Epidemiology and treatment of malaria in Kinshasa, Democratic Republic of the Congo

INAUGURALDISSERATION

zur

Erlangung der Würde eines Doktors der Philosophie

vorgelegt der

Philosophisch-Naturwissenschaftlichen Fakultät

der Universität Basel

von

Giovanfrancesco Ferrari

von Como, Italien

Basel, 2016

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät auf Antrag von Prof. Dr Christian Lengeler und Dr. Umberto D'Alessandro

Basel, den 23 Juni 2015

Prof. Dr. Jörg Schibler

The Dean of Faculty

To my family

Table of contents

List of figures **III**

List of tables **IV**

List of abbreviations **V**

Acknowledgements **VII**

Summary **IX**

Résumé **XIV**

1. Introduction 1

1.1 Global malaria distribution 1

1.2 Malaria disease and transmission 2

1.3 The *Anopheles* vector 3

1.4 Epidemiological measures of malaria risk 3

1.5 Vector control measures 4

1.6 Mapping malaria risk 5

1.7 A brief overview of the Democratic Republic of the Congo and its administrative and health system organisation 6

1.8 Epidemiological situation and parasite distribution in the Democratic Republic of the Congo 8

1.9 Malaria control interventions in the Democratic Republic of the Congo 9

1.10 Urbanisation and health 11

1.11 Epidemiology of urban malaria 11

1.12 Anaemia 13

1.13 Urban malaria control in Kinshasa 14

1.14 Epidemiology of severe malaria 15

1.15 A new treatment of severe malaria 16

1.16 Cost of severe malaria 16

2 Aims and objectives 17

3 A comprehensive malaria risk map in Kinshasa, Democratic Republic of Congo 19

3.1 Abstract 20

3.2 Background 22

3.3 Method 23

3.4 Results 31

3.5 Discussion 48

3.6 Conclusions 51

4 Identifying risk factors for *Plasmodium* infection and anaemia in Kinshasa, Democratic Republic of Congo 53
4.1 Abstract 54
4.2 Background 56
4.3 Method 57
4.4 Results 59
4.5 Discussion 68
4.6 Conclusions 71
5 An operational comparative study of quinine and artesunate for the treatment of severe malaria in hospitals and health centres in the Democratic Republic of Congo: The MATIAS study 72
5.1 Abstract 73
5.2 Background 75
5.3 Methods 76
5.4 Results 85
5.5 Discussion 91
5.6 Conclusions 94
6 Delayed anaemia after treatment with injectable artesunate in the Democratic Republic of Congo: a manageable issue 96
6.1 Abstract 97
7 Feasibility and acceptability of injectable artesunate for the treatment of severe malaria in the Democratic Republic of Congo 103
7.1 Abstract 104
7.2 Background 106
7.3 Methods 107
7.4 Results 112
7.5 Discussion 118
8 General discussion and conclusions 121
8.1 Prospects for future research on risk map data 122
8.2 Prospects for malaria control in Kinshasa 123
8.3 Prospects for the implementation of injectable artesunate in the DRC 126
8.4 Challenges to the introduction of injectable artesunate in the DRC setting 126
9 References 130
10 Curriculum vitae 147

List of figures

Figure 1-1: World malaria distribution in 2014 1
Figure 1-2: Administrative map of the Democratic Republic of the Congo 8
Figure 3-1: *Plasmodium falciparum* malaria prevalence (RDT positivity) by age group for the health zones of Selembao and Ngiri Ngiri 34
Figure 3-2: Standardized *Plasmodium falciparum* malaria prevalence in children aged 6-59 months, by health area 36
Figure 3-3: Interpolation results for standardized *Plasmodium falciparum* malaria prevalence in children aged 6-59 months, by health area 37
Figure 3-4: Standardized prevalence of anaemia (Hb<11g/dl) in children aged 6-59 months, by health area, surveys 2009 and 2011. 39
Figure 3-5: Standardized prevalence of severe anaemia (Hb<7g/dl) in children aged 6-59 months, by health area, surveys 2009 and 2011. 40
Figure 3-6: Percentage of children <5 years having slept under an ITN the night before the survey in 2009, by health area. 46
Figure 3-7: Percentage of children <5 years having slept under an ITN the night before the survey in 2011, by health area. 47
Figure 4-1: Left panel: ITN usage among children 6 to 59 months by transmission intensity. Right panel: ITN usage among individuals older than five years in Ngiri Ngiri (0.8%) and Selembao (26.8%) in Kinshasa, 2011. Light grey bars represent proportions of reported ITN usage in high transmission strata, and dark grey bars in low transmission strata. Error bars indicate 95% CI. 67
Figure 5-1: Map showing the location of the study sites and the selected health zones 78
Figure 6-1: Hb time course of persons with severe anaemia at follow-up visits, Democratic Republic of the Congo 101

List of tables

Table 3-1: List of the Health Zones in Greater Kinshasa surveyed in 2009 and 2011 and their corresponding populations. 27
Table 3-2: Characteristics of study households and individuals in the 2009 and 2011 surveys, Kinshasa, Democratic Republic of Congo 32
Table 3-3: Clinical outcomes, by health zone 42
Table 3-4: Malaria control indicators, by health zone. 45
Table 4-1: Number of children 6 to 59 months examined and the prevalence of *Plasmodium spp* in Kinshasa, by health zone and strata, 2011. 60
Table 4-2: Univariate and multivariable analysis of risk factors associated with malaria in children between 6 and 59 months of age in Kinshasa, stratified by malaria transmission zone, 2011. 62
Table 4-3: Univariate and multivariable analysis of risk factors associated with malaria in individuals aged > 5 years in Kinshasa, stratified by malaria transmission zone, 2011. 64
Table 4-4: Univariate and multivariable analysis of risk factors associated with anaemia in children between 6 and 59 months of age Kinshasa, stratified by malaria transmission zone, 2011 66
Table 5-1: Characteristics and clinical presentation of patients at recruitment 87
Table 5-2: Clinical examination at discharge 88
Table 5-3: Key time intervals 88
Table 5-4: Personnel time (in minutes) required to complete pre-administration tasks, by drug type 89
Table 5-5: Overall cumulative personnel time (in minutes) 89
Table 5-6: Mean cost (with SD) for treating one episode of severe malaria in patients admitted to hospitals and health centres in the Democratic Republic of Congo 90
Table 6-1: Summary data of patients presenting with life-threatening anaemia during follow-up visits, Democratic Republic of Congo 100
Table 7-1: Summary of interviews with health care providers 112
Table 7-2: Summary of interviews with patients/caretakers 117

List of abbreviations

ACT	Artemisinin-based combination therapy;
ADB	Asian Development Bank
AL	Artemether plus lumefantrine
ALU	Artemether plus lumefantrine (ALU).
AS-AQ	Artesunate plus amodiaquine
CDC	Centre for Disease Control
CI	Confidence interval
CRF	Case Report Form
DRC	Democratic Republic of the Congo
EKBB	Ethikkommission Beider Basel
GF	Global Fund
GFATM	Global Fund to fight AIDS TB and Malaria
GIS	Geographic Information System
GPS	Global Positioning System
HA	Health Area
Hb	Haemoglobin
HZ	Health Zone
IV	Intravenous
ITN	Insecticide-treated net
JICA	Japanese International Cooperation
KSPH	Kinshasa School of Public Health
MATIAS	MAlaria Treatment with Injectable ArteSunate
MMV	Medicines for Malaria Venture
MMWR	Morbidity and mortality weekly report
MoH	Ministry of Health
MSH	Management Science for Health
NGO	Non-governmental organization
NMCP	National Malaria Control Programme
NMSP	National Malaria Strategic Plan
PCT	Parasite Clearance Time
PMI	President's Malaria Initiative
PNLP	Programme National de Lutte contre le Paludisme
PSI	Population Services International

RDT	Rapid diagnostic test
SP	Sulfadoxine-pyrimethamine
UNICEF	United Nations International Children's Emergency Fund
USAID	United States Agency for International Development
WHO	World Health Organization

Acknowledgements

Foremost, I would like to express my special appreciation and sincere gratitude to my supervisor Christian Lengeler for the continuous support to this thesis until its completion, for the patient guidance, encouragement, advices he has provided throughout my time as his student, and for his flexibility and kindness.

I'm also grateful to Christian Burri for his constant and motivating support over the last years.

I also wish to express my gratitude to the staff of the Swiss TPH office in Kinshasa, in particular to Didier Kalemwa Mitembo for facilitating everyday life through the many difficulties of the Congolese bureaucracy and for his precious advices on my security (Papa Didier). Many thanks also to Jean Bosco Inyamwenyi, for all his support and friendship. I'd also like to thank Antoine Masendi Wmumbi, for his friendship, support and company during all this time and particularly during the long hours stuck together in the incredible traffic of Kinshasa. His great experience as a driver allowed data collection to take place in the most difficult and remote corners of Kinshasa.

I'm really grateful to some of the collaborators of the Kinshasa School of Public Health: to Professor Antoinette Tshefu Kitoto, director of the School of Public health, for her kindness and availability, valuable advices and continuous guidance in the field; to *mon ami* Henry Maggy Ntuku for all his help and patience throughout the duration of this PhD. Much of what I achieved would have not been possible without his constant help and presence. Many thanks go also to Pius Mafuta, for his laboratory skills and careful supervision of all laboratory related activities during the projects and to Dr Gaston Mwema for his invaluable friendship and medical advices (Grazie Gas!). Many thanks also to all the administrative staff, logisticians, and IT people of the School of Public Health who contributed to the realization of these projects.

My sincere gratitude goes also to some staff of the Biamba Marie Mutombo hospital, Marcel Benanduenga and Aline Diza for their friendship and great laboratory skills.

I also would like to thank Sandro Schmidlin (Moninga Sandrino) for his collaboration, friendship and invaluable support during the hard field work we conducted in Kinshasa.

Many thanks to all the people who contributed to the FEVERKIN and MATIAS studies, all supervisors, medical doctors, nurses, laboratory technicians for their excellent work. I am very grateful to all patients and their parents for their collaboration.

I'm also grateful to the Ministry of Health authorities and to the National Malaria Control Programme staff who facilitated the studies.

I'd like to thank the UBS Optimus Foundation for funding the FEVERKIN project and Medicines for Malaria Venture for funding the MATIAS project and for their technical support.

Many thanks also to the staff of Medicines Research department that with their frequent visits to Kinshasa have made my stay more pleasant and enjoyable.

I would like to thank my parents, brothers, my small nephews and nieces for their love and support during my long and extraordinary odyssey in the Democratic Republic of the Congo.

Summary

Sub-Saharan Africa is the region of the world with the highest burden of malaria (WHO, 2014), as well as some of the fastest growing cities. The 2011 United Nations report (UN, 2014a) estimated that by 2013 40% of the population of sub-Saharan Africa would live in urban areas, projected to become a total urbanised population of 760 million by 2030. Urbanisation has a significant impact on the economy, lifestyles, ecosystems and disease patterns (Omumbo et al., 2005).

Although historically considered a rural disease, malaria transmission does occur in urban Africa, causing specific challenges for inhabitants, notably the heterogeneous spatial distribution of risk and the low level of acquired immunity of citizens, who are therefore exposed to higher risk of severe disease (Robert et al., 2003). Kinshasa, the capital of the Democratic Republic of the Congo (DRC) and the third largest city in Africa, has undergone rapid urbanisation during the past decades, presenting today a heterogeneous pattern of land use. However, there is a paucity of information on the geographic distribution of malaria prevalence in urban Kinshasa and on the risk factors linked to its distribution.

The overall aim of the first part of this thesis was to update the distribution map of malaria in Kinshasa, since the latest epidemiological study was conducted in 2000 (Kazadi et al., 2004), and to update information on malaria control activities. Field research was initially conducted in 2009 by the School of Public Health of Kinshasa (KSPH), which sampled nine out of the 32 non-rural health zones (HZs); the remaining 23 were sampled during fieldwork conducted in 2011 by the Swiss Tropical and Public Health Institute (Swiss TPH) in collaboration with the KSPH. Two HZs already sampled in 2009 were resampled in the 2011 survey, bringing the total of HZs sampled in 2011 to 25.

In the first step (detailed in Chapter 3), which took place at the end of the 2011 rainy season, we undertook a cross-sectional survey to complete those urban and peri-urban HZs of Kinshasa that had not been sampled during the 2009 survey to assess the prevalence of malaria by Rapid Diagnostic Test (RDT) among children aged six to 59 months, anaemia and history of fever, and to obtain a comprehensive report on the state of key preventive malaria indicators. Point-referenced prevalence data from the two surveys and seasons were combined by indirect standardisation and mapped at the level of the health areas (HAs) by means of a geographic information system (GIS). The overall standardised malaria prevalence was found to be 11.7%, showing a decline over the previous two decades, with higher risk in the peri-urban areas compared to the more central urban areas. We observed considerable progress in key malaria indicators compared to the Demographic and Health Survey 2007 (DHS-DRC, 2007). Age groups with the highest prevalence were five to nine years and 15 to 19 years, an indication that malaria occurs more frequently in late childhood. This study presents the first comprehensive map of malaria risk in Kinshasa.

In the second step (discussed in Chapter 4), logistic regression with random effects was used to investigate predictors for malaria and anaemia among children aged six to 59 months and for malaria in individuals older than five years across zones in Kinshasa with a prevalence of malaria of less than 10% and 10% or greater. Accordingly, evidence was found of a different age pattern in the two transmission settings. The peak prevalence of malaria in children under five years was observed in the 48 to 59 months group in both transmission settings, but it increased more gradually for the lower transmission setting. In a separate analysis, in children over the age of five in two selected HZs, the peak prevalence was found to be in five to nine year-olds in the higher transmission setting and in 15 to 19 year-olds in the lower transmission setting. Reported fever in the last two weeks was associated with the risk of having malaria in all age groups in both transmission settings, with no evidence of a

difference in these associations; in children older than five years however, there was a significant interaction with a stronger association in the low transmission HZ. Insecticide-treated net (ITN) use was associated with a lower risk of malaria infection among children aged six to 59 months in the high transmission settings. Similar estimates were found in children over five years and the lower transmission HZ but the associations there were not significant. No evidence was found of a difference in these associations by strata. The risk of anaemia was found to decrease with increasing age, and to increase with malaria infection and reported fever among children aged six to 59 months. However, ITN usage did not show evidence of protection against anaemia. Low socioeconomic status was associated with malaria in high transmission settings in children aged six to 59 months and anaemia in low transmission settings.

The aim of the second part of this thesis was to demonstrate the operational feasibility of introducing injectable artesunate in the DRC as the preferred treatment for severe malaria, to provide a national cost estimate and to assess the acceptability of the new drug among health-care providers and patients. Furthermore, to assess the potential risk of delayed anaemia in patients, haemoglobin (hb) measurement was included at follow-up visits scheduled at days 14, 21 and 28 after treatment with injectable artesunate had been received.

In Chapter 5 we compare the implementation of injectable quinine and injectable artesunate in patients aged two months and older, through the evaluation of key components before (IV quinine) and after (IV/IM artesunate) the introduction of the new regimen. The time to discharge was lower in the artesunate compared to the quinine group. Similarly, the interval between admission and the start of intravenous treatment and the parasite clearance time were lower in the artesunate group. The overall staff pre-administration time and the personnel time spent on patient management were also lower in the artesunate group. In hospitals and health

centres, the mean total cost per patient treated for severe malaria was also found to be lower with injectable artesunate.

Chapter 6 discusses a sub-study, conducted within the MATIAS study, to assess the potential risk of delayed anaemia secondary to injectable artesunate in patients two months and older, through the measurement of hb levels at follow-up visits at days 7, 14, 21 and 28. Although the study was limited in its design (lack of follow-up information, no additional markers of haemolysis), we observed a decrease in hb levels in 23 (11.4%) out of 201 patients (out of 350, 57.4%) with complete hb measurements, from days 7 to 21. Of these 23 patients, five (2.5%) experienced a decrease in hb levels below 5 g/dl at at least one of the follow-up visits. At the day 28 clinical assessment, hb had recovered in all patients. These results point to the need for additional research to better understand the magnitude of delayed anaemia in African children.

In the last step (Chapter 7), qualitative methods were used to investigate the feasibility of implementing injectable artesunate from the perspective of health-care providers, as well as the acceptability of the new versus the old treatment from the perspective of both health-care providers and patients/caretakers. It was subsequently found that the use of artesunate was perceived to be easier by the health-care providers, with 75% of them reporting greater ease of use and preparation compared to quinine. Satisfaction with the injectable artesunate was high among both health-care providers (61.3%) and patients/caretakers (96.7%). Altogether, our work documented the feasibility, acceptability, greater operational simplicity and lower cost of injectable artesunate in the DRC, hence supporting its national deployment.

In conclusion, this thesis provides the first comprehensive map of malaria risk in Kinshasa and an overview of the state of malaria control activities. At the same time it allows for the identification of the health areas where malaria control interventions should be prioritised.

Furthermore, this thesis shows that introducing injectable artesunate in the routine care in the DRC is feasible and highly accepted by both health-care providers and patients/caretakers, thus providing the basis for practical recommendations for its rapid deployment within the country.

Résumé

L'Afrique subsaharienne est la région du monde qui supporte le fardeau le plus lourd en terme de cas de paludisme et de décès dus au paludisme (OMS, 2014) et avec quelques-unes des villes à croissance très rapide. Selon le rapport de 2011 des Nations Unies (ONU, 2014A), on estime que 40% de la population en Afrique subsaharienne vivaient dans des zones urbaines en 2013; c'est prévu qu'ils deviendront 760 millions d'ici 2030. L'urbanisation a un impact significatif sur l'économie, les modes de vie, les écosystèmes et les types de maladies (Omumbo et al., 2005).

Bien que historiquement considérée comme une maladie rurale, le paludisme en Afrique se transmet aussi dans les zones urbaines et qui cause des défis pour leurs habitants, notamment la distribution spatiale hétérogène du risque et le faible niveau de l'immunité acquise des citoyens donc exposés à un risque plus élevé de complications graves de la maladie (Robert et al., 2003). Kinshasa, capitale de la République Démocratique du Congo (RDC) et troisième plus grande ville d'Afrique a connu une urbanisation rapide au cours des dernières décennies, et elle présente aujourd'hui un pattern d'utilisation des terres très hétérogènes. Cependant, il y a pénurie d'informations sur la répartition géographique de la prévalence du paludisme dans la ville de Kinshasa et sur les facteurs de risque liés à sa distribution.

L'objectif de la première partie de cette thèse est de mettre à jour la carte de distribution du paludisme à Kinshasa, puisque la dernière étude épidémiologique a été menée en 2000 (Kazadi et *al.*, 2004) et aussi les informations sur les activités de lutte contre le paludisme. La recherche sur le terrain a été initialement réalisée en 2009 par l'Ecole de Santé Publique de Kinshasa (KSPH), qui a échantillonné 9 zones de santé (ZS) sur 32 non rurales, alors que les 23 autres ont été échantillonnées au cours de l'enquête menée en 2011 par le Swiss TPH en

collaboration avec le KSPH. Deux ZS déjà enquêtées en 2009 ont été reéchantillonnées en 2011, pour un totale de 25 ZS échantillonnées en 2011.

Dans une première étape (chapitre 3), à la fin de la saison des pluies 2011, nous avons conduit une enquête transversale pour compléter ces ZS urbaines et péri-urbaines de Kinshasa qui n'ont pas été échantillonnées au cours de l'enquête 2009, afin d'évaluer la prévalence du paludisme (par TDR) chez les enfants de 6 à 59 mois, l'anémie et l'histoire de la fièvre, et d'obtenir un rapport complet sur l'état des indicateurs clés de prévention du paludisme. Les données sur la prévalence du paludisme des deux enquêtes et de deux saisons ont été combinées par standardisation indirecte et cartographiées au niveau des zones de santé (HA) au moyen d'un système d'information géographique (SIG). La prévalence du paludisme standardisé était de 11,7% montrant une baisse au cours des deux dernières décennies, et avec un risque plus élevé dans les zones péri-urbaines par rapport aux zones centrales plus urbanisées. Nous avons observé des progrès considérables dans les indicateurs clés du paludisme par rapport à l'Enquête Démographique et de Santé 2007 (EDS-RDC, 2007). Les groupes d'âge avec la plus forte prévalence étaient les 5-9 et 15-19 ans, une indication que le paludisme survient de plus en plus à la fin de l'enfance. Cette étude présente la première carte du risque de paludisme à Kinshasa.

Dans une deuxième étape, (chapitre 4) une régression logistique avec des effets aléatoires a été utilisée pour étudier les facteurs de risque du paludisme chez les enfants de 6 à 59 mois et chez les individus âgés de plus de cinq ans et de l'anémie chez les enfants de 6 à 59 mois dans des zones à Kinshasa avec une prévalence du paludisme de moins de 10% et de plus de 10%. Il y avait des preuves d'un profil d'âge différent dans les deux zones de transmission. Le pic de prévalence du paludisme chez les enfants de moins de cinq ans a été observé dans le groupe d'âge 48 à 59 mois dans les deux zones de transmission, mais il a augmenté plus doucement pour les zones à transmission plus faible. Dans une analyse séparée chez les

enfants de plus de cinq ans dans deux ZS sélectionnées, la prévalence la plus élevée était chez le groupe d'âge 5-9 ans dans la ZS à transmission plus élevée et chez le 15-19 ans dans la ZS à transmission moins élevée. La fièvre rapportée au cours des deux dernières semaines a été associée au risque du paludisme dans tous les groupes d'âge dans les deux zones de transmission avec aucune évidence d'une différence de ces associations; par contre, chez les enfants âgés de plus de cinq ans il y avait une interaction significative avec une association plus forte dans la ZS à transmission plus faible. L'utilisation de la moustiquaire imprégnée d'insecticide (ITN) a été associée à un risque d'infection du paludisme inférieure chez les enfants de 6 à 59 mois dans les zones de forte transmission. Des estimations similaires ont été trouvées chez les enfants de plus de cinq ans dans les ZS à transmission plus faible, mais ces associations ne sont pas significatives. Il n'y avait aucune preuve d'une différence de ces associations par strates. Le risque d'anémie diminue avec l'âge, et augmente avec l'infection du paludisme et la fièvre rapporté chez les enfants de 6-59 mois. L'utilisation des MII n'a pas mis en évidence de la protection contre l'anémie. Le faible statut socio-économique a été associé à la malaria dans dans le ZS à forte transmission chez les enfants de 6-59 mois et à l'anémie dans les ZS à transmission plus faible.

L'objectif de la deuxième partie de cette thèse est de démontrer la faisabilité opérationnelle de l'introduction de l'artésunate injectable en RDC comme traitement privilégié pour le paludisme sévère, de fournir une estimation nationale des coûts, et d'évaluer l'acceptabilité du nouveau médicament chez les prestataires de soins de santé et les patients. En outre, pour évaluer le risque potentiel de l'anémie retardée chez les patients, la mesure de l'hémoglobine (hb) a été incluse lors de la visite de suivi aux jours 14, 21, 28 après le traitement par artésunate injectable.

Dans le chapitre 5, nous avons comparé la mise en œuvre de la quinine injectable avec l'artésunate injectable chez les patients de deux mois et plus, par l'évaluation des éléments clés

avant (quinine IV) et après (IV / IM artésunate) l'introduction du nouveau régime. Le temps de décharge était inférieur dans le groupe traité avec l'artésunate par rapport au groupe traité avec la quinine. De même, l'intervalle entre l'admission et le début du traitement par voie intraveineuse et le temps de clairance parasitaire était inférieure dans le groupe artésunate. Le temps total de pré-administration et le temps du personnel consacré à la gestion des patients étaient inférieurs dans le groupe artésunate. Dans les hôpitaux et les centres de santé, le coût total moyen par patient traité pour le paludisme sévère avec l'artésunate injectable était également inférieur avec l'artésunate injectable.

Le chapitre 6 est une sous-étude de l'étude MATIAS pour évaluer le risque potentiel de l'anémie retardée secondaire à l'artésunate injectable chez les patients de deux mois et plus, grâce à la mesure du taux d'hémoglobine lors des visites de suivi aux jours 7, 14, 21 et 28. Bien que l'étude ait été limitée dans la conception (manque des informations de suivi, aucun des marqueurs supplémentaires d'hémolyse), nous avons observé une diminution des niveaux d'hémoglobine dans 23 (11,4%) patients sur 201 (sur 350, 57,4%) chez les patients qui avaient complété toutes les mesures de l'hb à chaque visite de suivi, pendant les jours 7-21. Sur ces 23 patients, cinq (2,5%) ont connu une diminution des taux d'hb en-dessous de 5g / dl au moins une des visites de suivi. Chez tous les patients le taux d'hb avait récupéré à l'évaluation clinique au jour 28. Ces résultats soulignent la nécessité de recherches supplémentaires pour mieux comprendre l'ampleur de l'anémie retardée chez les enfants africains.

Dans une dernière étape (Chapitre 7), des méthodes qualitatives ont été utilisées pour étudier la faisabilité de la mise en œuvre de l'artésunate injectable du point de vue des prestataires de soins de santé ainsi que l'acceptabilité du nouveau traitement par rapport à l'ancien du point de vue des prestataires de soins de santé et patients/ gardiens. L'utilisation de l'artésunate était perçue comme plus facile par les prestataires de soins de santé, avec 75% d'entre eux

rapportant une plus grande facilité d'utilisation et préparation par rapport à la quinine. Le niveau de satisfaction avec l'artésunate injectable était élevé chez les prestataires de soins de santé (61,3%) et chez les patients/ gardians (96,7%). Au total, notre travail a documenté la faisabilité, l'acceptabilité, une plus grande simplicité d'utilisation et un coût moindre de l'artésunate injectable en RDC, d'où l'appui de son déploiement national.

En conclusion cette thèse fournit la première carte complète du risque de paludisme à Kinshasa et un aperçu de l'état des activités de lutte contre le paludisme. Dans le même temps, elle permet d'identifier les aires de santé où prioriser les interventions de lutte contre le paludisme.

