higher altitudes, malaria was only a significant source of febrile illness during epidemic outbreaks, but asymptomatic malaria infections were common in non-epidemic times.

CONCLUSIONS Malaria is once again endemic throughout the PNG highlands in areas below 1400–1500 m of altitude with a significant risk of seasonal malaria outbreaks in most area between 1400–1650 m. Ongoing control efforts are likely to result in a substantial reduction in malaria transmission and may even result in local elimination of malaria in higher lying areas.

keywords malaria, *Plasmodium falciparum*, *Plasmodium vivax*, Papua New Guinea, highlands malaria

Introduction

Although malaria is likely to have been present in low-lying intermountain valleys and in communities with links to the highly malarious lowlands prior to European contact (Radford *et al.* 1976), the problem of malaria in Papua New Guinea (PNG) highlands was first formally investigated after severe epidemic outbreaks in newly established tea and coffee plantations in Western Highlands Province (WHP) in the late 1940s and early 1950s (Spencer & Spencer 1955; Spencer *et al.* 1956). Of particular concern was the risk of introducing malaria into newly established coffee plantations through the extensive recruitment of highland labourers to work in lowland plantations (Radford *et al.* 1976).

The in-depth studies that followed these outbreaks (Peters *et al.* 1958; Peters & Christian 1960a,b) found seasonal low-level malaria and regular epidemics with significant morbidity and mortality in many of the densely populated highlands areas. Infections with *Plasmodium vivax* were predominant (68%), *Plasmodium falciparum* accounted for only 17% and *Plasmodium malariae* for 15% of all infections. Malaria transmission was highly seasonal, with regular epidemics occurring at the end of the rainy season (i.e. April–July). Overall, it was judged that the elimination of malaria from the PNG highlands was highly feasible (Peters 1960).

Subsequently, an elimination program based on indoor dichlorodiphenyltrichloroethane (DDT) spraying was started that by the early 1970s covered most of the

economically important, highly populated areas of the central PNG highlands (Parkinson 1974). Surveys conducted prior to the start of the DDT spraying operation found overall parasite rates of 5–10% in many highlands areas (Ewers & Jeffrey 1971), with strong seasonal fluctuation and predominance of *P. vivax* (Crane & Pryor 1971). As predicted, the DDT program was very successful, and by the end of the 1960s, parasite rates in the sprayed areas had dropped below 1% and malaria was considered close to eliminated (Black 1969; Ewers & Jeffrey 1971; Parkinson 1974; Crane *et al.* 1985). In remoter, lower-lying areas (<1200 m) such as in South Simbu, control was less successful and even after several years of control, parasite rates remained at 5–10% (McMahon 1973). When control programs ceased, rates rebounded quickly to levels near or above those seen before control (Crane *et al.* 1985; Mueller *et al.* 2005a). The control program never extended into the remote Southern and Western Highlands, and surveys in Enga (Sharp 1982) and Southern Highlands (Hii *et al.* 1997) in the late 1970s to early 1990s found rates of malaria comparable to those observed in pre-control surveys. In both areas, malaria prevalence strongly decreased with altitude, while the proportion of infection owing to *P. vivax* increased.

Since the stopping of large-scale vector control in the 1980s, the malaria situation in the PNG highlands has received very little attention, despite increasing reports of epidemic outbreaks. The large-scale epidemic that was observed during the 1997 El Niño event in the highlands of West Papua, Indonesia (Bangs & Subinato 1999), which also caused a massive increase in people seeking malaria treatment in all parts of the PNG highlands, once again alerted PNG health authorities of the malaria problem in the highland areas. In response, a series of rapid malariological surveys was conducted between 2000 and 2005 to assess the extent of malaria transmission throughout the central PNG highlands (Mueller *et al.* 2003a,b, 2004, 2006, 2007a,b; Maraga *et al.* 2011). Although primarily aimed at providing a basis for evidence-based planning and improved allocation of program resources at provincial level, together, these surveys allow the first in-depth investigation of the epidemiology of malaria in the PNG highlands for 40 years. In addition, they provide an essential pre-control baseline for the ongoing malaria control efforts undertaken by the PNG national malaria control program, with support from the Global Fund to Fight AIDS, Tuberculosis and Malaria. Here we present a thorough reanalysis of the combined dataset from all surveys that were previously published on a province-by-province basis (Mueller *et al.* 2003a,b, 2004, 2006, 2007a,b; Maraga *et al.* 2011), thereby providing a comprehensive summary of all data and additional comparative information on important aspects of PNG highlands malaria.

Materials and Methods

As part of an extensive assessment of the extent of malaria risk in PNG highlands, 153 surveys were conducted in 112 villages distributed through the central highlands range of PNG (Figure 1). Surveys were conducted on a province-by-province basis starting with Western Highlands (Mueller *et al.* 2003a) in 2000/2001, Eastern Highlands (Mueller *et al.* 2003b) in 2000–2002, Simbu in 2001/2002 (Mueller *et al.* 2004), Enga (Mueller *et al.* 2006) and highlands areas of Morobe (Mueller *et al.* 2007a) and Madang (Mueller *et al.* 2007b) in 2003/2004 and Southern Highlands in 2003–2005 (Maraga *et al.* 2011). Within each province, survey areas were selected based on altitudinal strata and accessibility, with individual villages within each area then selected at random. In all provinces, surveys were conducted both in the wet and dry season albeit not always in the same villages. Village locations were recorded by GPS, and elevations above sea level determined using a barometric altimeter. A detailed description of survey locations and underlying selection criteria are given elsewhere (Mueller *et al.* 2003a,b, 2004, 2006, 2007a,b; Maraga *et al.* 2011).

Despite the staged conduct of the surveys over 5 years, all surveys were conducted using a uniform method (Mueller *et al.* 2003a,b, 2004, 2006, 2007a,b; Maraga *et al.* 2011). To achieve a representative sample of the entire village population, a household-based sampling strategy was used. A number of households with a total population of approximately 200 people were selected. From each selected household, every member, who could be reached during the stay in the village, was included in the survey. If the village had fewer than 200 inhabitants, sampling of every resident was attempted. This approach allowed us to sample approximately 75% of all selected household members. Compared to data from demographic surveillance sites in PNG (Mueller & Hetzel, unpublished data), adolescents and infants are likely to be moderately under-represented in the sample thus obtained.

Demographical data were recorded from all household residents. From each household member that gave consent to participate, a thick and thin blood film was prepared, the spleen palpated in lying position and axillary temperature taken. Haemoglobin levels were measured using the HemoCue system (HemoCue AB, Ängelholm, Sweden). Symptomatic individuals were tested with rapid diagnostic test (Diamed, Cressier, Switzerland, or ICT Diagnostics, Durban, South Africa) and those found positive for malaria were treated according to standard treatment guidelines. A

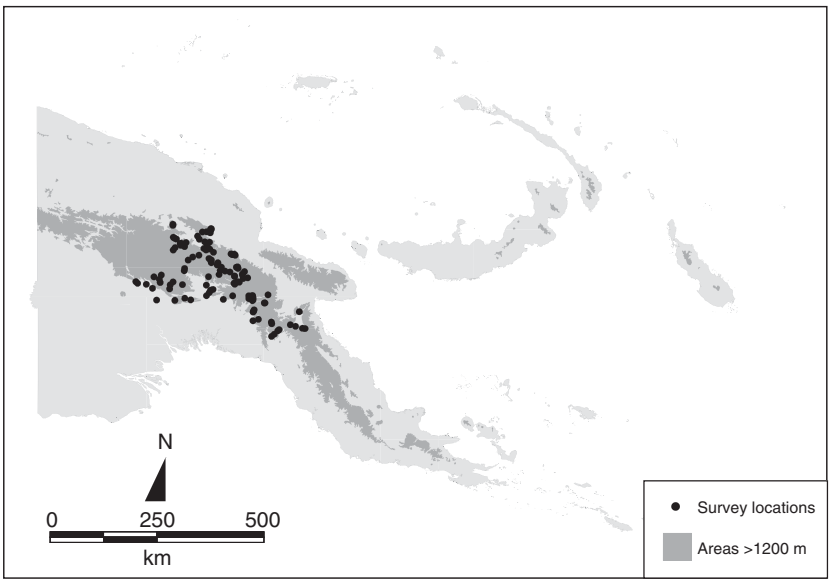

Figure 1 Location of all rapid malarialogical surveys conducted in Papua New Guinea highlands 2000–2004. For more detailed maps, see Mueller *et al.* (2003a,b, 2004, 2006, 2007a,b), Maraga *et al.* (2011).

short questionnaire on current symptoms, past malaria episodes and treatment, recent travel and other behavioural patterns (sleeping in garden houses, hunting and fishing) was administered to each participant or their guardian.

Giemsa-stained blood films were examined by light microscopy for 100 thick film fields under oil immersion before being declared negative. The parasite species in positive films were identified and densities recorded as the number of parasites per 200 white blood cells (WBC). Densities were converted to the number of parasites per μl of blood assuming 8000 WBC per μl. The slides were read independently by two experienced microscopists.

Statistical analyses were carried out using STATA 8.0 (Stata Corp., College Station, TX, USA) statistical software. Chi-square tests and logistic regression analyses were used for binary and categorical variables. Individual differences in haemoglobin levels were investigated using analyses of variance (ANOVA), while non-parametric tests were used to analyse differences in mean prevalence of infection and rates of enlarged spleens between surveys conducted at different altitude and season.

Results

Between December 2000 and July 2005, 153 surveys with a total of 22 485 participants were conducted in 112 villages distributed through the central highlands range of PNG (Figure 1). Thirty-six villages were surveyed twice, two villages three times. The majority of surveys were conducted in either the mid to late wet season [March–May: 55/153 (36%)] or early to mid dry season [June–August: 70/153 (46%)]. Nineteen surveys (12%) were in villages <1000 m, 22 (14%) at 1000–1199 m, 20 (13%) at 1200–1399 m, 47 (31%) at 1400–1599 and 45 (29%) at altitudes ≥1600 m. The number of participants ranged from 51 to 258 (median = 151) for non-epidemic (n = 135) and 38 to 249 (median = 121) for epidemic surveys (n = 19). Epidemic outbreaks were only observed at altitudes of 1250–1960 m [interquartile range (IQR) = 1520–1640] and between March and July.

Of all participants, 11 497 (51.1%) were female, 6442 (28.7%) children <10 years of age, 4256 (18.9%) adolescents (10–19 years) and 11 197 (49.8%) adults. The age of 560 (2.6%) participants could not be determined. Although there were significant differences in age distributions between non-epidemic and epidemic surveys (Table 1) or among surveys at different altitudes (data not shown), these differences were small (<3%) and thus unlikely to affect cross-survey comparisons. A more detailed description of survey characteristics is given elsewhere (Mueller *et al.* 2003a,b, 2004, 2006, 2007a,b; Maraga *et al.* 2011).

Altitude and malaria endemicity

The prevalence of malaria infections in the different surveys ranged from 0.0% to 41.8% (median 4.3%, IQR [0.1, 5.3]) in non-epidemic surveys and 6.6% to 63.2% (median 21.2%, IQR [10.3, 35.2], $P < 0.001$) in surveys conducted during epidemic outbreaks. Prevalence rates were highly negatively correlated with altitude rates in non-epidemic surveys (Figure 2, $\rho = -0.55$, n = 134,

$P < 0.001$) but not in endemic surveys ($\rho = -0.16$, $n = 18$, $P = 0.52$). The decrease was less pronounced for *P. vivax* than *P. falciparum* (Table 2) and the proportion of infections caused by *P. vivax* significantly increased with altitude ($\rho = 0.26$, $P = 0.002$). Consequently, while in areas below 1400 m, *P. falciparum* was more prevalent, and *P. vivax* dominated above 1600 m. While there was only limited seasonal difference in surveys conducted at altitudes <1200 m ($P = 0.15$), marked seasonality was observed at higher altitudes with the higher prevalence rates observed during the second half of the rainy season and the early dry season (i.e. March to July, median PR: 7.6% IQR [3.7, 21.9] compared to the main dry [August–October, 3.7%, IQR [2.2, 5.0] and the early wet season (November–February, 2.3%, IQR [1.3, 4.7]), $P = 0.008$]. These seasonal differences were exclusively found among *P. falciparum* infections (March–July: 4.3%, August–October: 1.5%, November–February: 0.8%, $P = 0.009$, *P. vivax*, $P = 0.22$) and owing to the high rates of *P. falciparum* infection during epidemic surveys (65.0% *vs.* 40%, $P = 0.1$). No significant seasonal differences were found if only non-endemic surveys >1200 m were considered ($P = 0.34$).

Enlarged spleens were commonly observed in children 2–9 years living in villages below 1000 m (34.4, IQR [14.2, 66.2]) indicating mesoendemic transmission. Spleen rates (SR) decreased significantly with increasing altitude (Table 2, $\rho = -0.56$, $P < 0.001$), but even at altitudes of 1000–1399 m, 18 of 41 (43.9%) surveys observed spleen rates >10%. Above 1400 m, enlarged spleens were rarely

Table 1 Basic demographic characteristics of study participants

	Non-epidemic (N = 20 082) (%)	Epidemic (N = 2403) (%)	P-value
Gender			
Female	10 139 (51.6)	1358 (50.4)	0.26
Age groups (years)			
<2	985 (4.9)	90 (3.7)	0.001
2–3	1351 (6.7)	155 (6.5)	
4–6	1869 (9.3)	216 (9.0)	
7–9	1575 (7.8)	201 (8.4)	
10–19	3819 (19.0)	437 (18.2)	
20–39	6266 (31.2)	799 (33.3)	
40+	3664 (18.2)	468 (19.5)	
NA	553 (2.8)	37 (1.5)	

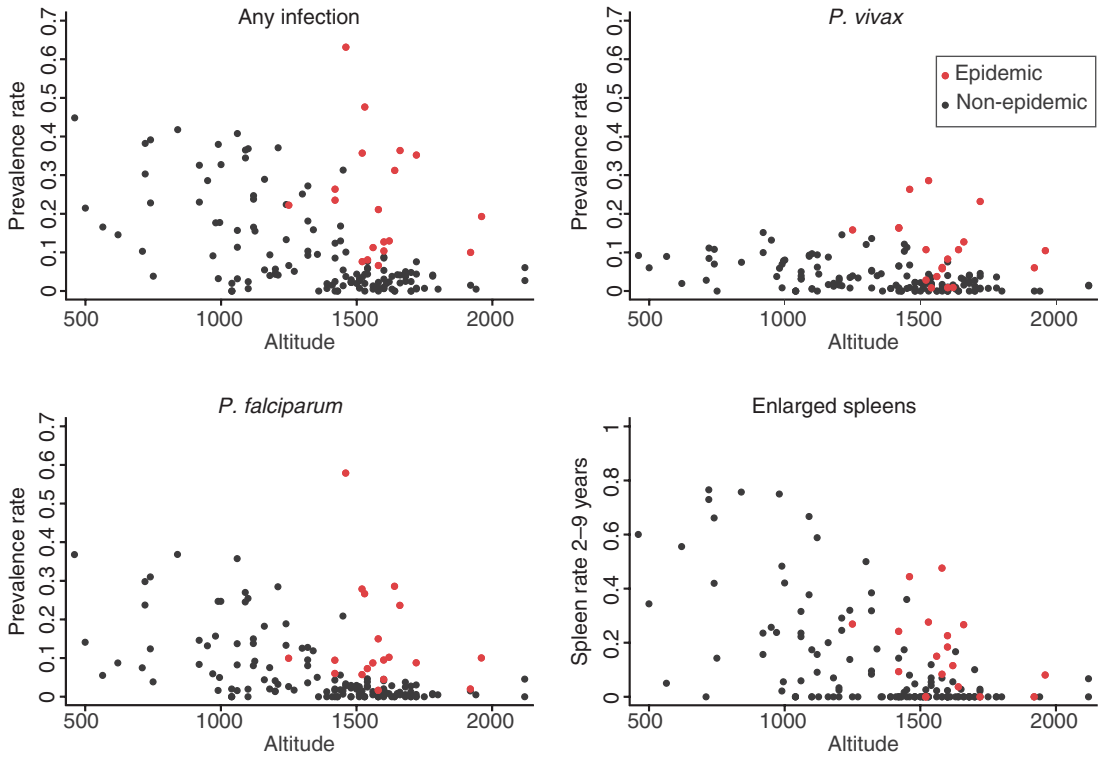

Figure 2 Association of altitude of survey location with prevalence of malarial infections and rate of enlarged spleens in children 2–9 years.

Table 2 Median prevalence and species composition of malarial infections in surveys conducted at different altitudes

		Prevalence of infection					Species composition†			Enlarged spleen	
Altitude (m)	N	Pf	Pv	Pm	Po	All	Pf	Pv	Pm	SR 2–9‡	IQR
Non-epidemic											
<1000	19	13.1	7.5	1.9	0	22.8	60.9%	27.3%	10.0%	34.4	14.2, 66.2
1000–1199	22	8.1	3.1	0.3	0	13.4	67.3%	25.5%	3.4%	8.0	0, 25.5
1200–1399	19	4.1	3.4	0	0	9.2	52.8%	36.5%	0.0%	8.9	0, 28.5
1400–1599	37	1.5	1.5	0	0	2.5	50.0%	50.0%	0.0%	0	0, 10.5
>1600	37	0.6	1.0	0	0	2.5	33.3%	66.6%	0.0%	0	0, 4.2
Spearman's rho		−0.61*	−0.42*	−0.45*	−0.30*	−0.55*	−0.24**	0.26**	−0.36*	−0.56*	
Epidemic											
1200–1399	1	9.7	16.3	0	0	22.2	36.0%	59.6%	4.4%	25.6	13.0, 40.0
1400–1599	7	8.8	5.6	0	0	11.3	67.5%	30.0%	0.0%	11.7	0, 32.6
>1600	8	9.8	9.4	1.4	0	16.1	55.5%	42.2%	3.5%	9.8	0.9, 21.5
Spearman's rho		−0.05	−0.27	0.25	0.02	−0.16	−0.01	−0.07	0.22	−0.37	

N, number of non-epidemic and epidemic surveys conducted at each altitude; IQR, Interquartile range. *$P < 0.001$, **$P < 0.01$.
†As a proportion of all infections.
‡SR 2–9: Proportion of children 2–9 years with enlarged spleen.

observed except during epidemic outbreaks (non-epidemic: 0, IQR [0, 4.5], epidemic 13.3, IQR [2.8, 26.7], $P < 0.001$). The prevalence of enlarged spleens was highly correlated with prevalence of malarial infections in non-epidemic (Figure 2, $\rho = 0.73$, $n = 134$, $P < 0.001$) but not in epidemic surveys ($\rho = 0.35$, $n = 18$, $P = 0.16$). Although the prevalence of infection was comparable in epidemic surveys ($P = 0.65$) and those conducted at altitudes below 1000 m, spleen rates were significantly lower in epidemic surveys (13.3% *vs.* 34.4%, $P = 0.02$).

Based on prevalence and spleen rates, the following altitudinal limits of malaria endemicity can be defined: >1000 m: moderately endemic (mesoendemic), 1000–1399: low endemic (hypoendemic) with risk of epidemic outbreaks, 1400–1599 m: epidemic malaria, and above 1600 m: no local malaria transmission although certain areas may be at risk of epidemics and imported cases may be present. Detailed maps and estimated proportion of villages in each stratum are given on a province-by-province basis in (Mueller *et al.* 2003a,b, 2004, 2006, 2007a,b; Maraga *et al.* 2011).

Individual risk factors for *Plasmodium* infection

The risk of malaria infection was very strongly age dependent. Overall (Figure 3), malarial infections were most common in children 2.0–3.9 (20.5%) and 4.0–6.9 (20.1%) years of age and lowest in adults (20–39: 8.0%, 40+: 6.1%, $P < 0.001$). With increasing altitude, a moderate shift in peak prevalence to older age groups was observed. In areas below 1400 m, peak prevalence was observed in children 2.0–3.9, shifting to children 4.0–6.9 at higher altitudes (Figure 3, test for age differences for individual age groups, χ^2-values 36.0–229.2, df = 6, $P < 0.001$). Significant differences in age-specific prevalence rates were observed for both *P. falciparum* (Figure 3, all *P*-values < 0.001, except >1600 m: $P = 0.064$) and *P. vivax* (all *P*-values < 0.001).

Whereas the relative age distribution in *P. vivax* infection was comparable during epidemic and non-epidemic surveys ($\chi^2 = 9.9$, df = 6, $P = 0.13$) in areas above 1200 m, significantly more *P. falciparum* infections were observed in adults during epidemics ($\chi^2 = 13.5$, df = 6, $P = 0.036$) with the median age of *P. falciparum*-positive participants rising from 12 years (IQR [5.0, 25]) in non-epidemic surveys to 16 years (IQR [6.4, 30], $P = 0.002$) in epidemic surveys.