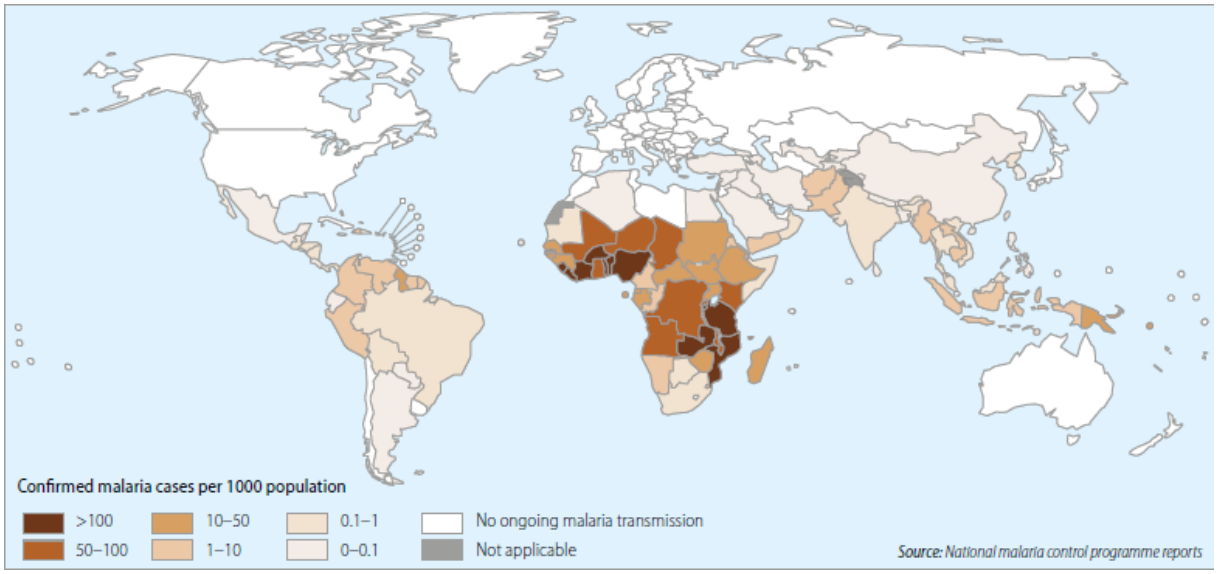
En outre, cette thèse montre que l'introduction de l'artésunate injectable dans les soins de routine en RDC est réalisable et hautement acceptée par les prestataires de soins de santé et les patients/ gardians, en apportant la base pour des recommandations pratiques pour son déploiement rapide dans le pays.

1. Introduction

1.1 Global malaria distribution

Malaria is recognised as one of the most prevalent and deadly parasitic diseases affecting humans worldwide. It is a major cause of morbidity and death in large areas of the developing world. According to the World Health Organization (WHO, 2014), at the end of 2013 there were 97 malaria-endemic countries (Figure 1.1) and 3.2 billion people were at risk of malaria. Guerra et al. (2008) reported that more than 70% of these people lived in areas at risk of *P. falciparum* transmission in 2007. Also according to the WHO, 198 million clinical cases of malaria were recorded globally in 2013 and 584,000 people died of the disease, representing a decrease in malaria case incidence and mortality rates of 30% and 47% respectively since 2000. Nevertheless, the African region alone still accounts for 81% of reported cases and 90% of all malaria deaths, primarily in children under the age of five (WHO, 2014).

Figure 1-1: World malaria distribution in 2014



1.2 Malaria disease and transmission

Malaria is an infectious disease caused by the single-celled parasite of the genus *Plasmodium,* of which four species have been identified that regularly cause disease in humans: *P. falciparum, P vivax, P ovale* and *P. malariae*. The general life cycle requires two separate cycles of asexual reproduction in the human host (one in the liver, called the exo-erythrocytic cycle, and one inside the red blood cells, known as the erythrocytic cycle) and a sexual reproductive stage inside the mosquito definitive host ("the vector").

When a human is bitten by an *Anopheles* mosquito infected with malaria, the parasite (sporozoite) is introduced into the body. Within 30 minutes, the sporozoites pass into the liver where they reproduce asexually (schizogony) in the liver cells (exo-erythrocytic cycle). The sporozoites mature into schizonts and release thousands of small offspring (merozoites) into the bloodstream. Merozoites invade the red blood cells in which they reproduce asexually. In the red blood cell the form of the parasite is now known as trophozoite. The trophozoite subsequently undergoes asexual reproduction leading to the formation of schizonts, which divide to form merozoites. The infected blood cell bursts and more merozoites are released in the blood from where they will penetrate new red blood cells and the cycle will continue. The lysis of the red blood cells is accompanied by fever, triggering the onset of symptoms. After some time, some of the merozoites transform into male or female gametocytes, necessary for the sexual reproduction of the parasite. If the gametocytes are ingested during a bite from an *Anopheles* mosquito, they will undergo sexual reproduction in the mosquito and will form a zygote. The zygote then matures into new sporozoites, which migrate to the salivary glands where they will be ready to be injected into a new human host to perpetrate the malaria life cycle.

1.3 The *Anopheles* vector

Malaria is transmitted by *Anopheles* mosquitoes. There are some 400 *Anopheles* species, 40 of which are major vectors thought to be of public health importance, while 28 are poor vectors. The *Anopheles gambiae* complex in Africa includes seven species of which *Anopheles gambiae sensu strictu, Anopheles arabiensis* and *Anopheles funestus* are excellent vectors. *An. gambiae sensu strictu* is generally anthropophagic (prefers feeding on humans) and endophilic (prefers to rest indoor). *An. Funestus* prefers permanent water bodies, feeds preferably on humans, both indoors and outdoors, and rests indoors. *An. arabiensis* is found in dry savannah, feeds on animals (zoophilic) and rests outdoors (Gillies & De Meillon, 1968).

1.4 Epidemiological measures of malaria risk

The risk of acquiring malaria depends on the interaction between the host, the parasite, the vector and the environment. When planning malaria control activities, governments need to quantify the risk of infection and the endemicity levels, which can be done by using either indirect or direct measures of malaria transmission.

Indirect methods measure the risk of malaria through surrogate markers of risk, such as rainfall, temperature, spleen rate and antibody titres. Direct methods measure the risk of malaria either clinically/microscopically or entomologically (Baird et al., 2002).

The first indirect method used to quantify malaria endemicity involves determining the spleen rate (percentage of people in a given population with an enlarged spleen) (Dempster, 1848), while the parasite rate is the most common direct measure. Malaria prevalence estimates obtained by measuring the spleen rate and the parasite rate have been used to classify endemicity levels as either holoendemic, hyperendemic, mesoendemic or hypoendemic (Metselaar & Van Thiel, 1959).

Another commonly used direct measure of malaria transmission intensity is the entomological inoculation rate (EIR), that is, the number of infective mosquito bites received per person per unit of time, usually one year (Macdonald, 1957). The EIR is expressed as the product of the "human biting rate" (the product of the anopheles mosquito density and the average number of persons bitten by one mosquito in a day) and the "sporozoite rate" (the proportion of mosquitoes with sporozoites in their salivary glands). Africa can support a wide range of EIRs, with mean annual EIRs of 121 infective bites, ranging from a maximum of 884 in Sierra Leone to close to 0 in Burkina Faso, the Gambia and Senegal (Hay et al., 2000). Although it can be challenging to measure the EIR, it remains the most effective method for assessing the effect of anti-vector control activities (Shaukat et al., 2010).

Incidence of malaria is the most direct measure of malarial disease. It measures the number of new malaria cases diagnosed during a specific period of time among all those who are susceptible to the disease. Incidence of malaria is useful in areas of low to moderate transmission rather than high transmission, where virtually everyone is infected to some extent at all times. Owing to the lack of laboratory confirmation of malaria, incidence of fever is often used as a proxy measure for incidence of malaria.

1.5 Vector control measures

Vector control methods can be grouped into measures directed against the parasite in the human host, measures against the vector and measures directed at preventing human–mosquito contact. The use of anti-malarial agents to treat clinical malaria or as chemoprophylaxis for the more vulnerable groups has proven to be difficult as a result of low compliance with self-medication and the inability to afford a full treatment, thus encouraging drug resistance.

The two currently most common and by far the most effective ways of controlling malaria transmission are indoor residual spraying (IRS) and insecticide treated nets (ITNs). Together they account for almost 60% of global investment in malaria control (WHO, 2012a), as they both reduce mosquito–human contact, mosquito density and longevity. In specific settings and under specific circumstances, they can be used in combination or complemented by other methods such as larval source management. Evidence of the effectiveness of ITNs comes from 22 randomised controlled trials conducted on ITN campaigns, reviewed by two Cochrane reviews (Lengeler, 2004; Ter Kuile et al., 2003), and showing a substantial impact on child morbidity and mortality. Although in principle IRS and ITNs could be considered suitable everywhere, there are essential differences between the two. ITNs provide individual personal protection, even at low population coverage, while in order to be effective IRS requires high coverage of all potential resting places.

Use of malaria control tools depends on the local malaria epidemiology and the availability of resources. In this context, risk maps of malaria are increasingly being recognised as a powerful tool to identify geographical areas for priority allocation of resources and to evaluate the progress of malaria intervention programmes.

1.6 Mapping malaria risk

The Pan-African Mapping Malaria Risk in Africa collaboration (MARA/ARMA, 1998) is based on 10,000 historical malaria parasite prevalence data collected from geographically positioned surveys in both published and unpublished sources, providing the first continental maps of malaria distribution and the first evidence-based burden of disease estimate.

The Malaria Atlas Project (MAP) represents the most advanced attempt to map the global endemicity of malaria. By gathering parasite rate surveys from historical data, the MAP

database was able to assemble close to 3,500 estimates of parasite prevalence from a multitude of sources, providing a substantial basis for global malaria endemicity mapping within defined spatial limits (Guerra et al., 2007).

Parasite prevalence data derived from nationally representative surveys such as the Demographic and Health Surveys (DHS), the Multiple Indicator Cluster Surveys (MICS) and Malaria Indicator Surveys (MIS) are often used to build maps for the period 2000 onwards.

An additional source of data for risk mapping is routine case statistics. Using clinical incidence data is particularly appropriate in areas of low transmission, where people will be more likely to develop symptoms. Such data must be based on a reliable disease surveillance system but unfortunately such systems are weak in many sub-Saharan countries. In addition, many new malaria cases are diagnosed solely on clinical signs and symptoms, with no parasitological confirmation.

As stated above, the EIR provides a direct measure of transmission, and hence a reflection of anti-vector control actions, by quantifying the parasite-infected mosquito pool to the human population (Shaukat et al., 2010). However, measurement of the EIR is based on protocols that are non-standardised and difficult to execute, making comparison among studies difficult.

1.7 A brief overview of the Democratic Republic of the Congo and its administrative and health system organisation

The Democratic Republic of the Congo (DRC) (Figure 1–2) is the second largest country in Africa (after Nigeria) with a surface area of 2,345,000 km^2, spanning the equivalent of two-thirds of the European Union. With an estimated population of 65 million people (National Statistics Institute, DRC), the majority of whom live in rural areas, the DRC is the third most populated country in Africa. Located in the central Africa, the DRC shares its borders with

nine countries and the Atlantic Ocean to the west. Administratively, the country is divided into 11 provinces, which are expected soon to become 26. The city province of Kinshasa is divided into 24 municipalities.

Since 2001, the DRC has been recovering from a long period of regional, inter-ethnic conflict, particularly in the eastern provinces where tensions and insecurity still continue. This has generated a vast humanitarian crisis resulting in the death of about five million people. The DRC continues to be a fragile country, characterised by political uncertainty and military instability.

The DRC is also one of the poorest countries in the world. It is estimated that 80% of Congolese live on less than USD 1 a day. In 2012, the DRC had the lowest human development index (HDI) value out of 187 countries (UNDP, 2014) and its per capita income stood at USD450 in 2013, among the lowest in the world (World Bank, 2013). Mortality rates are still high; out of every 1000 children born, 104 die, although this represents a considerable reduction on the previous rate of 158/1000 in 2010 (UNICEF, 2010).

The country is divided into 515 health zones (HZs). An HZ is the main operational unit of the health system, covering an average population of 150,000 in rural health zones and 250,000 in urban health areas. Conventionally, an HZ includes a general hospital of reference, some health centres, and dispensaries. An HZ is led by a team comprising the Central Board of Health Zone (CBHZ) which is responsible for the planning and management, implementation and reporting of health activities. It is split on average into 15 health areas (HAs) with between 5000 and 10,000 inhabitants. Kinshasa, the capital, has six health districts (Nsele, Funa, Lukunga, Kalamu, Gombe and Tshangu) and 35 HZs, including one rural (Maluku 2), four urban–rural (Maluku 1, Nsele, Mont Ngafula 1 and 2, Kisenso) and 30 urban.

Figure 1-2: Administrative map of the Democratic Republic of the Congo

1.8 Epidemiological situation and parasite distribution in the Democratic Republic of the Congo

The DRC is second only to Nigeria in terms of burden of malaria in the world. The WHO estimates that of the 128 million infections that occurred in 18 countries in sub-Saharan Africa in 2013, 14 million occurred in the DRC alone – 11% of all cases of malaria in sub-Saharan Africa. The preliminary findings of the Demographic and Health Survey 2013–2014 (DHS-DRC, 2013) indicate that 29.5% of Congolese children under the age of five had had a fever during the two weeks prior to the survey. Of these, 29.2% had received an antimalarial of which only 5% were artemisinin-based combination therapies (ACTs).

The Ministry of Health reports malaria as the first cause of mortality and morbidity in the country. Most of the DRC territory is very suitable for malaria transmission, with 97% of the population living in high transmission areas where *P. falciparum* is the prevalent species.

These include 1) the equatorial area where the transmission of malaria is intense and perennial with an EIR of up to 1000 infected bites per person per year. This allows for the early acquisition of natural immunity. In children under five years, 30 to 50% of fevers are attributed to malaria. Severe malaria in the form of cerebral malaria is found in children but only rarely in adults; and 2) the tropical area where transmission is seasonal with a rise in the rainy season which lasts five to eight months (EIR of 60 to 400). Semi-immunity appears later, morbidity is higher in the rainy season and severe malaria is described until early adulthood. Ninety-seven per cent of the Congolese population is exposed to these two epidemiological ecotypes, while 3% of the Congolese population is exposed to marginal malaria transmission in mountainous areas (between 1000 and 1500 m). In these areas, the transmission period is very short and there may even be years without transmission. Semi-immunity is low or even absent; severe malaria therefore occurs in the general population.

Messina et al. (2011) generated the first estimate of malaria prevalence among individuals aged 15 to 59 years across the DRC by employing high-throughput polymerase chain reaction (PCR) malaria analysis of dried blood spots left over from the 2007 DRC Demographic and Health Survey (DHS-DRC, 2007). Low prevalence was recorded in the central and east-central regions and near the urban areas of Kinshasa and Lubumbashi, whereas high prevalence was recorded in the northern regions of the country, and in the rural areas close to Kinshasa and Lubumbashi. Previously, the MAP identified very few parasite rate surveys in the DRC, highlighting the paucity of available epidemiological data (Guerra et al., 2007) for this region.

1.9 Malaria control interventions in the Democratic Republic of the Congo

The DRC has made considerable progress in the coverage of key interventions over the last five years. Malaria control activities are coordinated by the National Malaria Control Program

(NMCP), created in 1998. In 1999, the country endorsed the Roll Back Malaria (RBM) strategy, resuming and coordinating malaria control activities based on a National Malaria Strategic Plan (NMSP). The Strategic Plan is continuously updated to follow WHO and RBM recommendations, and the latest is the 2013–2015 strategic plan, which is aimed at reducing malaria morbidity and mortality by 75% by 2015. The current focus is to strengthen key interventions such as ITNs and IRS, the treatment of mosquito breeding sites, the prevention of malaria during pregnancy through intermittent preventive treatment (IPTp) and the improvement of early case management at all levels of the health system. In addition to these interventions, some major revisions of the 2013 strategic plan include 1) a change in the IPTp policy to add sulfadoxine-pyrimethamine (SP) at every antenatal care (ANC) clinic after the first trimester; 2) the introduction of artemether-lumefantrine (AL) as a second-line treatment for uncomplicated malaria; 3) the introduction of rectal artesunate for pre-referral treatment at community level; and 4) the introduction of injectable artesunate for all cases of severe malaria, consequently replacing quinine during a transition phase of three years (PMI, 2015; PNLP, 2013a).

Several donors contribute to malaria control efforts in the DRC: the Global Fund, the World Bank, the US President's Malaria Initiative (PMI), the UK Department for International Development (DfID), and a series of additional donors (UNICEF, KOJCA etc.). Each donor is covering a number of HZs, and soon the entire country will be covered with a minimum package of malaria services.

Following massive ITN distribution campaigns, national health indicators have improved steadily over the past decade. This increase has been further confirmed by the results of the MICS 2010 and DHS 2013. For example, in 2007 the use of insecticide-treated mosquito nets at national level was at 6% and 7% among children and women respectively; this has now risen to 56% and 60% respectively.

1.10 Urbanisation and health

The UN predicts that the world urban population will increase from 3,6 billion in 2011 to 6,3 billion in 2050 (UN, 2014c). Almost all of this growth will be concentrated in the cities and towns of the less developed regions. Africa in particular is experiencing a massive expansion of its urban population, which is projected to increase from 400 million in 2010 to 1,26 billion in 2050 (UN, 2014a).

The current urban planning of African cities has no resources to cope with the exceptionally high growth and the increased influx of migrants. In 2001, 924 million people lived in slums and informal settlements, with sub-Saharan Africa having the largest proportion of urban population residents in slums (91.7%) (UN-HABITAT, 2003). The epidemiology of individual diseases can differ according to specific urban dynamics and contexts. In industrialised countries, urbanisation and consequent improved health conditions have contributed to a change in disease patterns, with a rise in chronic diseases. In African cities, despite the increasing importance of non-communicable diseases, infectious diseases are still the leading cause of morbidity and mortality. The emergence of important socioeconomic disparities in urban areas also contributes to profound health inequalities (Alirol et al., 2011).

1.11 Epidemiology of urban malaria

The urban environment has been shown to be an unfavourable place for the proliferation of most species of *Anopheles*, owing to the lack of clean water collection pools, although some species have adapted to polluted waste water (Awolola et al., 2007; Barbazan et al., 1998; Batra et al., 2001; Sattler et al., 2005; Trape & Zoulani, 1987). Evidence of malaria transmission has been found in many African cities, although the levels were found to be generally lower than rural areas (Pond, 2013; Robert et al., 2003). It is estimated that of the 600 million people in Africa who are at risk of malaria, about 200 million are urban

inhabitants (Alirol et al., 2011; Keiser et al., 2004). Pond et al. (2013) documented a substantially lower prevalence of malaria in large African cities by analysing data from malaria indicator surveys, while Robert et al. (2003) found an inverse relationship between EIR and the level of urbanisation from a meta-analysis of malaria transmission in sub-Saharan Africa (from 7.1 infective bites per person per year in urban centres to 45.8 in peri-urban areas and 167.7 in rural areas). This is largely due to the fact that African cities tend to grow outwards with perimeters consisting of relatively underdeveloped settlements and often harbouring urban agriculture (Byrne, 2007). The most complete set of investigations on the effects of urbanisation on malaria transmission was conducted by Trape et al. (1987) in Brazzaville. They showed how the citizens living in different parts of the city were subjected to differences in malaria prevalence, from 3% in central areas to 81% in peripheral areas, reflecting in annual EIRs that varied from 1 to 100 (Robert et al., 2003; Trape, 1987).

In the middle of African cities it is common to find rice fields, market gardens, and large areas where urban agriculture is practised. Land use may influence malaria transmission in several ways. For example, larval habitats are gradually eliminated through the construction of new buildings and roads, although they may persist in areas where vegetation remains or where agriculture is practised. Urban agriculture provides optimal conditions for vector breeding and several studies have highlighted the increased malaria risk of citizens living nearby (Afrane et al., 2004). In addition, human influence such as tyre tracks and construction activities can contribute to the creation of artificial water collection reservoirs. Hence, vector density, sporozoite rate and EIR are influenced by the complex interplay between several biological and environmental factors, as well as socioeconomic status and coverage of malaria control measures. In addition, malaria transmission is continually changing in response to urbanisation and to environmental modifications.

The EIR is also affected by increased human population density. It is thought that high human density coupled with a low mosquito population reduces overall biting rates per person (Robert et al., 2003). High population density also limits the vector dispersal from breeding sites, thus localising bites, compared to areas of low population densities where female mosquitoes disperse over longer distances in search for a blood meal. In addition, the longevity of infective vectors appears to be reduced in the less favourable urban environment compared to rural areas (Coene, 1993).

The uneven distribution of malaria risk in urban areas leads to delayed acquisition of semi-immunity (Robert et al., 2003; Sattler et al., 2005; Trape et al., 1992; Trape & Zoulani, 1987). The first malaria infection often occurs late in childhood, exposing a much wider age range to severe forms of the disease (Lindsay & Martens, 1998; Modiano et al., 1998, 1999; Watts et al., 1990). Therefore, a large segment of the adult population in cities has no significant malaria immunity, representing a potential epidemic risk of great public health significance (Robert et al., 2003). Citizens with reduced immunity also have a higher risk of contracting malaria when travelling to rural areas, increasing demands on urban health services (Knudsen & Slooff, 1992; Martens & Hall, 2000).

An important operational parameter is that the risk of overtreatment is increased in urban areas. The reported history of fever in the last two weeks for predicting current or recent history of malaria has lower positive predictive value in most urban areas (Pond, 2013; Wang et al., 2006).

1.12 Anaemia

It is estimated that about 28% of the 260 million children suffering from anaemia are from sub-Saharan Africa (De Benoist et al., 2007), largely from malaria endemic countries. Besides

the contribution of malaria, anaemia can be caused by many additional factors. These include nutritional (iron deficiency) and non-nutritional ones (host factors, socio-cultural or comorbities) (Brooker et al., 2007; Ekvall, 2003; Hotez & Molyneux, 2008; Ong'echa et al., 2006; Owusu-Agyei et al., 2002). Important consequences of anaemia involve the slower physical and cognitive development of children (Biemba et al., 2000; Brabin et al., 2001; Marsh et al., 1995; Slutsker et al., 1994). Therefore it is important to determine the prevalence of anaemia in African cities and to untangle the fraction of anaemia caused by malaria. This will in turn guide the efficient and appropriate allocation of interventions such as nutrient supplements and fortified food in the most affected communities.

1.13 Urban malaria control in Kinshasa

Malaria in Kinshasa has been a known public health problem since colonial times (Kazadi et al., 2004; Mulumba et al., 1990; Ngimbi et al., 1982; Nguyen-Dinh et al., 1985; Peel & Van Hoof, 1948; Ward, 1977). The Program de Lutte Antipaludique was initially created as a pilot malaria project in 1976, in agreement with the United States Agency for International Development (USAID) and the government of Zaire, to implement malaria control activities in four HZs and in one rural area of Kinshasa. DDT was adopted as the primary measure of vector control followed by semi-annual blood surveys conducted in primary school children aged five to 15 years. The mean malaria prevalence found was 17% during the early school surveys conducted in 1981–1983. At the same time, a survey of infants at two hospitals confirmed ongoing malaria transmission in the city. Additional school surveys were then extended to other HZs. Mulumba et al. (1990) found the prevalence of malaria in six districts of Kinshasa to be 50% during 1986 and 1987, and higher in the peripheral districts. In 1998, the Program National de Lutte Contre le Paludisme [National Malaria Control Programme], was set up by the Ministry of Health and, in 1999, the DRC started implementing the RBM

strategy. The latest epidemiological survey, conducted in Kinshasa in 2000 among children aged five to nine, revealed a mean parasite prevalence of 34%, with much lower transmission in the city centre (parasite rate 4%) compared to the peri-urban area (46%) (Kazadi et al., 2004). Consequently, additional surveys to update prevalence figures in Kinshasa are urgently required.

1.14 Epidemiology of severe malaria

Uncomplicated malaria can lead to severe malaria and death. Several factors influence this progression including the species of the infecting parasite, the level of the innate and acquired immunity, and the timing of the treatment. Almost all severe forms of and deaths from malaria are largely but not entirely due to *P. falciparum* infections. Obtaining accurate incidence and distribution data of severe malaria is often problematic as a result of weak reporting by the health system and the fact that a large proportion of deaths occurs at home. It is estimated that 90% of deaths from severe malaria in children under five occur at home in the Gambia (Greenwood et al., 1987), and 47% in Zambia (Mudenda et al., 2011). In addition, severe malaria can be misdiagnosed (Reyburn et al., 2004; Taylor et al., 2004). Accounting for these considerations, the WHO estimated that the global annual incidence of severe malaria was approximately two million cases in 2012.

The risk of prognosis varies with age and according to the level of malaria transmission, and is associated with changes in the dominant clinical manifestation (Griffin et al., 2015; Idro et al., 2006; Okiro et al., 2013; Reyburn et al., 2005; Snow et al., 1994; Snow et al., 1997). In areas of high transmission, severe malaria is concentrated in young children over the age of six months and severe anaemia is the most common complication, while in areas of less intense or seasonal transmission, older children and adults are affected and cerebral malaria may predominate with higher case fatality rates (Griffin et al., 2015).

1.15 A new treatment of severe malaria

For many decades the mainstay for the treatment of severe malaria was quinine. Recently, two large open-label randomised controlled trials have been conducted to compare intravenous quinine with artesunate in malaria-endemic countries in Southeast Asia (SEAQUAMAT) and in Africa (AQUAMAT) (Dondorp et al., 2005; Dondorp et al., 2010). The evidence of the benefits of injectable artesunate versus quinine from these and additional small trials have led to a rapid change in the policy for the treatment of severe malaria. The WHO now recommends injectable artesunate for the treatment of severe malaria in children and adults and individual countries are adopting the new policy. Artesunate has been proven to reduce mortality and it is a fast-acting drug against several parasite stages, including gametocytes (Dondorp et al., 2005; Dondorp et al., 2010). However, the long-term safety profile is still of concern because of reported delayed severe anaemia (CDC, 2013).

In early 2012, the DRC changed its policy and injectable artesunate will be used in conjunction with quinine infusion for a transition period of three years. In this context, operational research is needed to support the policy change in order to establish the feasibility and acceptability of the new drug in the context of routine care.

1.16 Cost of severe malaria

Despite the clinical superiority of artesunate over quinine, concerns have been raised that artesunate could be costlier than quinine and that this might place a substantial economic burden on the healthcare system (Kyaw et al., 2014). However, modelling studies have shown that artesunate is highly cost-effective in the management of severe malaria, with an incremental cost per death averted of approximately USD150 (Lubell et al., 2010; Lubell et al., 2011). Cost estimates for the DRC are lacking and studies are required to establish procurement and operational costs.

2 Aims and objectives

The aims of this thesis were twofold: 1) to estimate the malaria risk and associated risk factors among different subgroups of the population of greater Kinshasa by means of a series of cross-sectional studies; and 2) to investigate through limited scope implementation studies how injectable artesunate may be best implemented as the preferred treatment for severe malaria in the DRC.

Specific objectives:

Part 1

(1) To establish a comprehensive and representative risk map of malaria transmission in the greater Kinshasa area on the basis of a malariometric survey in 25 health zones in children aged six to 59 months of age

(2) To assess the age profile of malaria risk in two health zones (one presumed low risk, one presumed high risk)

(3) To obtain a comprehensive report on the current malaria control activities in greater Kinshasa

Part 2

(4) To quantify the time from registration at the hospital to discharge for severe malaria patients and from initiation of injectable malaria treatment to initiation of oral treatment

(5) To evaluate the cost difference between the two treatment regimens (IV quinine vs IV/IM artesunate) from a provider perspective

(6) To quantify the cumulative staff time required for all steps of management of patients with severe malaria, including drug administration (quinine and artesunate) and material needed

(7) To evaluate the feasibility and acceptability of new treatment by administering a provider and patient/caretaker questionnaire.

3 A comprehensive malaria risk map in Kinshasa, Democratic Republic of Congo

Giovanfrancesco Ferrari[1,2], Henry M Ntuku[3], Sandro Schmidlin[1,2], Antoinette T Kitoto[3], Christian Lengeler[1,2]

[1] Swiss Tropical and Public Health Institute, P.O. Box, 4002, Basel, Switzerland

[2] University of Basel, Basel, Switzerland

[3] Kinshasa School of Public Health, Kinshasa, Democratic Republic of the Congo

This paper has been published in the *Malaria Journal* 2016, **15:**27.

3.1 Abstract

Background

In Kinshasa, malaria remains a major public health problem but its spatial epidemiology has not been assessed for decades now. The city's growth and transformation, as well as recent control measures, call for an update. To identify highly exposed communities and areas where control measures are less critically needed, detailed risk maps are required to target control and optimise resource allocation.

Methods

In 2009 (end of the dry season) and 2011 (end of the rainy season), two cross-sectional surveys were conducted in Kinshasa to determine malaria prevalence, anaemia, history of fever, bed net ownership and use among children 6–59 months. Geo-referenced data for key parameters were mapped at the level of the health area (HA) by means of a geographic information system (GIS).

Results

Among 7,517 children aged 6–59 months from 33 health zones (HZs), 6,661 (3,319 in 2009 and 3,342 in 2011) were tested for both malaria (by Rapid Diagnostic Tests) and anaemia, and 856 (845 in 2009 and 11 in 2011) were tested for anaemia only. Fifteen HZs were sampled in 2009, 25 in 2011, with seven HZs sampled in both surveys. Mean prevalence for malaria and anaemia was 6.4% (5.6–7.4) and 65.1% (63.7–66.6) in 2009, and 17.0% (15.7–18.3) and 64.2% (62.6–65.9) in 2011. In two HZs sampled in both surveys, malaria prevalence was 14.1% and 26.8% in Selembao (peri-urban), in the 2009 dry season and 2011 rainy season respectively, and it was 1.0% and 0.8% in Ngiri Ngiri (urban). History of fever during the preceding two weeks was 13.2% (12.5–14.3) and 22.3% (20.8–23.4) in 2009 and

2011. Household ownership of at least one insecticide treated net (ITN) was 78.7% (77.4–80.0) and 65.0% (63.7–66.3) at both time points, while use was 57.7% (56.0–59.9) and 45.0% (43.6–46.8), respectively.

Conclusions

This study presents the first malaria risk map of Kinshasa, a mega city of roughly 10 million inhabitants and located in a highly endemic malaria zone. Prevalence of malaria, anaemia and reported fever was lower in urban areas, whereas low coverage of ITN and sub-optimal net use were frequent in peri-urban areas.

Keywords: Malaria, Anaemia, mosquito nets, DRC, Democratic Republic of Congo, Kinshasa

3.2 Background

Malaria is the leading cause of morbidity and death in children under five years in the Democratic Republic of Congo (DRC), accounting for an estimated 40% of outpatient visits and 40% of overall mortality {Roll Back Malaria, 2014 #457}. Malaria is also a major public health issue in the capital city Kinshasa; an issue that has been studied since colonial times (Ngimbi et al., 1982). After Cairo and Lagos, Kinshasa is Africa's third largest city, with an estimated population of more than 10 million (Nations). In 1979–1980, the average malaria parasite rate in a representative sample of children was 33% (Ngimbi et al., 1982). Around the same time, malaria admissions comprised 29.5% of consultations in 1983, then 38.2% in 1985–86 (Greenberg et al., 1989). In 1986–1987, the mean prevalence rate of malaria in six districts of Kinshasa was 50%, with a higher prevalence in the peripheral districts (Mulumba et al., 1990). This reflected the distribution pattern of the main vector Anopheles gambiae, which was less present in the city centre than in the periphery (Karch et al., 1992; Coene, 1993). The latest study in 2000 confirmed the general prevalence distribution pattern, with lower prevalence in the city centre (parasite rate 4%) than in peri-urban areas (46%) (Kazadi et al., 2004).

A first insecticide treated net (ITN) distribution campaign in 2007 achieved a 15.9% rate of ITN ownership and a 12.6% rate of use among children under five (DHS-DRC, 2007). In 2008, the World Bank financed the acquisition and distribution of two million ITNs in Kinshasa through the PURUS project (*Programme d'Urgence de Réhabilitation Urbaine et Sociale*). The National Malaria Control Programme (NMCP), along with technical and logistic support from Population Services International (PSI), distributed two ITNs per household. Eight months after that distribution, the Kinshasa School of Public Health (KSPH) conducted a survey on basic malaria indicators to assess the impact of the intervention in 15

health zones (HZs) of the city. In 2011, the Swiss Tropical and Public Health Institute (Swiss TPH), in collaboration with the KSPH, conducted a second survey to evaluate the coverage and use of key malaria indicators, parasitaemia, anaemia and fever in the 23 HZs excluded from the 2009 survey. Kinshasa has expanded very rapidly in the past 20 years, thus updating and consolidating these data was urgently required for general malaria control purposes and for planning specific further research projects. Using geo-referenced prevalence data, this study aimed to generate the first map of malaria risk among children 6–59 months in Greater Kinshasa, down to the lowest level of the health system in DRC, the health area (HA). These maps will enable researchers and implementers to identify HZs of high priority for malaria control in Kinshasa.

3.3 Method

Study area

The study was conducted in Kinshasa, the capital of the DRC. The city is located along the southern bank of the Congo River, directly opposite the city of Brazzaville, capital of the Republic of the Congo. The climate is hot and humid (AW4 according to the Koppen classification), with a rainy season lasting from October to May (Kottek M, 2006). Characterised in the north by the Pool Malebo and by a marshy area in the north east along the river Congo, Kinshasa extends across a plain delimited to the south by hills with heights varying between 350 and 750 meters. The plain is crossed by three rivers (Ndjili, Nsele and Mai-Ndombe) and many smaller streams (D'Ascenzo, 2010; de Maximy, 1975). The northern and central parts of the city include the old colonial neighbourhoods (*ville*), some of which represent the most industrialised and commercial areas. To the south lies the *cité*, consisting of more recent, large, residential districts. The land use pattern is heterogeneous, with densely populated areas separated by large semi-rural areas where urban agriculture is practiced. The

most heavily inhabited area of Kinshasa covers 583 square km (Demographia, 2014), of which 80% is actually semi-rural. Administratively, Kinshasa has the status of a province, divided into four districts, which are further divided into 24 *communes* (municipalities). The organisation of the health system differs from the administrative system and comprises six health districts, divided into 35 health zones (HZs). These represent the primary operational units of the health system in DRC. An HZ usually covers a population of 100,000 to 150,000 inhabitants in rural areas and 200,000 to 250,000 in urban centres. They include a general referral hospital, some health centres and a dozen lower-level health facilities. Each HZ is further divided into 15 health areas (HAs), on average, which represent the lowest level of the health system. Each HA is clearly delimited and defined by the Ministry of Health and usually includes a population of 10,000 to 15,000 inhabitants. In Kinshasa Province, the three most eastern HZs are completely rural in nature, while the remaining 32 HZs are semi-rural or urbanised (http://www.kinshasa.cd). The study area only consisted of the 32 non-rural HZs because of the practical issues involved in including the three eastern HZs. Details of the sampled HZs can be found in Table 3.1.

Study design and sampling procedure

Two cross-sectional household surveys were conducted. The first survey was carried out at the end of the dry season between mid-September and end of October 2009 in 15 HZs, eight months after the first large ITN distribution campaign. The second survey was conducted at the end of the rainy season from mid-April to early June 2011 and covered 25 non-rural HZs. Seven HZs were sampled in both studies, including five HZs for which malaria prevalence was not measured in 2009 and two HZs for which prevalence was measured previously in 2009. The detailed list of HZs surveyed in 2009 and 2011 is presented in Table 3.1. For both

surveys, a multi-stage cluster sampling design was adopted to select households for inclusion, using the HZ as a primary sampling stage and the HA as a secondary sampling stage.

2009 survey

Fifteen HZs were selected using a probability proportional to size (PPS) sampling method, so that more populated HZs had a higher probability of being selected. Of these 15 HZs, 10 were selected by simple random sampling for the determination of malaria by rapid diagnostic test (RDT). In the remaining five HZs, only haemoglobin (Hb) was measured and malaria preventive measures were investigated using a pre-tested, structured questionnaire. In each HZ, data collection took place in half of the HAs, selected again with PPS. In case of an odd number of HAs per HZ, (n +1)/ 2 HAs were selected. In a third stage, a list of all streets with their approximate population number was obtained for each selected HA. Streets with fewer than 200 inhabitants were excluded and three streets were selected by simple random sampling. Households with at least one child aged 6–59 months were listed by community health workers (CHW) for each of the three streets. From this list, 25 eligible households were randomly selected, proportional to the size of each street. The target sample size of 325 children was calculated based on an estimated prevalence of anaemia of 69.2% in children aged 6–59 months. Assuming 1.3 children aged 6–59 months per household, a sample size of 260 households was set as the target per health zone (DHS-DRC, 2007).

2011 survey

From mid-April to early June 2011, the remaining 23 HZs were sampled, including the five HZs for which malaria prevalence had not been measured in the 2009 survey. In all 23 HZs, a questionnaire was administered to households and malaria parasite prevalence and the Hb concentration were measured in children aged 6–59 months. Two additional HZs already

investigated in 2009 were re-sampled in 2011 among children 6-59 months for both malaria and anaemia. To obtain the epidemiological age profile for all age groups in these latter HZs only, all individuals older than five were also included. In all, 25 HZs were sampled in the 2011 survey. The primary outcome measure was documented malaria in study children, as measured by RDT. The sample size was calculated based on the prevalence estimate for 2009 survey (6.4%) during the dry season, and increased to 10% to take into account the seasonal variation. In each HZ, the aim was to measure children's malaria with a precision of ± 8 absolute percent. The sample size calculation indicated the need for 55 children in each HZ. With a design effect accounting for clustering of two, this number increased to 110. With an average 1.3 children under five years in households in Kinshasa, 87 households needed to be selected (DHS-DRC, 2007). To account for losses in the study process, we aimed for 100 households in each of the 25 HZs. Hence, the total number of households sampled in Kinshasa in 2011 was 2,500, including 3,250 children aged 6–59 months. HA and household selection followed the same methodology applied in the 2009 survey (described above). An average of 25 households was set per HA.

Table 3-1: List of the Health Zones in Greater Kinshasa surveyed in 2009 and 2011 and their corresponding populations.

Health zone	Environment	Population	Year survey
Bandalungwa	Urban	147.252	2011
Barumbu	Urban	115.780	2011
Binza Meteo	Urban	325.446	2009†/2011
Binza Ozone	Urban	317.731	2011
Biyela	Urban	174.232	2009†/2011
Bumbu	Urban	316.188	2009
Gombe	Urban	22.732	2011
Kalamu I	Urban	112.915	2011
Kalamu II	Urban	100.782	2011
Kasa-Vubu	Urban	102.856	2009
Kikimi	Urban	198.997	2011
Kimbanseke	Urban	217.772	2011
Kingabwa	Urban	162.323	2009
Kingasani	Urban	171.538	2011
Kinshasa	Urban	135.665	2011
Kintambo	Urban	81.026	2011
Kisenso	Urban-rural	335.265	2009
Kokolo	Urban	336.086	2009
Lemba	Urban	249.292	2009†/2011
Limete	Urban	145.331	2009†/2011
Lingwala	Urban	66.595	2011
Makala	Urban	238.088	2011
Maluku I	Urban	149.040	Excluded
Maluku II	Rural	54.158	2009
Masina I	Urban	258.687	2011
Masina II	Urban	214.401	2009†/2011
Matete	Urban	223.248	2009
Mont Ngafula I	Urban-rural	196.810	2011
Mont Ngafula II	Urban-rural	111.921	2011
N'djili	Urban	249.310	2009
Ngaba	Urban	140.861	2011
Ngiri Ngiri§	Urban	125.634	2009/2011
Nsele	Rural	387.486	Excluded
Police	Urban	93.910	2011
Selembao§	Urban	269.498	2009/2011

***** KSPH/ NMCP 2009, KSPH/ Swiss TPH 2011; † Surveyed for malaria preventive indicators and prevalence of anaemia; § surveyed for malaria prevalence in both years and for all age groups in 2011.

Data collection

Household survey questionnaire

In 2009, survey data were collected using a paper-based questionnaire. In 2011, survey data were collected using smartphone technology. For the 2011 survey, a validated electronic semi-quantitative questionnaire was developed on an HTC smartphone running Google's Android operating system. Eight teams of three field workers (one interviewer, one laboratory technician, one community liaison person) were trained in using the electronic questionnaire, in general interviewing skills and in administering informed consent during simulated interviews sessions. Each of the eight teams visited, on average, 25 households per day in each selected HA. The 2011 questionnaire was a simplified version of the one used in 2009, which was adapted from the standard Malaria Indicator Survey Household Questionnaire from the Roll Back Malaria Partnership (www.RBM.org). All questions retained from the 2009 survey form were kept as they were in 2011 to ensure comparability between both surveys. The questionnaire was developed in French with oral translation into Lingala (the second *lingua franca* in Kinshasa) and field tested prior to the survey.

Prior to administering the questionnaire, a signed informed consent form was obtained from the head of the household or his/her representative. Participation was entirely voluntary. Respondents were asked about demographic information of usual residents, educational level, factors indicating the household's socio-economic status, household construction material, presence and type of mosquito bed net (verified by direct observation), use of mosquito bed net and ITN in the night prior to the survey, history of fever (past 2 weeks), whether fever was present on the day of the survey and health seeking behaviour in case of a fever episode. During the 2011 survey, the coordinates (longitude and latitude) of all investigated households were recorded on-site using the integrated Global Positioning System (GPS) of

the data collection devices. Households were revisited if no one was available for interview on the first attempt; if no one was available after two attempts, the interviewer continued to the next randomly selected household on the list until the desired number of households was obtained.

Blood testing

For each selected participant who gave signed informed consent, the same laboratory procedures as in 2009 were adopted during the 2011 survey. They included measuring axillary temperature with a digital thermometer, collecting peripheral blood by standard finger prick to test for malaria parasites with an RDT for *Plasmodium falciparum*-specific histidine rich protein 2 (HRP2) and other *Plasmodium species* (Pan pLDH for *P. vivax, P. malariae* and *P. ovale*) (Paracheck pf in 2009 and SD Bioline Malaria Antigen P.f/Pan in 2011) and assessing Hb level using a blood haemoglobin photometer (HemoCue 201 plus, Ängelholm, Sweden). In two HZs in 2011, Selembao and Ngiri Ngiri, individuals of all ages (not only children) were surveyed. RDTs were used for on–site diagnosis of malaria and treatment with artesunate-amodiaquine, the official first-line malaria treatment at the time of the survey, was offered as needed. The HemoCue was validated by running a weekly high and low Hb liquid control (HemoCue – HemoTrol).

Statistical analysis

To ensure consistency and integrity of data collected during the 2009 survey, all paper forms were rechecked by team supervisors in the field at the end of each day. Incomplete entries were sent back to be filled the next day. Questionnaires were first checked for completeness, and the information was manually coded and entered using EpiData and crosschecked using

EpiInfo (v. 6.04). Statistical analyses were performed using SPSS software for Windows (version 16.0), NCSS, and STATA (version 10).

Data collection devices used in the 2011 survey (HTC phones) were equipped with Open Data Kit (ODK) software (University of Washington & Google Foundation) to allow for data entry in the field. ODK programming also allowed for systematic range and consistency checks. Data in xml format were downloaded every evening from the HTC smartphones and then converted on the ODK Aggregate Server into tabular format (ODK aggregate).

Statistical analyses were performed using Stata version 12.1 (Stata Corp, College Station, Tx, USA). Analysis and mapping for the 2011 survey were based on geo-referenced prevalence data at the level of the HA. Since households could not be georeferenced in 2009, HA spatial coordinates were assigned to the HA's mean malaria prevalence. Maps were produced using ArcGIS version 10.0 (Environmental Systems Research Institute Inc. Redlands, USA). A centroid for every HA was first generated (for 2009 the centroid was generated at the centre of the HZ, since GPS coordinates of the households were not collected). The standardized prevalence data were then assigned to the centroids of the surveyed health zone. The next step involved using the IDW interpolation to get prevalence estimates at un-surveyed HZs. Lastly, the interpolated prevalence estimates were extracted using the centroids (points data) of the HZs. These estimates were subsequently used map out the prevalence at HZs level (polygons data). Boundaries (shape files) were initially available at the level of the HZ only, from the Health Monitoring Information System Unit of the Ministry of Health (MoH). By using images developed by the Japan International Cooperation Agency (JICA) and through collaborating with a team of experts from the *Institut géographique du Congo* (IGC), it was possible to develop shape files at the level of the HA. The most eastern rural HZs (Maluku I and II and Nsele) were excluded from the final map due to the great effort that drawing

boundaries in remote HAs would have entailed. This was beyond the means and the scope of this study.

Ethical consideration

For both surveys, ethical clearance was obtained from the Ethics Committee of the KSPH, at the University of Kinshasa. In addition, the 2011 survey received authorization from the ethical committees in Basel (Ethikkommission beider Basel, Basel-Stadt) as well as clearance from Swiss TPH's internal research commission.

Signed informed consent to participate was obtained from parents or guardians on behalf of the enrolled children or by the adult participants themselves. Precautions to minimise the risk of secondary infection during blood collection were taken. All tested participants with a positive RDT but no evidence of severe illness were diagnosed as having uncomplicated malaria and given a voucher for treatment, free of charge, as per the DRC national malaria treatment policy (artesunate-amodiaquine or artemether-lumefantrine), at the nearest health facility. Drugs were provided to the relevant facilities one day before the household visits started in the area, to ensure drug availability for treatment. Participants diagnosed with severe anaemia and those with severe illnesses were excluded from the study and immediately referred to the nearest health facility for diagnosis and management, as recommended by national guidelines.

3.4 Results

Characteristics of the study population

Household and individual characteristics of the study populations in 2009 and 2011 are shown in Table 3.2. A total of 3,896 households distributed throughout 15 HZs were included

in the 2009 survey, while 2,512 household in 25 HZs were sampled in 2011. The age distribution of individuals was similar between surveys, as were the proportions of men and women. Overall, 27,371 people were surveyed in 2009, including 12,761 men and 14,610 women. Of these, 47.1% were under 15 years of age, while the percentage of children 6–59 months was 24%. In addition, 302 pregnant women also participated. The 2011 survey included 15,005 people; 6,770 men and 8,235 women. Of these, 44.7% were under 15 years of age, while the percentage of children 6–59 months was 24.9%.

Table 3-2: Characteristics of study households and individuals in the 2009 and 2011 surveys, Kinshasa, Democratic Republic of Congo

	Survey 2009	**Survey 2011**
Household characteristics		
Number of households sampled	3896	2512
Mean (SD) household size	7.1	5.9 (2.1)
Individual characteristics		
Number of persons in sampled households	27371	15005
Median Age years (90% central range)	-	17
Age groups		
< 6 months	-	0.9
6-59 months (%)	24.0	24.9
5-9 years (%)	13.1	10.9
10-14 years (%)	10.0	8.9
15-19 years (%)	9.2	8.1
≥ 20 years (%)	43.7	46.2
Proportion of females (%)	53.4	54.9

Prevalence of *P. falciparum* by health zone

Table 3 gives the proportion of children 6–59 months who tested positive for malaria with RDT, by sampled HZs. A total of 3,319 children 6–59 months in 10 HZs were tested for malaria by RDT in the 2009 survey, whereas 3,342 were tested in 25 HZs in 2011. Prevalence of confirmed malaria was 6.4% (5.6–7.4) at the end of the 2009 dry season, ranging from 1.0% (0.3–2.6) in Ngiri Ngiri (urban centre) to 14.1% (10.6–18.2) in Selembao (peri-urban). At the end of the 2011 wet season, malaria prevalence was 17.0% (15.7–18.3),

ranging from 0.7% (0.0–4.1) in Kinshasa and Lingwala (urban centre) to 46.0% (37.1–55.1) in Biyela (peri-urban). *P. falciparum* accounted for 52% (95% CI: 47.4-55.8) of infections in 2011 survey, non-falciparum infections for 0.3% (95% CI: 0.0-1.3) while mixed infection (were not distinguished) prevalence was 48% (95% CI: 43.9-52.3).

In the two HZs sampled in both 2009 and 2011, prevalence of malaria in children aged 6–59 months was 1.0% (0.3–2.6) and 0.8% (0.0–4.2) in Ngiri Ngiri, and 14.1% (10.6–18.2) and 26.8% (19.9–34.7) in Selembao. Age-specific rates (Figure 3.1) show that prevalence in Ngiri Ngiri in 2011 was highest among individuals aged 15–19 years (14.0%), followed by the groups aged 5–9 years (4.8%), > 20 (4.2%), 10–14 (1.4%) and 6–59 months (0.0%). In Selembao, malaria prevalence was highest among the groups aged 5–9 (34.2%) and 15–19 (28.3%) years, followed by those 6–59 months (26.2%), 10–14 years (25.0%) and over 20 years (17.6%). All-ages malaria prevalence was 3.8% (2.4–5.8) in Ngiri Ngiri and 23.8% (20.4–27.6) in Selembao.

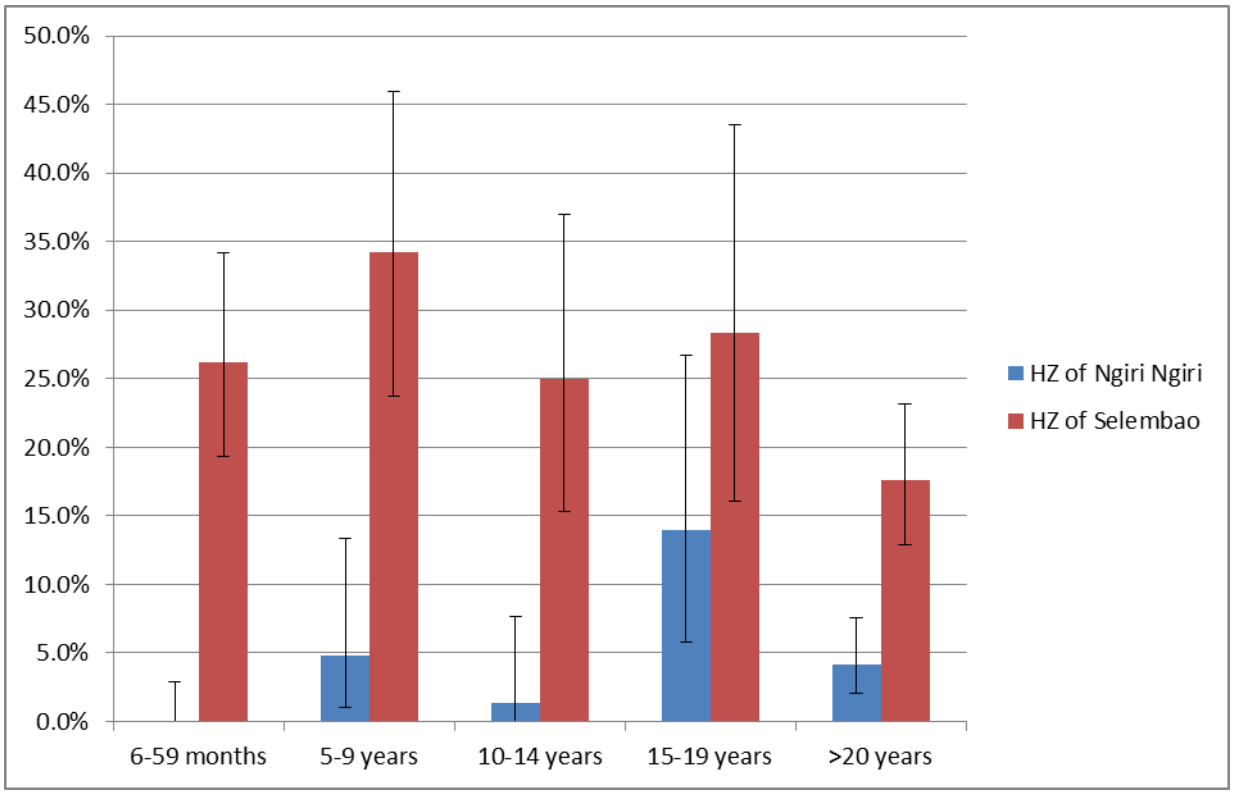
To prepare the data for mapping, direct standardisation was used to make prevalence rates of malaria comparable between surveys, accounting for the two different years and seasons. The standardisation was done according to the following formula:

$$P_s = \frac{p_1 n_1 \bar{p}_1 \sum n_1 + p_2 n_2 \bar{p}_2 \sum n_2}{(\sum n_1 + \sum n_2)}$$

where, P_s is the overall standardized prevalence for surveys 2009 and 2011, p_1 is the prevalence rate in survey 1 (2009), p_2 is the prevalence rate in survey 2 (2011), n_1 is the number of study participants in survey 1, n_2 is the number of study participants in survey 2,

$\bar{p}_1$ is the overall prevalence rate for survey 1, $\bar{p}_2$ is the overall prevalence rate for survey 2 and $\sum$ is the total number of study participants (per survey).