At the time of the surveys, bed net use was relatively low overall with only 11.7% of participants having slept under a net the previous night. Use was most common in villages <1000 m (31.1%) and least common in those >1600 m (4.9%, $P < 0.001$). When taking into account differences in prevalence with altitude and age, bed net use was independently associated with a significant reduction in risk of malarial infections [odds ratio (OR) = 0.80, $P = 0.002$] in non-epidemic surveys. The protective effect of bed nets was exclusively against infection with *P. falciparum* (OR = 0.73, $P < 0.001$) with no effect at all against infection with *P. vivax* ($P = 0.97$). During epidemic surveys, there was also a tendency for insecticide treated nets (ITN) use to be associated with protection against *P. falciparum* infections (OR = 0.49, $P = 0.055$) but not against *P. vivax* infections ($P = 0.25$).

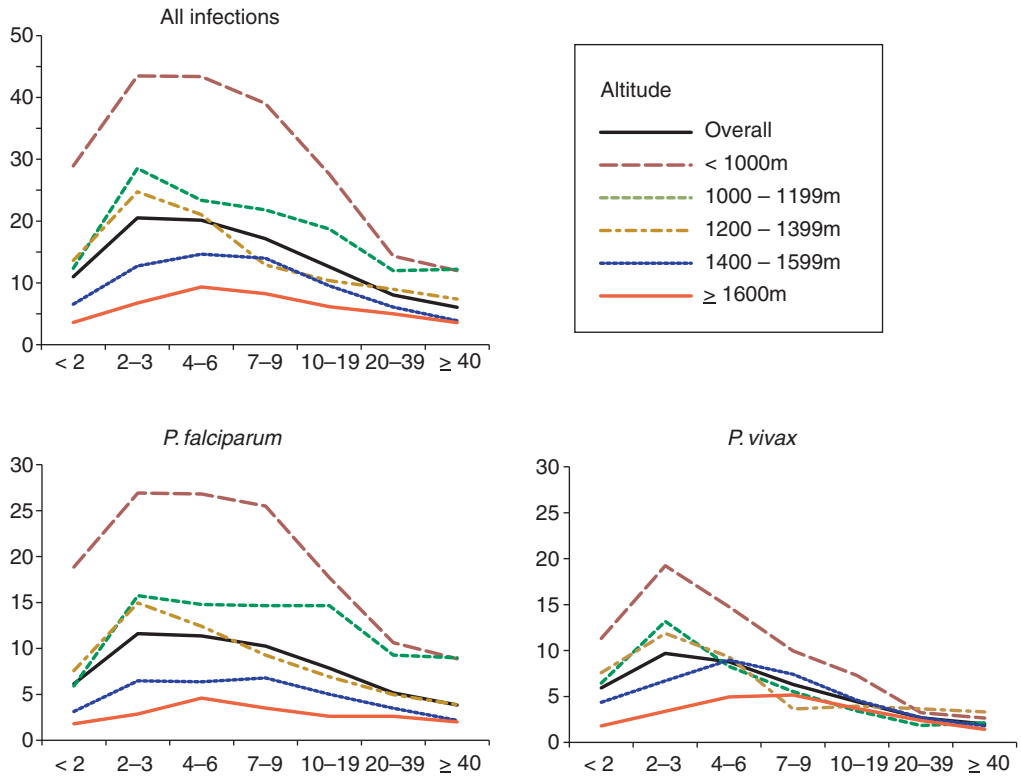

Figure 3 Age-specific prevalence of malarial infections at different altitudes.

In non-epidemic surveys, people reporting sleeping regularly in a garden house had a higher risk of both *P. falciparum* (Table 3, OR = 1.37, $P < 0.001$) and *P. vivax* infections (OR = 1.21, $P = 0.032$), whereas a recent history of travel to the lowlands was associated only with significantly higher risk of *P. falciparum* (OR = 2.38, $P < 0.001$) but not *P. vivax* infections ($P = 0.12$).

Malaria-associated morbidity

Although malaria infections were associated with substantial morbidity throughout the highlands, the burden of malaria-attributable fevers (MAF) was strongly related to transmission levels (Table 4). Whereas malaria was a significant source of febrile illness in moderate to low endemic areas (i.e. altitude <1400 m), at higher altitude malaria was only a significant source of febrile illness during epidemic outbreaks. Outside epidemics, 29.5% of participants living below 1400 m with a history of fever during the last 72 h had concurrent malarial infections, compared to only 7.7% ($P < 0.001$) among those reporting a febrile illness in surveys conducted above 1400 m. Similarly, if only cases with manifest fever (i.e. axillary temperature >37.5 °C) were considered, 40.0% and 15.6% ($P < 0.001$), respectively, were positive for malaria upon blood slide examination. In children <10 years, malaria-attributable cases of febrile illness increased to 42.8% (history) and 54.7% (temp >37.5 °C) for low to moderately endemic areas (<1400) and 13.1% and 19.6% at altitudes above 1400 m ($P < 0.001$). In the high transmission season (i.e. March to August), malaria was a more common cause of illness in both endemic (35.0% *vs.* 12.1%, $P < 0.001$) and non-endemic areas (8.7% *vs.* 4.3%, $P = 0.010$). During epidemic outbreaks, malaria was, however, the major source of morbidity. Not only do significantly more people report a febrile illness (1400 m: 24.4% *vs.* 12.2%, $P < 0.001$), the fevers are also much more likely to have concurrent malarial infections (38.8% *vs.* 7.7%, $P < 0.001$).

While malaria was an important source of illness, the majority of *Plasmodium* spp. infections were asymptomatic with only 26.9% people with malarial infection reporting to be suffering from febrile illness (Table 4, 'sympt'). In non-epidemic surveys, there was no difference in rate of reported febrile illness (in last 72 h) associated with a malaria infection among people living at different

Table 3 Individual, multivariate risk factors for malaria infections in non-epidemic and epidemics surveys

	Any infections		*Plasmodium falciparum*		*Plasmodium vivax*	
	OR [CI_{95}]	*P*	OR [CI_{95}]	*P*	OR [CI_{95}]	*P*
Non-epidemic surveys						
Altitude (m)						
<1000						
1000–1199	0.60 [0.52, 0.69]		0.69 [0.59, 0.81]		0.56 [0.45, 0.70]	
1200–1399	0.38 [0.32, 0.44]		0.38 [0.32, 0.46]		0.59 [0.46, 0.75]	
1400–1599	0.15 [0.12, 0.17]		0.11 [0.08, 0.13]		0.35 [0.28, 0.45]	
≥ 1600	0.09 [0.08, 0.11]	<0.001	0.06 [0.04, 0.08]	<0.001	0.25 [0.19, 0.32]	<0.001
Age (years)						
<2						
2.0–3.9	2.05 [1.57, 2.69]		1.85 [1.32, 2.59]		1.83 [1.27, 2.63]	
4.0–6.9	1.99 [1.57, 2.57]		1.76 [1.27, 2.44]		1.63 [1.57, 2.31]	
7.0–9.9	1.62 [1.24, 2.12]		1.63 [1.17, 2.28]		1.04 [0.71, 1.53]	
10.0–19.9	1.18 [0.93, 1.52]		1.26 [0.93, 1.71]		0.78 [0.55, 1.11]	
20.0–39.9	0.64 [0.50, 0.82]		0.72 [0.53, 0.98]		0.42 [0.29, 0.60]	
≥40	0.53 [0.41, 0.70]	<0.001	0.60 [0.43, 0.84]	<0.001	0.36 [0.24, 0.54]	<0.001
Garden house	1.34 [1.20, 1.50]	<0.001	1.37 [1.20, 1.57]	<0.001	1.21 [1.02, 1.45]	0.032
Lowland travel	1.80 [1.34, 2.37]	<0.001	2.38 [1.74, 3.24]	<0.001		
ITN use	0.80 [0.70, 0.93]	0.002	0.73 [0.61, 0.87]	<0.001		
Epidemic surveys						
Altitude (m)						
<1600						
≥1600	0.75 [0.58, 0.95]	0.020			0.44 [0.30, 0.65]	<0.001
Age (years)						
<2						
2.0–3.9	1.93 [1.03, 3.62]		2.42 [0.94, 6.20]		1.25 [0.59, 2.64]	
4.0–6.9	2.50 [1.38, 4.55]		3.64 [1.49, 8.90]		1.49 [0.73, 3.03]	
7.0–9.9	1.96 [1.07, 3.60]		2.72 [1.09, 6.74]		1.22 [0.59, 2.50]	
10.0–19.9	1.19 [0.67, 2.11]		1.93 [0.80, 4.64]		0.77 [0.39, 1.52]	
20.0–39.9	0.98 [0.56, 1.71]		1.77 [0.75, 4.18]		0.54 [0.28, 1.05]	
≥40	0.65 [0.36, 1.17]	<0.001	1.17 [0.48, 2.86]	<0.001	0.33 [0.16, 0.71]	<0.001
Garden house					0.53 [0.36, 0.77]	0.001
ITN use			0.49 [0.22, 1.06]	0.055		

altitudes ($P = 0.326$). The proportion was significantly higher during epidemic (43.6%) than non-epidemic surveys (22.6%, $P < 0001$) and for *P. falciparum* (29.1%) than *P. vivax* (25.2%, $P = 0.033$). An additional 16.9% reported having suffered from 'sik malaria' (local vernacular for febrile illness without cough) during the last 2 weeks. In total 37.7% and 65.2% ($P < 0.001$) of people with concurrent *Plasmodium* infections thus reported ongoing or recent febrile illness during non-epidemic and epidemic surveys, respectively.

Irrespective of endemicity, malaria infections were associated with an increased risk of anaemia (Table 4). In non-epidemic surveys, concurrent malarial infections resulted in a 0.58–1.29 g/dl reduction (all *P*-values < 0.001) in age- and sex-specific mean haemoglobin (Hb) levels. The effect of concurrent infections on Hb levels was significantly smaller at low altitudes ($P < 0.001$). However, as population mean Hb levels increased significantly with increasing altitude (and thus decreasing malaria endemicity, Table 4), moderate-to-severe anaemia (i.e. Hb < 8 g/dl) was only commonly found in moderate and low endemic area with 7.4% and 4.2% of participants, respectively.

Discussion

The surveys conducted from 2000 to 2005 show that malaria is once again endemic throughout the PNG highlands in areas below 1400–1500 m of altitude and that a significant risk of seasonal malaria outbreaks exists in most areas between 1400–1650 m. As such, the malaria situation at the start of the 20th century is remarkably similar to that of the 1950s and 1960s prior to the last elimination campaign (Black 1954; Spencer & Spencer

Table 4 Morbidity associated with malarial infections at different altitudes: observed fevers, reported febrile illness and haemoglobin levels

		Axillary temperature >37.5 °C			Reported fever lasts 3 days			Anaemia			
										ΔHB (adj)§	
Altitude (m)	*N*	Febrile*	MAF†	Sympt‡	Febrile*	MAF†	Sympt‡	Mean g/dl	Hb < 8 g/dl	g/dl	CI_{95}
Non-epidemic											
<1000	3018	5.6%	40.5%	8.9%	15.0%	35.4%	20.9%	11.1	7.4%	0.58	0.42, 0.73
1000–1199	3340	3.8%	34.7%	8.0%	13.6%	26.4%	21.9%	12.0	4.8%	0.95	0.76, 1.14
1200–1399	3072	2.2%	49.2%	9.3%	11.9%	25.7%	26.3%	12.2	3.4%	1.29	1.07, 1.24
1400–1599	5272	1.8%	18.1%	7.0%	12.8%	9.6%	24.9%	12.9	1.4%	1.06	0.84, 1.29
>1600	5551	0.9%	10.6%	3.1%	11.7%	5.8%	22.3%	13.5	0.5%	0.90	0.66, 1.14
		$\chi^2_4 = 215.6$ $P < 0.001$	$\chi^2_4 = 31.9$ $P < 0.001$	$\chi^2_4 = 7.2$ $P = 0.13$	$\chi^2_4 = 22.6$ $P < 0.001$	$\chi^2_4 = 211.7$ $P < 0.001$	$\chi^2_4 = 4.7$ $P = 0.32$	$F_{4,17950} = 702.3$ $P < 0.001$	$\chi^2_4 = 389.5$ $P < 0.001$	$LR_{4,17939} = 8.5$ $P < 0.001$	
Epidemic											
1200–1399	171	5.9%	60.0%	15.8%	25.2%	46.5%	52.6%	11.9	1.8%	1.34	0.74, 1.94
1400–1599	1260	4.5%	50.1%	10.0%	26.2%	37.0%	42.1%	12.3	4.8%	1.42	1.13, 1.71
>1600	972	3.1%	63.3%	9.6%	21.6%	41.6%	44.1%	12.7	2.7%	1.48	1.15, 1.81
		$\chi^2_2 = 4.5$ $P = 0.11$	$\chi^2_2 = 1.3$ $P = 0.52$	$\chi^2_2 = 2.1$ $P = 0.50$	$\chi^2_2 = 6.6$ $P = 0.04$	$\chi^2_2 = 2.1$ $P = 0.36$	$\chi^2_2 = 1.5$ $P = 0.46$	$F_{2,2158} = 11.5$ $P < 0.001$	$LR_2 = 8.4$ $P = 0.02$	$F_{2,2118} = 0.8$ $P = 0.32$	

*Febrile: Prevalence observed fevers (axillary temperature >37.5°C) and reported febrile illness (in last 3 days) among participants in surveys.
†Malaria-attributable fevers (MAF): Proportion of participants with observed fevers and reported febrile illness that have concurrent *Plasmodium* spp. infections (positive on thick film).
‡Sympt: Proportion of participants with *Plasmodium* spp. infections that have observed or reported febrile symptoms.
§Reduction in mean haemoglobin levels associated with concurrent *Plasmodium* spp. infection.

1955; Spencer *et al.* 1956; Peters *et al.* 1958; Peters & Christian 1960a,b; Ewers & Jeffrey 1971; Sharp 1982) with the same areas once again endemic for malaria and current transmission and risk of epidemic equally peaking in the late dry to early wet season (i.e. March to June).

The one major change is the increased prevalence of *P. falciparum* both in endemic areas as well as a cause of potentially severe epidemic outbreaks (Mueller *et al.* 2005b). This is most likely due to the increased travel between the highlands and highly endemic areas in Madang and Morobe provinces after the construction of a sealed highway (Radford *et al.* 1976). Recent travel to the lowlands, often to market highland grown vegetable in coastal towns, was found to be a significant risk factor for infection with *P. falciparum* but not *P. vivax*. Such travel constitutes a source of introduction of coastal parasites into highlands area that can cause epidemic outbreaks of *P. falciparum* malaria in areas that are otherwise climatically suited only for endemic transmission of *P. vivax* (Mueller *et al.* 2002).

Altitude and seasonal rainfall patterns are the major determinants of malaria transmission in the PNG highlands. At altitudes below 1400 m, temperatures allow endemic malaria transmission with *P. falciparum* as the predominant parasite. Given its shorter developmental cycles in the mosquito and thus lower minimum temperature (Gilles & Warrell 2002), endemic *P. vivax* transmission can occur locally until at least 1600 m, where *P. falciparum* transmission happens mainly during epidemic outbreaks (Mueller *et al.* 2005b). As a consequence, the relative proportion of *P. vivax* infection increased with altitude. Interestingly, the altitudinal limits for stable malaria transmission in PNG are lower than those reported from the East African (Ghebreyesus *et al.* 2000; Shanks *et al.* 2005; Gahutu *et al.* 2011) but comparable to those in the Malagasy highlands (Romi *et al.* 2002). The reasons for this are unclear.

As already shown by Peters *et al.* in the late 1950s (Peters & Christian 1960b), transmission in highlands areas is closely linked to seasonal rainfall patterns. Following the onset of the rainy season in November, it takes several months for mosquito numbers to build up sufficiently for malaria transmission to take off. As the rains wane and breeding sites start drying up in April–May, mosquito numbers also start dropping and malaria transmission falls until it practically stops at the height of the dry season. The seasonal pattern of malaria transmission thus showed a 2-month lag compared to the observed rainfall patterns (Peters & Christian 1960a). Although recent entomological studies in the PNG highlands are lacking, the seasonal difference in prevalence as well as the timing of epidemic outbreaks indicate the same still holds true today.

Despite its wide geographical distribution, malaria is not a major source of febrile illness in PNG highland areas

above 1400 m. Except during epidemic outbreaks that can carry a very high burden of morbidity (Mueller *et al.* 2005b), fewer than one in 10 participants with reported and one in six with measured axillary temperature >37.5 had concurrent *Plasmodium* infections. Given the syndromic nature of fever treatment at PNG health facilities, most of these febrile, parasite-negative patients would nevertheless be treated with antimalarials. The new 2009 PNG malaria treatment guidelines (PNG Department of Health 2009) for the first time include parasitological diagnosis of all fever cases with rapid diagnostic tests (RDTs) or light microscopy and advocate treatment only for parasitologically confirmed cases. If properly implemented, these new guidelines could reduce the number of malaria treatments dispensed in areas >1400 m by up to 90%, thus limiting the amount of overtreatment and reducing the risk of drug resistance.

As transmission levels are low at altitudes >1400 m, it was surprising that even at these higher altitudes the majority of *Plasmodium* infections were not associated with febrile symptoms. Equally intriguing, although the age of peak prevalence shifting into older age groups with increasing altitude, at all altitudes children had a higher risk of being infected than adults (Figure 3). Both observations challenge the notion that PNG highlanders are 'non-immune' to malaria and together with recent observations of predominantly asymptomatic communities in remote island (Harris *et al.* 2010) and East African Highlands (Baliraine *et al.* 2009) indicate that a significant level of clinical immunity to malaria may be achieved even at low levels of transmission. Several factors could contribute to the apparent level of antimalarial immunity. Firstly, studies of Indonesian transmigrants found that adults acquire significant clinical immunity after as few as three *P. falciparum* infections, whereas children require many more infections to acquire a comparable level of immunity (Baird *et al.* 1991, 2003). Secondly, as a result of the high levels of overtreatment with antimalarials, many highlanders are likely to have residual antimalarial drug levels in their blood, which may suppress infecting *Plasmodium* parasites at low levels allowing the development of strong, protective immune responses (Roestenberg *et al.* 2009). Last but not least, parasites in low transmission settings tend to be genetically less diverse (Anthony *et al.* 2005) making it easier to acquire immunity to locally circulating parasites. Irrespective of the reasons, the presence of large number of asymptomatic infections will limit the effectiveness of a malaria control strategy that is primarily based on case-management.

Future perspective for malaria in the PNG Highlands

In 2004, PNG received a first grant from the Global Fund to Fight AIDS, Tuberculosis and Malaria that allowed a country-wide distribution of 1.35 million long-lasting insecticide-treated bed nets (Global Fund to Fight AIDS, Tuberculosis and Malaria, 2011). With continued Global Fund support for 2009–2013, the national malaria control program is conducting additional long-last insecticide treated nets (LLIN) distributions and will roll out of artemisinin-based combination therapy and RDT throughout PNG. The experience from the DDT control program in the 1960s and 70s (Black 1969; Parkinson 1974) as well as the significant protection associated with the use of (mostly untreated) bed nets in the current study indicates that the ongoing control efforts are likely to result in a substantial reduction in malaria transmission and may even lead to local elimination of malaria in specific areas. Given the increasing mobility of many highlands populations, there thus remains a significant risk of re-introduction of malaria with potential for epidemic outbreaks (Mueller *et al.* 2005b) should malaria control measures be neglected or abandoned. Continued monitoring of the malaria transmission, identification of residual transmission hotspots as well as surveillance for potential outbreaks therefore need to be important components of any malaria control and elimination program in the PNG Highlands.

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MAJOR ARTICLE

Relapses Contribute Significantly to the Risk of *Plasmodium vivax* Infection and Disease in Papua New Guinean Children 1–5 Years of Age

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Background. *Plasmodium vivax* forms long-lasting hypnozoites in the liver. How much they contribute to the burden of *P. vivax* malaria in children living in highly endemic areas is unknown.

Methods. In this study, 433 Papua New Guinean children aged 1–5 years were Randomized to receive artesunate (7 days) plus primaquine (14 days), artesunate alone or no treatment and followed up actively for recurrent *Plasmodium* infections and disease for 40 weeks.

Results. Treatment with artesunate-primaquine reduced the risk of *P. vivax* episodes by 28% ($P = .042$) and 33% ($P = .015$) compared with the artesunate and control arms, respectively. A significant reduction was observed only in the first 3 months of follow-up (artesunate-primaquine vs control, −58% [$P = .004$]; artesunate-primaquine vs artesunate, −49% [$P = .031$]) with little difference thereafter. Primaquine treatment also reduced the risk of quantitative real-time polymerase chain reaction– and light microscopy–positive *P. vivax* reinfections by 44% ($P < .001$) and 67% ($P < .001$), respectively. Whereas primaquine treatment did not change the risk of reinfection with *Plasmodium falciparum*, fewer *P. falciparum* clinical episodes were observed in the artesunate-primaquine arm.

Conclusions. Hypnozoites are an important source of *P. vivax* infection and contribute substantially to the high burden of *P. vivax* disease observed in young Papua New Guinean children. Even in highly endemic areas with a high risk of reinfection, antihypnozoite treatment should be given to all cases with parasitologically confirmed *P. vivax* infections.

In areas that are coendemic for *Plasmodium falciparum* and *Plasmodium vivax*, the burden of infections and disease caused by *P. vivax* peaks at an earlier age than that due to *P. falciparum* [1–6]. In Papua New Guinea (PNG), highly endemic for malaria caused by all 4 *Plasmodium* species that infect humans [7], *P. vivax* is the most common cause of malarial illness in infants [8] and toddlers [9], but its incidence decreases rapidly after that age and clinical disease is rare in children >5 years old [3], even though *P. vivax* infections remain common throughout childhood and into adulthood [10, 11]. The burden of *P. falciparum,* on the contrary, continues to rise through early childhood, with incidence of *P. falciparum* malaria peaking in children 3–7 years old [9, 12, 13] and *P. falciparum* infections remaining prevalent in school-aged children [10, 11].

In PNG, *P. vivax* and *P. falciparum* are transmitted by the same mosquito vectors and studies in different PNG lowlands population reported comparable sporozoite rates for *P. falciparum* and *P. vivax* in the local vector populations [3, 14, 15]. An important characteristic of *P. vivax* is related to its capacity to generate long-lasting liver stages (ie, hypnozoites) that after varying periods of dormancy [16] can cause relapsing malaria infection and clinical disease. As a consequence of this ability, a single mosquito inoculation may result in several blood-stage infections, during the

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following months or even years. Although such relapsing infections are an important source of illness in nonimmune travelers [17], it is unclear how much they contribute to the burden of *P. vivax* malaria in perennially exposed children living in (highly) endemic countries.

Currently primaquine is the only licensed radical treatment for hypnozoites [18, 19]. Because of the concern that primaquine can cause potentially life-threatening hemolysis in Glucose-6-phosphate dehydrogenase (G6PD)-deficient individuals [20], the lack of reliable parasitological diagnosis at most PNG health facilities, and the prevailing perception that given the high transmission level treating hypnozoites may be of little benefit, meant that primaquine treatment was not formally adopted as part of the PNG treatment guidelines until 2010. As a consequence, up to 87% of children with *P. vivax* malaria experience a recurrent *P. vivax* infection within 6 weeks of treatment [21] with approximately 25% of these infections associated with clinical symptoms. It has been suggested that relapses are responsible for the vast majority of these recurrent infections [22] and that in addition to being the predominant cause of blood-stage infections, they may contribute significantly to *P. vivax* clinical malaria and transmission [16].

The development of high-throughput genotyping methods [23, 24] has greatly increased our ability to study the longitudinal dynamics of *P. falciparum* infections [25, 26] and differentiate new from ongoing or recrudescent infections [27, 28]. Although similar methods now exist for *P. vivax* [29], genotyping cannot differentiate relapses from new infections [30], because relapses are usually genetically distinct from the primary infection [31, 32]. It is therefore not possible to directly quantify the contribution of relapses to the burden of *P. vivax* reinfection and disease by genotyping individual infections in longitudinal studies of participants living in areas of high transmission and thus high reinfection risk. Such a direct estimation is possible only with use of an imaginative study design, wherein relapses are deliberately prevented in a portion of study subjects. Therefore, to assess the contribution of hypnozoites to the burden of *P. vivax* reinfection and disease, we conducted a longitudinal cohort study in children aged 1–5 years old in a hyperendemic area of PNG, where we selectively treated preexisting hypnozoites in a subset of the children.

MATERIALS AND METHODS

Study Description

This study was conducted in 11 villages in the Ilaita area of Maprik District, East Sepik Province, a highly endemic area where all 4 human malaria species coexist, with *P. falciparum* the most common parasite in all age groups except among children ≤4 years, in whom *P. vivax* predominates [9, 11]. Malaria transmission is moderately seasonal, with transmission peaking in the early wet season (ie, December through March) [25]. The study area is serviced by a single health subcenter and an aid post. A more detailed description of the study areas is given elsewhere [9].

All children aged 1–5 years living in study villages, whose parents consented to their participation, were tested for G6PD deficiency (OSSMR-D G6PD Assay; R&D Diagnostics). All G6PD-normal children were subsequently randomized to 1 of the 3 groups: (1) artesunate (4 mg/kg/d for 7 days) plus primaquine (0.5 mg/kg/d for 14 days), (2) artesunate alone (4 mg/kg/d for 7 days), or (3) no treatment (control). Owing to a concurrently ongoing mass-distribution of long-lasting insecticide-treated nets (LLINs), which resulted in nearly universal LLINs coverage, treatment of the cohort was delayed until after the LLIN campaign finished in early April 2008.

Immediately before treatment administration, children were assessed for symptoms of febrile illness, a detailed history of bed net use and recent antimalarial treatment was obtained, and a venous blood sample was collected for immunological and molecular studies. Children in control and artesunate arms found to be parasitemic were treated with arthemeter-lumefantrine (Coartem; Novartis). All treatment doses for the cohorts were administered as direct observed therapy and monitored for side effects.

After completion of treatment children were followed up for the presence of febrile illness actively every 2 weeks and passively throughout the study at the local health center and aid post for the duration of the follow-up (40 weeks). Finger-prick blood samples were collected every 2 weeks for the first 12 weeks and every 4 weeks thereafter from all children seen during active follow-up (active detection of infection, see Supplementary Figure 1). Malaria infection was investigated in all symptomatic children using a rapid diagnostic test (RDT) for malaria (ICT Diagnostics) and 250-μL finger-prick blood samples were collected for confirmation of infection by light microscopy (LM), and quantitative real-time polymerase chain reaction (qPCR). Only RDT-positive and LM-confirmed, RDT-negative symptomatic children were treated with arthemeter-lumefantrine . All other illness episodes detected were referred to local health center and treated in accordance with PNG treatment guidelines.

The study received ethical clearance by the PNG Institute of Medical Research Institutional Review Board (IRB 07.20) and the PNG Medical Advisory Committee (07.34).

Laboratory Methods

All blood films were read independently by 2 expert microscopists. Slides with discrepant results were reread by a third microscopist. Thick blood films were examined for 100 thick-film fields (under ×100 oil immersion lens) before being declared negative for infection. Parasite densities were recorded

as the number of parasites per 200 white blood cells and converted to parasites per microliter of blood, assuming counts of 8000 white blood cells/μL [33]. Slides were scored as LM positive for an individual *Plasmodium* species if the species was detected independently by ≥2 microscopists and/or if subsequent qPCR diagnosis confirmed the presence of the species. Densities were calculated as the geometric mean densities of all positive readings.

Plasma and peripheral blood mononuclear cells were collected from all venous blood samples. The remaining red blood cells were pelleted and aliquoted. Finger-prick blood samples were separated into plasma and cell pellets. DNA was extracted from the cell pellet fraction of all samples using the QIAamp 96 DNA Blood kit (Qiagen), and *Plasmodium* sp. infections were detected using a 4-species qPCR assay [34]

Statistical Analyses

For analysis purposes, clinical malaria was defined as fever (axillary temperature ≥37.5°C) or history of febrile illness within the last 48 hours in the presence of a concurrent *Plasmodium* sp. infection of any density or *P. falciparum* >2500/μL and *P. vivax* >500/μL [35]. The associations between the incidence of clinical malaria and treatment as well as other covariates were assessed by negative binomial regressions. Children were considered at risk from the first day after the last primaquine or artesunate dose until they withdrew, were lost to follow-up, or completed the study. Children were not considered at risk for 14 days after each recurring or new episode. The time to the first *P. vivax* episode or infection and its association with treatment and covariates were modeled using Cox regression and the proportional-hazards assumption was checked using the test based on the Schoenfeld residuals. The log-rank test was used to test differences between survival curves. In all survival analyses, children were considered at risk until they reached the end point of interest, withdrew, were lost to follow-up or completed the study. Differences between treatment groups at baseline were investigated using χ^2 and Fischer's exact tests for categorical characteristics and the Kruskal–Wallis test for continuous variables. Tests were 2 tailed, and the confidence level was set at 95%. All analyses were performed using Stata 12 software (StataCorp 2011, release 12; StataCorp).

RESULTS

Of 463 children screened, 449 (97.0%) G6PD-normal children were randomized to artesunate (7 days), artesunate plus primaquine (14 days), or no treatment (Figure 1). Sixteen children were withdrawn from the study or migrated out of the study area between randomization (late January) and the start of the study (mid-April). Therefore, a total of 433 children 1.1–5.6 years old were treated and followed up actively and passively for 40 weeks.

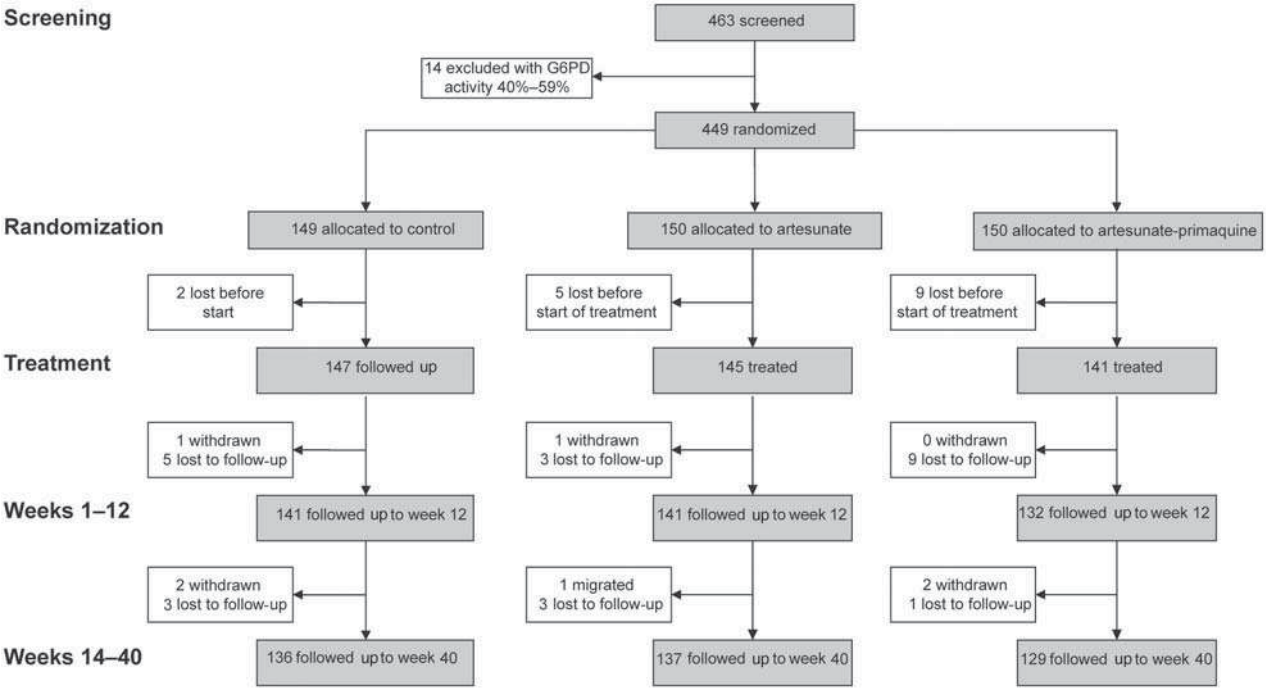

Figure 1. CONSORT study design, randomization, and retention of study participants during follow-up.

Table 1. Demographic and other Key Characteristics of Treatment Groups Before Start of Treatment

Characteristic	Artesunate		Artesunate-Primaquine		Control		*P*
Male patients, No.	75	52	68	49	75	51	.79
Age, mean (SD), y	3.1	(1.1)	3.2	(1.2)	3.3	(1.2)	.57
Village of residence, No. (%)							.64
Ilaita 1	5	3	5	4	8	5	
Ilaita 2–4	27	19	29	21	27	18	
Ilaita 5	8	6	3	2	5	3	
Ilaita 6	5	3	6	4	6	4	
Ilaita 7	5	3	8	6	13	9	
Ingamblis	31	21	26	19	19	13	
Kamanokor	14	10	21	15	17	12	
Sunuhu	38	26	28	20	37	25	
Utamup	12	8	14	10	14	10	
Currently ill, No. (%)	17	12	31	22	27	19	.059
Slept under net, No. (%)	133	94	120	86	130	90	.053

Abbreviation: SD, standard deviation.

No significant differences in demographic characteristics and infection status were observed at baseline (ie, before the start of treatment) between treatment groups, nor was there any difference in the distribution of children in each group among study villages (Table 1). There was a tendency for a higher LLIN use in the artesunate group than in the artesunate-primaquine and control groups ($P = .053$) at baseline. Reported rates of LLIN use during follow-up were comparable ($P = .74$).

During follow-up, 92% (range 74%–98%; interquartile range [IQR], 92%–95%) of children were seen at active detection of infection time points (Figure 1). There was no difference in the average number of study contact between treatment arms (likelihood-ratio (LR), 0.21; $df = 2$; $P = .90$).

During 40 weeks of follow-up, a total of 271 febrile episodes with *P. vivax* of any density (incidence rate [IR], 0.89) and 115 episodes with *P. vivax* >500/μL (Incidence rate (IR), 0.37) were detected; 132 children (30%) had 1 *P. vivax* malaria episode (any density), and 60 (14%) had ≥2 episodes (maximum, 4). The incidence of *P. vivax* malaria decreased strongly with age (Incidence rate ratio (IRR) for *P. vivax* episodes of any density, 0.81 [95% confidence interval (CI), .73–.91; $P < .001$]; IRR for *P. vivax* >500/μL, 0.60 [95% CI, .50–.72; $P < .001$]) and varied between villages (LR for episodes of any density, 16.0; $df = 8$; $P = .042$).

The incidence of *P. vivax* malaria of any density differed significantly between the 3 treatment arms (Table 2). Treatment with artesunate-primaquine reduced the risk of *P. vivax* episodes of any density during 40 weeks of follow-up by 28% (95% CI, 1%–52%; $P = .042$) compared with the artesunate arm and by 33% (95% CI, 8%–52%; $P = .015$) compared with the control arm. The differences were almost entirely due to a strong reduction in incidence in the first 3 months of follow-up (Figure 2) (IRR for artesunate-primaquine vs control, 0.42 [95% CI, .23–.76; $P = .004$]; IRR for artesunate-primaquine vs artesunate, 0.51 [95% CI, .27–.94; $P = .031$]), with little or no difference during the rest of the follow-up (Table 2). In multivariate analyses, only treatment and age were significant predictors of risk of malaria, and adjustment for age did not alter the observed differences between treatment arms (Supplementary Table 1). Similar differences were observed for the time to first or only *P. vivax* episode (Table 2; Figure 2). Interestingly, neither treatment resulted in a significant reduction in the incidence of *P. vivax* malaria episodes with a density >500/μL (Table 2).

In children in the artesunate-primaquine and artesunate arms who successfully cleared preexisting blood-stage infections, differences in the time to first *P. vivax* infection were investigated (Table 3; Figure 2). When diagnosed with qPCR, new *P. vivax* blood-stage infections were detected very rapidly, with 50% of children in artesunate and artesunate-primaquine groups infected by day 23 (IQR, 14–30 days) and day 30 (IQR, 15–56 days), respectively. It took significantly longer until infection became patent by LM, with the difference between treatment arms becoming even more pronounced (median, 29 days for artesunate [IQR, 16–55 days] vs 78 days for artesunate-primaquine [IQR, 42–280]). Overall, the elimination of liver stages through primaquine treatment was found to reduce the risk of qPCR- and LM-positive recurrent blood-stage parasitemia by 44% (95% CI, 28%–57%; $P < .001$) and 67% (95% CI, 55%– 75%; $P < .001$), respectively. The risk of *P. vivax* parasitemia did not vary with age (LR for qPCR, 1.93 [$df = 1$; $P = .16$]; LR for LM, 0.53 [$df = 1$; $P = .47$]) but differed significantly among children living in different villages

Table 2. Incidence of *Plasmodium vivax* and *Plasmodium falciparum* Malaria in Treatment Groups

	Placebo			Artesunate				Artesunate-Primaquine				
	Events	PYR	Incidence	Events	PYAR	Incidence	IRR (95% CI)	Events	PYAR	Incidence	IRR (95% CI)	*P*
P. vivax malaria												
Any density												
9-mo follow-up	105	102.2	1.03	99	103.9	0.93	0.91 (0.69– 1.24)	67	97.8	0.69	0.67 (.48–.92)	.037
0–3 mo	37	33.3	1.11	31	33.9	0.91	0.82 (.51–1.31)	15	32.3	0.46	0.42 (.23–.76)	.009
>3 to 9 mo	68	68.9	0.99	68	70.0	0.97	0.98 (.70–1.39)	52	65.4	0.76	0.81 (.56–1.16)	.446
P. vivax >500/μL												
9-mo follow-up	42	104.6	0.40	42	106.1	0.40	0.98 (.62–1.57)	31	99.1	0.31	0.78 (.47–1.28)	.549
P. falciparum malaria												
Any density												
9-mo follow-up	69	103.6	0.67	55	105.4	0.52	0.79 (.52–1.18)	34	98.9	0.34	0.51 (.32–.81)	.015
0–3 mo	11	34.4	0.32	8	34.9	0.23	0.71 (.28–1.83)	2	32.9	0.06	0.19 (.04–.87)	.041
>3 to 9 mo	58	69.2	0.84	47	70.6	0.67	0.80 (.51–1.25)	32	66.0	0.48	0.57 (.35–.94)	.083
P. falciparum >2500/μL												
9-mo follow-up	42	104.6	0.40	32	106.3	0.30	0.75 (.47–1.20)	21	99.5	0.21	0.53 (.31–.89)	.053
First or only malaria episode												
P. vivax, any density	73	73.3	1.00	74	78.1	0.95	0.95 (.69–1.31)	45	82.2	0.55	0.55 (.38–.80)	<.001
P. vivax >500/μL	34	91.8	0.37	35	92.3	0.38	1.04 (.59–1.83)	25	90.3	0.28	0.70 (.38–1.29)	.379
P. falciparum, any density	50	88.0	0.57	39	92.7	0.42	0.74 (.49–1.13)	30	90.6	0.33	0.58 (.37–.92)	.017
P. falciparum >2500/μL	35	94.4	0.37	29	96.4	0.30	0.81 (.50–1.33)	19	94.8	0.20	0.54 (.31–.95)	.085

Abbreviations: IRR, ; PYAR, ; PYR.

(LR for qPCR, 19.1 [$df = 8$; $P = .008$]; LR for LM, 49.4 [$df = 8$; $P < .001$]). Adjustment for village differences did not significantly change the treatment effects.

The treatment had no significant effect on the likelihood of being reinfected with *P. falciparum,* as detected with either qPCR (Table 3) ($P = .85$) or LM ($P = .40$). During follow-up, only 158 children experienced febrile episodes with any concurrent *P. falciparum* parasitemia (IR, 0.51), and 95 with *P. falciparum* >2500/μL (IR, 0.30). Thirty children had >1 *P. falciparum* episode (any density). Children in the artesunate-primaquine arm were significantly less likely to become ill with *P. falciparum* malaria than those in the control arm (Table 2) (IRR for all *P. falciparum,* 0.51 [95 CI , .32–.81; $P = .004$]; IRR for *P. falciparum* >2500/μL, 0.53 [95% CI, .31–.89; $P = .018$]), but not those in the artesunate arm (all *P. falciparum,* $P = .61$; *P. falciparum* >2500/μL, $P = .22$). The incidence of *P. falciparum* malaria of any density varied significantly among villages ($P < .001$) but showed no association with age ($P = .86$), whereas *P. falciparum* >2500/μL showed a nonlinear association with age ($P = .005$) but did not vary among villages. Adjustment for village of residence or age did not significantly change the associations of treatment with incidence of *P. falciparum* malaria (data not shown).