Figure 3-1: *Plasmodium falciparum* malaria prevalence (RDT positivity) by age group for the health zones of Selembao and Ngiri Ngiri

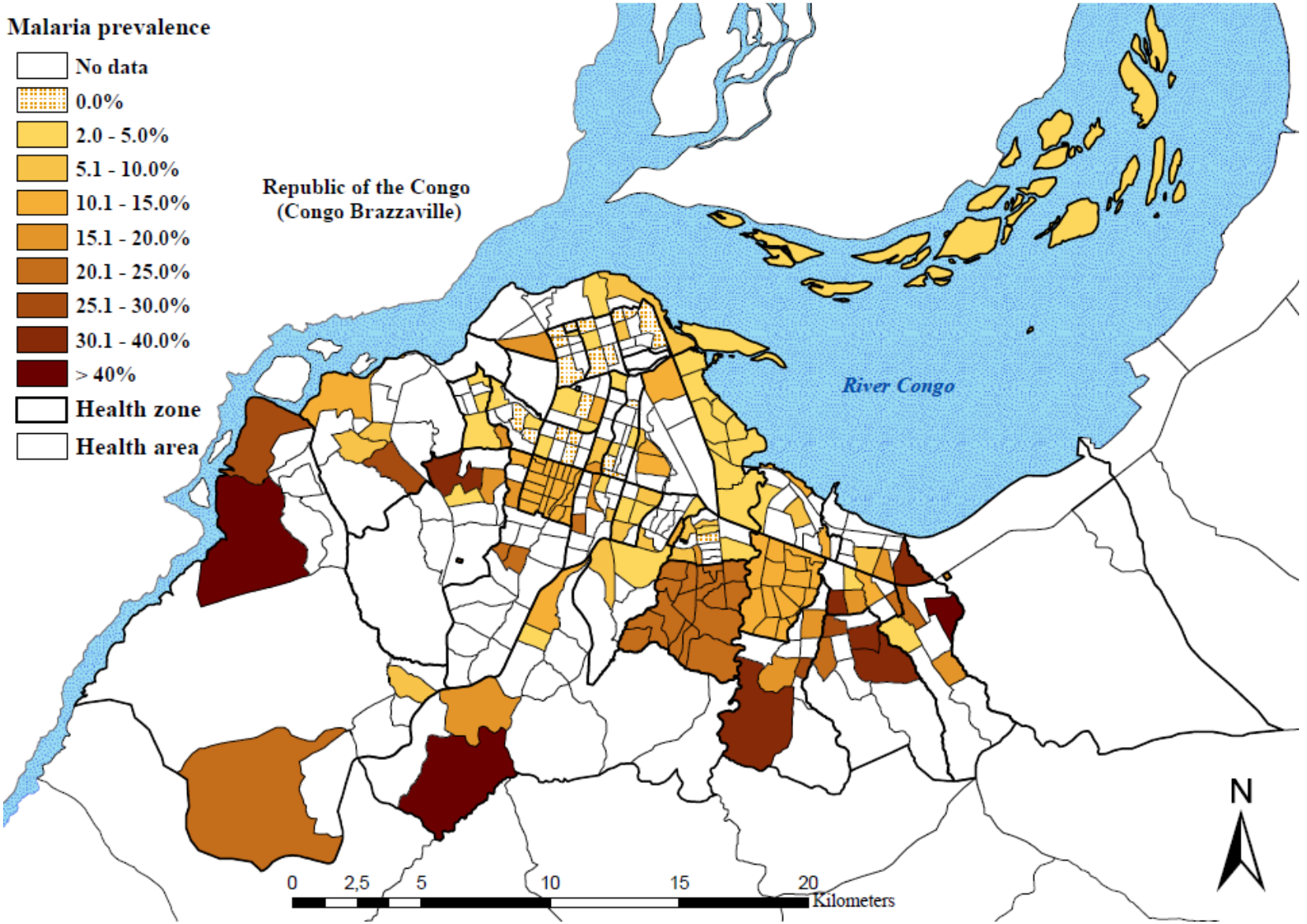


Geographical distribution of *P. falciparum* malaria

Results from the two surveys were used to produce a representative and standardised map of risk for malaria in children aged 6–59 months. Figure 3.2 shows the spatial distribution of the standardised prevalence rates of *P. falciparum* from the 2009 and 2011 surveys, at the level of the HA. Interpolated standardised prevalence rates are presented in Figure 3.3. Based on this risk map, three zones could be approximately defined; low risk in the central north part of the city, where prevalence rates were generally low (≤ 5%); intermediate risk in the central southern part of the city, where prevalence rates were between >5% and ≤30%; and high

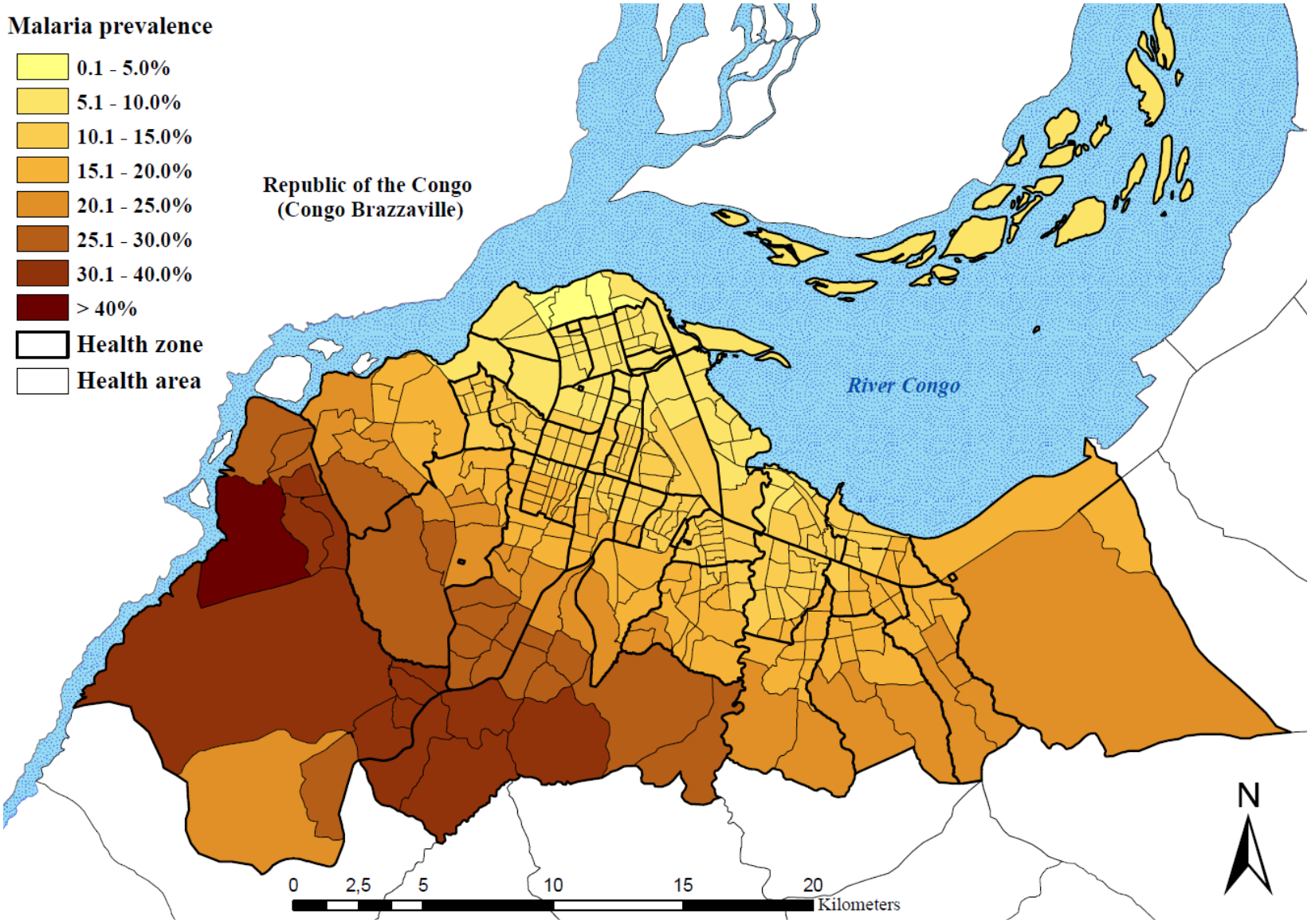
risk in the south western and eastern zones, where prevalence rates were higher (>30%) and, in general, more homogeneously distributed.

Figure 3-2: Standardized *Plasmodium falciparum* malaria prevalence in children aged 6-59 months, by health area



The 2009 data for the health zones of Bumbu, Kingabwa, Kisenso, Kokolo and Ndjili were only available at the level of the health zones.

Figure 3-3: Interpolation results for standardized *Plasmodium falciparum* malaria prevalence in children aged 6-59 months, by health area



Note: The data of Figure 3.2 were used for an inverse distance weighting (IDW) interpolation and then a mean prevalence value was calculated for every health area.

Geographical distribution of anaemia

A total of 4,164 and 3,353 children aged 6–59 months were tested for anaemia in the 2009 and 2011 surveys, respectively. The mean prevalence of anaemia (Hb < 11g/dl) was similar between surveys: 65.1% (63.7–66.6) in 2009 and 64.2% (62.6–65.9) in 2011. Results also show that the prevalence of moderate (7.0-9.9 g/dl) and severe (< 7.0 g/dl) anaemia was 34.2% and 1.9% in 2009, and 30.1% and 1.9% in 2011 (Table 3.3).

The formula given above was used to standardise the prevalence of anaemia and of severe anaemia. The spatial distribution of the standardised prevalence of anaemia for both surveys is shown in Figure 4. The risk of anaemia was consistently high across the entire study area, with maximal mean prevalence rates (> 70%) in the HZs of Kingabwa, Matete and Biyela. A map showing the standardised prevalence of severe anaemia is presented in Figure 5.

Figure 3-4: Standardized prevalence of anaemia (Hb<11g/dl) in children aged 6-59 months, by health area, surveys 2009 and 2011.

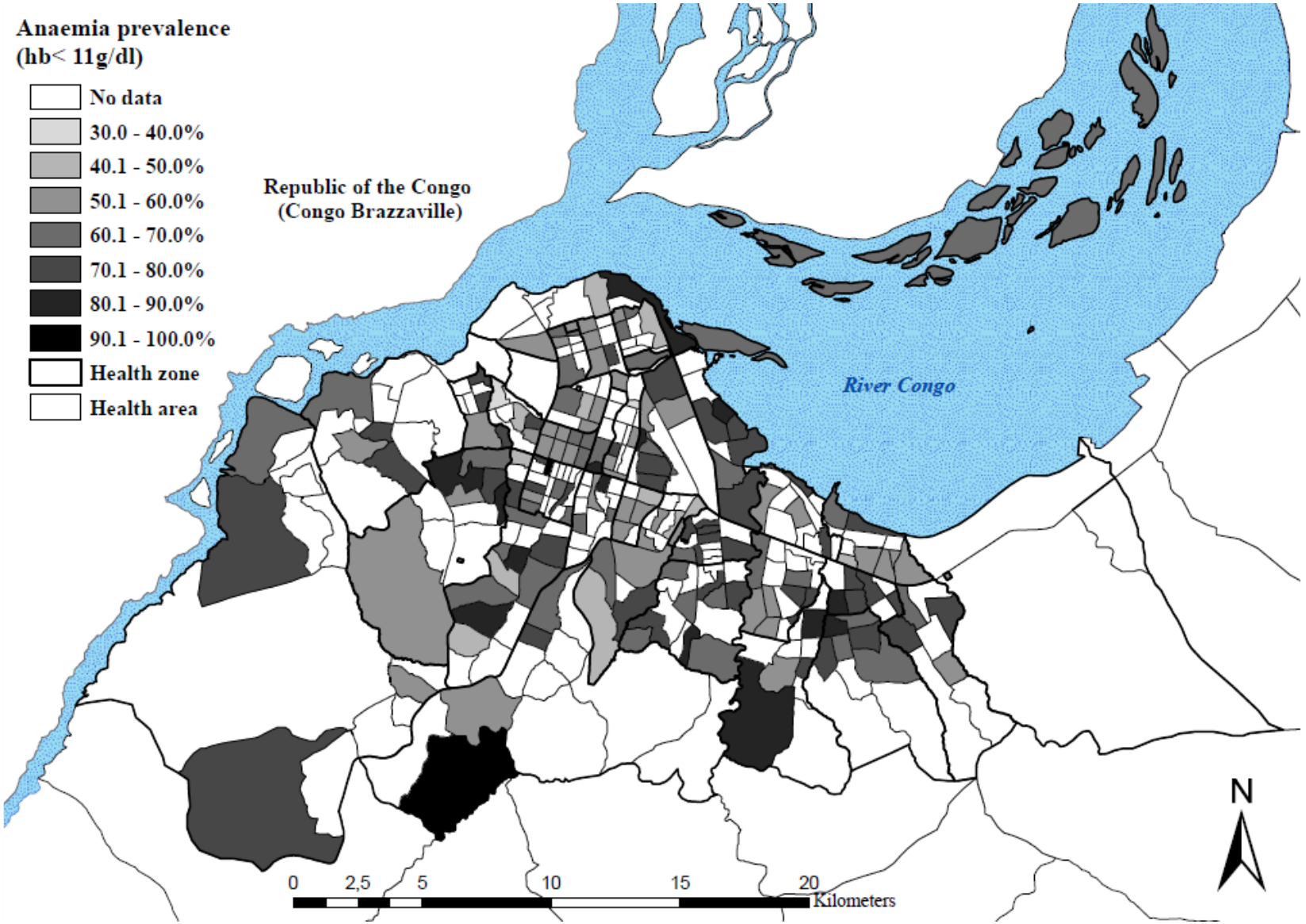
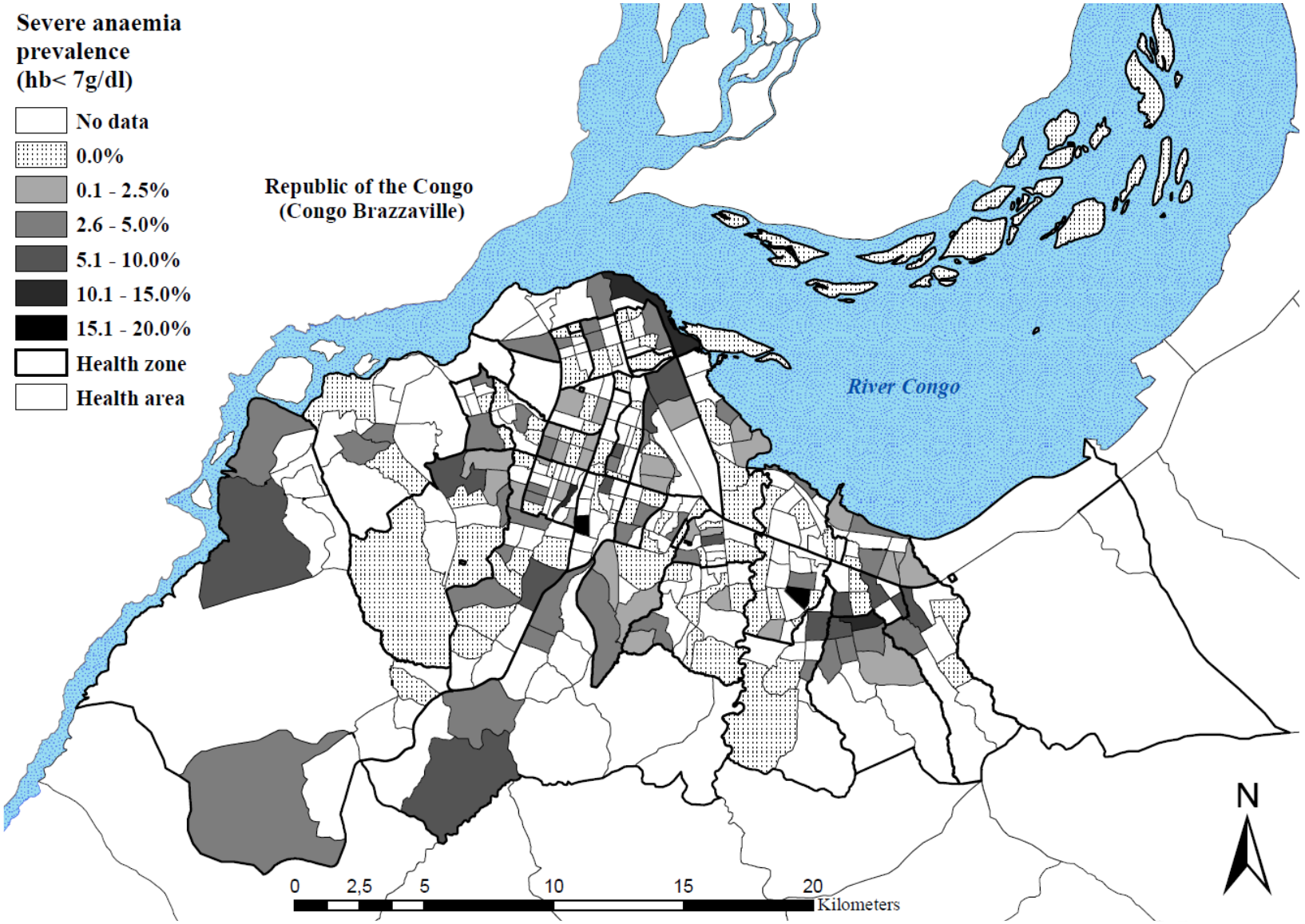


Figure 3-5: Standardized prevalence of severe anaemia (Hb<7g/dl) in children aged 6-59 months, by health area, surveys 2009 and 2011.



History of fever

The proportion of children aged 6–59 months reporting a history of fever in the two weeks preceding the survey was 13.2% (12.5–14.3) in 2009 and 22.3% (20.8–23.4) in 2011. On the day of the 2011 survey (data not available for 2009), 3.2% (106/3348) were febrile (defined as temperature > 37.5 C). The positive predictive value (PPV) of history of fever among children with a positive RDT was 29.7% (26.3–32.7) in 2011. Health seeking behaviour in case of fever was high in 2011 (data not available for 2009): overall, 91.4% (770/842) of children sought some type of care. In all, 53.9% sought modern treatment at home by a family member, whereas 36.1% were taken to a health facility. Very few (0.5%) made use of traditional medicine. Private facilities were the most common provider of treatment among those who sought care outside the home, covering 65.4% of the cases, whereas 22.9% consulted a public facility and 11.8% consulted a confessional structure. In case of home treatment, drug outlets represented the principal source of treatment (96.3%). Unfortunately, only 4.3% of the antimalarials purchased were the recommended combination of artesunate-amodiaquine. As a result, only 3.6% received the recommended treatment at home within the 24 hours. In 66.5% of fever cases, treatment was sought within 24 hours regardless of whether treatment was recommended or not.

Table 3-3: Clinical outcomes, by health zone

Health zone	Malaria prevalence in children aged 6-59 months							Anaemia prevalence in children aged 6-59 months							Children <5 years with a fever episode in the 2 weeks before the survey			
	Survey 2009 (dry season)			Survey 2011 (wet season 2011)			Standardised prevalence	Survey 2009 (dry season)			Survey 2011 (wet season 2011)				Survey 2009 (dry season)		Survey 2011 (wet season 2011)	
	%	[95% CI]	N	%	[95% CI]	N	%	% severe (<7g/dl)	% any (<11g/dl)	N	% severe (<7g/dl)	% any (<11g/dl)	N		%	N	%	N
Bandalungwa				1.5	[0.2-5.3]	134	1.0				0.7	51.5	134				22.8	149
Barumbu				2.4	[0.5-6.9]	125	1.7				0.8	57.8	128				19.6	143
Binza Météo				24.8	[17.0-34.0]	109	17.1	1.3	70.6	238	1.7	65.3	118		20.4	339	24.4	131
Binza Ozone				19.1	[12.9-26.7]	136	13.2				1.5	66.2	136				19.0	158
Biyela				46.0	[37.1-55.1]	126	31.7	4.5	64.9	313	2.4	78.6	126		9.5	422	24.7	150
Bumbu	7.4	[4.7-10.9]	299				13.6	3.6	69.2	308					9.3	407		
Gombe				11.5	[6.7-18.0]	139	7.9				3.6	65.5	139				29.6	159
Kalamu I				16.2	[8.4-27.1]	68	11.2				0.0	73.5	68				28.8	73
Kalamu II				2.5	[0.8-5.7]	200	1.7				1.5	56.7	203				19.5	221
Kasa Vubu	2.8	[1.2-5.4]	286				5.1	1.6	55.4	242					10.2	352		
Kikimi				32.8	[24.9-41.6]	131	22.6				2.3	64.9	131				18.5	151
Kimbanseke				36.1	[27.9-44.9]	133	24.9				2.3	75.9	133				22.7	154
Kingabwa	2.6	[1.2-4.9]	345				4.8	1.5	74.3	315					9.8	386		
Kingasani				25.0	[18.3-32.7]	152	17.2				3.3	76.2	151				24.6	175
Kinshasa				0.7	[0.0-4.0]	136	0.5				1.5	61.2	134				30.7	150
Kintambo				11.7	[7.0-18.1]	145	8.1				1.5	68.2	132				24.8	165
Kisenso	11.2	[8.0-15.1]	331				20.5	1.0	69.3	267					8.3	348		
Kokolo§	9.3	[6.5-12.9]	353				17.0	0.6	66.7	36					10.3	39		
Lemba				7.7	[3.8-13.7]	130	5.3	1.8	59.4	276	3.1	53.8	130		25.8	357	15.3	150
Limete				17.3	[11.3-24.8]	133	11.9	1.8	69.5	334	3.0	72.2	133		14.3	399	29.1	148
Lingwala				0.7	[0.0-4.1]	135	0.5				0.7	63.0	135				27.3	154
Makala				17.9	[11.8-25.5]	134	12.3				4.5	69.4	134				20.6	155
Maluku II*	8.0	[5.0-12.0]	261				14.7	1.2	54.2	260					11.1	342		
Masina I				12.3	[7.3-19.0]	138	8.5				0.7	66.7	138				20.2	163

Masina II				24.8	[17.7-33.0]	133	17.1	2.5	57.6	321	2.3	60.9	133	15.6	458	21.7	161
Matete	3.5	[1.8-6.0]	344				6.4	2.1	74.0	334				6.6	394		
Mont Ngafula I				33.6	[25.7-42.2]	134	23.2				3.7	69.4	134			20.8	154
Mont Ngafula II				35.3	[27.3-44.1]	133	24.4				3.0	68.4	133			19.0	158
Ndjili	6.3	[4.0-9.3]	366				11.5	2.4	61.3	287				16.7	412		
Ngaba				7.5	[3.6-13.3]	134	5.2				1.5	50.7	134			28.8	153
Ngiri Ngiri	1.0	[0.3-2.6]	387	0.8	[0.0-4.2]	124	1.5	1.3	62.4	314	0.8	58.8	131	15.3	428	12.9	140
Police§				17.0	[11.1-24.5]	135	11.7				0.7	53.3	135			13.4	164
Selembao	14.1	[10.6-18.2]	347	26.8	[19.9-34.7]	145	23.6	1.9	67.1	319	1.3	65.3	150	15.2	387	19.1	162
TOTAL	**6.4**	**[5.6-7.4]**	**3319**	**17.0**	**[15.7-18.3]**	**3342**	**11.7**	**1.9**	**65.1**	**4164**	**1.9**	**64.2**	**3353**	**13.2**	**5470**	**22.3**	**3841**

* The HZ of Maluku II although surveyed in 2009 was excluded from the final risk map since the choice was to map only HZs of non-rural character. § The HZs Kokolo and Police consist of military and police camps scattered in the city.

Coverage of malaria control measures

Eight months after the 2009 distribution campaign, ITN coverage (measured by the possession of at least one ITN per household) reached 78.7% (77.4–80.0). In 2011, it was 57.7% (56.0–59.9). In 2009, coverage ranged from 51.7% in Biyela (peri-urban) to 92.7% in Maluku II (peri-urban) (Table 3.4), with a mean number of 2.0 ITNs per household. In 2011, ITN coverage ranged from 34.4% in Biyela to 81.8% in Kinshasa, with a mean number of 1.9 ITNs per household (1.1).

The most common reasons for not owning an ITN, as given by households in both the 2009 and 2011 surveys, included not having obtained the ITN during the mass distribution campaign (38.7% and 23.8%), either because they were absent (26.6%, 2009) during the campaign or because the stock had been sold out (3.8% and 18.8%). A high proportion of respondents reported having discarded or destroyed their ITN because of rumours (7.4% and 23.8%). Other reasons given were heat (2.4% and 10.6%) and the absence of mosquitoes at home (9.4% in 2009).

The proportion of respondents who reported that their child slept under an ITN the night before the survey decreased from 65.8% (63.5–66.0) in 2009 to 45.0% (43.6–46.8) in 2011. Figures 3.6 and 3.7 show the geographical distribution of ITN usage among children under five, geo-referenced and mapped at the level of the HA for both surveys. Use rate decreases progressively towards the periphery in both surveys, with markedly lower use rates (<30%) in the south-eastern and western health zones of Biyela (30.8%, 2009; 17.3%, 2011), Selembao (53.3% 2009; 28.4% 2011), Kikimi (27.2% 2009) and Kimbanseke (20.1% 2011).

A higher proportion of pregnant women, 83.1% (77.5–87.7), reported using an ITN in 2009, than in 2011, were reported use decreased to 43.1% (37.5–48.9). Again, the HZs on the outskirts of the city showed the lowest use rates.

Table 3-4: Malaria control indicators, by health zone.

Health zone	Children < 5 years having slept under an ITN the night before the survey [95% CI]				Households that possess at least one ITN [95% CI]			
	Survey 2009		Survey 2011		Survey 2009		Survey 2011	
	%	N	%	N	%	N	%	N
Bandalungwa			36.2	149			61.0	100
Barumbu			55.9	143			70.7	99
Binza Météo	63.4	331	34.4	131	79.0	200	47.0	100
Binza Ozone			50.0	158			58.0	100
Biyela	30.8	422	17.3	150	51.7	259	34.4	90
Bumbu	81.2	393			91.2	260		
Gombe			57.9	159			68.0	100
Kalamu I			53.4	73			60.0	50
Kalamu II			64.3	221			72.7	150
Kasa Vubu	78.5	302			82.8	263		
Kikimi			27.2	151			37.0	100
Kimbanseke			20.1	154			31.0	100
Kingabwa	68.7	371			66.7	252		
Kingasani			40.8	174			58.0	112
Kinshasa			73.2	149			81.8	99
Kintambo			61.8	165			73.5	102
Kisenso	50.7	341			72.3	242		
Kokolo	72.2	36			92.0	25		
Lemba	66.1	301	36.0	150	83.4	259	60.0	100
Limete	69.0	390	51.4	148	71.8	262	51.0	100
Lingwala			53.2	154			70.0	100
Makala			44.5	155			52.0	100
Maluku II	84.1	292			92.7	260		
Masina I			47.9	163			57.0	100
Masina II	60.7	425	37.3	161	82.1	257	46.0	100
Matete	66.1	387			80.4	260		
Mont Ngafula I			48.1	154			55.0	100
Mont Ngafula II			37.3	158			56.0	100
Ndjili	59.8	381			79.5	258		
Ngaba			67.1	152			74.0	100
Ngiri Ngiri	82.1	418	48.6	140	91.1	259	67.9	106
Police			33.5	164			50.0	102
Selembao	53.3	379	28.4	159	68.1	257	51.0	102
TOTAL	**65.0 [63.7-66.3]**	**5169**	**45.0 [43.6-46.8]**	**3835**	**78.7 [77.4-80.0]**	**3896**	**57.7 [56.0-59.9]**	**2512**

Figure 3-6: Percentage of children <5 years having slept under an ITN the night before the survey in 2009, by health area.