DISCUSSION

By selectively removing liver stages from some but not all children, we demonstrated that relapses cause approximately 50% of infection and more than 60% of clinical episodes in the first 3 months of follow-up, with little effect thereafter. The Chesson strain of *P. vivax* [36] that is present in the Southwest Pacific is known to have a short relapse frequency

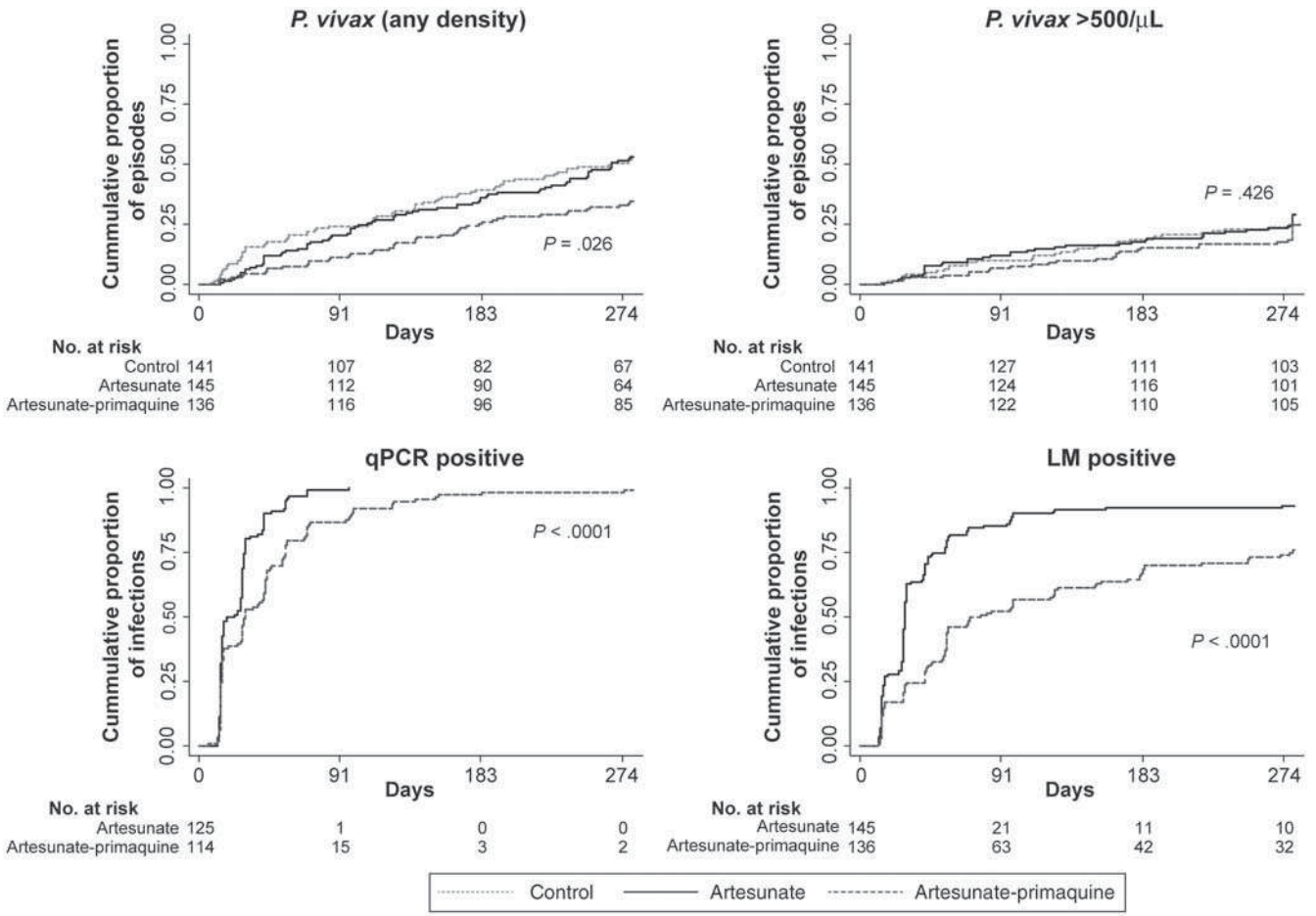

Figure 2. Time to first *Plasmodium vivax* clinical episode (any density) and reinfection as demonstrated by quantitative real-time polymerase chain reaction (qPCR) and light microscopy (LM). Differences between groups were tested by log-rank tests.

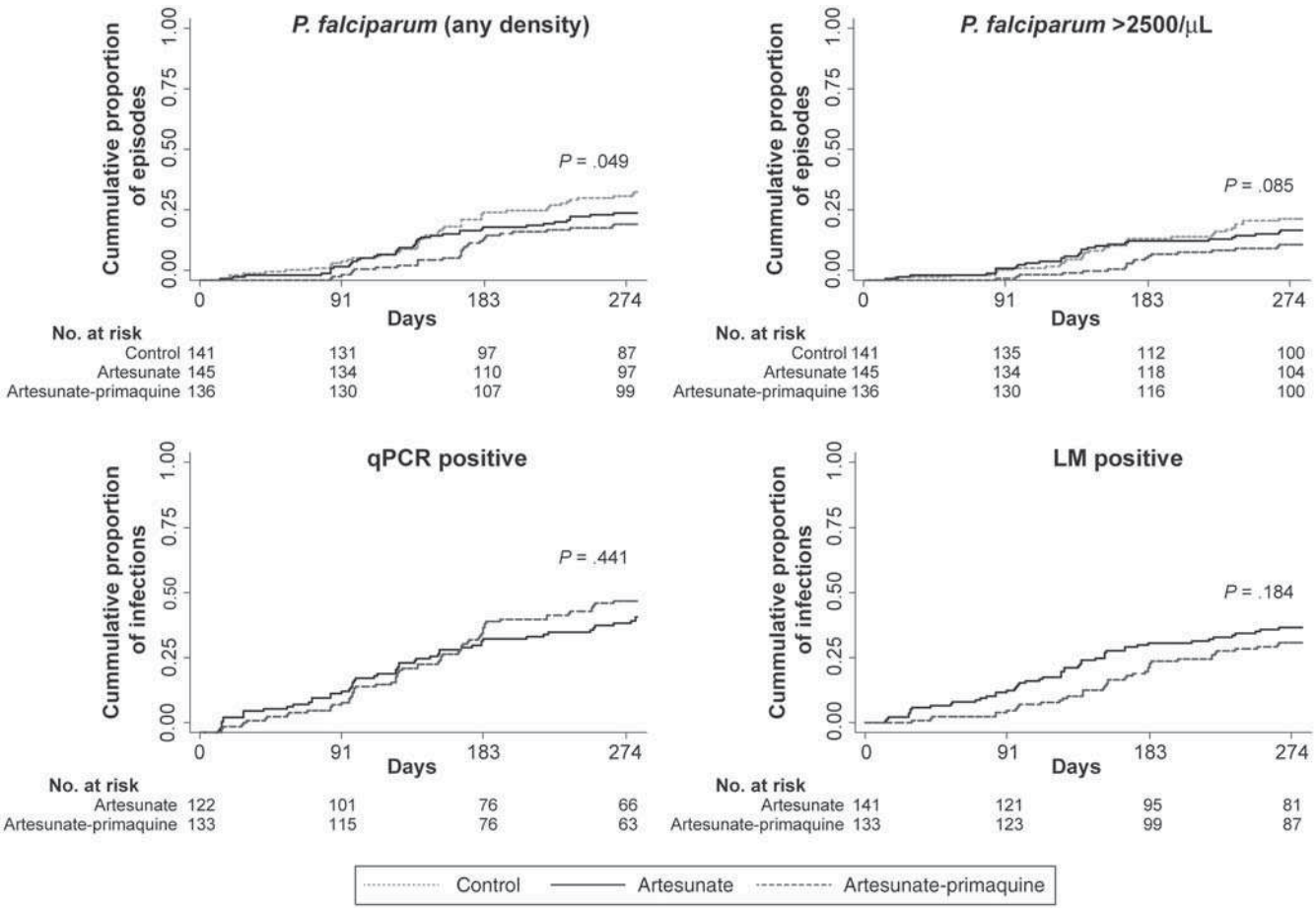

Figure 3. Time-to-first *Plasmodium falciparum* clinical episode (any density) and reinfection as demonstrated by quantitative real-time polymerase chain reaction (qPCR) and light microscopy (LM). Differences between groups were tested by log-rank tests.

Table 3. Incidence of New *Plasmodium vivax* and *Plasmodium falciparum* Infections in Artesunate and Artesunate-Primaquine Treatment Groups

	Artesunate				Artesunate-Primaquine					
	No.	PYR	ST, Median, d	Incidence	No.	PYAR	ST, Median, d	Incidence	HR	*P*
Incidence of first or only *P. vivax* (re)infection										
LM	145	21.7	30	6.14	139	45.9	79	2.18	0.43 (0.33–0.56)	<.001
PCR	125	8.5	17	14.62	117	12.2	28	9.16	0.66 (0.50–0.86)	.002
Incidence of first or only *P. falciparum* (re)infection										
LM	141	83.5	…	0.61	136	85.0	…	0.45	0.73 (0.48–1.12)	.147
PCR	122	64.3	…	0.85	135	70.6	253	0.92	1.06 (0.74–1.52)	.750

Abbreviations: d, days; HR, Hazard ratio; LM, light microscopy; PYAR, Person-year-at-risk; PYR, Person-year-at-risk; qPCR, quantitative real-time polymerase chain reaction; ST, median: median survival time.

(approximately 1 month [16, 37]). Consequently, in the artesunate-only arm, 71% and 85% of children had recurrent LM-detectable *P. vivax* infection by 6 and 12 weeks respectively. This finding resembles previous reports of the rate of recurrent *P. vivax* parasitemia after arthemeter-lumefantrine treatment [21], suggesting that most children in the cohort were likely to have had hypnozites in their livers at the time of treatment. This fast relapse pattern of residual hypnozoites in the artesunate and control arms plus the acquisition of new infections (and consequent establishment, or reestablishment, of new cohorts of hypnozoites) could explain the limited effect of primaquine beyond 3 months.

Interestingly, primaquine reduced the incidence of only low-density but not high-density clinical infection. Although past genotyping studies showed that relapses are often genetically different from primary infections [31, 32] (but see [38]), in PNG where the mean multiplicity of *P. vivax* infection is approximately 3 [39], sexual recombination in the mosquito is likely to be common, and therefore different blood-stage infections that originate from a single infected mosquito bite are genetically often related. Partial immunity acquired against a related primary infection may therefore allow children to better control blood-stage parasite densities, as in observations of sequential, homologous infections among patients receiving malaria therapy [40], resulting in mild and probably mostly self-limiting clinical episodes (in patients receiving malaria therapy, reinfection with homologous strains resulted only in a few transient symptoms). Therefore, outside a research setting, many of these episodes might not lead to treatment seeking and might not be treated.

Although we showed directly for the first time the substantial contribution of relapses to the burden of *P. vivax* infection and disease, the estimated burden caused by relapses is subject to several potential uncertainties. First, local PNG *P. vivax* strains are relatively resistant to primaquine and thus require higher doses of primaquine to prevent relapses [18, 41]. Although the recommended daily primaquine dose was used [19], concurrent treatment with active schizonticide drugs, such as chloroquine or quinine [18], is required to be effective against Chesson strain parasites. Even then, 14-day high-dose primaquine has an efficacy of only approximately 80% against New Guinea vivax strains [41]. Although artesunate is a highly effective schizonticide, the efficacy of the artesunate-primaquine combination is unknown. As indicated by the much faster recurrence of *P. vivax* compared with *P. falciparum* blood-stage infections in the primaquine arm, it is therefore possible that the chosen treatment did not eliminate all hypnozoites and that the burden of hypnozoite-derived infections is underestimated.

Population-wide distribution of LLINs took place immediately before the study. Compared with a study conducted 2 years earlier [9], we observed an approximately 50% lower incidence of clinical malaria. The hypnozoites present at the time of the treatment would therefore have been acquired mostly under the higher transmission present before LLINS distribution. Similarly, the delayed LLINS distribution meant that the study was started toward the end of the high–transmission season and continued through the low-transmission season [9,25]. Both factors could have resulted in overestimating the contribution of relapses.

As expected, treatment with primaquine had no effect on the risk of acquiring new *P. falciparum* infections. However, significantly fewer of the *P. falciparum* infections in the artesunate-primaquine group were associated with clinical illness. Unfortunately, the low number of *P. falciparum* episodes and thus limited statistical power precluded a more in-depth investigation of this intriguing observation. Confirmation in a larger study will be required.

The demonstrated large contribution of relapses to the burden of *P. vivax* infections and (mild) disease not only leads to a better understanding of *P. vivax* epidemiology but also has important implications for clinical practice and formulation of treatment guidelines. The high rate of relapses is almost certainly the principal reason for the higher prevalence,

multiplicity, and incidence of *P. vivax* infection and disease in early childhood [8, 9, 39], contributing substantially to the much faster acquisition of immunity to *P. vivax* compared with *P. falciparum* [3]. Furthermore, relapses may significantly contribute to transmission, because *P. vivax* gametocytemia closely follows asexual parasitemia. It will therefore be difficult to achieve a sustained reduction in *P. vivax* transmission, leading to local elimination, without targeting the hypnozoite reservoir [42, 43]. Although relapses seem to be predominantly associated with mild disease, without appropriate antirelapse therapy, children will be exposed to chronic blood-stage infections (or reinfections) with *P. vivax* that can lead to severe anemia in their cumulative effect [44, 45].

These findings have important public health relevance: even in areas with intense transmission and thus high risk of reinfection, strong efforts should be made to eradicate *P. vivax* hypnozoites in all cases of parasitologically confirmed *P. vivax* infection. The only currently available drug that effectively attacks the dormant hepatic reservoir is primaquine. Although the effect of primaquine against hypnozoites has been known for >50 years [18] and radical cure with primaquine is part of World Health Organization and many national treatment guidelines [19], concerns about its safety in persons with (severe) G6PD deficiency have hampered its programmatic implementation. The recent development of RDTs that specifically detect *P. vivax* will facilitate the recognition and diagnosis of this species. Poor adherence to the current 14-day primaquine schedule, the lack of therapeutic alternatives, and the lack of reliable, point-of-care (rapid) tests for G6PD deficiency remain major obstacles, which urgently need to be addressed if the recent reductions in global *P. vivax* burden are to be sustained and local elimination achieved [43, 46].

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Tolerability and safety of primaquine in Papua New Guinean children 1 to 10 years of age.

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Tolerability and Safety of Primaquine in Papua New Guinean Children 1 to 10 Years of Age

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Primaquine is currently the only drug available for radical cure of *Plasmodium vivax* and *P. ovale* liver infection stages, but limited safety data exist for children <10 years of age. Detailed daily assessments of side effects in glucose-6-phosphate dehydrogenase (G6PD)-normal children treated with 14 days of primaquine plus chloroquine (3 days; *n* = 252) or artesunate (7 days; *n* = 141) (0.5 mg/kg of body weight) showed that both treatments are well tolerated, do not lead to reductions in hemoglobin levels, and can thus safely be used in children 1 to 10 years of age.

Primaquine (PQ), one of the oldest synthetic antimalarial drugs, remains to date the only licensed product that can eliminate the hepatic dormant stages—the hypnozoites—of the two *Plasmodium* species (*P. vivax* and *P. ovale*) capable of producing relapses. The latest World Health Organization (WHO) recommendations for the prevention of hypnozoite-derived relapses state that the drug should be used contemporaneously with an effective blood schizontocidal for 14 days at a dosage of 0.25 mg/kg of body weight (in a single daily dose) (11). In areas where tolerance to primaquine has been observed, such as in Oceania and Southeast Asia, this dose should be doubled to 0.5 mg/kg. While strongly endorsing this recommendation, the WHO explicitly states that it is based on limited evidence.

Despite over 60 years of continuous use, primaquine still carries a reputation of being an "unsafe" drug. Side effects can be summarized into three main categories, the most important of which is the array of potentially life-threatening hemolytic side effects that it can cause among glucose-6-phosphate dehydrogenase (G6PD)-deficient individuals (12). This enzymatic deficiency, of which ~140 different variants exist (3), is an absolute contraindication for the use of primaquine when the enzyme's activity is below the threshold of 5%, but the drug can be used in milder cases with the provision of spreading the treatment on a weekly basis over a 2-month-long schedule of 0.75 mg/kg (11). The second important side effect is an increase in the level of methemoglobin, which is mild and generally well tolerated (5) unless the patient has an inborn deficiency of the methemoglobin reductase metabolic pathway. Finally, primaquine can cause dose-dependent abdominal discomfort when taken on an empty stomach (4) and, as a consequence, is best taken with food (11). Apart from the aforementioned side effects, primaquine is usually safe and well tolerated in patients without inborn deficiencies (1, 2).

There are, however, virtually no published data available on the safety and tolerability of primaquine in children, and the WHO therefore maintains that primaquine is contraindicated in children less than 4 years of age (11), even though children in that age group suffer the brunt of *P. vivax* disease in areas of high endemicity such as the southwest Pacific (6–8). A recent study that included the coadministration of a single dose of 0.75 mg/kg primaquine with artesunate plus sulfadoxine-pyrimethamine (SP) to children 1 to 12 years of age as part of a mass-administration trial resulted in significantly reduced hemoglobin (Hb) levels 7 days after treatment (10). The Hb reduction was largest in children with G6PD deficiency but was also present in G6PD heterozygote- and homozygote-normal children, raising concerns that PQ may cause moderate anemia when coadministered with artemisinins and that excluding individuals based on G6PD status alone may not be sufficient to prevent PQ-induced hemolysis.

There is thus an urgent need for a more extensive evaluation of PQ's safety and tolerability in young children, in particular if coadministered with an artemisinin. Here, we report the results from two different pediatric cohorts treated with different primaquine-containing antimalarial schedules as part of a wider epidemiological study, for the evaluation of their tolerability and safety.

This study was performed in two cohorts and was conducted in the Maprik region of the East Sepik province in Papua New Guinea (PNG). The study region is an area of hyperendemicity for both *P. falciparum* and *P. vivax*, with *P. vivax* the predominant source of infection and disease in the first 3 years of life and a progressive replacement by *P. falciparum* as the main cause of disease, extending even to adulthood (6, 8, 9). Since 2011, PNG has adopted artemether-lumefantrine for the treatment of uncomplicated malaria, irrespective of the species. If an infection by *P. vivax* or *P. ovale* is confirmed, an additional 14-day course of primaquine at a dose of 0.25 mg/kg/day is recommended but rarely implemented.

Two different pediatric cohorts of children 5 to 10 and 1 to 5 years of age, arranged to assess the epidemiology of malaria according to age, were treated with antimalarials at the beginning of a 9-month-long follow-up. All children living the study villages whose parents agreed to their participation were tested for G6PD deficiency using a G6PD assay kit (Dojindo Laboratories, Japan) and OSMMR-D G6PD assay (R&D Diagnostics, Greece) accord-

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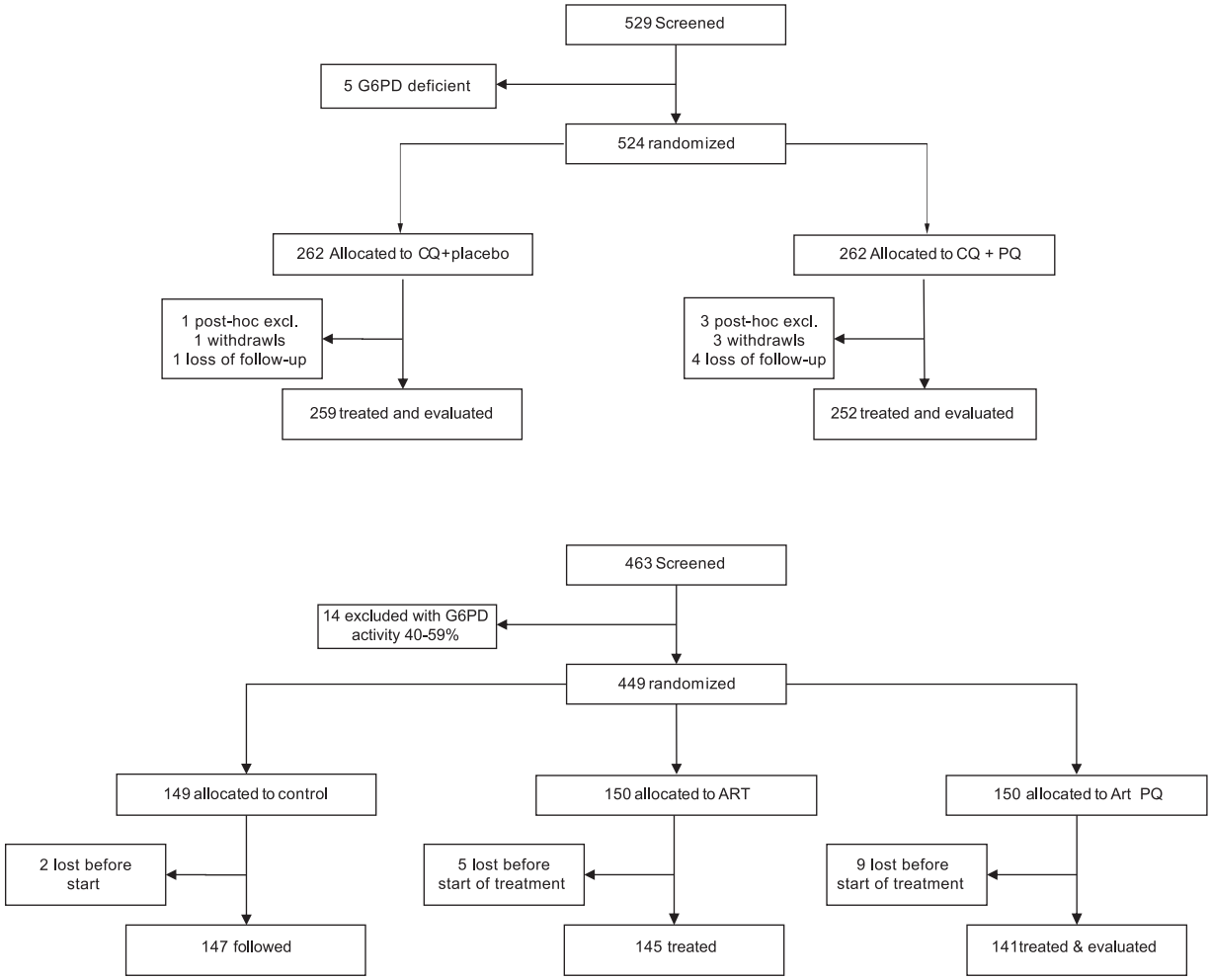

FIG 1 Trial design and drugs administered in the two different study cohorts. (Upper panel) Albinama cohort (5 to 10 years of age). (Lower panel) Ilaita cohort (1 to 5 years of age).

ing to the manufacturers' instructions, with all G6PD-deficient children excluded prior to enrollment and drug treatment. The first community-based cohort included 529 children 5 to 10 years of age recruited in Albinama; 524 (99.1%) were G6PD normal and randomized to receive either chloroquine (standard 25 mg/kg/total dose, divided over 3 days) and high-dose primaquine (0.5 mg/kg/day for 14 days [group 1; $n = 262$]) or chloroquine and placebo (3 or 14 days, respectively [group 2; $n = 262$]) (Fig. 1). Three children in the placebo arm and 10 in the primaquine arm were excluded *post hoc* due to protocol violations. The study was double blinded, with randomization performed using preallocated, block-randomized treatment codes. The second cohort was recruited among children aged 1 to 5 years of age, in Ilaita, an area neighboring Albinama. A total of 463 children were screened for G6PD deficiency, and 449 (97.0%) were randomized to receive 7 days of artesunate treatment (4 mg/kg/day) and 14 days of high-dose primaquine treatment (0.5 mg/kg/day, starting on day 1 of artesunate treatment; Fig. 1), only artesunate monotherapy for 7 days, or no antimalarial treatment. A total of 9 children allocated to the artesunate-plus-primaquine arm were lost in the 3 months between randomization and the start of treatment.

All primaquine doses were administered under supervision; those given to children 5 to 10 years of age were given with a snack, while parents or guardians of children 1 to 5 years of age were advised to make sure children had a meal or were breastfed prior to drug administration. Children in both arms of the cohort of those 5 to 10 years of age and the artesunate-plus-primaquine arm in the cohort of those 1 to 5 years of age were followed up on a daily basis. The occurrences of different signs and symptoms and adverse events and the general tolerability of the study drugs were recorded daily using standardized questionnaires by specifically trained study nurses. Illnesses detected at recruitment were treated according to PNG national guidelines. The proportions of children with reported signs or symptoms were compared using Fisher's exact or χ^2 tests, and (paired) *t* tests were used to compare differences in hemoglobin levels at baseline and day 8 (5 to 10 years of age) and day 14 (1 to 5 years of age).

Table 1 summarizes the presence of different signs and symptoms at baseline and the cumulative incidence of adverse events throughout the 14-day follow-up period. Among the children 5 to 10 years of age, no significant differences were observed at baseline between the primaquine and placebo groups for any of the signs and symptoms (Table 1; $P > 0.41$). Symptoms were rare during the 14 days of follow-up (frequency $< 7\%$), and no differences in

TABLE 1 Prevalence at baseline and cumulative occurrence of different signs and symptoms according to treatment group and cohort[a]

Sign, symptom, or parameter	Children age 5–10 yr						Children age 1–5 yr		
	Baseline			Days 1–14 (cumulative)					
	n/252 (%) for group 1 (CQ + PQ)	*n*/259 (%) for group 2 (CQ + placebo)	*P* value[b]	*n*/252 (%) for group 1 (CQ + PQ)	*n*/259 (%) for group 2 (CQ + placebo)	*P* value[b]	*n*/141 (%) at baseline for group 3 (ART + PQ)	*n*/141 (%) at days 1–14 for group 3 (ART + PQ)	*P* value[c]
Signs and symptoms									
Fever[d]	29 (11.5)	24 (9.3)	0.41	11 (4.4)	10 (3.9)	0.83	26 (18.4)	11 (7.8)	0.16
Yellow sclera				0	0			3 (2.1)	0.043
Dizziness	0	1 (0.4)	1.00	0	2 (0.8)	0.50		1 (0.7)	0.36
Headache	5 (2.0)	6 (2.3)	0.80	8 (3.2)	10 (3.9)	0.81	2 (1.4)	4 (2.8)	0.85
Earache				2 (0.8)	0	0.24		0	
Tiredness			1.00	5 (2.0)	7 (2.7)	0.77		5 (3.6)	0.35
Itchiness				3 (1.2)	1 (0.4)	0.37		1 (0.7)	1.00
Skin rash				1 (0.4)	1 (0.4)	1.00		2 (1.3)	1.00
Chest tightness				1 (0.4)	0	0.49		0	
Cough	13 (5.2)	13 (5.0)	0.94	19 (7.5)	16 (6.2)	0.60	16 (11.4)	17 (12.1)	0.15
Shortness of breath	1 (0.4)	3 (1.2)	0.62	1 (0.4)	1 (0.4)	1.00	2 (1.4)	2 (1.4)	0.30
Nausea	1 (0.4)	0	0.49	8 (3.2)	5 (1.9)	0.42	4 (2.8)	4 (2.8)	0.85
Vomiting	0	1 (0.4)	1.00	6 (2.4)	3 (1.2)	0.33	0	2 (1.4)	0.72
Stomachache	0	0		1 (0.4)	0	0.49	2 (1.4)	6 (4.3)	0.010
Diarrhea	0	0		1 (0.4)	2 (0.8)	1.00	4 (2.8)	2 (1.4)	0.30
Myalgia				1 (0.4)	0	0.49		2 (1.4)	0.30
Any sign or symptom	30 (11.9)	25 (9.7)	0.41	29 (11.5)	26 (10.0)	0.67	30 (21.3)	27 (19.2)	0.05
Laboratory measures									
Mean hemoglobin (gl/dl)[e]	10.8	10.9	0.71	10.3	10.5	0.007	9.31	9.81	
ΔHb				−0.56	−0.34	0.24		0.48	
CI_{95}				−0.29 to −0.81	−0.59 to −0.08			0.30 to 0.66	

[a] ART, artesunate; CQ, chloroquine; PQ, primaquine; CI_{95}, 95% confidence interval. Only limited clinical assessment was conducted at baseline for the cohort of children 1 to 5 years of age.
[b] *P* value for comparison of groups 1 and 2.
[c] *P* value for comparison of groups 1 and 3 for days 1 to 14 (cumulative).
[d] Axillary temperature >37.5°C or reported fever.
[e] Hemoglobin levels assessed at baseline and day 8 (5 to 10 years of age) and day 14 (1 to 5 years of age) of follow-up.

the occurrence of new symptoms and their cumulative incidence during the 14 days of follow-up were observed between the groups ($P > 0.24$).

Among the younger children, the prevalence of adverse events at baseline was higher (21%) due to a higher prevalence of malarial fevers (1 to 5 years of age, 14.9%; 5 to 10 years of age, 5.3% [$P < 0.001$]). Similarly, the occurrence of new symptoms and their cumulative incidence during the 14 days of follow-up were higher (Table 1). However, most of the symptoms were related to febrile illness and/or cough. Although a somewhat higher rate of stomachaches was observed ($P = 0.010$), the rates of nausea and vomiting were comparable to those observed in the older children who received a snack with each of the primaquine doses. No treatments had to be discontinued due to poor tolerability or repeated vomiting in either cohort.

Among the older children, a marginally larger (0.56 versus 0.33 g/dl; $P = 0.24$) but clinically and statistically insignificant drop in mean hemoglobin (Hb) levels (measured by Hemacue, Angholm, Sweden) was observed at day 8 in the primaquine group versus the placebo group. Similarly, equal numbers of children in both groups experienced clinically relevant drops of >2 gl/dl (22/247 versus 22/257; $P = 0.89$). Among the children 1 to 5 years of age treated with artesunate plus primaquine, Hb levels did not change in the first 3 days of treatment (9.31 versus 9.35 g/dl; $P = 0.77$) and then increased by 0.48 g/dl after 14 days of treatment (9.31 versus 9.81 g/dl; $P < 0.001$). Only 1 child experienced a drop in Hb of 2 g/dl, with no clinical evidence of hemolysis. Both primaquine schemes were therefore well tolerated and safe.

This report of these two cohorts provides the first published detailed evidence of acceptable safety and tolerability of the 14-day high-dose (0.5 mg/kg) primaquine schedule in G6PD-normal children 1 to 10 years of age. In the placebo-controlled study of children 5 to 10 years of age, the side effects were observed infrequently and were thought to be associated with the primaquine treatment only in one child. The moderately higher rate of adverse events in the younger cohort was almost entirely due to the higher levels of preexisting illness observed at baseline. The well-known gastrointestinal side effects of primaquine were rare even in children 1 to 5 years of age when the drug was not coadministered with food. As some children may not have had a meal at home prior to treatment, the tolerability could have been even better if food had been given as part of the primaquine treatment itself. The low level of side effects noticed and the lack of any notable reduction in Hb levels after 7 days of concurrent high-dose primaquine (0.5 mg/kg) and artesunate daily treatment indicate that the combination of these two drugs is safe and that primaquine can be safely given to G6PD-normal Melanesian children 1 to 5 years of age. Given the good tolerability in both cohorts, it might be possible to investigate shorter courses (<14 days) of higher doses of primaquine (>0.5 mg/kg) that might improve compliance with primaquine treatment (2). While further pediatric safety studies need to be conducted in other populations, the WHO recommendations for primaquine use should be regularly reviewed to assess the adequacy of primaquine treatment in G6PD-normal patients >1 year of age.

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Pharmacokinetic properties of single-dose primaquine in Papua New Guinean children: Feasibility of abbreviated high-dose regimens for radical cure of vivax malaria.

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Pharmacokinetic Properties of Single-Dose Primaquine in Papua New Guinean Children: Feasibility of Abbreviated High-Dose Regimens for Radical Cure of Vivax Malaria

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Since conventional 14-day primaquine (PMQ) radical cure of vivax malaria is associated with poor compliance, and as total dose, not therapy duration, determines efficacy, a preliminary pharmacokinetic study of two doses (0.5 and 1.0 mg/kg of body weight) was conducted in 28 healthy glucose-6-phosphate dehydrogenase-normal Papua New Guinean children, aged 5 to 12 years, to facilitate development of abbreviated high-dose regimens. Dosing was with food and was directly observed, and venous blood samples were drawn during a 168-h postdose period. Detailed safety monitoring was performed for hepatorenal function and hemoglobin and methemoglobin concentrations. Plasma concentrations of PMQ and its metabolite carboxyprimaquine (CPMQ) were determined by liquid chromatography-mass spectrometry and analyzed using population pharmacokinetic methods. The derived models were used in simulations. Both single-dose regimens were well tolerated with no changes in safety parameters. The mean PMQ central volume of distribution and clearance relative to bioavailability (200 liters/70 kg and 24.6 liters/h/70 kg) were within published ranges for adults. The median predicted maximal concentrations (C_{max}) for both PMQ and CPMQ after the last dose of a 1.0 mg/kg 7-day PMQ regimen were approximately double those at the end of 14 days of 0.5 mg/kg daily, while a regimen of 1.0 mg/kg twice daily resulted in a 2.38 and 3.33 times higher C_{max} for PMQ and CPMQ, respectively. All predicted median C_{max} concentrations were within ranges for adult high-dose studies that also showed acceptable safety and tolerability. The present pharmacokinetic data, the first for PMQ in children, show that further studies of abbreviated high-dose regimens are feasible in this age group.

Primaquine (PMQ) is an 8-aminoquinoline drug used for primary (causal) and terminal malaria prophylaxis, radical cure of *Plasmodium vivax* and *P. ovale*, and as a gametocytocidal agent in *P. falciparum* infections (1–4). It remains the only FDA-approved drug for elimination of liver stages (hypnozoites and schizonts) of *P. vivax* and *P. ovale* (2, 4, 5). In many non-African tropical countries, such as Papua New Guinea (PNG), there is hyperendemic transmission of *P. vivax* (5–7). Children carry the burden of repeated *P. vivax* infections in this geoepidemiologic setting (5, 8, 9). Therefore, a safe and effective radical cure would benefit personal well-being, growth, and development and lessen the economic impact of the infection on the community (10).

Primaquine is conventionally administered as a 14-day course for terminal prophylaxis and radical cure (2), but this regimen can be problematic due to poor compliance (1, 5). An abbreviated regimen would have advantages (11, 12) as long as it was safe and well tolerated. Pharmacokinetic studies of PMQ to date have been conducted only in adults (6). As the pharmacokinetic and pharmacodynamic profiles of antimalarial drugs can differ between adults and children (13), there is a need for a study of PMQ disposition in the pediatric age group (14, 15). The conventionally recommended PMQ regimen for radical cure of 0.5 mg/kg daily for 14 days is well tolerated in glucose-6-phosphate dehydrogenase (G6PD)-normal PNG children (12). To characterize the pharmacokinetic properties of PMQ and facilitate the development of higher-dose, shorter-course PMQ treatment in this patient population, we conducted an intensive-sampling pharmacokinetic study with 0.5 or 1.0 mg/kg of body weight given as a single dose to healthy PNG children aged 5 to 12 years.

MATERIALS AND METHODS

Study site, approvals, and subjects. The present study was based at the Alexishafen Health Centre, Madang Province, on the north coast of PNG, where there is hyperendemic transmission of *P. falciparum* and *P. vivax* (8). The study was approved by the PNG Institute of Medical Research Institutional Review Board and the Medical Research Advisory Committee of the PNG Health Department. Subjects were recruited between August and September 2010 from local villages, where an explanation of study aims and procedures was given to community members. After written informed consent had been obtained from parents/guardians, eligible children were screened for G6PD status (WST-8 [lyophilized] method; Dojindo Molecular Technologies Inc., Japan), their demographic and anthropometric data were recorded, a hemoglobin concentration was measured (HemoCue, Ängelholm, Sweden), and a blood slide was taken. Children who had (i) normal G6PD status, (ii) a blood slide that was negative for malaria, (iii) no clinical features of illness, (iv) no history of PMQ allergy, (v) no evidence of severe malnutrition (weight-for-age nutritional

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Z score [WAZ], <60th percentile), and (vi) a hemoglobin concentration of ≥80 g/liter were recruited and admitted to the Alexishafen Health Center for the first 2 days of study procedures.

Baseline assessment, treatment allocation, and clinical procedures. At enrollment, a detailed history and symptom questionnaire were completed with the assistance of the parents/guardians and a full physical examination was performed, including body weight, height, axillary temperature, supine and erect blood pressure and pulse rate, respiratory rate, mean upper arm circumference, and spleen size. A urine sample was tested for the presence of protein, blood, and/or glucose. Baseline methemoglobin levels were determined by pulse oximetry (Masimo Rad-57 pulse oximeter with SpMet; Masimo, Australia), and an electrocardiogram was taken. An intravenous cannula was inserted, and a venous blood sample was drawn for a full blood count (Coulter counter; Beckman Coulter, Australia). The remainder of the sample was centrifuged and the plasma stored at <−20°C for subsequent drug and biochemical assays. The red cell pellet was retained for parasite genotyping.

Each child was randomized to a single oral dose of PMQ at either (i) 0.5 mg/kg body weight (group A) or (ii) 1.0 mg/kg (group B) PMQ base given as diphosphate tablets (Shin Poon Pharmaceuticals, Seoul, South Korea). Participants were not required to fast. The drug was administered with water under direct observation, and to minimize adverse effects of PMQ when taken on an empty stomach, all participants were given a packet of crackers to consume directly afterwards. If a child vomited within 1 h of dosing, the same dose was to be readministered and the time documented. After dosing, all participants had additional 2-ml venous blood samples drawn at 1, 2, 3, 4, 6, 8, 12, 18, 24, 36, 48, 72, 120, and 168 h for drug assay. Aliquots of plasma taken at baseline and at 72 and 168 h were retained for biochemical analysis. All children were reassessed clinically on days 1, 2, 3, and 7 for symptoms and vital signs, as well as hemoglobin concentration (HemoCue), methemoglobin saturation (Masimo), and blood slide.

Laboratory methods. Screening for G6PD deficiency was performed according to the manufacturer's instructions. In brief, a 250-μl finger prick blood sample was collected into a cooled tube containing EDTA as the anticoagulant. A 5-μl aliquot of whole blood was then mixed with buffer-dye solution, wrapped in aluminum foil, and incubated at 37°C for 25 min. A 10-μl aliquot of hydrochloric acid then was added, and the color change was assessed visually in reference to a standard color chart. Each sample was then loaded into a 96-well plate and read on a microplate reader at an absorbance of 460 nm to confirm visual interpretation. Participants who had G6PD deficiency were withdrawn from the study, and the result was recorded in the child's health book with a recommendation that PMQ therapy not be administered in the future. Other biochemical tests were performed at PathWest Laboratory Medicine, Fremantle Hospital, Western Australia, Australia, using methods that have been described previously (16).

After initial microscopy in the field, all blood films were reexamined independently by two skilled microscopists in a central laboratory with discrepancies adjudicated by a third microscopist. Parasite densities were calculated from the number of parasites per 200 or 500 white blood cells depending on parasitemia and an assumed total peripheral white cell count of 8,000/μl. Reinfection and recrudescence were distinguished by comparing PCR-restriction fragment length polymorphism-generated genotype patterns of merozoite surface protein 2 to PCR genotype patterns of merozoite surface protein 1 and glutamate-rich protein in pairs of samples obtained at enrollment and on the day of reappearance of parasitemia (17).

Plasma concentrations of PMQ and its principal metabolite, carboxyprimaquine (CPMQ), were determined simultaneously using a validated liquid chromatography-mass spectrometry (LC-MS) method as described previously (18). Intra- and interday precision for both PMQ and CPMQ were <10% across the concentration range of 5 to 1,000 μg/liter, with >85% recovery and sensitivity of 1 to 2 μg/liter. All venous blood samples for drug assay were centrifuged for 5 min at 3,000 × *g*, and the plasma was separated from the red cell pellet and stored in a foil-covered tube at <−20°C until assay within 6 months of sample collection. Primaquine content was determined in 20 tablets selected randomly from the single batch used in the present study. The stated content was 26.3 mg PMQ diphosphate (or 15 mg PMQ base), which was comparable to the assayed mean ± standard deviation (SD) content of 25.71 ± 0.85 mg.

Pharmacokinetic modeling. Log_e plasma concentration-time data sets for PMQ and CPMQ were analyzed by nonlinear mixed-effect modeling using NONMEM (v 7.2.0; ICON Development Solutions, Ellicott City, MD) with an Intel Visual FORTRAN 10.0 compiler. The first-order conditional estimation (FOCE) with interaction estimation method was used. The minimum value of the objective function (OFV), conditional weighted residual (CWRES) plots, and condition number (<1,000) were used to choose suitable models during the model-building process. Allometric scaling was employed *a priori*, with volume terms multiplied by $(\text{WT}/70)^{1.0}$ and clearance terms by $(\text{WT}/70)^{0.75}$, where WT is body weight in kg (19). Two structures for residual variability (RV), equivalent to proportional and combined RV structures on the normal scale, were used for the log-transformed data. Secondary pharmacokinetic parameters, including area under the curve ($\text{AUC}_{0-\infty}$) and elimination half-lives ($t_{1/2}$) for the participants, were obtained from *post hoc* Bayesian prediction in NONMEM using the final model parameters. Models were parameterized using k_a (absorption rate constant), V_C/F (central volume of distribution [V_c] relative to bioavailability [F]), CL/F (clearance), and V_P/F and Q/F (peripheral volumes of distribution[s] and their intercompartmental clearance[s], respectively).

PMQ was initially modeled alone using one- and two-compartment models (ADVAN 2 and 4, respectively) with first-order absorption and with and without lag time. Once a suitable model for PMQ was obtained, CPMQ and PMQ data sets were modeled simultaneously. All PMQ was assumed to be converted to CPMQ to allow identifiability in the model. One-, two- and three-compartment models were tested for CPMQ using user-defined linear mammillary models (ADVAN 5). Once the structure of the models was established, interindividual variability (IIV) and correlations between IIV terms were estimated when supported by the data. Finally, relationships between model parameters and the covariates dose group, dose (mg/kg), age, and sex were identified using correlation plots and subsequently evaluated within NONMEM.

Model evaluation and simulations. A bootstrap using Perl speaks NONMEM (PSN) with 1,000 samples was performed, and the parameters derived from this analysis were summarized as median and 2.5th and 97.5th percentiles (95% empirical confidence intervals [CI]) to facilitate evaluation of final model parameter estimates. In addition, prediction-corrected visual predictive checks (pcVPCs) were performed with 1,000 data sets simulated from the final models, and these were stratified according to treatment group for PQ. The observed 10th, 50th, and 90th percentiles were plotted with their respective simulated 95% CIs. Numeric predictive checks (NPCs) were performed to complement the pcVPCs in assessing the predictive performance of the model.

Once a final model had been established, simulations were performed to assess three different multiple-dosing treatment regimens on the peak concentration (C_{max}) of PMQ and CPMQ. These were (i) 0.5 mg/kg daily for 14 days, (ii) 1.0 mg/kg daily for 7 days, and (iii) 1.0 mg/kg twice daily for 7 doses (3.5 days). Each treatment regimen had the same total dose (7.0 mg/kg). C_{max} was determined from simulated subjects, and their drug concentrations were obtained at 6-min intervals. For the simulations, 1,000 male and female subjects for each age between 5 and 10 years were used. Weights for each age group were based on sex and simulated from WHO weight-for-age data (20). Simulated data for male and female children were combined, and the resulting median for each dosing regimen was plotted against age. Simulated concentrations across all age groups were pooled, and the median concentration with 95% prediction intervals versus time was plotted for both PMQ and CPMQ for the three dosing regimens.