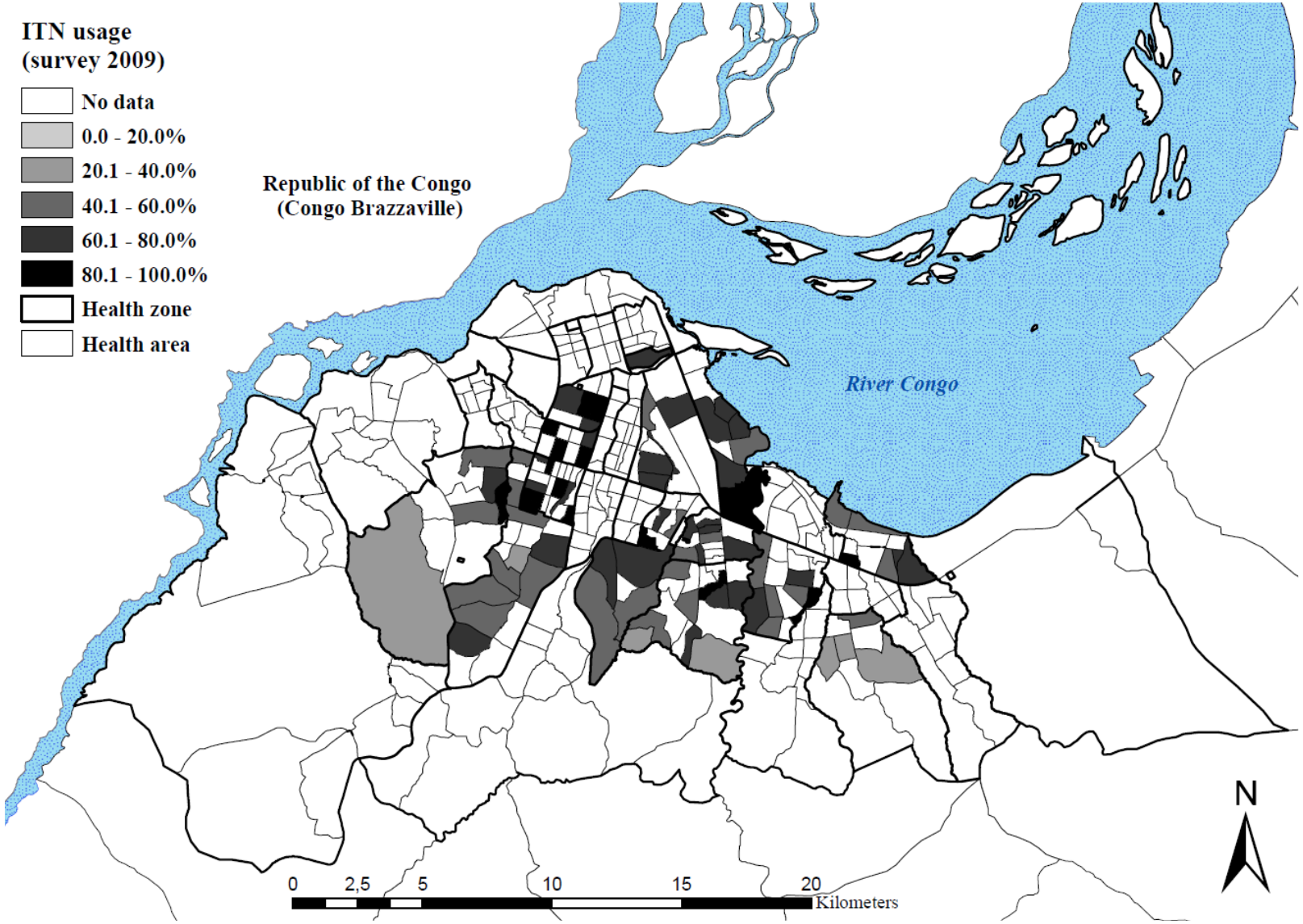
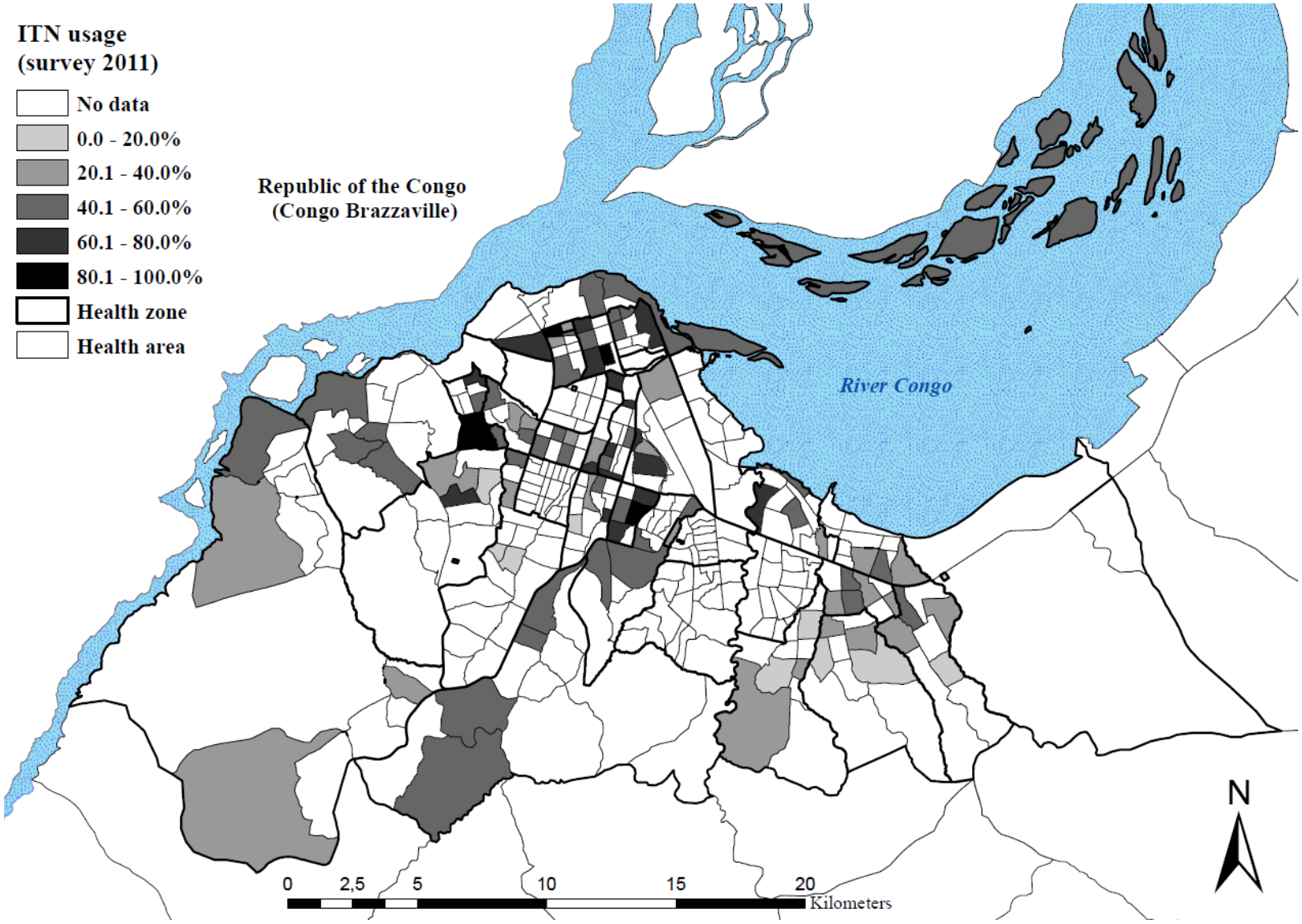


Figure 3-7: Percentage of children <5 years having slept under an ITN the night before the survey in 2011, by health area.



3.5 Discussion

Kinshasa falls within the perennial transmission in the classification of the Mapping Malaria Risk in Africa (MARA) project. This study created the first malaria risk map at the scale of a health area, the lowest level of the health system in DRC. Although the mean endemicity level seems to have declined over the last 30 years in Kinshasa, the results from the 2009 and 2011 cross-sectional surveys show that malaria was still a public health concern (Kazadi et al., 2004; Mulumba et al., 1990; Ngimbi et al., 1982). The geographic pattern of endemicity is comparable to that identified in earlier studies done in Kinshasa (Ngimbi et al., 1982; Mulumba et al., 1990; Kazadi et al., 2004). Prevalence is clearly highest in the more densely populated and less urbanised districts in the periphery, although marked variations in rates are apparent, even over a few kilometres. These findings are consistent with a recent meta-analysis that used data on the prevalence of malaria parasitaemia to document an analogous situation in other cities in Sub-Saharan Africa (Pond, 2013). A similar reduction in the annual *P. falciparum* entomological inoculation rates (APfEIR) has been observed in the more urbanised central areas, with a tendency to increase gradually towards the peri-urban areas (Keiser et al., 2004; Hay et al., 2000; Robert et al., 2003). The relative reduction in the APfEIR in urban areas was also reported in Kinshasa and in Brazzaville (Coene et al., 1993; Karch et al., 1992; Trape et al., 1987). Trape JF et al. suggested a relationship between levels in transmission in certain districts of Brazzaville and prevalence of malaria reported (Trape et al., 1987). The existence of a linear correlation between APfEIR and prevalence was also confirmed by Hay SI *et al*. (Hay et al., 2005).

In this study, the overall standardised malaria prevalence was 11.9% in children 6–59 months, ranging from 0.5% in the downtown health zones of Kinshasa and Lingwala, to 31.7% in Biyela, a semi-rural peripheral district extending south east. Results also show that

spatial heterogeneity is high in the central and northern urbanised HZs, whereas in the western and south-eastern HZs, more homogeneous levels of high risk can be found (Figure 2). It is likely that the proximity to productive breeding sites could account for the uneven distribution of malaria risk, together with socio-economic stratification and level of control measures (Machault et al., 2009; Clark et al., 2008; WHO, 2007). Kazadi et al. observed that initial urbanisation might increase levels of malaria transmission through increased human density and the creation of breeding sites favourable to *Anopheles gambiae*, the main local vector (Kazadi et al., 2004; Coene et al., 1993). In a second phase, the densification of human habitations reduces potential mosquito breeding sites and hence transmission levels. In Kinshasa, the increase in the density of dwellings in older urban districts has progressively eliminated the last remaining open spaces, contributing to the scarcity of *Anopheles* breeding sites through elimination and pollution. However, exceptions exist, especially where urban agriculture and gardens persist. In particular, the districts extending towards the south-east and west maintain a semi-rural character. Various studies have documented the presence of higher prevalence or transmission rates in areas close to agriculture fields (Klinkenberg et al., 2008; Yadouleton et al., 2010; Stoler et al., 2009; Matthys et al., 2006; Afrane et al., 2004; Clark et al., 2008). Kinshasa is crossed by rivers from north to south, creating large flood zones where much of the gardening is practised. This characteristic is particularly evident in the large semi-rural areas south west of the *boulevard* Lumumba, encompassing the health zones of Kingasani, Biyela, Kimbanseke and Kikimi. The areas favour *Anopheles sp.* breeding sites and are consistent with the more homogeneous transmission pattern observed in the areas on the outskirts of the city, compared to the more urbanised zones.

Additional factors, such as the use of personal protection against mosquitoes or socio-economic status, should also be considered as important determinants explaining the

distribution of disease prevalence. A spatial regression analysis linking malaria prevalence to risk factors for malaria in Kinshasa will be published separately (Ferrari et al., 2016).

Not surprisingly, the age groups with the highest prevalence, independent from the level of endemicity in both urban and semi-rural areas (Ngiri Ngiri and Selembao), were those aged 5–9 and 15–19. Hence, in Kinshasa, malaria infections seem to occur more frequently late in childhood. This could be in part explained by the age specific ITN usage across age groups, with highest use in younger children in the low endemicity setting (percent of usage in Ngiri Ngiri 49% compared to 28% in Selembao) as compared to lower and similar utilization rates among age groups in the high transmission setting (Ferrari et al., 2016). Higher malaria prevalence rates in older children were also found in school surveys carried out in the 1980s in Kinshasa and in Brazzaville. At that time, the finding was attributed to the increased use of antimalarials in early childhood (Trape, 1987; Sexton et al. 1984).

A concerted effort to scale-up ITN coverage through a free distribution in Kinshasa led to an ITN ownership rate of 78.7% of households in 2009. This represented a 395% increase in household possession of ≥1 ITN over the 2007 estimate of only 15.9% (DHS-DRC, 2007). However, 24 months after the distribution campaign, ITN ownership had decreased to 57.7% of households. Clearly, this points to the need for stronger programmes for routine ITN distribution as it occurs in most endemic settings, in addition to the campaigns, (Grabowsky et al., 2007).

The prevalence of anaemia was high in 2009 (65.1% in children 6 to 59 months) and in 2011 (64.2%). This is consistent with the 69.2% prevalence reported by the DHS 2007 (DHS-DRC, 2007) Furthermore, the distribution of anaemia across Kinshasa was highly

heterogeneous as shown in Figure 4, and the absence of a spatial trend seems in favour of the role of additional factors other than malaria in the aetiopathogenesis of this condition. Multiple factors account for anaemia and their contributions can vary according to the setting (Hall et al., 1982). In Kinshasa, 23% and 9% of children suffer from chronic and severe forms of malnutrition (DHS-DRC, 2007) and sickle cell anaemia is widespread (Tshilolo et al., 2009).

The maps we present for different variables reflect survey results from two distinct time periods and seasons. Since malaria transmission is neither constant throughout the year nor between years, this has likely introduced some mistakes. To account for these differences, we tried standardising prevalence rates, but that is an imperfect means of accounting for such differences. Moreover, surveys were based on detection of cases of uncomplicated malaria and, therefore, it is not possible to draw strong conclusions about the prevalence of anaemia, which is more often related to severe malaria.

3.6 Conclusions

This study provides the first comprehensive risk map of malaria at the level of the health areas in Kinshasa, a mega-city in a highly endemic malarious zone. Overall malaria prevalence has undoubtedly decreased over the last 30 years, but it is impossible to quantify the effect given the lack of representative historical data. As expected, prevalence rates were lower in the central urban districts compared to the more peripheral and more rural districts (Kazadi, 2004 et al.). The penetration of malaria control measures, especially ITNs, remains insufficient and is less successful in less developed and less accessible HZs on the outskirts of the city. Hopefully, this gap can be closed in the years to come with renewed efforts by the National Malaria Control Programme and its partners. Despite the methodological

limitations, the risk map provides a good baseline assessment against which to assess the effect of future control efforts.

4 Identifying risk factors for *Plasmodium* infection and anaemia in Kinshasa, Democratic Republic of Congo

Giovanfrancesco Ferrari[1,2], Henry M Ntuku[3], Amanda Ross[1,2], Sandro Schmidlin[1,2], Didier M Kalemwa[1,2], Antoinette T Kitoto[3], Christian Lengeler[1,2]

[1] Swiss Tropical and Public Health Institute, P.O. Box, 4002, Basel, Switzerland

[2] University of Basel, Basel, Switzerland

[3] Kinshasa School of Public Health, Kinshasa, Democratic Republic of the Congo

This paper has been published in the *Malaria Journal* 2016, **15:**362.

4.1 Abstract

Background

There is little data on the risk factors for malaria infection in large cities in central Africa and in all age groups. There may be different associations with the risk factors for areas with different malaria transmission intensities such as the effect of fever or age. This study aimed at identifying risk factors associated with *Plasmodium* infection and anaemia among children 6-59 months and individuals aged older than five years in Kinshasa, a large city with heterogeneity in malaria prevalence.

Methods

This study analysed data from 3342 children aged 6-59 months from 25 non-rural health zones (HZs) and for 816 individuals aged older than five years from two HZs in Kinshasa (non-rural), collected during a cross sectional malaria survey in 2011. Logistic regression with random effects was used to investigate predictors for malaria and anaemia. Differences in risk factors in areas with a prevalence of less than 10% and 10% or greater were investigated.

Results

There was evidence of a different age-pattern in the two transmission settings. For children under five years, the highest prevalence of malaria was observed in the 48-59 months group in both transmission settings but it increased more gently for the lower transmission HZs (p=0.009). In a separate analysis in children over five years in two selected HZs, the peak prevalence was in 5-9 years old in the higher transmission setting and in 15-19 years old in the lower transmission setting. Reported fever was associated with malaria in both transmission strata, with no evidence of a difference in these associations (p=0.71); however in children older than five years there was a significant interaction with a stronger association in the low transmission HZ. Insecticide-treated net (ITN) use was associated with a lower risk of malaria infection in children 6-59 months in the

high transmission HZs. Similar estimates were found in children over five years and the lower transmission HZ but the associations there were not significant. There was no evidence of a difference in these associations by strata. The risk of anaemia decreased with increasing age in all strata, whereas it increased with malaria infection and reported fever. ITN use did not show evidence of protection against anaemia. Low socio-economic status was associated with malaria in high transmission setting in children 6-59 months and anaemia in low transmission setting.

Conclusions

This study shows that in areas of low transmission in Kinshasa, the peak prevalence occurs in older age groups however ITN use was highest in children under five years. Targeted distribution of ITN to all age groups should be continued. For most risk factors, there was no evidence of an interaction with transmission intensity however the associations with age and with fever in the last two weeks did vary significantly.

Keywords: Malaria, Malaria risk, Anaemia, Fever, ITN use, ITN ownership, Democratic Republic of Congo, Kinshasa

4.2 Background

Urbanization, widespread use of malaria control measures and effective treatment in recent years have had a significant impact in reducing the prevalence of malaria in many African cities, as well as contributing to the more heterogeneous risk in malaria observed in many urban areas (Keiser et al., 2004; Matthys et al., 2006; Staedke et al., 2003; Wang et al., 2005; Keiser et al., 2004; Caldas de Castro et al., 2004). In Kinshasa, the capital of the DRC malaria has considerably decreased during the past thirty years. A survey conducted in 2011, showed a prevalence of 17% among children 6-59 months of age, and the existence of a gradient of prevalence from the centre (lower risk) to the periphery (higher risk) (Ferrari et al., 2016; Kazadi et al., 2004; Mulumba et al., 1990; Ngimbi et al., 1982). Moreover, traditionally attention has focused on high risk groups, and little attention has been put on older children and adults. Understanding the complex heterogeneity of risk factors that can contribute to increased risk of malaria in urban settings across different age groups will enable a more effective use of control measures.

In addition, many studies have shown that control of malaria can reduce the prevalence of childhood anaemia (Korenromp et al., 2004; Lengeler et al., 2004; Premji et al., 1995; Premji et al., 1995; Mathanga et al., 2010). Anaemia, in particular due to iron deficiency, is a major public health challenge in paediatric populations in sub-Saharan Africa, and in DRC it is among the top five leading causes of years lived with disability (YLDs) (GBD 2010). In Kinshasa the current rate of anaemia (Hb<11 g/dl) among pre-school aged children was 65% in 2011 (Ferrari et al., 2016).

The present study investigated individual and household risk factors for *Plasmodium falciparum* infection and anaemia in Kinshasa in children aged 6-59 months in 25 non-rural HZs, as well as in individuals aged five years and older in a separate analysis in two HZs.

4.3 Method

Study area and recruitment of study participants

This study used cross-sectional data from a survey conducted in 2011 in Kinshasa, the capital city of the DRC, which is described in detail in Ferrari et al. 2016 (Ferrari et al., 2016). In summary, data collection took place from April to June 2011, before the end of the rainy season and included 2512 households selected through a multi stage sampling procedure to obtain a sample of 3342 children aged 6 to 59 months from 25 health zones (HZs) and 816 individuals aged five years or older from two HZs selected out of the 25 (Ngiri Ngiri and Selembao). The HZ represents the primary operational unit of the health system in DRC, and covers about 150.000 inhabitants. It includes a general referral hospital, health centres and lower-level health facilities. Each HZ is further divided in health areas. In Kinshasa malaria transmission is ensured by *Anopheles gambiae s.l.*, and usually peaks during the long rainy season from September to May (Coene et al., 1993). From each participant a finger-prick blood sample was collected to test for malaria by rapid diagnostic test (RDT) (SD Bioline Malaria Antigen P.f/Pan), providing an immediate on-site diagnosis. The level of haemoglobin (Hb) was measured with a HemoCue 201 plus+ photometer (Ångelholm, Sweden). Axillary temperature was measured using a digital thermometer and the individual's history of fever in the preceding two weeks was also recorded. A standardized electronic survey questionnaire was administered to all heads of eligible household using an HTC smartphone running with Android OS. The survey questionnaire was an adaptation of the standard Malaria Indicator Survey Questionnaire from the Roll Back Malaria Partnership (www.RBM.org) created with the Build component of the Open Data Kit (ODK) software (University of Washington & Google Foundation). Respondents were asked about demographic information of the residents, educational level, assets owned (such as television and bicycle), presence of insecticide treated bed net (ITN) and use of ITN the night prior to the survey.

Assessing risk factors of *Plasmodium* infection and anaemia

The analysis was stratified according to malaria transmission intensity, based on the prevalence of malaria infection measured in 2011 among children 6-59 months (Ferrari et al., 2016). The prevalence ranged from 0.7 % to 46% in children aged 6 to 59 months. Two strata were defined at the HZ level: a prevalence of infection below 10% or a prevalence above 10%. The 10% prevalence cut-off was an arbitrary selection to allow enough observations in each strata. *Plasmodium* infection and anaemia were assessed for their association with a number of variables. For individuals aged older than five years, data collection took place in only two HZs with different transmission intensities (Ngiri Ngiri, 0.8% and Selembao, 26.8% in children younger than 5 years); these data were analysed separately.

The primary outcomes of the study were the presence or absence of *Plasmodium* malaria as measured by rapid diagnostic test (RDT) and the anaemia test results. A child aged 6 to 59 months was defined as anaemic if his/her Hb was below 11.0 g/dl. Therefore, the outcomes variables were dichotomous. Recorded explanatory variables were: age, gender, educational level of the respondent, occupation of the respondent, insecticide mosquito-net use and reported fever during the last two weeks and wealth index. A wealth index, calculated according to the method of Filmer et al. (2001), was constructed for each household based on ownership of household assets (having a television, a radio, etc.) and house characteristics (having electricity, drinking water, toilet type, roof and ground material) (Filmer et al., 2001). Three categories were generated to classify households ranging from the poorest to the least poor in the community.

Statistical methods

The proportions with malaria infection and with anaemia were analysed using a logistic regression model with random effects to take account clustering by health zone and health

area. All analysis were performed separately for children (6 to 59 months) and individuals older than five years since they were sampled from different HZs. The analysis was carried out using STATA version 13 (Stata Corporation College Station, TX, USA).

Ethics approval and consent to participate

Ethical approval of the study was obtained from the ethics committee of the Kinshasa School of Public Health University of Kinshasa, in DRC, as well as the ethical committee in Basel (Ethikkommission beider Basel, Basel-Stadt). Individual written informed consent was obtained by parents or guardian on behalf of their children (until the age of 10) or by the adults study participants themselves. In addition, assent was obtained from children over 10 years of age. Every precaution to minimize the risk of infection during blood sampling was taken. All patients who tested positive for malaria by RDT were treated for free by the nationally recommended therapy combination with artemisinin, artesunate plus amodiaquine (ASAQ), previously placed at the health centre of reference of the corresponding health area.

4.4 Results

Data collection took place in 2512 households, in the 25 HZs that were visited. A total of 3342 children aged 6-59 months were included in the analysis, 1118 and 2224 in the low and high transmission setting respectively. A similar number of males (50%) and females were included; the median age was 30 months (90% central range 9–55). Table 4-1 shows the number of children examined, by HZ and by transmission strata. For individuals above 5 years, data collection took place in two HZs only and included 816 individuals, of which 34% were males and the median age was 22 years (90% central range 6–62).

Table 4-1: Number of children 6 to 59 months examined and the prevalence of *Plasmodium spp* in Kinshasa, by health zone and strata, 2011.

Health zone	Malaria prevalence in children aged 6-59 months [95% CI]					
	<10%			>10%		
	%		N	%		N
Bandalungwa	1.5	[0.2-5.3]	134			
Barumbu	2.4	[0.5-6.9]	125			
Binza Météo				24.8	[17.0-34.0]	109
Binza Ozone				19.1	[12.9-26.7]	136
Biyela				46.0	[37.1-55.1]	126
Gombe				11.5	[6.7-18.0]	139
Kalamu I				16.2	[8.4-27.1]	68
Kalamu II	2.5	[0.8-5.7]	200			
Kikimi				32.8	[24.9-41.6]	131
Kimbanseke				36.1	[27.9-44.9]	133
Kingasani				25.0	[18.3-32.7]	152
Kinshasa	0.7	[0.0-4.0]	136			
Kintambo				11.7	[7.0-18.1]	145
Lemba	7.7	[3.8-13.7]	130			
Limete				17.3	[11.3-24.8]	133
Lingwala	0.7	[0.0-4.1]	135			
Makala				17.9	[11.8-25.5]	134
Masina I				12.3	[7.3-19.0]	138
Masina II				24.8	[17.7-33.0]	133
Mont Ngafula I				33.6	[25.7-42.2]	134
Mont Ngafula II				35.3	[27.3-44.1]	133
Ngaba	7.5	[3.6-13.3]	134			
Ngiri Ngiri	0.8	[0.0-4.2]	124			
Police				17.0	[11.1-24.5]	135
Selembao				26.8	[19.9-34.7]	145
TOTAL N			1118			2224

Risk factors for *Plasmodium* infection in children aged 6-59 months (25 HZs)

The risk factors for *Plasmodium* infections in children 6-59 months are shown in Table 4-2. There was an increase in the proportion with malaria infection with age in both transmission strata. The greatest risk was in children 48-59 months: an odds ratio (OR) of 5.86 (95% confidence interval (CI) 1.62-21.17) for the 36-47 months group and an OR of 15.53 (95% CI 4.26-56.64) for the 48-59 months group, compared to the youngest age group. The effect was also seen in higher transmission strata, although the OR was lower: an OR of 1.73 (95% CI 1.36-2.20) for the 36-47 months group and an OR of 2.54 (95% CI 1.93-3.35) for the 48-59

months group compared to the youngest age group. The interaction between age and transmission intensity was significant (p=0.009).

Treated net use was found to significantly lower malaria infection risk in the higher transmission strata with 38% protection (OR=0.62, 95% CI 0.50 – 0.77), however the effect was not significant in the lower transmission strata. Children who reported fever in the last two weeks had a significantly elevated risk of malaria infection in both strata.

Higher education levels showed a trend towards being protective in both transmission settings (Table 2). However there was no evidence of an association with the occupation of the respondent. Finally, children living in the wealthiest tertile were significantly less likely to have a malaria infection compared to the children from the poorest tertile in strata of high transmission (OR=0.27, 95% CI 0.20-0.38, p<0.001). No evidence was found in the HZs with less than 10% prevalence (OR=0.82, 95% CI 0.31-2.13, p=0.83), however the interaction between socioeconomic status and transmission was not significant (p=0.14).

Table 4-2: Univariate and multivariable analysis of risk factors associated with malaria in children between 6 and 59 months of age in Kinshasa, stratified by malaria transmission zone, 2011.

	< 10% prevalence								> 10% prevalence								
			Univariate analysis			Multivariate analysis					Univariate analysis			Multivariate analysis			Interaction by transmission zone
Variable	***n***	**(%)**	**OR**	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**	***n***	**(%)**	**OR**	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**	***P*-value**
Sex																	
Male	521	3.1	1			1			1141	23.6	1			1			
Female	527	2.7	0.86	0.42-1.8	0.687	0.87	0.41-1.88	0.731	1162	23.3	1.01	0.84-1.23	0.886	0.98	0.80-1.21	0.857	0.670
Age																	
6-35 months	446	0.7	1			1			958	17.8	1			1			
36-47 months	416	3.1	4.76	1.35-16-84		5.86	1.62-21.17		872	25.5	1.57	1.26-1.97		1.73	1.36-2.20		
48-59 months	186	7.5	12.02	3.41-42.34	< 0.001	15.53	4.26-56.64	< 0.001	473	31.1	2.08	1.61-2.68	< 0.001	2.54	1.93-3.35	< 0.001	0.009
Reported treated bed net use																	
No	446	3.6	1			1			1342	27.6	1			1			
Yes	596	2.3	0.65	0.31-1.34	0.240	0.82	0.38-1.76	0.606	961	17.6	0.56	0.46-0.69	< 0.001	0.62	0.50-0.77	< 0.001	0.705
Fever in the last two weeks																	
No	798	1.6	1			1			1744	18.3	1			1			
Yes	245	6.9	4.50	2.15-9.41	< 0.001	5.53	2.52-12.11	< 0.001	559	39.4	2.89	2.34-3.56	< 0.001	2.94	2.36-3.68	< 0.001	0.254
Education of the respondent																	
No education	24	8.3	1			1			220	32.3	1			1			
Primary	390	3.6	0.41	0.09-1.92		0.35	0.07-1.82		1135	26.5	0.76	0.55-1.03		0.90	0.65-1.26		
Secondary	471	2.8	0.31	0.07-1.47		0.28	0.05-1.50		740	19.7	0.52	0.37-0.72		0.78	0.54-1.14		
Superior and above	163	0.6	0.07	0.01-0.78	0.080	0.05	0.00-0.68	0.084	208	10.6	0.25	0.15-0.42	< 0.001	0.47	0.26-0.86	0.056	0.754
Occupation of the respondent																	
Without occupation	720	2.8	1			1			1523	23.2	1			1			
Manual labour	86	2.3	0.83	0.19-3.63		0.98	0.21-4.47		212	27.8	1.27	0.92-1.76		1.29	0.91-1.84		
Self employed	104	3.8	1.40	0.47-4.18		1.58	0.49-5.11		275	24.7	1.08	0.80-1.46		1.01	0.74-1.38	0.236	
Employed	138	2.9	1.04	0.35-3.11	0.931	1.79	0.51-6.31	0.742	293	20.1	0.83	0.61-1.13	0.229	1.35	0.95-1.94		0.860
Wealth tertile																	
Poorest	196	4.1	1			1			1175	31.6	1						
Middle	298	3.0	0.73	0.28-1.93		0.72	0.26-2.04		575	19.5	0.52	0.41-0.67		0.54	0.42-0.70		
Wealthiest	546	2.4	0.57	0.23-1.40	0.488	0.82	0.31-2.13	0.828	540	9.44	0.23	0.17-0.31	< 0.001	0.27	0.20-0.38	< 0.001	0.142

Risk factors for *Plasmodium* infection in individuals older than 5 years (2 HZs)

The risk factors for *Plasmodium* infection in individuals aged older than five years are shown in Table 4-3. The association between age and malaria infection was strong. The highest prevalence was observed in the 15-19 years age group in the low transmission HZ of Ngiri Ngiri with an OR of 7.11 (95% CI 1.17-43.05) compared to the 5-9 years-old. In the higher transmission HZ of Selembao however, ORs were lower and more homogeneously distributed across all age groups, compared to the 5-9 years-old group which showed the highest prevalence. The interaction between age and transmission intensity however was not significant (p=0.11).

ITN use was not found to significantly lower the prevalence of malaria infection, although the estimates were in the direction of being protective. Individuals aged five years and older who reported fever in the last two weeks had an elevated risk of having malaria infection in both sites, and the association was stronger for the lower transmission: OR = 38.71 (95% CI 11.08-135.23), and OR = 2.05 (95% CI 1.07-3.95) in Selembao, with a highly significant interaction term (p<0.0001). There was no evidence of an effect of higher education levels, occupation of the respondent or socio-economic status.

Table 4-3: Univariate and multivariable analysis of risk factors associated with malaria in individuals aged > 5 years in Kinshasa, stratified by malaria transmission zone, 2011.

	Ngiri Ngiri: 0.8% prevalence								Selembao: 26.8% prevalence								
			Univariate analysis			Multivariate analysis					Univariate analysis			Multivariate analysis			Interaction by transmission zone
Variable	***n***	**(%)**	**OR**	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**	***n***	**(%)**	**OR**	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**	***P*-value**
Sex																	
Male	142	5.6	1.0			1.0			143	28.7	1.0			1.0			
Female	257	4.3	0.75	0.29-1.91	0.548	0.74	0.23-2.37	0.616	274	20.1	0.62	0.39-1.00	0.050	0.66	0.40-1.08	0.102	0.733
Age																	
5-9 years	62	4.8	1.0			1.0			76	34.2	1.0			1.0			
10-14 years	68	1.5	0.29	0.03-2.90		0.22	0.01-3.39		68	25.0	0.64	0.31-1.32		0.79	0.37-1.72		
15-19 years	48	14.6	3.36	0.82-13.75		7.11	1.17-43.05		46	28.3	0.76	0.34-1.68		0.85	0.37-1.96		
> 20	221	3.6	0.74	0.19-2.87	0.022	1.09	0.21-5.72	0.009	227	17.6	0.41	0.23-0.74	0.021	0.45	0.24-0.83	0.042	0.105
Reported treated bed net use																	
No	244	6.1	1.0			1.0			315	25.7	1.0			1.0			
Yes	155	2.6	0.40	0.13-1.24	0.089	0.33	0.09-1.21	0.075	102	14.7	0.50	0.27-0.91	0.017	0.57	0.30-1.09	0.078	0.746
Fever in the last two weeks																	
No	366	2.2	1.0			1.0			361	21.6	1.0			1.0			
Yes	33	33.3	22.38	8.17-61.27	< 0.001	38.71	11.08-135.23	< 0.001	54	33.3	1.81	0.98-3.37	0.066	2.05	1.07-3.95	0.036	< 0.001
Education of the respondent																	
No education	8	12.5	1.0			1.0			35	34.3	1.0			1.0			
Primary	120	5.0	0.37	0.04-3.50		0.18	0.01-2.49		186	26.9	0.70	0.33-1.52		0.82	0.36-1.87		
Secondary	189	4.8	0.35	0.04-3.16		0.27	0.02-3.48		145	18.6	0.44	0.19-0.99		0.49	0.20-1.19		
Superior and above	82	3.7	0.27	0.02-2.91	0.802	0.17	0.01-2.87	0.647	51	13.7	0.30	0.11-0.88	0.041	0.32	0.09-1.13	0.115	0.865
Occupation of the respondent																	
Without occupation	244	5.7	1.0			1.0			200	26.0	1.0			1.0			
Manual labourer	37	2.7	0.46	0.06-3.58		0.82	0.09-7.91		64	23.4	0.87	0.45-1.68		1.17	0.58-2.37		
Self employed	27	3.7	0.63	0.08-5.00		1.33	0.13-13.64		56	16.1	0.55	0.25-1.19	0.398	0.56	0.24-1.28	0.425	
Employed	91	3.3	0.56	0.16-2.00	0.696	0.59	0.11-3.20	0.913	97	20.6	0.74	0.41-1.33		1.12	0.56-2.23		0.911
Wealth tertile																	
Poorest & middle [a]	162	4.9	1.0			1.0			201	25.4	1.0			1.0			
Wealthiest	237	4.6	0.94	0.37-2.38	0.891	1.31	0.18-9.64	0.618	99	19.2	0.74	0.42-1.30	0.293	0.91	0.48-1.73	0.779	0.676

[a] Combined due to low number of observations

Risk factors for anaemia in children aged 6-59 months (25 HZs)

The risk of having anaemia was found to decline progressively with increasing age (Table 4-4) in both low and high transmission strata ($p<0.001$). Although there was no evidence that malaria infection increased the risk of having anaemia in the low transmission strata (OR=2.01, 95% CI 0.89-4.51), this effect was significant in the higher transmission strata (OR=3.40, 95% CI=2.60–4.44). There was no evidence that reported ITN use was protective for the anaemia status in either strata. There was also no evidence of an association with fever, nor with education or occupation. Belonging to the wealthiest tertile was borderline significantly associated with the risk of having anaemia in both low transmission (OR=0.68, 95% CI=0.47-0.99) and high transmission strata.

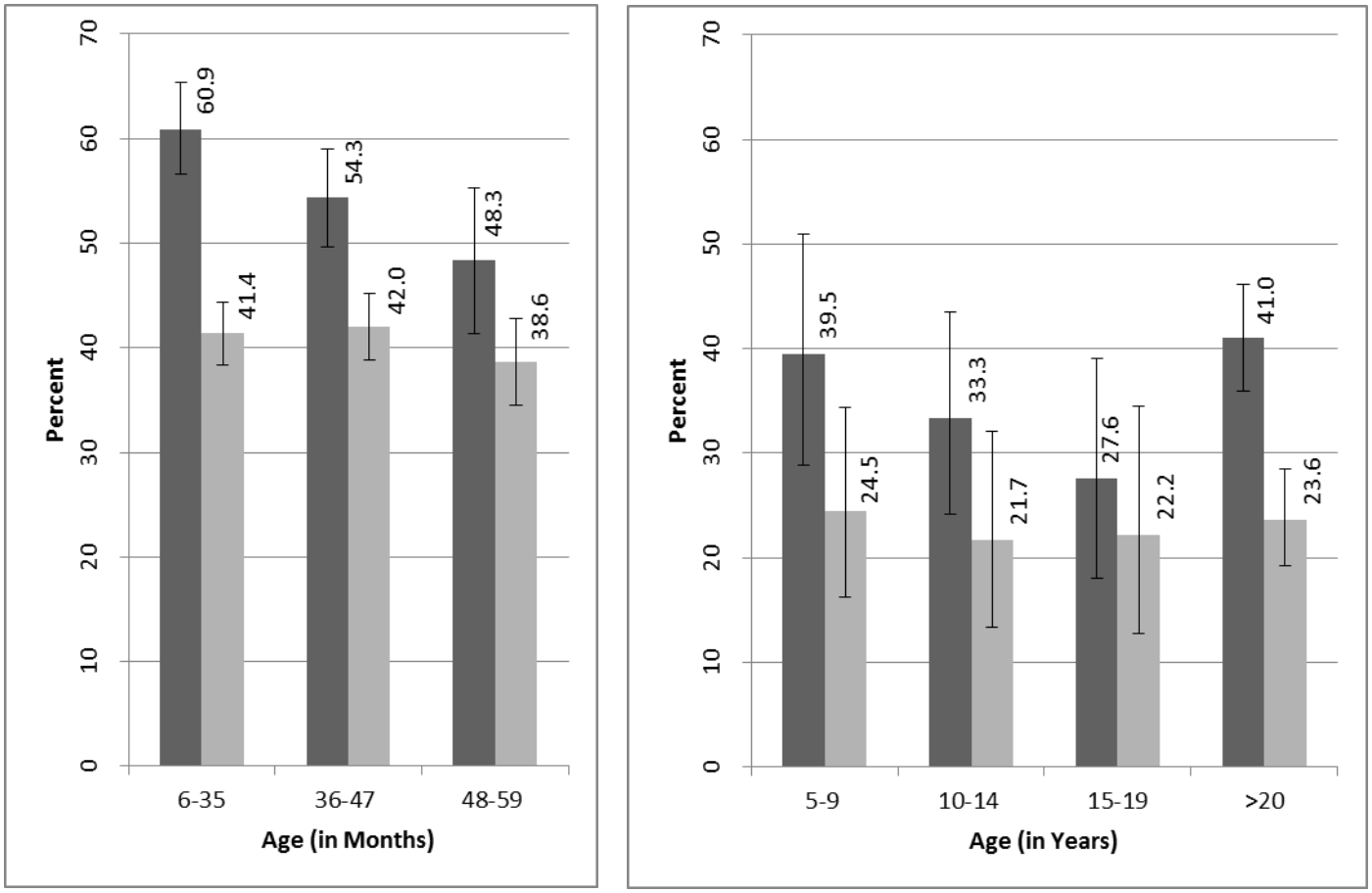
Table 4-4: Univariate and multivariable analysis of risk factors associated with anaemia in children between 6 and 59 months of age Kinshasa, stratified by malaria transmission zone, 2011

	< 10%								> 10%								
			Univariate analysis			Multivariate analysis					Univariate analysis			Multivariate analysis			Interaction by transmission zone
Variable	***n***	**(%)**	**OR**	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**	***n***	**(%)**	**OR**	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**	***P*-value**
Sex																	
Male	521	55.5	1.0			1.0			1161	69.0	1.0			1.0			
Female	526	59.7	1.19	0.93-1.52	0.167	1.21	0.93-1.58	0.160	1142	66.5	0.89	0.75-1.07	0.210	0.93	0.77-1.12	0.355	0.064
Age																	
6-35 months	445	73.0	1.0			1.0			957	80.6	1.0			1.0			
36-47 months	416	50.5	0.38	0.28-0.50		0.38	0.28-0.51		873	61.6	0.39	0.31-0.48		0.35	0.28-0.43		
48-59 months	186	36.6	0.21	0.15-0.31	< 0.001	0.19	0.13-0.28	< 0.001	473	53.3	0.28	0.22-0.35	< 0.001	0.23	0.18-0.29	< 0.001	0.473
Education of the respondent																	
No education	24	70.8	1.0			1.0			220	75.0	1.0			1.0			
Primary	390	62.1	0.67	0.27-1.66		0.64	0.24-1.70		1135	69.4	0.76	0.54-1.05		0.83	0.58-1.20		
Secondary	471	57.7	0.56	0.23-1.38		0.57	0.21-1.50		740	64.9	0.62	0.44-0.86		0.78	0.53-1.15		
Superior and above	162	44.4	0.33	0.13-0.84	< 0.001	0.35	0.13-0.99	0.037	208	61.5	0.53	0.35-0.81	0.004	0.80	0.49-1.31	0.629	0.412
Occupation of the respondent																	
Without occupation	720	60.7	1.0			1.0			1523	68.8	1.0			1.0			
Manual labourer	86	39.5	0.42	0.27-0.67		0.41	0.25-0.68		212	69.3	1.03	0.75-1.40		0.97	0.69-1.36		
Self-employed	104	60.6	1.00	0.65-1.52		1.03	0.65-1.63		275	65.1	0.85	0.64-1.11		0.77	0.58-1.03		0.010
Employed	137	50.4	0.66	0.46-0.95	< 0.001	0.94	0.61-1.44	0.005	293	63.8	0.80	0.62-1.04	0.268	0.92	0.68-1.24	0.313	
Net use																	
No	446	57.2	1.0			1.0			1341	68.3	1.0			1.0			
Yes	595	57.5	1.01	0.79-1.30	0.922	0.91	0.70-1.20	0.515	962	67.0	0.94	0.79-1.13	0.524	1.09	0.90-1.32	0.512	0.653
Malaria infection																	
No	1017	57.3	1.0			1.0			1762	63.1	1.0			1.0			
Yes	30	66.7	1.49	0.69-3.21	0.302	2.01	0.89-4.51	0.078	540	83.1	2.89	2.26-3.69	< 0.001	3.40	2.60-4.44	< 0.001	0.119
Fever in the last two weeks																	
No	798	54.6	1.0			1.0			1744	65.0	1.0			1.0			
Yes	244	66.4	1.64	1.22-2.21	< 0.001	1.30	0.93-1.80	0.197	559	76.4	1.74	1.40-2.17	< 0.001	1.32	1.04-1.67	0.039	0.755
Wealth tertile																	
Poorest	196	62.2	1.0			1.0			1176	72.9	1.0			1.0			
Middle	298	65.8	1.17	0.80-1.70	0.423	1.14	0.76-1.71		574	64.1	0.66	0.54-0.82	0.000	0.78	0.62-0.99		
Wealthiest	545	51.9	0.66	0.47-0.92	0.013	0.68	0.47-0.99	0.003	540	60.7	0.58	0.46-0.71	0.000	0.77	0.60-0.99	0.073	0.022

ITN use

There were some age-specific differences in ITN usage (Figure 4-1), with highest use in younger children (p-value=0.006) in the low transmission strata. In areas of high transmission, ITN usage although lower appeared more homogeneously distributed across age groups (Figure1). No significant differences in the utilization were found among individuals age more than five years, in both low and high transmission strata (Figure 1).

Figure 4-1: Left panel: ITN usage among children 6 to 59 months by transmission intensity. Right panel: ITN usage among individuals older than five years in Ngiri Ngiri (0.8%) and Selembao (26.8%) in Kinshasa, 2011. Light grey bars represent proportions of reported ITN usage in high transmission strata, and dark grey bars in low transmission strata. Error bars indicate 95% CI.



4.5 Discussion

The identification of risk factors for malaria infection and anaemia, provides information on the local malaria epidemiology and has the potential to lead to a more effective and targeted use of malaria control measures. This study presents the results of an analysis of the association of a number of variables that alone or in combination could affect the risk of acquiring *Plasmodium* infection and anaemia, in a city with diverse malaria transmission patterns. The key results from this analysis are the association between malaria infection and age, with older age groups being exposed to higher risk of malaria in low transmission settings and a lower and more homogeneous risk across all age groups in high transmission settings. Shift in the age of peak prevalence towards the older groups has been described for malaria and other infectious diseases and is consistent with exposure-related acquired immunity (Woolhouse et al., 1998; Snow et al., 2002). In zones of low transmission, children are less exposed to infective malaria, hence delaying the age of the first infection and the acquisition of immunity (Winskill et al., 2011). Clearly, in Kinshasa the risk of *Plasmodium* infection seems to occur later in childhood, which is consistent with areas of rather low levels of transmission. The prevalence rates by HZ shown in Table 1 (range: 0.7-46.0), with most HZs below 30% confirm that Kinshasa overall has a moderate endemicity level. Recent school surveys done in Kimbanseke, a relatively high prevalence HZ southeast of Kinshasa, found similar results, showing children aged 10-13 at higher risk of malaria and a high prevalence of asymptomatic infections (Matangila et al., 2014).

The relationship of ITN use by the different age groups could also influence the age pattern of risk that was observed: children in the youngest age group, 6-35 months, were significantly more likely to sleep under an ITN in the low transmission setting, whereas utilization was similar among age groups in high transmission setting. A similar shift in age of peak of prevalence towards the older children has been observed elsewhere with an increase in net

coverage (Wang et al., 2006). Only 44% of children 6-59 months used an ITN the night preceding the survey, which is still far from universal coverage. In individuals > 5 years, overall ITN use was even lower, with only 38% and 23.3% using an ITN in Ngiri Ngiri and Selembao, respectively. In addition, less than 58% of HHs owned enough ITNs to cover all household members in 2011. These low values are of concern.

Higher malaria prevalence in older children has also been attributed to increased use of antimalarials in early childhood (Trape et al., 1987). In case of fever in Kinshasa, it is common practice by the caregivers to initially treat their child at home (54% of the cases) although only 4.3% of the children treated for fever receive a recommended combination therapy containing artemisinin (unpublished data).

Results indicated that sleeping under an ITN the previous night reduced the risk of *Plasmodium* infection by 38% (OR=0.62, 95% CI 0.50 – 0.77) among children 6-59 months of age in areas of high transmission, consistent with the vast body of evidence supporting the efficacy and effectiveness of ITN in protecting against malaria (Lengeler et al., 2004). In low transmission areas, however, there was no evidence of such an association, presumably because the overall risk of infection was lower.

Reported history of fever was associated with malaria infection overall. There was evidence of a difference in this association with transmission level among individuals aged older than five years ($p<0.001$). The weaker association of reported fever with malaria in areas of high transmission could be explained by differences in the levels of acquired immunity.

The data confirm that anaemia is frequent in urban Kinshasa, with 65% prevalence among children 6-59 months, 30% moderate (7.0-9.9 g/dl) and 1.9% severe (< 7.0 g/dl). ITN use in Kinshasa did not appear to be associated with benefits in lower anaemia risk, contrary to what has been documented in other settings (Premji et al., 1995; Lengeler et al., 2004; Korenromp

et al., 2004; Mathanga et al., 2010). These findings are consistent with a Kenyan study that found only a small difference in prevalence of anaemia between villages with and without ITNs (Mathanga et al., 2010). Anaemia has many causes in addition to malaria (nutrition (UNICEF, 2010), soil transmitted infections (STH) and schistosomes), and in Kinshasa these are likely to also be contributors to this morbidity. A recent study revealed a high prevalence of STH infections among primary school children in Kinshasa (32.8%) (Matangila et al., 2014). Nevertheless, the estimated odds of anaemia in zones of high transmission were 3.5 times (95% CI 2.70-4.62) higher in malaria infected children.

In this study, the risk of anaemia was shown to decrease with increasing age in both low and high transmission strata. These results are consistent with studies conducted in West Africa, showing a significant reduction in the mean haemoglobin level in children aged 2-5 years compared to children aged 1-2 (Soares Magalhaes et al., 2011).

This study also showed differences in the effect in malaria risk or anaemia risk by socioeconomic status, consistent with previous studies carried out in sub-Saharan Africa (De Beaudrap et al., 2011; Gahutu et al., 2011; Winskill et al., 2011; Ayele et al., 2012), and as documented in a multi country analysis of DHS data (Balarajan et al., 2011).

This study however has some limitations. Foremost, the analysis draws on cross-sectional data; hence the causal nature of associations should be viewed with a certain caution. A second most important limitation of the study relates to the smaller sample size for individuals over five years compared to that of children 6-59 months, limiting our ability to potentially detect important differences and interactions between risk factors and transmission. Furthermore, the low proportion of males for this survey (34%) may have triggered a gender-response bias, with consequences on the prevalence and associations found. The direction and

magnitude of a possible bias remain unknown. Lastly, RDTs are limited in sensitivity to detect low density parasitaemia and their use may have led to an underestimation of the true proportion of people infected with *Plasmodium falciparum*. The underestimation may have differed with acquired immunity affecting the age pattern (Harris et al., 2010; Alves et al., 2002; Nicastri et al., 2009; Wu et al., 2015).

4.6 Conclusions

For the most part, there was no evidence of an interaction between malaria infections and the risk factors with transmission intensity; however the associations with age and with fever in the last two weeks did vary significantly. The results also show that school-aged children are the least protected with ITN, across the different transmission settings, hence representing an important reservoir for infection. The observation of a shift in the peak age of risk for malaria to older groups is consistent with areas of low transmission and highlights the need for a more equal distribution of ITN in Kinshasa to target all age groups and not only the traditional high-risk group of young children.

5 An operational comparative study of quinine and artesunate for the treatment of severe malaria in hospitals and health centres in the Democratic Republic of Congo: The MATIAS study

Giovanfrancesco Ferrari[1,2], Henry M Ntuku[3], Christian Burri[1,2], Antoinette T Kitoto[3], Stephan Duparc[4], Pierre Hugo[4], Didier K Mitembo[1,2], Amanda Ross[1,2], Philippe L Ngwala[5], Joseph N Luwawu[6], Papa N Musafiri[7], Symphorien E Ngoie[8], Christian Lengeler[1,2]

[1] Swiss Tropical and Public Health Institute, P.O. Box, 4002, Basel, Switzerland

[2] University of Basel, Basel, Switzerland

[3] Kinshasa School of Public Health, Kinshasa, Democratic Republic of the Congo

[4] Medicines for Malaria Venture, Geneva, Switzerland

[5] Zone de Santé de Kimpese, Kimpese, Democratic Republic of the Congo

[6] Zone de Santé Rurale de Kisantu, Kisantu, Democratic Republic of the Congo

[7] Zone de Santé de Maluku, Maluku, Democratic Republic of the Congo

[8] Centre Hospitalier Roi Baudouin 1[er] Masina, Kinshasa, Democratic Republic of the Congo

This paper has been published in the *Malaria Journal* 2015, **14:**226

5.1 Abstract

Background

The Democratic Republic of the Congo (DRC) has the highest number of severe malaria cases in the world. In early 2012, the National Malaria Control Programme (NMCP) changed the policy for treating severe malaria in children and adults from injectable quinine to injectable artesunate. To inform the scaling up of injectable artesunate nationwide, operational research is needed to identify constraints and challenges in the DRC's specific setting.

Methods

The implementation of injectable quinine treatment in 350 patients aged two months or older in eight health facilities from October 2012 to January 2013 and injectable artesunate in 399 patients in the same facilities from April to June 2013 was compared. Since this was an implementation study, concurrent randomised controls were not possible. Four key components were evaluated during each phase: 1) clinical assessment, 2) time and motion, 3) feasibility and acceptability, and 4) financial cost.

Results

The time to discharge was lower in the artesunate (median = 2, 90% central range 1 – 9) compared to the quinine group (3 (1 – 9) days; p <0.001). Similarly, the interval between admission and the start of intravenous (IV) treatment (2 (0 – 15) compared to 3 (0 – 20) hours; p <0.001) and parasite clearance time (23 (11 – 49) compared to 24 (10 – 82) hours; p <0.001) were lower in the artesunate group. The overall staff pre-administration time (13 (6 – 38) compared to 20 (7 – 50) minutes; p <0.001) and the personnel time spent on patient management (9 (1 – 24) compared to 12 (3 – 52) minutes; p <0.001) were lower in the artesunate group. In hospitals and health centres, the mean (standard deviation, SD) total cost

per patient treated for severe malaria with injectable artesunate was USD 51.94 (16.20) and 19.51 (9.58); and USD 60.35 (17.73) and 20.36 (6.80) with injectable quinine.

Conclusions

This study demonstrates that injectable artesunate in the DRC is easier to use and that it costs less than injectable quinine. These findings provide the basis for practical recommendations for rapid national deployment of injectable artesunate in the DRC.

5.2 Background

The Democratic Republic of the Congo (DRC) has the highest severe malaria burden in the world (WHO, . Geneva: World Health Organization. 2011). The combination of artesunate plus amodiaquine (AS-AQ) was adopted as a first-line treatment for uncomplicated malaria in 2005, with a second ACT, artemether plus lumefantrine (AL), added in 2010. Meanwhile, injectable quinine remained the recommended first-line drug for cases of treatment failure and for severe malaria.

In 2010, the AQUAMAT trial demonstrated that treating severe malaria with artesunate reduced the case fatality rate in African children (<15 years) by 22.5% compared to treatment with injectable quinine (Dondorp et al., 2010). Previously, the benefit of artesunate compared to quinine had been demonstrated in adults in the SEAQUAMAT trial carried out in Southeast Asia (Dondorp et al., 2005). These results led to the recommendation of injectable artesunate as the treatment of first choice for severe malaria in children and adults in the WHO guidelines in 2011 (WHO, 2011a).Nevertheless, cases of delayed haemolytic anaemia secondary to injectable artesunate administration were reported and the causative role of artesunate is still controversial. Its long-term safety profile is under evaluation. In addition to its efficacy, injectable artesunate offers a number of programmatic advantages over quinine, such as eliminating the need for rate-controlled infusions or cardiac monitoring, and the risk of induced hypoglycaemia (WHO, 2011a).

In 2012, the National Malaria Control Programme (NMCP) of the DRC, with support from the relevant ministry departments, decided to adopt the revised WHO severe malaria treatment guidelines, which strongly recommended injectable artesunate in preference to

quinine or artemether as first-line treatment for severe malaria. An implementation period of three years to scale up injectable artesunate was included in the national strategic plan.

This transition will require many operational and clinical adaptations. To support this process, there is a need for locally derived operational experience addressing constraints and challenges, something that all implementing countries will have to consider. These data are essential for three reasons: 1) better planning of the implementation of the new treatment based on quantified operational parameters; 2) identifying constraints and pitfalls to guide the training of health care providers; and, 3) providing strong and locally relevant arguments in situations where the health staff are reluctant to accept the change of treatment.

The present MATIAS study ('MAlaria Treatment with Injectable ArteSunate') aims to support the national introduction of injectable artesunate as the first-line treatment of severe malaria in the DRC by assessing four key components: 1) clinical safety, 2) time and motion, 3) feasibility and acceptability, and 4) cost.

5.3 Methods

Study design

The MATIAS study was an observational implementation study of patients aged two months and older with severe malaria and included two successive phases. In the first phase, between October 2012 and January 2013, severe malaria patients were treated with intravenous (IV) quinine. Then, between April and June 2013, severe malaria patients were treated with IV artesunate.