TABLE 1 Admission details for the children in each primaquine dose group

Parameter	Value for dose group[a]: A ($n = 15$)	B ($n = 13$)
Age (mo)	97 (72–120)	80 (70–90)
Sex (no. [%] male)	9 (60)	6 (46)
Body wt (kg)	19.3 ± 3.5	17.5 ± 2.2
Height (cm)	115 ± 10	111 ± 7.1
Upper arm circumference (cm)	15.9 ± 1.4	15.7 ± 1.6
Axillary temp (°C)	36.6 ± 0.2	36.5 ± 0.4
Supine systolic/diastolic blood pressure (mmHg)	82 (80–90)/54 (50–60)	90 (80–92)/60 (53–61)
Standing systolic/diastolic blood pressure (mmHg)	90 (80–100)/60 (50–64)	90 (80–95)/60 (58–61)
Systolic/diastolic postural blood pressure change (mmHg)	−2 (−10–2)/0 (−6–0)	−2 (−4–0)/−1 (−5–0)
Respiratory rate (breaths/min)	22 ± 3	25 ± 4*
Supine pulse rate (beats/min)	83 ± 11	84 ± 13
Hemoglobin (g/liter)	117 ± 12	115 ± 12
Methemoglobin (% of total hemoglobin)	1.0 ± 0.2	1.1 ± 0.5
Rate-corrected electrocardiographic QT interval ($ms^{0.5}$)	440 (414–447)	431 (417–440)
White cell count ($\times 10^9$/liter)	9.0 ± 2.7	9.5 ± 5.2
Platelet count ($\times 10^9$/liter)	275 ± 67	216 ± 74*
Serum alanine aminotransaminase (U/liter)	9 (6–13)	9 (8–16)
Serum total bilirubin (μmol/liter)	1.9 (1.8–2.9)	2.1 (1.8–2.9)
Serum creatinine (μmol/liter)	47 (41–51)	41 (40–46)

[a] Data are means ± SD or medians (IQR). *, $P < 0.05$ versus a single dose of 0.5 mg/kg PMQ.

Data analysis. Data are, unless otherwise stated, summarized as means and SD or medians and interquartile ranges (IQR). General linear modeling for repeated measures was used to determine whether variables differed significantly over time or by treatment group and whether there was a treatment group-time interaction.

RESULTS

Patient characteristics. Thirty children were recruited, but two in group B were found to be parasitemic on review of the baseline blood smear and were excluded. Details of the remaining 28 children are summarized in Table 1. The two groups were well matched for demographic, anthropometric, and clinical characteristics. No child had a baseline value for hemoglobin, methemoglobin, or biochemical assays that was outside reference ranges for these analytes (16).

Safety and tolerability. Both doses were well tolerated. No child vomited after drug administration. There were no changes in symptoms and their severity during follow-up in either group, including nausea and abdominal pain, and no severe adverse events were reported. No children developed abnormal hepatorenal function on day 3 or 7 after treatment. Hemoglobin concentrations declined initially and then increased (trend $P = 0.033$) (Fig. 1), with no between-group differences (mean difference, −2 [−11 to 8]; $P = 0.69$). After pooling dose groups, the mean hemoglobin concentration at day 2 was significantly lower than that at day 7 ($P < 0.023$, Bonferroni corrected). There were no significant changes in methemoglobin levels in participants over time or between groups (trend $P = 0.81$; between-group mean difference [95% confidence interval], −0.1 [−0.2 to 0.1]; $P = 0.29$).

Pharmacokinetic modeling. There were 246 PMQ and 360 CPMQ individual plasma concentrations available for analysis. Of these, 6 (2.4%) and 3 (0.8%) were below the limit of quantification for PMQ and CPMQ, respectively. For PMQ, a 1-compartment model with first-order absorption and no lag time adequately described the plasma concentration-time coordinates. An additional compartment did not improve the CWRES plot or result in a significant decrease in the objective function value (ΔOFV, −5.767; $P > 0.05$; df, 2). For CPMQ, a model with two CPMQ-specific compartments, central and peripheral (P1), was superior to a single additional compartment with an improved CWRES plot accompanied by a decrease in the OFV of 254.163 ($P < 0.01$; df, 2). The addition of a third compartment for CPMQ did not result in further improvement (ΔOFV, −1.435; $P > 0.05$; df, 2). Therefore, the structural model parameters were k_a, V_C/F_{PMQ}, CL/F_{PMQ}, V_C/F^*_{CPMQ}, V_{P1}/F^*_{CPMQ}, CL/F^*_{CPMQ}, and Q_2/F^*_{CPMQ}, where F* represents the combination of the bioavailability of PMQ and the fraction of metabolic conversion of PMQ to CPMQ. As k_a was poorly estimated in the combined model with a relative standard error (RSE) of >80%, it was fixed as the value obtained from modeling PMQ alone. IIV was estimable on k_a, V_C/F_{PMQ}, and CL/F_{PMQ}. V_C/F^*_{CPMQ} and CL/F^*_{CPMQ} and the correlation between V_C/F_{PMQ} and CL/F_{PMQ}, as well as that between V_C/F^*_{CPMQ} and CL/F^*_{CPMQ}, were estimated. None of the tested covariates significantly improved the model.

The final model parameter estimates and the bootstrap results for PMQ and CPMQ are summarized in Table 2. Most (94%) bootstrap runs were successful. Bias was <5% for all structural and random model parameters. The condition number for the final model was 631. Figures 2 and 3 show goodness-of-fit plots for PMQ and CPMQ, respectively, and pcVPCs are shown in Fig. 4. From the NPCs, 10 and 9% of the data points were below and 7.5 and 8% of the data points were above the 80% prediction interval for PMQ and CPMQ, respectively.

The simulated C_{max} for PMQ and CPMQ for the three different dosing regimens are depicted by age in Fig. 5. Variability between the age groups was low (<7%) for both PMQ and CPMQ. Simu-

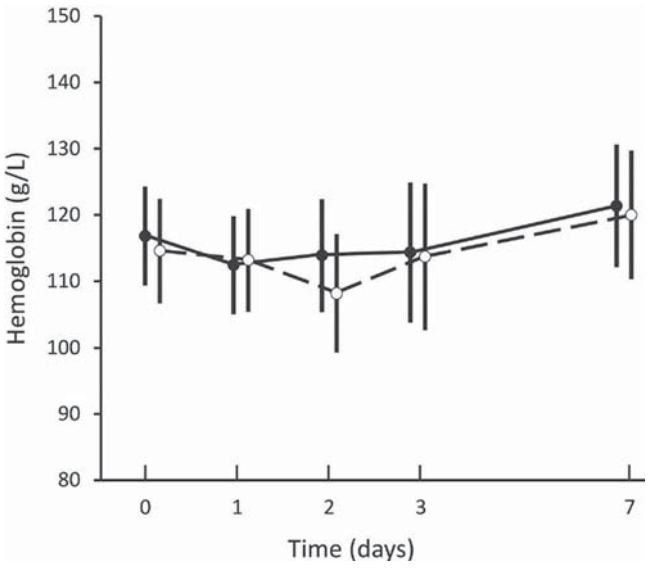

FIG 1 Means and 95% confidence intervals for hemoglobin concentrations measured before and for 7 days after dosing of 0.5 mg/kg (closed circles) and 1.0 mg/kg (open circles).

TABLE 2 Final population pharmacokinetic estimates and bootstrap results for PMQ and CPMQ

Parameter	Mean	RSE (%)	Bootstrap median (95% CI)
Objective function value	−790.147		−810.879 (−978.555-−668.075)
Structural model			
k_a[a] (/h)	2.18	30	2.2 (1.2–6.3)
CL/F_{PMQ} (liters/h/70 kg)	24.6	7	24.5 (21.7–28.1)
V_C/F_{PMQ} (liters/70 kg)	200	6	199 (175–226)
$CL/F^{\star}_{CPMQ}$ (liters/h/70 kg)	1.15	8	1.14 (0.98–1.34)
$V_C/F^{\star}_{CPMQ}$ (liters/70 kg)	7.19	27	7.08 (4.04–10.74)
$Q/F^{\star}_{CPMQ}$ (liters/h/70 kg)	3.59	15	3.59 (2.77–4.47)
$V_P/F^{\star}_{CPMQ}$ (liters/70 kg)	14.2	8	14.2 (12.6–16.3)
Variable model (% shrinkage)			
IIV in k_a (%)	138 (25)	22	140 (89–245)
IIV in CL/F_{PMQ} (%)	33 (2)	12	32 (24–39)
IIV in V_C/F_{PMQ} (%)	31 (6)	16	31 (20–41)
IIV in $CL/F^{\star}_{CPMQ}$ (%)	95 (3)	14	94 (70–124)
IIV in $V_C/F^{\star}_{CPMQ}$ (%)	40 (1)	16	39 (27–49)
$r(CL/F_{PMQ}, V_C/F_{PMQ})$	0.820	32	0.829 (0.633–0.934)
$r(CL/F_{CPMQ}, V_C/F_{CPMQ})$	0.829	26	0.837 (0.409–1.62)
RV for PMQ (%)	25.0	6	24 (21–27)
RV for CPMQ (%)	20	9	20 (16–24)
RV for CPMQ (μg/liter)	2.39	39	2.32 (1.09–4.77)

[a] k_a was fixed in the combined model. RSE and bootstrap values were from the PMQ-only model. *r* is the Pearson product-moment correlation coefficient for the two variables in parentheses. The % shrinkage for IIV is a measure of parameterization of the data, with low percentages indicating an acceptable number of model parameters.

lated plasma PMQ and CPMQ concentrations, together with 95% prediction intervals for the three different dosing regimens, are shown in Fig. 6 and are summarized in Table 3. The median C_{max} for both PMQ and CMPQ after the last dose of the 1.0 mg/kg 7-day regimen were approximately double those at the end of 14 days of 0.5 mg/kg daily, while the 1.0 mg/kg twice-daily regimen resulted in a 2.38 and 3.33 times higher C_{max} for PMQ and CPMQ, respectively.

DISCUSSION

The present study was designed to generate novel pharmacokinetic data that could be used to develop an abbreviated PMQ dosing regimen for radical cure of pediatric vivax malaria. Both 0.5 mg/kg (conventional) and 1.0 mg/kg (double) doses were safe and well tolerated. Frequent blood sampling and a validated LC-MS assay allowed simultaneous population pharmacokinetic analysis of PMQ and CPMQ plasma concentration-time profiles. Based on those single-dose data, published efficacy and tolerability studies of high-dose PMQ in adults (21–23), and practical considerations, two short-course PMQ regimens were simulated, specifically 1.0 mg/kg daily for 7 days and 1.0 mg/kg twice daily for 3.5 days. The predicted plasma PMQ and CPMQ concentrations achieved during these two regimens were no greater than those seen in previous pharmacokinetic studies of adults, suggesting that both could be further assessed in safety and efficacy field studies.

A major barrier to the control of malaria in countries where vivax malaria is endemic, such as PNG, is the ability of the parasite to relapse from dormant hypnozoites (7), including in the aftermath of successful treatment of falciparum malaria (17). Repeated vivax infections contribute to substantially increased morbidity and mortality (5, 8, 9) and have an adverse socioeconomic impact (10). Radical cure of vivax malaria in this geoepidemiologic situation has, however, been hampered by several factors. First, PMQ induces hemolysis in G6PD-deficient patients, mandating pretreatment testing for G6PD status or the use of low-dose PMQ regimens that may not be effective, especially against the Chesson strain in PNG (24). The development of cost-effective point-of-care tests (25, 26) should help to overcome this problem. Second,

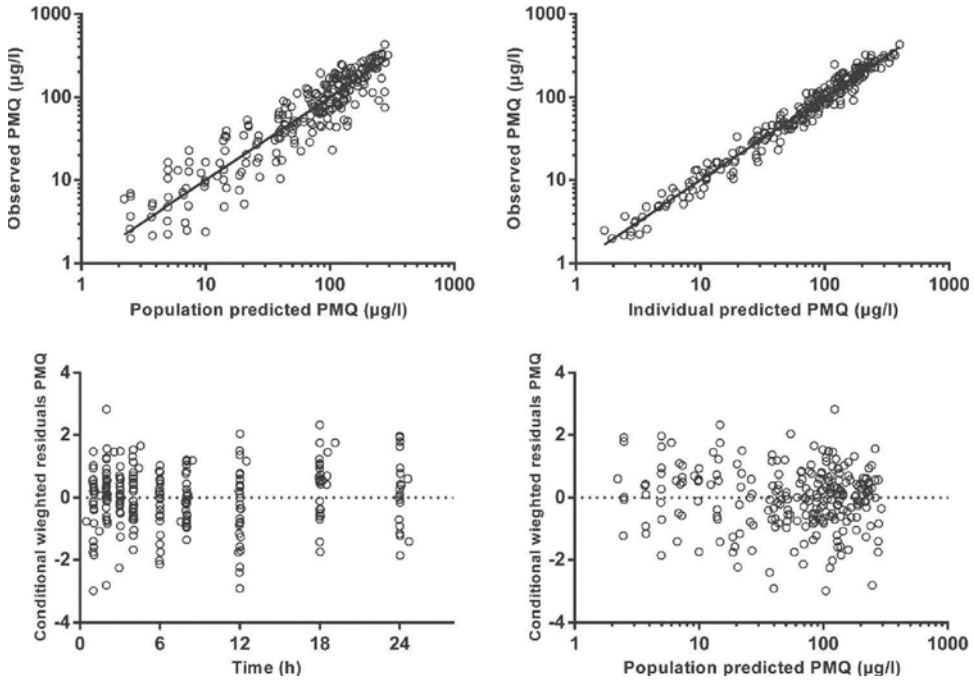

FIG 2 Goodness-of-fit plots for primaquine.

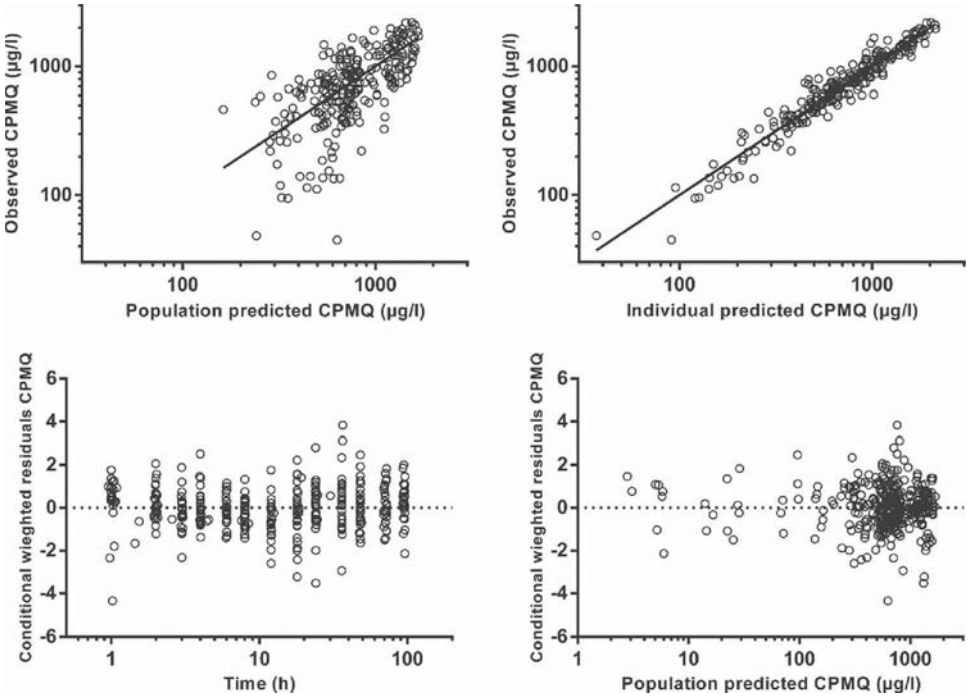

FIG 3 Goodness-of-fit plots for carboxyprimaquine.

prolonged treatment courses are associated with poor compliance (27, 28). Since the total dose of, rather than duration of exposure to, PMQ determines the efficacy of radical cure (2, 29, 30), abbreviated treatment courses have been developed. Third, PMQ is associated with gastrointestinal side effects that are related to dose but which are attenuated by coadministration with food (31) and also causes methemoglobinemia which is typically mild and transient (6).

Several adult studies have examined the safety and efficacy of abbreviated high-dose PMQ courses. In Caucasians taking 0.5 mg/kg twice daily with food for 7 days, side effects were generally nonsevere, although 5% of the subjects had methemoglobinemia sufficient to cause peripheral cyanosis without respiratory compromise (21). However, in Thai patients with vivax malaria, this same regimen was as well tolerated as the conventional 14-day, once-daily 0.5 mg/kg regimen (23). In another Thai study, patients with vivax malaria who received 1.0 mg/kg daily for 7 days had the same adverse effect profile as those receiving 0.5 mg/kg daily for 7 days, but *P. vivax* relapses were significantly fewer (22).

Extrapolation of the findings from available adult studies (21–23) suggests that a 7-day regimen of 1.0 mg/kg daily in children should also be well tolerated and effective. Because it has an elimination half-life of 4 to 6 h (32), there is no significant PMQ accumulation with daily dosing. Therefore, the C_{max} and area under the plasma concentration-time curve at the beginning and end of a 14-day course of daily PMQ doses are similar (1, 33). This lack of time/dose-dependent kinetics improves the validity of extrapolation from single to multiple dosings in our simulations. The mod-

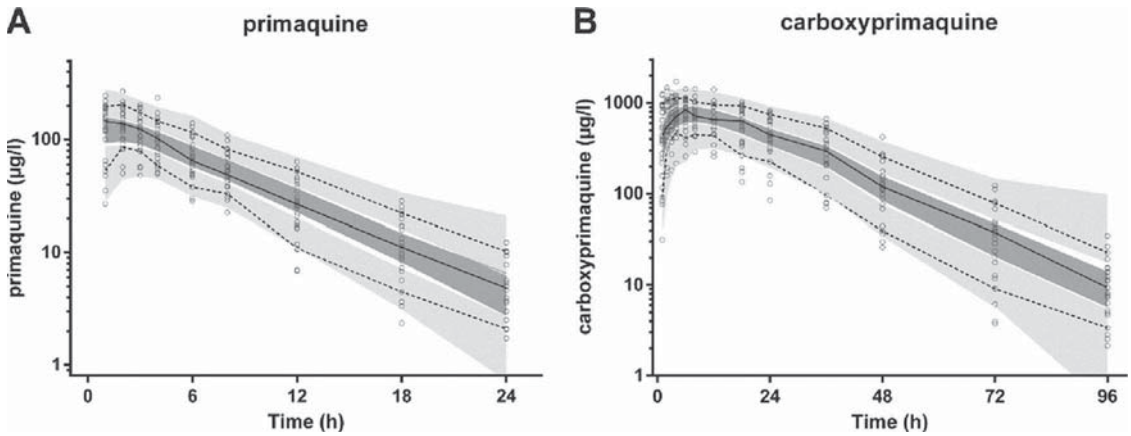

FIG 4 Prediction-corrected visual predictive check with observed 50th (solid line), 10th, and 90th (dotted lines) percentiles within their simulated 95% CI (gray shaded areas) for primaquine (A) and carboxyprimaquine (B) (μg/liter on a $\log_{10}$ scale) overlying the data points (○).

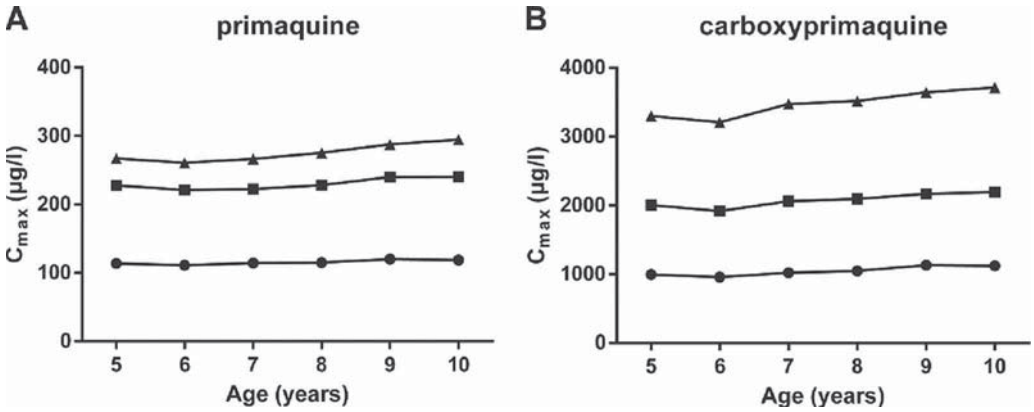

FIG 5 Simulated C_{max} for PMQ (A) and CPMQ (B) for children aged 5 to 10 years for three different dosing regimens: (i) 0.5 mg/kg daily for 14 days (●), (ii) 1 mg/kg daily for 7 days (■), and (iii) 1 mg/kg twice daily for 7 doses (3.5 days; ▲).

el-derived mean CL/F of 24.6 liters/h/70 kg in our children is within the range for adults of 20 to 40 liters/h, as is the mean V_C/F of 200 liters/70 kg versus 200 to 300 liters in adults (34). These data suggest that the median PMQ C_{max} predicted after the last dose of this regimen in our children (230 μg/liter) would be similar to that in adults. There are no adult C_{max} data for single or multiple 1.0 mg/kg doses, but in one of the first papers to detail the pharmacokinetic properties of single-dose PMQ in adults (35), the C_{max} increased from 53 μg/liter at 0.25 mg/kg to 104 μg/liter at 0.5 mg/kg and 176 μg/liter at 0.75 mg/kg, a trend consistent with our predicted C_{max}.

Although no adult pharmacokinetic studies have utilized a PMQ dose as high as 2.0 mg/kg given as a once-daily or divided twice-daily dose, the 95% prediction intervals for PMQ C_{max} after the last dose of the 3.5-day, 1.0 mg/kg twice-daily regimen (142 to 508 μg/liter) lie within the range of concentrations found in healthy Thai adults who were given a single 45-mg (0.75 mg/kg) dose (113 to 532 μg/liter) (36). This emphasizes the wide interindividual variability in PMQ disposition. In any case, single PMQ doses of up to 240 mg (6.0 mg/kg) in adults are not associated with significant gastrointestinal side effects as long as the drug is taken with food (32, 37). In addition, in the study of Caucasians who took 0.5 mg/kg twice daily for 7 days, the presence of peripheral cyanosis was not related to plasma PMQ or CPMQ concentrations (21).

Carboxyprimaquine, the principal metabolite of PMQ (38), has substantially less potent antimalarial and hemolytic activity than its parent compound (39, 40). Its relatively slow elimination means that it accumulates during multiple daily or twice-daily dosing. However, this should not have clinical consequences, even after a high-dose abbreviated regimen, since other PMQ metabolites are considered responsible for toxicity, and they appear to be minor and highly labile (41). In any case, the predicted median CPMQ C_{max} after the last dose of the 1.0 mg/kg twice-daily regi-

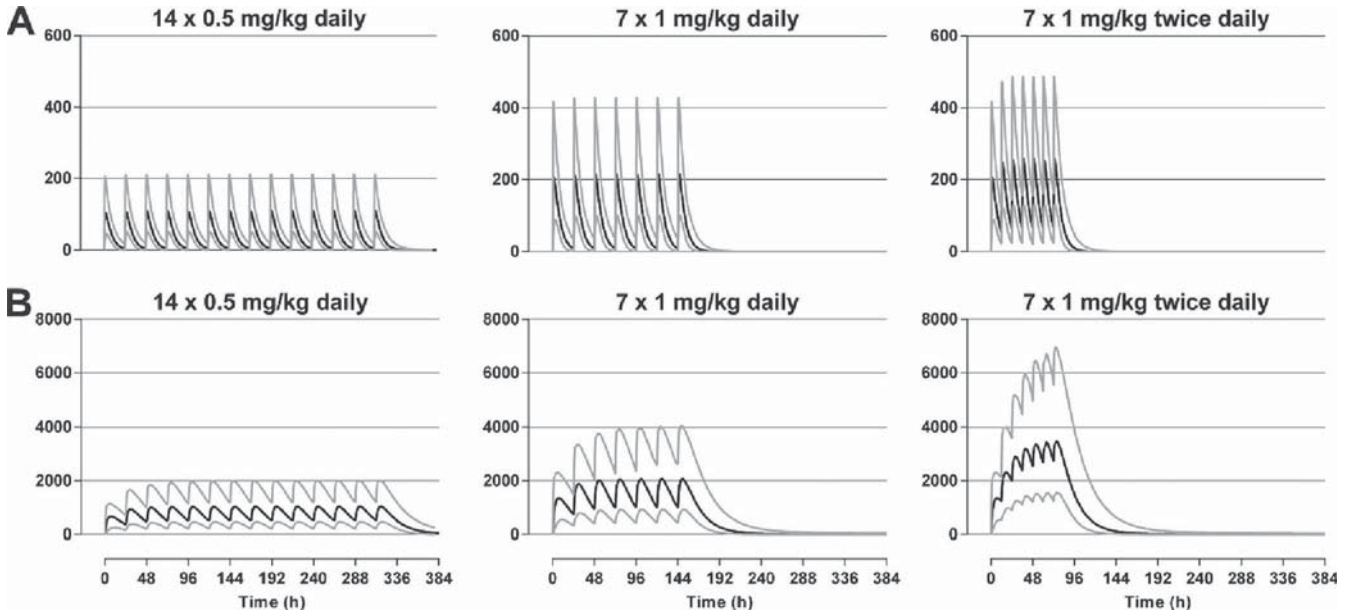

FIG 6 Simulated median (black lines) and 95% prediction intervals (gray lines) for plasma PMQ (A) and CPMQ (B) concentrations (μg/liter) for three different dosing regimens.

TABLE 3 Median intervals for C_{max} of PMQ and CPMQ for three different treatment regimens simulated for children aged 5 to 10 years

Treatment regimen	Median (95% prediction) intervals for C_{max} of:	
	Primaquine	Carboxyprimaquine
0.5 mg/kg daily for 14 days	115 (56.5–226)	1,046 (478–2,032)
1 mg/kg daily for 7 days	230 (112–442)	2,073 (968–4,143)
1 mg/kg twice daily for 3.5 days	275 (142–508)	3,477 (1,638–7,001)

men given over a 3.5-day period in the present study (3,477 μg/liter) were within the absolute range of equivalent median C_{max} from healthy Vietnamese adults given 30 mg PMQ base daily for 14 days (42) and similar to those seen in Caucasians at the end of a week of 0.5 mg/kg twice daily (means ± SD, 3,824 ± 624 μg/liter) (21).

In the present study, the higher 1.0 mg/kg single dose was as well tolerated as the conventional 0.5 mg/kg dose. In particular, there was no between-group difference in hemoglobin concentrations over time. In a larger-scale Tanzanian study, single-dose PMQ was associated with a mild mean fall in hemoglobin of 5 g/liter in children who were G6PD replete (43), raising the possibility that longer courses promote the development of anemia even when G6PD deficiency has been excluded. In the Thai study of 0.5 mg/kg twice daily for 7 days in adults (22), there was a similar proportionate fall in hematocrit by day 3, with a plateau thereafter and recovery after cessation of drug, a pattern that was also seen in the conventional 14-day 0.5 mg/kg daily regimen. If this between-group similarity applies to PNG children, the 5 g/liter mean fall in hemoglobin observed with a conventional 14-day 0.5 mg/kg daily regimen (12) would be no greater with higher-dose abbreviated regimens, but this needs to be assessed in further studies.

Conventional 14-day primaquine therapy elevates methemoglobin levels to around 4% of total hemoglobin in healthy subjects, but levels of up to 20% are usually asymptomatic (32). There may be racial differences in the propensity to methemoglobinemia, since short-course high-dose PMQ caused peripheral cyanosis in 5% of Caucasians (21) but no Thai subjects were affected in two separate similar studies (22, 23). Although there appears to be no relationship between plasma PMQ or CPMQ concentrations and methemoglobinemia (21), which may reflect the activity of transient toxic metabolites (41), future studies of short-course, high-dose PMQ regimens should include serial methemoglobin monitoring to ensure that such regimens are safe.

Due to a lack of published data on the safety and tolerability of PMQ in very young children, the WHO recommends that PMQ not be given to children under the age of 4 years (12, 44). Since vivax malaria is a common infection in this age-group in countries where it is endemic, such as PNG, including severe cases and deaths (45), consideration should be given to including these children in future trials.

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We have no conflicts of interest to declare.

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Primaquine treatment for *Plasmodium vivax* – An essential tool for malaria Control and Elimination in Papua New Guinea.

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Primaquine treatment for *Plasmodium vivax* – An essential tool for malaria control and elimination in Papua New Guinea

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SUMMARY

Plasmodium vivax is a major cause of malarial infection and disease in young children in Papua New Guinea. Recent increase in funding for malaria control has improved accessibility to preventative measures, diagnosis and artemisinin combination therapies. Yet, the current treatment and control measures are more effective against *P. falciparum* than against *P. vivax* and *P. ovale* due to the biological differences in the liver stage life-cycle of these parasites. *P. vivax* and *P. ovale* have a dormant liver stage called hypnozoites. The artemisinin combination therapies, while highly effective against the blood stages of all plasmodium species causing human malarial illness, have no effect upon the hypnozoites in the liver and the stage V gametocytes of *P. falciparum*. Currently, primaquine is the only licensed drug shown to be effective against both, hypnozoites of *P. vivax* and *P. ovale* in the liver, and the stage V gametocytes of *P. falciparum*. Primaquine has a high associated risk of life-threatening haemolytic anaemia when administered to glucose-6-phosphate dehydrogenase (G6PD) deficient persons. The lack of cheap, reliable point-of-care testing for the diagnosis of G6PD deficiency remains a major obstacle to the widespread use of primaquine in clinical and public health practice. Furthermore, there is paucity of primaquine safety and tolerability data, especially in young children with the highest *P. vivax* disease burden. For malaria control and elimination efforts to be effective, interventions such as mass drug administration must include primaquine. This opinion paper highlights the need to eradicate hypnozoites in the liver of the human host with primaquine treatment for radical cure of malarial illness, and the challenges in the use of primaquine as a public health tool for malaria control and elimination programs in countries such as Papua New Guinea.

Introduction

Papua New Guinea (PNG) exhibits one of the highest endemicity levels of *Plasmodium vivax*, the most widely distributed plasmodium species, with an estimated 2.5 billion at risk population globally (1). The current treatment regimens and malaria control measures are more effective against *Plasmodium falciparum* than *P. vivax,* as shown in countries like Brazil (2) and Thailand (3) where sustained malaria control and case management programs lead to a proportional increase in the prevalence of *P. vivax,* replacing *P. falciparum* as the predominant species. *P. vivax* is more difficult to control due to the dormant liver stage in its life-cycle called hypnozoites.

Biology of hypnozoite relapses in *P. vivax* and *P. ovale*

In contrast to *P. falciparum*, *P. vivax* and *P. ovale* both have the ability to develop into hypnozoites that can remain dormant in the parenchymal cells of the host liver following an acute infection. After a period of time, which varies in duration depending on the geographical area, hypnozoites can spontaneously reactivate, causing the release of new merozoites into the blood stream triggering a new reproduction cycle. *P. vivax* strains from tropical regions cause relapses more frequently at approximately three-weekly intervals and more often than strains from temperate regions which take about 8 to 10 months after the initial infection (4). The risk of relapse within a month of the primary parasitemia often exceeds 50% and multiple relapse episodes (three or more) can occur following the first relapse episode (5). The latent phase of *P. vivax* in the liver is therefore an important source of new arising infections, even more so as the current control measures and treatment regimens may not effectively address this particular source of new clinical episodes. Little is known of *P. ovale,* often associated with mixed infections in PNG (6). *P. ovale* relapse malaria cases of African origin have been seen in travellers almost five months after returning from Africa (7). Clinically, relapses may present as a new malaria episode, indistinguishable from a new infection, and with the potential to further transmit, through the development of gametocytes, the infection to a new mosquito and eventually to a new human host.

Primaquine hypnozoiticidal therapy for *P. vivax* and *P. ovale*

Historical context

The radical cure of *P. vivax* and *P.ovale* infections requires the treatment of both blood and liver stages of the parasite. For over 60 years, the only drug known to have any effect in eradicating the liver stages of both *P. vivax* and *P. ov*ale has been primaquine (PQ).

PQ is an analogue of pamaquine (plasmoquine), an 8-aminoquinoline drug produced in Germany during the 1920s (8). It is the only licensed drug, currently available for radical cure (elimination) of hypnozoites The current recommendation for PQ implying a 14-day long treatment course, was adopted from work by Sinton and Bird in 1928 (9) on pamaquine, which in combination with quinine seemed to adequately cure *P. vivax* infections, provided the treatment duration was sufficiently long. Studies in healthy non-immune volunteers have shown that provided an adequate total dose was delivered, the dosing schedule did not affect the overall efficacy of PQ (10). The administration of 60 mg (base) of PQ daily for 7 days was as effective as the 30 mg daily for 14 days in preventing relapses in glucose-6-phosphate dehydrogenase (G6PD) normal adult volunteers infected with the Chesson strain of *P. vivax* (10). The Chesson strain is an isolate from PNG which is relatively resistant to PQ and thus requires higher doses of PQ to prevent relapses (11). Overall, these past observations and recent studies and reviews support the so-called total dose effect with the curative activities of PQ being equally effective at the same total dose over 7 and 14 days (5,10). The total dose effect is probably a correlate of the key pharmacokinetic index of area under the curve (AUC).

Toxicity: the problem of G6PD deficiency

There is an important major drawback of implementation of PQ in routine clinical practice for the treatment of hypnozoites. PQ has a high associated risk of side

effects, particularly among people with G6PD deficiency, an X-chromosome-linked hereditary disorder (more common in males compared to females) due to mutations in the G6PD gene (12). There are many biochemical and clinical phenotypes due to the functional variants arising from the mutations in the G6PD gene. This enzymatic deficiency, of which about 140 different variants exist, is an absolute contraindication for the use of PQ when the enzyme's activity is below the threshold of 5%, but the drug can be used in milder cases with the provision of spreading the treatment on a weekly basis during a two month-long 0.75 mg/kg schedule. G6PD deficiency is frequent in malaria-endemic areas such as PNG. Other drugs known to have an association with haemolysis in persons with G6PD deficiency, and which are widely used in PNG, are: sulfamethoxazole; dapsone; nitrofurantoin, and co-trimoxazole. The rarity of reported cases of haemolytic anaemia associated with the use of these drugs in PNG, suggest this clinical phenotype to be either rare or subclinical. Further work is needed in this area.

Dosing: pharmacological considerations

Experimental challenges carried out in the 1950's showed synergy between blood schizonticides, such as chloroquine (CQ) or quinine with PQ in preventing relapses while CQ or quinine administered alone have been shown to have no effect upon the hypnozoites (13). Furthermore, therapeutic efficacy of weekly administration of PQ was increased when used concurrently with CQ on healthy G6PD deficient subjects infected with the Chesson strain of *P. vivax* (14). CQ has been the choice of treatment for *P. vivax* infection for a long time but the emergence of drug resistance has forced changes to treatment regimens and research is needed to find effective combination antimalarial therapies. The risk of relapse after treatment with CQ alone begins after 35 days reaching 58% by 60 days; while with quinine therapy relapses are encountered earlier, starting at 17 days, with 60% of patients relapsing by day 35 (5). This effect is due to its slower elimination profile leading to more prolonged therapeutic concentrations of CQ, and to the fact that CQ has a longer half-life when compared to quinine (30-60 days vs. a few hours, respectively) (15).

Primaquine for gametocyte therapy

Besides its activity against hypnozoites, PQ is highly effective in killing the sexual forms of all *Plasmodium spp*. parasites (i.e. the gametocytes) (16). This is particularly important in the treatment of *P. falciparum*, whose stage V gametocytes are relatively resistant to treatment with most other blood-stage antimalarials (17,18). Consequently, *P. falciparum* gametocytes are commonly seen for up to 4 weeks after successful treatment of asexual forms and can contribute both to transmission and to potentially faster spread of drug resistance (19). For these reason, a single dose of 0.75 mg/kg PQ was included in the PNG national treatment guidelines until 2000 when the PQ single dose was dropped in conjunction with the switch from CQ or Amadiaquine (AQ) mono-therapy to CQ/AQ plus SP combination therapy. Following a large consultative process, WHO has recently issued a recommendation for the inclusion of a single dose of 0.75 mg of PQ for treatment of *P. falciparum* malaria irrespective of G6PD status in places in which there is a threat of artemisinin resistance or where elimination programs are in place (20). PNG should therefore consider re-introducing such a single PQ dose in its national treatment guidelines.

Primaquine in Papua New Guinea and the Melanesian Western Pacific

To-date, several molecular studies to characterise G6PD deficiency have been conducted in PNG (21,22), Solomon Islands (23) and Vanuatu (24) . There are also reports of population based screening of G6PD deficiency in the Solomon Islands (25). However, epidemiological screening of markers of G6PD deficiency in any given population is of only limited value (12), as long as it does not allow an estimation of the prevalence of clinically relevant phenotypes (allelic mutations associated with haemolysis) present at the individual level. Studies are therefore needed to identify the allelic mutations in Melanesian populations that are associated with clinically significant risks of severe/life-threatening haemolysis. The absence of such evidence presents a major obstacle for the implementation of a PQ treatment policy and the use of PQ in mass drug administration for malaria elimination in *P. vivax* endemic countries such as PNG. For these reasons, the National Malaria

Control Program Strategic Plan (26) clearly states the need to test for G6PD deficiency at the hospital level and for adopting the use of only a low dose of PQ (0.25 mg/kg) as treatment for confirmed *P. vivax* cases in all health facilities until further information is available. However a review of 18 studies published since 1950 (27) showed that PQ ≤0.25 mg/kg effectiveness was no different compared to that patients with no PQ treatment.

Efficacy data of *P. vivax* treatment and the contribution of hypnozoites to *P. vivax* infection

For a long time, the effective treatment for *P. vivax* was CQ. *P. vivax* resistance to CQ, first reported in 1989 from PNG (28), has later become widespread throughout the Island of New Guinea (29,30), requiring new studies to reassess the effectiveness of the old and new treatment regimens against relapses. Following reports of increasing resistance of *P. falciparum* and *P. vivax* against CQ and sulfadoxine-pyrimethamine (SP) (31), a trial of combination antimalarial therapies in children was carried out in Madang (32). The standard treatment for uncomplicated malaria in PNG was changed from AQ/CQ + sulfadoxine-pyrimethamine (SP) to artemether-lumefantrine (AL) in 2009, in the wake of global trends to move towards artemisinin-based combination therapies (ACT). However, the efficacy of AL for preventing recurrent *P. vivax* infections was not substantially better than that of CQ + SP, with 87.0% and 69.7% of participants in the CQ + SP and the AL groups, respectively, showing recurrent *P. vivax* infections during 6 weeks of follow-up ($p = 0.06$) (32). Recently, genotyping of the same samples showed most of the infections in the AL group were of different genotypes, suggesting new *P. vivax* infections rather than recrudescence of the initial infection from the same genotype observed at baseline (33).

The observations from these studies suggest that the new standard treatment of AL, while effective against acute clinical *P. vivax* malaria episodes, does not prevent late treatment failures (34), therefore, may have limited effect on the prevalence and transmission of *P. vivax.* Indeed, both new infections and/or relapses from hypnozoites in the liver allow *P. vivax* to re-establish blood stage infection very

rapidly following treatment with AL, particularly because the half-life of lumefantrine (the only drug remaining in the bloodstream after the disappearance of the short-lived artemisinin component) is relatively short (4-5 days), and thus the post-treatment prophylactic effect conferred by the use of this drug combination is shorter than that of other combinations. As a consequence, the new PNG standard treatment protocol for confirmed (or suspected) *P. vivax* malaria adopted in 2009 includes the prescription of PQ at 0.25 mg/kg daily for 14 days after three days of AL (35).

Only recently, studies on PQ safety, tolerability and its effect on hypnozoites were performed in cohorts of PNG children aged 1 to 10 years old living in areas of high transmission and thus high re-infection risk (36,37). The results show PQ to be safe and effective when used in combination with artesunate in G6PD normal children. Pre-treatment with artesunate plus PQ (14d, 0.5mg) reduced incidence of *P. vivax* malaria by 49% for the initial 3 months ($p = 0.031$) and 19% for months 4-9 ($p = 0.25$), and reduced time to first light microscopy and PCR-positive infections by 57% and 48%, respectively ($p < 0.001$), when compared to a group treated only with artesunate (37). The effect of artesunate +PQ was limited to the first 3 months of follow-up and 30% of the children in the artesunate +PQ group had re-infection by 2 weeks of follow-up. Even though the artesunate +PQ combination may not be optimally efficacious in eradicating hypnozoites completely, most likely due to the short half-life of artesunate, PQ use had a significant impact in reducing the incidence and burden of relapse malarial disease when compared to artesunate alone.

Alternatives to primaquine for hypnozoiticidal therapy

Tafenoquine

Although the improved use of PQ could result in a significant improvement of current treatment of *P. vivax* malaria and reduce transmission of any *Plasmodium* spp., the need for G6PD testing as well as problems with adherence to the long 14-day treatment schedule are considerable obstacles to a large-scale roll-out of PQ therapy. Consequently, there is a great need to develop alternative anti-hypnozoite drugs. One such novel drug is tafenoquine (38,39). As another 8-aminoquinoline it

shares with PQ the problem of potential haemolysis in G6PD deficient individuals; however, due to its long half-life, it can be given as a single dose or 3-day long treatment and can thus potentially be combined with standard 3-day blood-stage regimens. Tafenoquine is currently undergoing phase II/III testing.