Four components were evaluated in each phase: 1) clinical safety, assessed on the basis of limited routine patient information; 2) time and motion parameters; 3) feasibility and acceptability; and 4) financial costs. The results of the feasibility and acceptability component required additional in-depth studies and are reported elsewhere (Ntuku et al., personal communication).

Participants (population, inclusion, exclusion criteria)

The study population consisted of patients admitted with severe malaria to one of the study sites between October 2012 and June 2013. Patients were included in the study if they were older than two months, fulfilled the WHO criteria of severe *Plasmodium falciparum* malaria (WHO, 2013b), had either a positive rapid diagnostic test (RDT) for *P. falciparum* (SD Bioline Malaria Antigen P.f/Pan Standard Diagnostics Inc, Yongin, South Korea) and/or a positive Giemsa-stained thick blood smear on admission, and they or their relative or guardian gave informed written consent. Patients were excluded if they had a known serious adverse reaction to quinine and/or artemisinin derivatives, or if there was a history of adequate anti-malarial treatment for more than 24 hours before admission. Women with known or suspected pregnancy in all trimesters during the second (artesunate) phase were not included and were treated with quinine according to the national guidelines (PNLP, 2012). Pregnancy status was determined by details from the patient's history and/or by a positive pregnancy test.

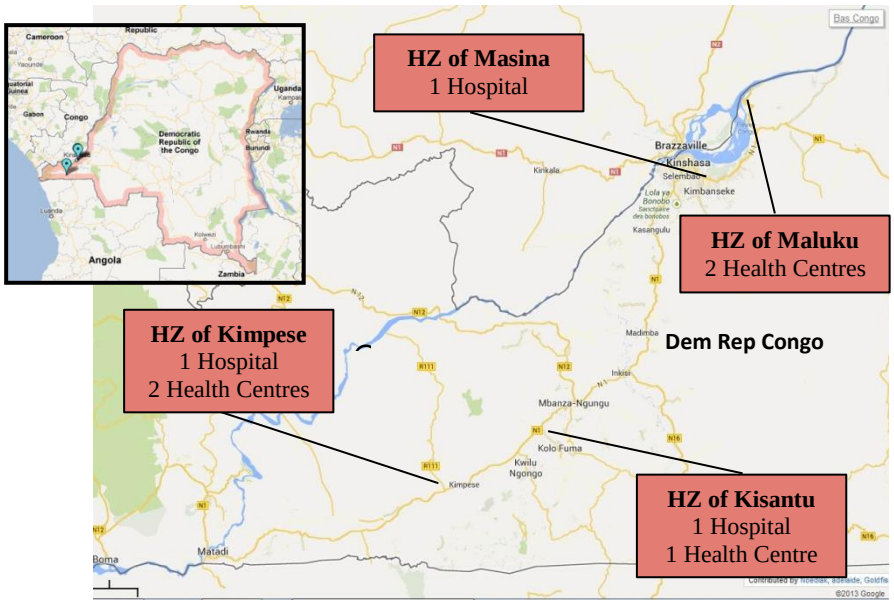
Signed informed consent for participation was obtained in French or in the local language from all participants or from their relatives or guardians. Because of the life-threatening nature of the disease, an initial consent was obtained from the accompanying relative or guardian on behalf of the patient, if necessary, and final consent was solicited as soon as the

patient was able to decide and respond. Since this was an observational study, investigators did not intervene in patient management, which was left to the discretion of the attending physicians. Ethical clearance for the study was obtained from the Ethics Committee of both Cantons of Basel, Switzerland (EKBB, Ref No 201/12) and from the Ethics Committee of the Kinshasa School of Public Health (KSPH Ethics Commission, Ref No 057/12), University of Kinshasa, DRC. The study was registered in ClinicalTrials.gov (Identifier: NCT01828333).

Study settings

The study sites consisted of three hospitals and five health centres in one urban and three rural health zones (HZs) in the DRC, representative of typical health facilities in the country (Figure 1). The sample included a large public health hospital (Institut Médical Evangélique, Kimpese, Bas Congo); one medium-sized, non-profit, missionary hospital (St Luc Kisantu); and a medium-sized, government hospital (Centre Hospitalier Roi Baudouin). In addition, five rural health centres were selected within the same HZ (Health Centre Bita, Health Centre Menkao, Health Centre Ngeba, Health Centre CECO, Health centre La Famille) (Figure 5-1 and see additional file 1).

Figure 5-1: Map showing the location of the study sites and the selected health zones



Interventions

During the first phase, patients receiving IV quinine were treated according to the national treatment guidelines. An initial loading dose of 20 mg of quinine salt/kg in 5 – 10 ml isotonic glucose solution (5%) per kg body weight was infused over four hours. Following a rest period of eight hours and 12 hours after administration of the loading dose began, a maintenance dose of 10 mg of quinine salt/kg was given. The maintenance dose was repeated every 12 hours until the patient was able to swallow the oral treatment (PNLP 2012; WHO, 2013b). Patients receiving artesunate (Guilin Pharmaceuticals, Shanghai, China) received doses intravenously at 2.4 mg/kg on admission, at 12 and 24 hours, and then once daily until oral treatment could be swallowed (WHO, 2013b). The content of each 60 mg vial of artesunate powder was dissolved in 1 ml of sodium bicarbonate and then diluted with normal saline solution or dextrose 5% before IV injection (WHOPARs) . At least three doses of artesunate had to be given before switching to a full course of oral treatment. The drugs used for the study were provided for free by the manufacturer (Artesunate, Guilin Pharmaceutical Co. Ltd, Shanghai, China) and by the funding agency, Medicines for Malaria Venture (MMV) (quinine).

Study outcomes

Outcome measures were defined for each of the four study components. For the clinical assessment component, the outcomes were: 1) duration of hospitalisation, defined as the time from hospital registration to discharge, (this was the primary study endpoint); 2) time from hospital admission to start of parenteral treatment; 3) time from initiation of parenteral treatment to initiation of oral treatment; 4) parasite clearance time (PCT), defined as the time from the initiation of a patient's parenteral treatment until the patient's first negative blood film; and 5) clinical status at discharge. For the time and motion component, the main

outcome measure was the cumulative staff time required for all steps of drug preparation, administration and patient management. For the feasibility and acceptability component, the main outcomes were health-provider perceived feasibility of patient management, perceived ease of applying drug treatment, and perceived quality of case management by patient/caretaker. These results are reported separately (Ntuku *et al*. in prep). For the financial cost component, the main outcome was the total financial cost of patient management, including treatment.

Sample size calculation

The study sample size was calculated based on seven centres, a mean hospitalisation of 2.23 days (standard deviation of 1.64) (Dondorp et al., 2005), 80% power and an assumed 20% shorter hospital stay with injectable artesunate. This calculation yielded 25 patients per centre and study period. Under the assumption of an effect variation by centre with a standard deviation of 0.05, the required number per centre was corrected to 27. This effect was presumed to be moderate, as each centre acted as its own control in the study. The two-phase study design was selected to fit the implementation strategy in this area. To ensure a safety margin and to aid disaggregation of the data by centre, the number of patients to be recruited was finally set to 50 patients of all ages from each centre and per study phase. One of the sites initially selected was removed due to difficulties in initiating the study. However, due to recruitment numbers slightly below expectations during the quinine phase, two additional study centres were added. This amendment increased the number of treatment centres to eight.

Statistical methods

Continuous outcomes were described using their mean and standard deviation, or median and 90% central range if the distribution was skewed. Dichotomous outcomes were summarised as proportions. Clinical characteristics are presented by age groups <five years and ≥five years. Skewed data, such as the time to event outcomes, were compared using the non-parametric Wilcoxon rank sum test. The paper-based questionnaires were double-entered and validated in EpiData version 3.1 software (The EpiData Association, Odense, Denmark) and analysed in Stata version 12.1 (Stata Corp, College Station, TX, USA).

Key procedures

Prior to the first study phase, all investigators and staff involved in the study in each hospital/health centre participated in a three-day training on study procedures. Laboratory technicians received a refresher course on thick blood smear preparation/reading and, before the second phase, a refresher course on haemoglobin (Hb) measurement with the HemoCue 201 plus system (Angelholm, Sweden). Simulated interviews were conducted to practice obtaining informed consent. Local principal investigators took part in practical sessions on filling in the case report forms (CRF). Nurses and doctors attended a separate training on reporting serious adverse events (SAE). Nurses also participated in piloting the time and motion study tool, which included observing and timing the activities related to drug preparation/administration prior to the first data collection. Upon completion of the first phase, hospital and health centre personnel involved in the study convened in Kinshasa for a two-day training on preparing and administering injectable artesunate. Job aids and training tools developed by MMV were used for this training. In addition, each site received ten doses of injectable artesunate for training purposes, allowing health care providers to become

familiar with the new drug prior to patient recruitment. Weekly supervision visits to each site throughout the duration of the study ensured regular monitoring of the study team.

Patient assessment

Demographic information and limited routine clinical history data were collected for each patient and local study physicians (hospitals) or nurses (health centres) performed basic routine clinical assessments. A Giemsa-stained thick blood smear was performed and examined every 12 hours during the first 24 hours and then every 24 hours until negative or until patient discharge. For PCT calculations, thick blood smears were later reread for quality control by experienced microscopists at the KSPH, blinded to the results of the first reading and to the RDT results. Hb levels were systematically assessed with a HemoCue 201 plus+ photometer (Angelholm, Sweden) during the second study phase, at hospital admission, at discharge and at follow-up visits on days 7, 14, 21, and 28. The HemoCue testing resulted in a change in study protocol because of reports of haemolytic anaemia following artesunate treatment (CDC, 2013). The results of that extension are presented elsewhere (Burri et al., 2014). To ensure the proper functioning of the photometer, high and low Hb liquid controls (HemoCue Eurotrol HemoTrol) were run weekly at each site. Given the observational nature of the study, laboratory tests were not systematically performed and were left to the discretion of the physician or treating nurse, except for parasitological tests required for inclusion in the study and the Hb assessment during the second phase. Time of admission, time of start and end of parenteral treatment, and time to discharge were also recorded for every patient during both phases.

Parenteral treatment was completed by administering a full course of the recommended first-line, oral, combination therapy AS-AQ or AL in the artesunate phase, or with quinine tablets

or the standard treatment practiced by the centre in the quinine phase. The first dose of the oral treatment was administered at the health facility in the presence of the nurse responsible. Subsequent doses were administered at home, according to the instructions given to parents and guardians. Patients were discharged at the discretion of the attending physician/nurse, after a final clinical assessment. During the first study phase, patients were asked to return to the hospital/health centre for follow-up seven days after discharge to assess their clinical status and their adherence to oral therapy. In the second study phase, patients were asked to return on days 7, 14, 21, and 28 after discharge to assess the clinical status and adherence to oral therapy and to determine their Hb levels at these time points.

Time and motion

The time and motion methodology consisted of 1) dividing a process into key tasks, and 2) observing each task to assess the average time required to perform it. The sum of the average times spent on each task was used to compute the total average time to complete the process. In each of the three participating hospitals, an external study nurse supervised the time and motion component and was present throughout the study. In the five health centres, the health centre personnel were responsible for the measurements. Therefore, the number of patients followed up was limited as a second nurse was not always available. Observed activities included: 1) pre-administration tasks (preparation of all materials and injectable solution, searching for the vein, setting the infusion in case of quinine), 2) drug administration, and 3) all other activities related to patient management. Observations were made by the nurses using digital stopwatches and a checklist to record the time taken for each task. Inter-observer agreement was not formally assessed. Materials required for all tasks were also recorded on the same observer checklist and this information was used later to calculate financial costs.

Cost of treatment component

A financial cost analysis was carried out from the provider's perspective, accounting only for costs incurred by the hospitals and the health centres. Complete unit cost data on resources used were recorded for 386 patients under quinine and for 333 patients under artesunate. To estimate the mean unit cost, the 2014 average exchange rate (USD 0.00107 to the Congolese Franc) was adopted (OANDA). Health care costs were divided into four main categories: 1) drug costs (parenteral quinine and artesunate, oral therapy), 2) diagnostic costs (blood smear), 3) administration equipment costs (infusion set, IV solution, syringes), and 4) in-patient costs (consultation cost, bed occupancy, blood transfusion, and nursing care). Administration equipment, blood smear and parenteral quinine unit costs were estimated from the hospital/health centre price lists, as well as in-patient costs. The full dose costs for both parenteral quinine and artesunate were applied, since the recommendation given in the study was to avoid re-using the drug once it was opened, and hence partially used ampoules had to be discarded. Artesunate was used in the 60-mg vial, the WHO pre-qualified formulation at the time. Costs of oral treatment with AS-AQ/AL were included in the analysis despite being subsidised by the Global Fund to Fight AIDS, TB and Malaria (GFATM) in the selected health facilities. Costs of artesunate and of AS-AQ/AL were obtained from the Management Sciences for Health (MSH) *International Drug Price Indicator Guide* (MSH, 2014).

Additional treatments and diagnostic costs, other than the parenteral drug and the thick blood smear, were not included in the analysis. Specific costs associated with co-morbidities, with the exception of blood transfusions (severe anaemia), were not considered in the analysis because they would have required a level of clinical monitoring that was not possible in this study. In two sites (referral hospital Saint Luc and Health Centre Ngeba), a lump sum health care payment system was in place, thus unit costs were unavailable. The decision was made

to reflect as closely as possible the local practice and to generate nationally relevant data rather than internationally, fully costed estimates. Hence, the lump sum estimates were taken for this analysis. However, the two sites were analysed separately to take these differences into account, since lump sums are likely to underestimate the full cost of treatment, especially if there is a central subsidy by an external donor, as in the case of these two facilities.

5.4 Results

Clinical assessment

A total of 749 patients were recruited from eight sites, 399 in the quinine group from October 2012 to January 2013 (study phase one), and 350 in the artesunate group from April to July 2013 (study phase two). The quinine group consisted of 248 (62%) children between two and 59 months, and 151 (38%) individuals aged five years and above. The artesunate group consisted of 215 (61%) children between two and 59 months and 135 (39%) individuals aged five years and above. The demographic and baseline characteristics were similar for the two study groups (Table 5.1). All patients tested positive for malaria, either by thick blood smear or RDT on the day of inclusion. Overall mortality was 2.8% (21/749), with 3.8% for patients treated with quinine (15/399) and 1.7% for patients treated with artesunate (6/350) (p=0.110). The majority of deaths (13 of 21, 62%) occurred within the first 24 hours after admission, of which nine of 15 were in the quinine group (with two dying before receiving the treatment) and four of six were in the artesunate group (zero before receiving the treatment). Of the eight deaths that occurred after 24 hours, six occurred in the quinine group and two in the artesunate group. Prostration was the most frequent manifestation of severe malaria at admission in children between two to 59 months in the quinine (204/248, 82%) and artesunate groups (171/215, 80%), as well as in individuals five years and above (122/151, 81% and 120/135, 90%). Respiratory distress and convulsions were also frequent symptoms

at admission in both groups. The total number of patients who received a blood transfusion was 214 (29%), with 128 (32%) and 88 (25%) in the quinine and artesunate groups, respectively. Five per cent of the patients under the quinine regimen had persistent symptoms at discharge, compared to 3% under the artesunate regimen (Table 5.2). A decrease in Hb levels at one of the follow-up visits was a frequent SAE reported during the artesunate regimen (Burri et al., 2014). A seven-day oral quinine course was the most frequently prescribed oral medication to complete treatment after the initial injectable quinine regimen (92%), whereas AS-AQ was the most prescribed oral medication (97%) after injectable artesunate for all ages. Patient adherence was assessed by the duration of oral treatment and the reported number of tablets taken. Following injectable quinine and injectable artesunate, 236 (85%) and 308 (99%) patients fully adhered to the treatment, respectively.

The time to discharge was slightly lower in the artesunate group compared to the quinine group, with a median of two (90% central range 1 – 9) *versus* three (1 – 9) days, respectively ($p < 0.001$). Given that mortality was slightly higher in the quinine group, this would have led to a shorter hospital stay but the effect would be minimal because of the low case fatality rate. The interval between admission and start of parenteral treatment was significantly shorter in the artesunate group compared to the quinine group, two (0 – 15) *versus* three (0 – 20) hours ($p < 0.001$). The interval from beginning parenteral treatment initiating oral treatment was slightly longer in the artesunate group (45 (32 – 56) *versus* 39 (12 – 67) hours in the quinine group, $p < 0.001$). Parasite clearance time was 23 (11 – 49) hours for artesunate *versus* 24 (10 – 82) hours for quinine ($p < 0.001$) (Table 5.3).

Table 5-1: Characteristics and clinical presentation of patients at recruitment

	Quinine (N = 399)		**Artesunate (N = 350)**	
	2-59 months (N = 248)	**>5 years** (N = 151)	**2-59 months** (N = 215)	**>5 years** (N = 135)
Sex				
Female	122 (49%)	72 (48%)	115 (53%)	71 (53%)
Age	24 (7-53)	10 (5-48)	24 (7-48)	8 (5-48)
Medical history (past 30 days)				
Other malaria episode	18 (7%)	7 (5%)	16 (7%)	16 (12%)
Fever (N=398)	90 (37%)	78 (52%)	59 (27%)	76 (56%)
Pre-treatment with anti-malarial	31 (12%)	20 (13%)	20 (9%)	20 (15%)
Other treatment(s) received	113 (46%)	75 (50%)	98 (46 %)	83 (61%)
Other major health problem(s)	6 (2%)	3 (2%)	0 (0%)	4 (3%)
Episode of convulsion (N=394)	34 (14%)	10 (7%)	13 (6%)	11 (8%)
Known hypersensitivity to other drugs	0 (0%)	5 (3%)	2 (1%)	5 (7%)
Signs and symptoms on admission				
Fever	220 (89%)	129 (85%)	197 (92%)	121 (90%)
Fever before enrolment (days and range)	3 (2-4)	3 (2-5)	3 (1-7)	3 (1-7)
Vomiting	100 (40%)	78 (52%)	113 (53%)	78 (58%)
Coma	23 (9%)	12 (8%)	5 (2%)	11 (8%)
Reported convulsions	72 (29%)	19 (13%)	59 (27%)	14 (10%)
Blantyre coma score (8-24 months)	3 (2-5)	-	4 (3-8)	-
Glasgow coma score (>2 years)	10.5 (5-13)	10 (8-15)	7.5 (4-5)	NA
Pallor	NA	NA	77 (36%)	20 (15%)
Jaundice	3 (1%)	4 (3%)	7 (3%)	2 (1%)
Shock	10 (4%)	2 (1%)	2 (1%)	2 (1%)
Respiratory distress	128 (52%)	58 (38%)	96 (45%)	64 (47%)
Severe anaemia (<5 g/dl) (N=326 Q; 334 A)	8.1 (3%)[a]	9.1 (2%)[a]	15 (7%)[b]	1 (1%)[b]
Parasite count (per µl); geometric mean (95 % CI)	17068 (12119-24038)[c]	12022 (7040-20527)[c]	22289 (15498-32057)[c]	12346 (7812-19511)[c]
Prostration	204 (82%)	122 (81%)	171 (79%)	120 (89%)
Urine colouration (N=391)	2 (1%)	2 (1%)	10 (5%)	8 (6%)
Clinical examination on admission				
Weight (kg and SD)	11.1 (3.0)	33.2 (17)	11.2 (4)	27.6 (15)
Temperature (°C and SD) (N=398)	38.1 (1)	38.3 (1)	38.1 (1)	38.4 (1)
Pulse	125 (70-180)	102 (64-148)	119 (60-171)	94 (60-140)
Respiratory rate per minute	42.5 (28-72)	39.0 (24-60)	40 (24-72)	40 (20-58)
Co-morbidity	82 (34%)	51 (34%)	80 (37%)	58 (43%)

Data are summarised as numbers (%), median (90% central range) or mean (SD); NA = Not available;
[a] Clinical assessment only; [b] HemoCue; [c] The initial parasitaemia was calculated only for those patients for whom the biological confirmation was done by thick blood smear

Table 5-2: Clinical examination at discharge

	Quinine		**Artesunate**	
	2-59 months (N = 226)	**>5 years (N = 144)**	**2-59 months (N = 208)**	**>5 years (N = 131)**
Weight (kg)	11.1 (3.0)	32.8 (16.7)	11.2 (4.3)	27.2 (14.9)
Temperature (°C)	36.7 (0.5)	36.6 (0.5)	36.7 (0.4)	36.5 (0.4)
Pulse	100 (70-128)	90 (41-120)	90 (64 - 124)	85.4 (18.8)
Respiratory rate per minute	35 (22-40)	28 (16-48)	31.8 (6.6)	29.2 (8.0)
Persistence of signs at discharge	12 (5.4%)	6 (4.3%)	7 (3.4%)	4 (3.0%)

Data are summarised as numbers (%), median (90% central range) or mean (SD).

Table 5-3: Key time intervals

	Quinine	**Artesunate**	**p-value**
Time to discharge (days)	3 (1-9)	2 (1-9)	p <0.001
Interval between admission and beginning of parenteral treatment (hours)	3 (0-20)	2 (0-15)	p <0.001
Interval between beginning of parenteral treatment and oral treatment (hours)	39 (12-67)	45 (32-56)	p <0.001
Parasite clearance time (hours)	24 (10-82)	23 (11-49)	p <0.001

Median and 90% central range

Time and motion study

Administration times by task are shown in Tables 5.4 and 5.5. There was a reduction in the staff time required for all tasks during the artesunate phase. The total median personnel time for pre-administration and patient management tasks was 33 (10 – 60) for artesunate and 36 (13 – 92) minutes for quinine. The median cumulative staff time for observed drug pre-administration tasks per patient per drug session was 13 (6 – 38) for artesunate and 20 (7 – 50) minutes for quinine. Cumulative median personnel time spent for patient management was 9 (1 – 24) for artesunate and 12 (3 – 52) minutes for quinine.

Table 5-4: Personnel time (in minutes) required to complete pre-administration tasks, by drug type

Quinine (N = 832)		**Artesunate (N = 795)**	
Material preparation	6 (2-18)	Material preparation	4 (1-10)
Drug preparation	4 (1-14)	Reconstitution	3 (1- 8)
Search for the vein	5 (1-14)	Dilution	2 (1-10)
Perfusion regulation	4 (1-10)	Dose verification	2 (1-6)
-		Search for the vein	3 (1-10)

Median and 90% central range

Table 5-5: Overall cumulative personnel time (in minutes)

	Quinine	**Artesunate**	**p-value**
Overall personnel pre-administration time	20 (7-50)	13 (6-38)	p <0.001
Overall personnel patient management time	12 (3-52)	9 (1-24)	p <0.001
Overall personnel time	36 (13- 92)	33 (10-60)	p <0.001

Median and 90% central range

Cost analysis

In hospitals and health centres, the mean (SD) total costs per patient treated for severe malaria with injectable artesunate were USD 51.94 (16.20) and 19.51 (9.58); and USD 60.35 (17.73) and 20.36 (6.80) with injectable quinine. Costing details for individual study sites are given in Table 5.6.

Table 5-6: Mean cost (with SD) for treating one episode of severe malaria in patients admitted to hospitals and health centres in the Democratic Republic of Congo

Hospital/ Health centre	Mean length of stay, days (SD)		Blood smear unit cost		Mean injectable drug cost		Mean oral drug cost[a]		Mean administration cost		Mean inpatient cost		Mean total cost per patient	
	QNN	ART	QNN	ART	QNN	ART	QNN	ART	QNN	ART	QNN	ART	QNN	ART
Kimpese referral hospital	7.12 (4.43)	6.26 (5.01)	2.94	2.94	0.45 (0.22)	7.72 (3.28)	0.66 (0.38)	0.48 (0.06)	1.89 (0.83)	1.39 (0.48)	49.56 (18.04)	47.25 (19.82)	61.58 (18.72)	59.57 (20.97)
Centre Hospitalier Roi Baudouin	4.09 (3.41)	3.72 (2.17)	3.21	3.21	0.78 (0.17)	3.24 (1.51)	0.97 (0.30)	0.56 (0.21)	6.59 (1.56)	0.90 (0.83)	38.60 (12.99)	38.76 (8.32)	53.29 (7.86)	46.58 (8.55)
Hôpital St Luc Kisantu	3.13 (1.06)	6.68 (4.00)	NA	NA	NA	3.87(1.72)	0.70 (0.23)	0.50 (0.18)	NA	NA	NA	NA	50.34 (9.98)[b]	55.44 (11.81)[b]
Health Centre CECO	3.96 (2.35)	4.28 (3.36)	1.07	1.07	0.57 (0.14)	7.19 (2.51)	1.00 (0.49)	0.48 (0.13)	2.10 (0.34)	1.62 (0.45)	40.28 (10.32)	41.62 (14.47)	32.53 (14.25)[c]	28.21 (9.41)[c]
Health Centre La Famille	3.80 (1.54)	2.58 (1.50)	1.07	1.07	0.94 (0.43)	7.26 (3.95)	1.58 (0.59)	0.66 (0.30)	3.89 (1.59)	1.22 (0.45)	10.48 (3.63)	8.19 (3.21)	19.35 (4.46)	18.21 (5.02)
Health Centre Bita	2.18 (0.68)	1.99 (0.11)	1.07	1.07	1.49 (0.33)	7.30 (2.68)	1.99 (0.78)	0.49 (0.17)	6.48 (1.23)	1.51 (0.23)	6.71 (2.07)	6.39 (0.30)	21.97 (2.73)	16.56 (2.87)
Health Centre Menkao	1.78 (0.97)	1.27 (1.34)	1.07	1.07	1.81 (0.72)	9.12(5.09)	1.05 (0.45)	0.63 (0.24)	5.10 (1.19)	2.60 (0.73)	3.15 (1.29)	2.49 (1.76)	13.92 (2.59)	15.66 (5.84)
Health Centre Ngeba	4.6 (2.59)	2.7 (0.98)	NA	NA	NA	5.87 (1.95)	0.97 (0.30)	0.43 (0.04)	NA	NA	NA	NA	6.86 (0.84)[b]	4.47 (0.10)[b]

In 2014 USD; NA = Not available

[a] Mean cost for oral quinine and AS-AQ

[b] Unit costs not available. Lump sum payment system. All exams and drugs other than anti-malarial are included. Patients pay a part of the total costs; the rest is supported by a partner.

[c] Among health centres, blood transfusion was only performed in CECO. To allow cost comparison with the other health centres, costs of blood transfusion were not included in the total costs. Total costs for CECO under ART and QNN are USD47.47 (9.41) and USD51.79 (14.25) respectively if blood transfusion is included.

5.5 Discussion

This study is the first to quantify key operational parameters in the management of patients with severe malaria treated with injectable artesunate. Injectable artesunate was superior to quinine for almost all of the parameters assessed. Furthermore, from the provider's perspective, overall costs were lower for injectable artesunate in hospitals and similar in health centres. The aim of the study was to assess operational aspects rather than safety and efficacy. However, there was no indication for any of the outcomes obtained from available clinical charts that patients fared worse with injectable artesunate compared to parenteral quinine, concurring with available data on the efficacy and safety of the use of injectable artesunate in the DRC (Dondorp et al., 2010).