Research priorities

Even though tafenoquine will address the problem of poor compliance with current PQ regimens, additional anti-hypnozoite drugs that can be safely given in G6PD deficient individuals are a high priority on the malaria elimination research agenda (40). It is also essential to carry out studies that may contribute to a better understanding of the role of relapses as sources of newly arising infections, and also in this contextthe potential efficacy and safety of PQ in preventing or delaying such relapses, particularly in children. A safety, tolerability and pilot efficacy of short course, high dose PQ treatment for *P. vivax* in children 5 to 10 years old is currently in progress in PNG, comparing the standard 14-day to a 7-day regimen. If shown to be safe, proceeding to a 3 ½-day dose regimen will be considered.

Health policy considerations for PNG

The clinical implementation of PQ alongside a blood-schizonticidal drug in primary health care settings is challenging. Uncertainties remain around the appropriate application of PQ in PNG, particularly in the presence of G6PD deficiency. Potentially as a consequence of ambiguous recommendations, the prescription of PQ to patients has been inconsistent in recent years (41).

Point of care testing for G6PD

The available methods of testing for G6PD deficiency, such as the Motulsky dye decolouration test (42), NADPH fluorescent spot test (43,44) and variations of the MTT formazan methods (45-48) require specialized equipment and have therefore not been successfully implemented in clinical settings in malaria-endemic areas (45,49). More recently, the FDA approved the BinaxNOW G6PD test (Inverness

Medical, Switzerland) and the Dojindo G6PD WST-8 Assay Kit (Dojindo Molecular Technologies, Japan) (45,50,51), which have provided a more suitable alternative but challenges to their implementation in malaria-endemic areas remain. These are qualitative diagnostic tests, dependent on visual interpretation of colour change within a specified time and temperature range. The optimal temperature for BinaxNOW® G6PD ranges from 18 to 25ºC. Both tests are temperature/light sensitive, rather expensive (approximately US$8 and US$5 per test, respectively), not stand-alone kits (i.e. requiring additional equipment) and require a higher level of training to perform and interpret than the commonly utilised malaria rapid diagnostic tests (RDTs). An alternative would be the new G6PD test kit called CareStart™ (Access Bio, New Jersey, USA), which is still undergoing development. It was recently tested for the first time under field conditions to assess its performance (52). The CareStart™ is an RDT-format test which could be used together with current RDT testing for malaria diagnosis once fully developed and approved as point-of-care, easy to use diagnostic tool for G6PD deficiency testing.

Until such challenges are overcome and routine G6PD screening is implemented at outpatient health services in PNG, *P. vivax* malaria and relapses from the dormant stages in the liver will remain a challenge for the PNG National Malaria Control Programs as the low dose PQ treatment recommended in the absence of G6PD testing is unlikely to be effective against circulating vivax strains present in PNG (5,27).

The hypnozoites in the liver represent an important source of re-infection, disease and transmission of *P. vivax*. In order for malaria prevention and control programs to be effective, treatment options for eradication of the liver stages of *P. vivax* must be evaluated and implemented together with other control measures such as ACTs and insecticide-impregnated bed nets.

Recommendations

For elimination of malaria to become an eventual reality, all confirmed cases of malaria, including *P. falciparum* monoinfections will need to be treated with hypnozoiticidal doses of PQ 0.5 mg/kg or higher. A single dose of 0.75 mg/kg PQ that was included in the PNG national treatment guidelines prior to 2000 should be reintroduced as routine gametocidal treatment for clinical cases of *P. falciparum* and a treatment policy to include PQ for asymptomatic infections of all plasmodium species. Asymptomatic carriers of all human *Plasmodium spp*. contribute to disease, transmission and development of resistance to antimalarial drugs (53). Mass drug administration for malaria control and elimination must include PQ (54) with an ACT such as dihydroartemisinin-piperaquine (55) that has a partner drug with a long terminal elimination phase (56). Finally, knowledge and distribution of G6PD deficiency variants throughout PNG and the Melanesian Pacific, that are associated with clinically significant risk of severe haemolytic anaemia, and a point-of-care easy to use diagnostic test for G6PD deficiency will be needed to eliminate malaria.

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9. Summary of results and conclusions

Study 1(Reanalysis of published surveys)

Epidemiology of malaria in the Papua New Guinean highlands.

Results

- 153 house-hold based, rapid malaria population surveys were conducted in 112 villages throughout the central PNG highlands
- Prevalence of malaria infections ranged from 0.0% to 41.8% (median 4.3%) in non-epidemic surveys and 6.6% to 63.2% (median 21.2%, $P < 0.001$) during epidemics in the different surveys
- *P. vivax* infection was predominant at altitudes >1600 m
- Below 1400 m and during epidemics, *P. falciparum* was the predominant infection
- Outside of epidemics, the prevalence decreased significantly with altitude
- Prevalence was reduced with bed net use [odds ratio (OR) = 0.8, $P < 0.001$]
- For people sleeping in the gardens, the prevalence increased (OR = 1.34, $P < 0.001$)
- Below 1400 m malaria was a significant source of febrile illness
- Above 1400 m, malaria was only a significant source of febrile illness during epidemic outbreaks.
- Asymptomatic malaria infections were common in non-epidemic times

Conclusions

- Malaria is once again endemic throughout the PNG in areas below 1400 – 1500 m
- There is a significant risk of seasonal malaria outbreaks in most areas between 1400 – 1600 m.
- Ongoing malaria control efforts are likely to result in substantial reduction in malaria transmission
- Malaria control efforts may even result in local elimination

Relapses contribute significantly to the risk of *P. vivax* infection and disease in Papua New Guinean children 1-5 years of age

Recruitment

- After screening for G6PD deficiency, 449 of the 463 eligible children aged 1 to 5 years from 11 study villages were recruited and randomized into three groups.
- 150 children allocated to receive ART (4mg/kg for 7 days) plus PQ (0.5mg/kg for 14 days)
- 150 children to receive ART alone (4mg/kg for 7 days)
- 149 children to receive no treatment as the control group
- Of the 449 randomised children, 16 withdrew or moved out of the study area before commencement of the study
- A total of 433 children were treated and followed up actively and passively from 40 weeks
- There were no significant differences in the demographic characteristics and infections status were observed before start of treatment between the three pre-treatment groups.
- The use of LLIN was comparable between the groups
- There was no difference in the average number of study contact between the three treatment arms

P. vivax episodes among study children

- 271 febrile episodes with *P. vivax* of any density were detected during the 40 weeks of follow up
- 115 episodes of *P. vivax* with densities >500µL/ml were detected among the study children
- 132 children (30%) had 1 *P. vivax* episode of any density
- 60 children (14%) had ≥2 episodes (maximum 4)

- The incidence of *P. vivax* malaria decreased strongly with age for *P. vivax* episodes of any density and varied between villages for episodes of any density

Treatment Effect

- The incidence of *P. vivax* malaria of any density detected during the 40 weeks of follow up differed significantly between the three treatment groups:
    - ❖ Treatment with ART+PQ reduced the risk of *P. vivax* episodes of any density by 28% (95% CI, 1%-52%; *P* = 0.042) as compared to the ART only, arm.
    - ❖ ART+PQ treatment reduced the risk of *P. vivax* episodes by 33% (95% CI, 8%-52%; *P* = 0.015) as compared to the control arm.
- The difference in the risk reduction between the treatment groups was limited to the first three months with little or no difference during the entire 40 weeks of follow up:
    - ❖ IRR for ART+PQ vs. ART alone, 49%, [95% CI, 6%-73%; *P*=0.013] at 3 months of follow up
    - ❖ IRR for ART+PQ vs. control, 58%, [95% CI, 24%-77%; *P*= 0.004] at three months of follow up

Time to first *P. vivax* infection by qPCR and LM diagnosis

- The first *P. vivax* infection after pre-treatment diagnosed by qPCR occurred very quickly among children in the treatment groups:
    - ❖ 50% of children in the ART only, group had *P. vivax* infection detected by day 23 of follow up (IQR, 14-30 days)
    - ❖ 50% of children in the ART+PQ group had *P. vivax* infection detected by day 30 (IQR, 15-56 days)
- Diagnosis by LM of first *P. vivax* infection among the children in the treatment groups showed a more pronounced difference:
    - ❖ Median 29 days (IQR, 16-55 days) for ART only group
    - ❖ Median 78 days (IQR, 42-280) for ART+PQ group

- Overall the elimination of *P. vivax* liver stages through primaquine treatment was found to reduce qPCR- and LM-positive recurrent blood-stage parasitaemia:
    - ❖ 44% reduction in risk (95% CI, 28%-57%; $P < 0.001$) by qPCR diagnosis
    - ❖ 67% reduction in risk (95% CI, 55%-75%; $P < 0.001$) by LM diagnosis

Conclusions

- ❖ *P. vivax* relapses from hyponzoites contribute significantly to the high burden of infection and clinical illness in children <5yrs old
- ❖ Pre-treatment with PQ reduced the incidence of *P. vivax* infections by 57% in the first 3 months
- ❖ Treatment of *P. vivax* should include eradication of hypnozoites from the liver

Tolerability and safety of primaquine in Papua New Guinean children 1 to 10 years of age

Recruitment

- The children in this study were part of two paediatric cohorts aged 5-10 and 1-5 years, designed to assess the effect of PQ pre-treatment upon liver- and blood-stages of *P. vivax* infection and disease burden.
- All the children were screened for G6PD deficiency after informed consent for the parent or legal guardian.
- Only G6PD normal children were enrolled into the two longitudinal cohort studies and randomized to pre-treatment groups.
- The first cohort of 524 G6PD normal children aged 5-10 years was a randomized, double blind placebo controlled study.
- Children were randomized to receive either, CQ+PQ (standard 25mg/kg/total dose for 3 days + 0.5mg/kg PQ for 14 days) or, CQ+Placebo (standard 3d+14d):
    - ❖ 247 children received CQ+PQ
    - ❖ 257 children received CQ+Placebo
    - ❖ 10 children from CQ+PQ and 3 children from the CQ+Placebo were excluded post-hoc due to protocol violation.
- The second cohort of 449 G6PD normal children aged 1-5 years recruited and randomized into three groups.
    - ❖ 150 children allocated to receive ART (4mg/kg for 7 days) plus PQ (0.5mg/kg for 14 days)
    - ❖ 150 children to receive ART alone (4mg/kg for 7 days)
    - ❖ 149 children to receive no treatment as the control group
    - ❖ Of the 150 children in the ART+PQ group, 9 withdrew or moved out of the study area before commencement of the study

- All drug doses were administered as DOT with food in the cohort of 5-10 years old while in the younger cohort of 1-5 years old, parents were advised to feed their children prior to treatment.

Safety and Tolerability

- Children were followed up daily for 14 days of DOT for drug side effects
- There were no significant differences between PQ and placebo groups for signs and symptoms at baseline among the 5-10yrs old children (P>0.41)
- Drug related symptoms were rare during the 14 days of DOT (frequency < 7)
- There were no differences in occurrence of new symptoms and their cumulative incidence during the 14 days of follow-up between the PQ and placebo groups (P>0.24)
- Among the younger children aged 1-5yrs, prevalence of adverse events was higher due to higher prevalence of malarial fevers (1-5 yrs: 14.9% vs. 5-10yrs: 5.3%, P<0.001)
    - ❖ The occurrence of new symptoms and their cumulative incidence among the 1-5yrs old during the 14 days of follow-up was higher
    - ❖ Most of the symptoms were related to febrile illness and/or cough
    - ❖ There was a higher rate of stomach-ache among the 1-5yrs old (P<0.010)
- The rates of nausea and/or vomiting were comparable among the two cohorts of children; the older 5-10yrs old who received a snack and the younger 1-5yrs old fed by their parents prior to drug ingestion
- No treatment had to be discontinued due to poor tolerability or repeated vomiting in either cohort
- A marginally larger but clinically and statistically insignificant drop in haemoglobin was observed at day 8 in the older 5-10yrs old children, for PQ vs. Placebo group (Hb: 0.56 vs. 0.33g/dl, P=0.24)

- Equal number of children in the two cohorts experienced a clinically significant drop in haemoglobin >2g/dl (22/247 vs. 22/257, *P*=0.89)
- Among the 1-5yrs old treated with ART+PQ, the haemoglobin did not change in the first 3 days of treatment (9.31 vs. 9.35g/dl, *P*=0.77)
- The 1-5yrs old children's haemoglobin increased by 0.48g/dl after 14 days of treatment (9.31 vs. 9.81g/dl, *P*<0.001)
- Only one child experienced a haemoglobin drop of 2g/dl with no clinical evidence of haemolysis

Conclusions

- PQ dose of 0.5mg/kg was well tolerated and safe in PNG children 1-10yrs old

Pharmacokinetic properties of single-dose primaquine in Papua New Guinean children: Feasibility of abbreviated high-dose regimens for radical cure of vivax malaria

Recruitment

- 30 G6PD normal children aged 5-12yrs old were recruited and randomized to a single oral dose of PQ at either, 0.5mg/kg (group A) for 14 days or 1mg/kg (group B) for 7 days
- 2 children in group B were excluded due to positive blood slides upon review at baseline
- The two groups were well matched for demographics, anthropometrics and clinical characteristics
- All the children had baseline values for haemoglobin, methaemoglobin and biochemistry parameters within the normal ranges of analytes

Safety and Tolerability

- Both PQ doses of 0.5mg/kg and 1.0mg/kg were well tolerated
- No child vomited after each PQ dose administration
- There were no changes in symptoms and their severity during follow up in either group including nausea and abdominal pain
- No severe adverse events were observed or reported
- No child developed abnormal hepatorenal functions on days 3 or 7 after treatment
- Haemoglobin concentrations declined initially then increased (Trend *P*=0.033) with no between group difference (mean difference= -2(-11 to 8), *P*=0.69)
- On pooling dose groups, mean haemoglobin concentrations at day 2 was significantly lower than at day 7 (Bonferroni-corrected *P*<0,023)
- There were no significant changes in methaemoglobin levels in participants over time or between groups (Trend *P*=0.81); between group mean difference (95% CI, -0.1(-0.2 to 0.1), *P*=.29)

Pharmacokinetic Modelling

- The mean PQ central volume of distribution and clearance relative to bioavailability (200 litres/70kg and 24.6 litres/h/70kg) were within published ranges for adults
- The median predicted maximal concentration (C_{max}) for both PQ and carboxyprimaquine (CPQ) after last dose of a 1mg/kg 7 day PQ regimen were approximately double those at the end of 14 day of 0.5mg/kg daily dose
- A 1mg/kg twice daily regimen for 3.5 days resulted in a 2.38 and 3.33 times higher C_{max} for PQ and CPQ, respectively
- All predicted median C_{max} concentrations were within ranges in adult high-dose studies that also showed acceptable safety and tolerability

Conclusions

- The present pharmacokinetic data, the first for PQ in children, show that further studies of abbreviated high-dose regimen are feasible in this age group

Primaquine treatment for Plasmodium vivax – An essential tool for malaria Control and Elimination in Papua New Guinea.

- It highlights several key challenges for treatment, control and elimination of *P. vivax*, including:.
  - ❖ biology of the *P. vivax* hypnozoites
  - ❖ the difficulties of malaria elimination without the eradication for the hypnozoites from the liver with PQ
  - ❖ lack of cheap, point-of-care test for G6PD deficiency
- The implementation of public health programs for routine use of PQ including MDA for malaria control and elimination are hindered by the PQ associated haemolytic anaemia in persons with the severe variants of G6PD deficiency.
- In PNG and other Melanesian countries the higher dose of 0.5mg/kg PQ should be administered to *P. vivax* confirmed cases as the Chesson strain present in these countries are relatively resistant to PQ.

10. Discussion

The studies (articles 2-4) in this thesis were designed to generate several novel paediatric data on the epidemiology and treatment of *P. vivax* in PNG children. By an innovative approach to study design, where the liver-stages of *P. vivax* infection were cleared in some study children but not all (with 0.5mg/kg PQ treatment) a significant contribution of hypnozoites to the burden of *P. vivax* infection and disease was demonstrated in children 1 to 10 years old. The cohort studies also provided an ideal opportunity to gather field-based safety and tolerability data on the use of 0.5mg/kg high dose PQ, which was shown to be safe and well tolerated in G6PD normal PNG children aged 1-10 years old. A pharmacokinetic profiling of properties of single doses of 0.5mg/kg and 1.0mg/kg PQ as a feasibility study for further abbreviated high-dose PQ regimen showed, all predicted median C_{max} concentrations were within ranges in adult high-dose studies and the single high doses of PQ were safe and well tolerated in PNG children aged 5 to 12 years old.

In PNG, *P. viva*x infection and malarial illness is mainly confined to children <3 years old as reported by several published studies [25, 26, 50]. The contribution of dormant hypnozoites of *P. vivax* to infection and disease burden from relapses malaria has been shown to be substantial [132]. Moreover, due to the different periodicity of hypnozoite activation in different geographic areas and climatic settings [21], it represents a major barrier to treatment, control and elimination of *P. vivax* malaria in endemic countries such as PNG. The current recommended treatment guidelines by PNG NDoH and WHO for confirmed *P. vivax* malaria illness [43, 131], are inadequate due to little or no available data on the use of PQ in children. The field-base safety and tolerability data on the use of 0.5mg/kg in 1 to 10 years [133] adds to this knowledge gap, while the pharmacokinetic profiling of single high doses of PQ provide the basis for further evaluation of abbreviated high-dose PQ regimens.

A major drawback for public health use of PQ has been related to its associated side effect of life-threatening haemolytic anaemia in G6PD-deficient persons with the severe variant forms, of which 140 variants exist in different populations [83]. The lack of cost-effective point-of-care test to identify persons with these

severe forms of G6PD deficiency is a major obstacle to PQ routine use and in MDA for malaria elimination efforts in impoverished countries with high malaria disease burden.

Further research is needed for hypnozontocidal drugs such as Tafenoquine and RDTs for G6PD testing for mass screening. Work is currently ongoing in PNG on short course, high dose, PQ regimen in children and molecular studies to characterize the prevalence of severe variants of G6PD deficiency in different PNG ethnic populations. The finding from these studies may improve compliance for PQ and improve its routine public health use and for MDA, as PNG moves towards malaria elimination in the future. Without an effective drug for eradicating *P. vivax* hypnozoites in the liver and a reliable, and cheap easy-to-use, RDT based test kit for G6PD testing, the goal of malaria elimination and its eradication from the globe will be difficult to achieve in *P. vivax* endemic countries such as PNG.

11. General conclusions

Study 1

- Malaria is endemic throughout the PNG highlands in areas below 1400 – 1500 m with significant risk of seasonal malaria outbreaks in most areas between 1400 – 1600 m
- Ongoing malaria control efforts are likely to result in substantial reduction in malaria transmission and possibly result in local elimination

Study 2

- Relapses from hypnozoites in the liver contribute significantly to the high burden of *P. vivax* infection and clinical disease in PNG children aged 1-10 years old.
- *P. vivax* strains (Chesson) in PNG relapse quickly within 12 weeks of clearance of hypnozoites from the liver
- Older children experience less clinical illness, due to faster acquisition of natural *P. vivax* clinical immunity
- All confirmed clinical cases of *P. vivax* malaria must receive the 0.5mg/kg PQ regimen for radical treatment to clear the hypnozoites from the liver
- Asymptomatic *P. vivax* parasite carriers should be treated with a blood-stage antimalarial and PQ for radical cure
- The partner drug for PQ must have a long terminal elimination phase to effect post-treatment prophylaxis in order to stop the first or second phase *P. vivax* relapses to prevent reinfection and relapse malarial illness

Study 3

- PQ high dose of 0.5mg/kg is safe and well tolerated in PNG children 1 to 10 years old
- PQ treatment for confirmed *P. vivax* clinical cases should use 0.5mg/kg instead of the low dose 0.25mg/kg which may not be effective in eradicating the tropical strains (Chesson) of *P. vivax* hypnozoites from the liver

Study 4

- The pharmacokinetic profiling of single high doses of 0.5mg/kg and 1mg/kg were within the ranges in adult high-dose published studies that also show acceptable safety and tolerability
- Since the highest *P. vivax* infection and disease burden is in children <5 years old and WHO recommends PQ not to be used in this age group, future studies should involve children 1 to 5 years to generate safety and tolerability data.

Article 5 (Opinion paper)

- For elimination to become an eventual reality, all confirmed cases of malaria, including *P. falciparum* monoinfections will need to be treated with hypnozoiticidal doses of PQ 0.5mg/kg or higher
- A single dose of 0.75mg/kg PQ that was included in the PNG national treatment guidelines prior to 2000 should be reintroduced as routine gametocidal treatment for clinical cases of *Plasmodium falciparum;* and a treatment policy to include PQ for asymptomatic infections of all plasmodium species
- MDA for malaria control and elimination must include PQ and a partner ACT drug with a long terminal elimination phase such as DHAPQ

- Finally, knowledge and distribution of G6PD deficiency variants throughout PNG and the Melanesian Pacific, that are associated with clinically significant risk of severe haemolytic anaemia and a point-of-care easy to use diagnostic test for G6PD deficiency will be needed to eliminate malaria.

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