A major reason for conducting the study in two phases was the need for comparative operational data between the new regimen and the old regimen. Because many aspects in health services are setting-specific, it was thought that the best controls would be the facilities themselves. The strongest study design would include a randomised concurrent control trial with enough health facilities to account for inter-facility variability, however, time and logistical reasons precluded such an approach for the current study. The design outlined here was the best suited to the Ministry of Health's current plan for scaling up artesunate. The operational parameters of treating severe malaria are unlikely to be sensitive to seasonal effects, and also unlikely to change much in a given facility over time periods equal to that of the study. Hence, although not randomised, this design allowed a reasonable comparison of the two regimens in real-world implementation settings. Although injectable quinine has been the mainstay for treating severe malaria for many years, there are virtually no existing data in the literature quantifying the operational parameters of interest.

In this study, patients admitted with severe malaria experienced a median delay of three hours before receiving their initial quinine dose compared to two hours with artesunate (Table 3). This time delay depended on several factors that should be further investigated. In particular, it could reflect the difficulties of promptly and safely administering quinine via IV. Although comparable in its preparation, quinine is a difficult drug to administer because of its unfavourable safety profile; it requires correct dose calculation, taking into account previous quinine treatment to avoid overdosing and serious consequences for the patient.

In the AQUAMAT trial (Dondorp et al., 2010), the risk of children dying while waiting to receive quinine was almost four times higher than the risk in children treated with artesunate. This delay adds to the time needed for referral, during which the condition of the patient can deteriorate (WHO, 2010). In this study, two patients died before receiving quinine compared to none in the artesunate group. Although this delay is still critical for both regimens, it can be expected to decrease further for injectable artesunate as skills and confidence are acquired through repeated administration and preparation by health personnel.

The well-known difficulties in administering quinine may also explain the difference observed in the time interval between the beginning of the parenteral treatment and the initiation of oral treatment. Lack of confidence or uncertainty in reconstructing the history of previous treatments with quinine could potentially limit the number of doses a patient receives. According to the national DRC directives on the treatment of severe malaria (PNLP, 2012), the number of doses of quinine administered should be minimised until the patient can tolerate an oral medication. Under the artesunate regimen in this study, the WHO's recommendations of a minimum of three injections during the first 24 hours, irrespective of the patient's ability to tolerate oral medication were strictly followed. This is one possible explanation for the prolonged time interval to the initiation of oral therapy.

The artesunate regimen achieved parasite clearance faster than the quinine regimen, which likely accounts for the shorter hospital stay. The reduction in median hospital stay by a day reduces costs of malaria treatment and minimises socio-economic impacts on patients and their families. This is especially important for poorer and more vulnerable segments of the population.

The estimated costs of treating a patient with severe malaria in this study are similar to those calculated in previous studies (Lubell et al., 2011; Lubell et al., 2009), although lower than those reported by Kyaw et al., which used a more detailed cost analysis approach (Kyaw et al., 2014). The costs were highly variable, depending on the level and type of facility (public, private or missionary). The mean pooled estimate total cost was found to be similar for artesunate compared to quinine in health centres, USD 19.51 (9.58) and 20.36 (6.80), while lower in hospitals, USD 51.94 (16.20) to USD 60.35 (17.73). Inpatient costs were the major driver costs for the difference observed between hospitals and health centres. Less standardized inpatient costs are established by each hospital and health centre and take into account a number of parameters, which include cost of labour, and the organisation of the health service. Since it was not possible to analyse all patient costs, particularly the cost related to supportive measures and the presence of co-morbidities, the total treatment costs are clearly underestimated. For the purpose of this study, a new vial of quinine was used for every dose, but this is not necessarily the case in the real world. As a result, drug costs were likely overestimated. However, not all sessions of drug preparation and administration were included due to understaffed health centres and the inability to reliably observe the most severe cases in need of prompt treatment.

The results show that the overall time spent on pre-administration tasks and on direct post-treatment patient care was slightly lower in the artesunate compared to the quinine group.

Although statistically significant, this time difference is smaller than expected considering that artesunate is easier to use. This could be explained by the fact that health personnel had a limited time to get used to preparing and administering artesunate before starting patient enrolment in the second phase. Therefore, it could be that the overall difference in the pre-administration times will increase over time, in favour of artesunate. The overall personnel time spent on patient care was lower with artesunate administration compared to quinine. This is likely to have resulted in more time to care for other patients, leading to a positive effect on the overall quality of care. This was consistent with health care providers' higher satisfaction when using artesunate, as described elsewhere (Ntuku *et al*, in prep).

5.6 Conclusions

This study provides for the first time descriptive evidence of the effectiveness and practicability of using injectable artesunate for treating severe malaria in hospitals and health centres in the DRC. For most operational and cost parameters, injectable artesunate was found to be superior to injectable quinine. Combined with its higher efficacy, these findings support the rapid switchover in the country. These findings also provide some useful operational and cost data for national authorities and for local health care managers involved in planning the transition.

Training health personnel is obviously a key factor for a successful transition, including a change in the attitudes and behaviours of providers.

The MATIAS study has contributed further evidence that injectable artesunate is a better treatment option than injectable quinine for patients with severe malaria. The findings suggest that transition to the new drug should be accelerated as quickly as possible. The Ministry of

Health of the DRC is currently scaling up the use of injectable artesunate in the public sector, with the support of the GFATM and the other partners, which will enable 100% coverage of in-patient cases within a three-year period.

6 Delayed anaemia after treatment with injectable artesunate in the Democratic Republic of Congo: a manageable issue

Christian Burri[1,2], Giovanfrancesco Ferrari[1,2], Henry Maggi Ntuku[3], Antoinette Tshefu Kitoto[3], Stephan Duparc[4], Pierre Hugo[4], Didier Kalemwa Mitembo[1,2], and Christian Lengeler[1,2]

[1] Swiss Tropical and Public Health Institute, P.O. Box, 4002, Basel, Switzerland

[2] University of Basel, Basel, Switzerland

[3] Kinshasa School of Public Health, Kinshasa, Democratic Republic of the Congo

[4] Medicines for Malaria Venture, Geneva, Switzerland

This paper has been published in the *American Journal of Tropical Medicine* 2014, **91:**821-823

6.1 Abstract

Cases of delayed haemolytic anaemia have been described after treatment with injectable artesunate, the current World Health Organization (WHO)–recommended first-line drug for the treatment of severe malaria. A total of 350 patients (215 [61.4%] < 5 years of age and 135 [38.6%] ≥ 5 years of age) were followed-up after treatment with injectable artesunate for severe malaria in hospitals and health centres of the Democratic Republic of the Congo. Complete series of hemoglobin (Hb) measurements were available for 201 patients. A decrease in Hb levels between 2 and 5 g/dL was detected in 23 (11.4%) patients during the follow-up period. For five patients, Hb levels decreased below 5 g/dL during at least one follow-up visit. All cases of delayed anaemia were clinically manageable and resolved within one month.

Acute *Plasmodium falciparum* malaria and improperly treated uncomplicated malaria can progress rapidly, especially in young children and almost invariably results in death (WHO, 2006). The second edition of the WHO guidelines for the treatment of malaria updated in April 2011 recommends injectable artesunate for the management of severe malaria in all age groups and epidemiologic settings (WHO, 2011a, 2012b)

In early 2012, the National Malaria Control Program of the Democratic Republic of the Congo adopted the new guidelines. To assess the feasibility and acceptability of use of the new drug in the Democratic Republic of the Congo, we conducted a longitudinal operational study (Malaria Treatment with Injectable Artesunate [MATIAS]).

In mid-January 2013, during the recruitment phase of MATIAS, the Centres for Disease Control and Prevention (CDC) reported 19 cases of delayed haemolytic anaemia in hyperparasitaemic travellers in the second and third weeks after treatment with parenteral

artesunate (CDC, 2013). The haemolysis resolved and Hb levels improved in all patients within 4–8 weeks after artesunate therapy.

In view of the large scale deployment of parenteral artesunate at national level, these results warranted further investigations. On the basis of this report, the MATIAS protocol was amended to extend the follow-up of patients from 7 to 28 days after parenteral treatment with artesunate and to include weekly measurements of Hb levels. We present the results of a sub-study conducted within the MATIAS study in eight sites in the Democratic Republic of the Congo.

A total of 350 patients who fulfilled the WHO criteria for severe *P. falciparum* malaria were treated with intravenous artesunate (Guilin Pharmaceuticals, Shanghai, China). Details of history and clinical assessment obtained by local physicians and nurses were collected for all patients. A thick blood smear was prepared and a rapid diagnostic test was conducted at admission for each patient. Thereafter, a thick blood film was prepared every 12 hours during the first 24 hours and then every 24 hours until results were negative or patient discharge. The patients were treated with injectable artesunate at a dose of 2.4 mg/kg given at admission, at 12 hours, at 24 hours, and then once a day until the patient was able to tolerate oral medication, in accordance with WHO recommendations. Parenteral treatment was completed by giving a full course of the recommended first-line oral combination therapy: artesunate plus amodiaquine or artemether plus lumefantrine.

Hb levels were assessed by using a HemoCue (Ängelholm, Sweden) Hb201 plus photometer at hospital admission, at discharge, and at follow-up visits on days 7, 14, 21, and 28. To ensure proper functioning of the photometer, high and low liquid controls were tested weekly at each site. No additional biologic or serum markers were investigated.

Of 350 patients treated with intravenous artesunate, 61.4% were children 2–59 months of age. Four (1.1%) patients were referred to another health facility and six (1.7%) died during the treatment period. A total of 270 (77.1%) patients made all four scheduled follow-up visits and successfully completed the full clinical assessment. A total of 41 (11.7%) patients made only one follow-up visit, either at day 21 or at day 28, and 29 (8.3%) did not make any visit.

Of the 270 patients seen at all planned follow-up visits, complete series of Hb measurements (admission, discharge, and four follow-up visits) were available for 201 (57.4%) patients. The proportion of severe anaemia cases at admission was 5.5% (11 patients). Among 201 patients with full follow-up, a delayed decrease in the Hb level (2–5 g/dL) was observed in 23 patients (11.4%) between day 7 and day 21 (95% confidence interval = 3.1–36.2%) treated with injectable artesunate. The wide confidence interval highlights the uncertainty of this estimate.

Of these 23 patients, 5 showed a decrease in Hb levels below 5 g/dL on least one of the follow-up visits (Table 6.1). This decrease was observed eight days after the first dose of artesunate for 1 patient, 15 days after the first dose for 2 patients, and 16 days after the first dose for 1 patient. All four patients received a blood transfusion. A decrease was observed 23 days after the first dose for 1 patient. All five patients had received three doses of artesunate except for one of the patients with anaemia on day 15, who had received 5 doses. All patients had completed the parenteral treatment with a full course of artesunate plus amodiaquine. For those patients, Hb levels increased by the day 28 clinical assessment.

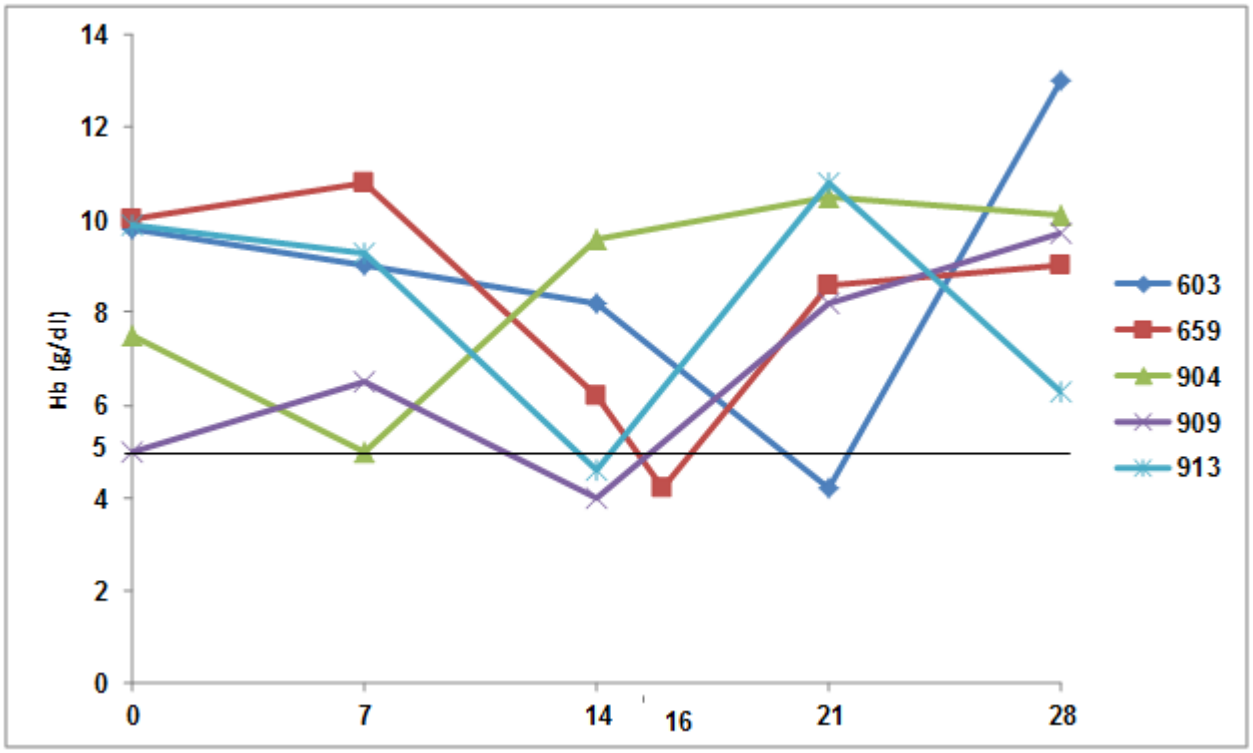
Table 6-1: Summary data of patients presenting with life-threatening anaemia during follow-up visits, Democratic Republic of Congo

Case no	Age	PP[1]	PCT[2]	Hb admission (g/dl)	Hb nadir (g/dl)	Day of lowest Hb drop	Hb at day 28
603	3 years	435'815	NA	9.8	4.2	23	13.0
659	30 months	199'299	NA	10	4.2*, **	16	9.0
904	26 months	NA	24	7.5	5.0*	8	10.5
909	6 months	53'293	23.6	5.0*	4.0*	15	9.7
913	8 months	NA	24	9.9	4.6*	15	6.3

NA=not available
[1]*Peak parasitaemia (parasites/ µl)*
[2]*Parasite Clearance Time (hours)*
**The patients received a blood transfusion*
***The patient had an abscess at injection site and the Hb dropped after the incision*

We observed in our patients a pattern similar to that reported[4] with respect to the time of occurrence of anaemia (median minimum Hb level at approximately 15 days) (Figure 6.1). The Hb pattern of patient 904 resembled the natural Hb course associated with malaria; the Hb level decreased during the first week post-treatment (early onset). Four patients showed a delayed decrease in the Hb level during the second or third week post-treatment with artesunate. Three patients showed a tendency to normalization of the Hb level by day 28. For patient 913, the Hb level normalized after day 28. Two of the three patients for whom parasitaemia results at admission were available were hyperparasitaemic, a condition reported to be a potential prognostic factor in a high proportion of patients with delayed haemolytic anaemia (CDC, 2013). No deaths were observed, and all patients fully recovered at the end of the follow-up period.

Figure 6-1: Hb time course of persons with severe anaemia at follow-up visits, Democratic Republic of the Congo



A limitation of this study was that we had no follow-up information for 29 patients because the follow-up was only expanded from 7 to 28 days after the start of the study and no resources were available for home visits. We could therefore not ascertain whether there were severe haemolytic events or deaths in this group. Half of these patients came from one site near the border with Angola, and some of these patients might have crossed the border. However, the site with the largest number of patients enrolled (n = 80) had a follow-up rate of 100%, and no cases of severe delayed anaemia or deaths were reported. In addition, three other sites that had 104 patients enrolled had follow-up rates of 95% and no deaths.

Another limitation of the study was that no control group was available and no markers of haemolysis were investigated beyond the Hb level. Thus, it is not possible to associate the delayed decrease in the Hb level observed during the follow-up period with artesunate

treatment or disease-specific pathologic processes. In addition, the lack of a control group did not enable measuring the contribution of potential confounders, such as soil-transmitted helminth infections, or reinfections with *P. falciparum* within the 28-day follow-up period.

In a recent study conducted in Gabon and Ghana, delayed haemolysis occurred in 7% of children treated with injectable artesunate (Rolling et al., 2014), which is consistent with our findings. Given that artemisinin derivatives could increase the cumulative total dose of artemisinins, thus contributing to an increment in the risk of delayed haemolysis, the risk versus benefit of using alternative antimalarial drugs should be considered. Unfortunately, data for hematologic follow-up of patients after antimalarial treatment are still scarce, including for quinine treatment. A study of imported malaria cases in the United Kingdom reported a decrease in Hb levels in 61% of cases 5–21 days after treatment with quinine (Ladhani et al., 2005).

The proportion of patients with severe anaemia in our study groups was below 1% for the whole duration of the follow-up period. In all cases, delayed anaemia was clinically manageable with appropriate and prompt care. The outcomes of this study supports recent WHO recommendations (WHO, 2013a) for continued use of injectable artesunate as a life-saving treatment and that healthcare professionals should be made aware of the potential for delayed haemolytic anaemia for up to one month post-treatment and encouraged to actively monitor patients during this period. Our data support the need for strengthening pharmacovigilance systems as recommended by a meeting convened by Medicines for Malaria Venture that involved malaria experts working in the field of severe malaria (Medicines for Malaria Venture (MMV)).

7 Feasibility and acceptability of injectable artesunate for the treatment of severe malaria in the Democratic Republic of Congo

Henry Maggi Ntuku[1], Giovanfrancesco Ferrari[2,3], Christian Burri[2,3], Antoinette Tshefu Kitoto[1], Didier Kalemwa Mitembo[2,3], Christian Lengeler[2,3]

[1] Kinshasa School of Public Health, Kinshasa, Democratic Republic of the Congo.

[2] Swiss Tropical and Public Health Institute, P.O. Box, 4002, Basel, Switzerland

[3] University of Basel, Basel, Switzerland.

This paper has been published in the *Malaria Journal* 2016, **15:**18.

7.1 Abstract

Background

The Democratic Republic of the Congo (DRC) changed its national policy for the treatment of severe malaria in both children and adults in 2012 from intravenous quinine to injectable artesunate. The country is now planning to deploy nationwide injectable artesunate as the preferred treatment for the management of severe malaria. To support this process, we assessed the feasibility and acceptability of the use of injectable artesunate in the context of the DRC, from the perspective of both health care providers and patients/caretakers.

Methods

Questionnaires and observations were used to collect information from health care providers and patients/guardians in 8 health facilities in the Province of Kinshasa and in the Province of Bas-Congo

Results

A total of 31 health care providers and 134 patients/care takers were interviewed. Seventy five percent (75%) of health care providers found it less difficult to prepare injectable artesunate compared to quinine. None of them encountered problems during preparation and administration of injectable artesunate. The large majority of care providers (93%) and patients/care takers (93%) answered that injectable artesunate took less time than quinine to cure the symptoms of the patients. Twenty six (84%) health care providers reported that the personnel workload had diminished with the use of injectable artesunate. Seven (22.6%) health workers reported adverse drug reactions, of which a decrease in the hemoglobin rate was the most common (56%). All care providers and the majority of patients/guardians (96%, N=128) were either satisfied or very satisfied with injectable artesunate.

Conclusions

Our findings show that the use of injectable artesunate for the treatment of severe malaria is feasible and acceptable in the context of DRC, with appropriate training of care providers.

Both care providers and patients/guardians perceived injectable artesunate to be effective and safe, thus promoting acceptability.

7.2 Background

In the Democratic Republic of the Congo (DRC), malaria is one of the leading causes of death in children under 5 years of age, with an estimated 9,000,000 cases and 22,000 deaths reported in 2012 (PNLP, 2013a). As a result, the DRC is the second country in the world in terms of burden of malaria (PNLP, 2013b; WHO, 2011c). For severe malaria, the case fatality is reaching 10% (Likwela et al., 2012).

Severe malaria is obviously a medical emergency, and reducing its burden is currently the highest priority of malaria control, as evidenced by the Roll Back malaria (RBM) target of near-zero deaths by 2015 (Roll Back Malaria Partnership, 2011).

For the management of severe malaria cases, comparative clinical trials between quinine and injectable artesunate have demonstrated that the treatment with artesunate was associated with a substantial reduction of case fatality in both children and adults (A. Dondorp et al., 2005; A. M. Dondorp et al., 2010; Sinclair et al., 2012). In addition, intravenous artesunate offers a number of programmatic advantages over quinine in terms of not requiring rate-controlled infusion or cardiac monitoring (WHO, 2013b). These results led to a change in the WHO guidelines for the treatment of severe malaria in 2011, recommending intravenous artesunate as the preferred treatment for severe malaria in children and adults (WHO, 2011a). As a result of this change, an additional 195,000 deaths could be averted every year in Africa (MMV, 2012).

Following the new WHO guidelines, the *Programme National de Lutte contre le Paludisme* (PNLP) of the DRC changed the national policy for the treatment of severe malaria in both children and adults from intravenous quinine to injectable artesunate in 2012 (PNLP, 2012).

However, this policy change requires a number of clinical and operational adaptations, as quinine has been the treatment of choice for many decades.

One important element for a successful transition, besides logistical aspects, is that there is good acceptability of the new treatment by the health care providers, as well as by the patients. Finally, there is also a need to determine the perceived effectiveness and safety of the new treatment. These factors are a prerequisite for achieving a successful rollout and therefore high public health impact. Here we investigate the feasibility and acceptability of the use of injectable artesunate in the context of the DRC, in order to identify arising issues and propose solutions before the start of the national roll-out.

Although a number of studies have investigated the efficacy of Injectable artesunate for the treatment of severe malaria as well as some issues related to its use (Burri et al., 2014; Rolling, Agbenyega, et al., 2013; Rolling, Wichmann, et al., 2013), to our knowledge, none has focused so far on the feasibility of the implementation of the new IV/IM antimalarial drug from the perspective of care providers, as well as its acceptability from the perspective of the patient/caretaker.

7.3 Methods

Study sites and population

This study was conducted between January and July 2013 as part of the MATIAS study (Treatment of severe malaria – An operational comparative study for the treatment of severe malaria between quinine and artesunate in Hospitals and Health Centres of Kinshasa and Bas Congo province). The MATIAS study was a non-controlled longitudinal observational comparative descriptive implementation study conducted in children and adults admitted with severe malaria to hospital and health centres.

The study was implemented in 8 health facilities, of which 3 hospitals and 5 health centres in Greater Kinshasa, the capital of the DRC (Referral Hospital Roi Baudoin, Health Centre Bita,

Health Centre Menkao) and in the Province of Bas Congo (Referral Hospital Saint Luc Kisantu, Health Centre Ngeba, Referral hospital of Kimpese, Health centre Ceco, Health centre La Famille). Figure1 shows the location of the study sites.

Kinshasa sites were visited by urban and semi-rural populations, whereas the Bas-Congo sites served largely a rural population. All sites are hyper to holoendemic for malaria and transmission is perennial with seasonal variation (Malaria Atlas Project, 2010; S. M. Taylor et al., 2011). At the time the study started, injectable artesunate had not been deployed to public health facilities and was not available in the private sector.

The study was conducted in two consecutive phases; in the first phase, in the 8 selected study sites, a target number of 350 patients was recruited over three months, from October 2012 to January 2013, with intravenous quinine as the treatment drug. In the second phase, following the introduction of injectable artesunate the same target number of patients was recruited over the following three months period, from April to July 2013. Between the two phases a three months interval was kept in order to train the hospital and health centres healthcare providers in the preparation and administration of the new drug.

The MATIAS study included four key components: (1) clinical assessment of patients, (2) a time and motion study, (3) financial costs, (4) feasibility and acceptability assessments through provider and patient/caretaker questionnaires

The results of the first three components are reported elsewhere (Ferrari et al., 2015), while the results of the fourth component are reported here.

All interviews for the feasibility and acceptability assessment were conducted during the second phase (Artesunate phase), since the aim was chiefly to have comparative quinine versus artesunate assessments. Participants belonged to two groups with separate questionnaires: (1) Health care providers who prescribed or administered the drug (2) Patients or guardians of patients who were treated with injectable artesunate in one of the study sites.

Training and implementation of injectable artesunate

In preparation for the first part of the study (quinine treatment), a three-day training on study procedures was given to all investigators and staff involved in patient's clinical management in each hospital and health centre. The training included an update of knowledge on malaria diagnosis and management.

Before starting the second phase, a two-day training on the preparation and administration of injectable artesunate was given to all staff involved in clinical management in the study sites. During these sessions we used a new training tool kit provided by the Medicines for Malaria Venture (MMV) product development partnership (MMV). This kit consisted of a very detailed user guide, an explicit and straightforward job aid and a practical training video. Prior to patient recruitment, health care providers were allowed some time to become familiar with handling of the new drug under supervision.

Injectable artesunate (Guilin Pharmaceutical Co, Ltd, Shanghai, China) was packed in boxes containing each 1 vial of 60 mg of artesunate powder for injection, 1 ampoule of sodium bicarbonate and 1 ampoule of sodium chloride. The following steps were necessary prior to drug injection: (1) calculation of number of vials required based on patient weight, (2) reconstitution of artesunate solution with sodium bicarbonate solution, (3) dilution of the solution with sodium chloride.

Artesunate was given intravenously at a dose of 2.4mg/kg bodyweight at 0, 12 and 24 hours, and then once a day. In line with the WHO recommendations (WHO, 2013b), parenteral treatment was given for a minimum of 24h irrespective of the patient's ability to tolerate oral medication. After completion of the injectable treatment, the patient was given a full course of a recommended oral artemisinin-based combination therapy. Parenteral artesunate was given for a maximum of 7 days, until oral treatment could be taken reliably.

Patients were followed up at day 7, day 14, day 21 and day 28 after discharge. Artesunate was provided free of charge by the manufacturer (Guilin Pharmaceuticals, Shanghai, PDR China)

while the costs of quinine were covered by the study. Hence the treatment was free for all patients. In each site the study recruited local clinicians to manage patients, while the research team carried out a weekly supervision at each study site throughout the duration of the study.

The National Malaria Control Program provided political support. All authorizations for drug importation were obtained from the Ministry of Health through the National Drug Authority. All relevant authorities were actively involved in the planning of the study and preliminary results of the study were shared and discussed during stakeholders meetings. Unpublished preliminary results of the study were used by the National Malaria Control Program to develop training manuals for healthcare providers and communication tools in prevision of deployment of injectable artesunate.

Data collection

Two questionnaires were used to collect data. Interviews were conducted by nine trained interviewers recruited from the local community. Two of them were physicians, 4 were nurses and 3 were social workers. These interviewers were supervised by study field scientists. A three-day training was given to all interviewers prior to data collection. The training included familiarization with the study tools and practicing interviews. Basic techniques of probing and recording responses were also discussed during the training. Interview guides were developed and pre-tested prior to use.

Interviews with care providers focused on ease of application and drug handling, perceived safety of the treatment, quality of the patient management, perception of old versus new treatment on staff work load, and level of satisfaction with the new treatment. The core questions of the interviews compared injectable artesunate and quinine. While obviously there could have been a recall bias due to the fact that the interviews were done during the artesunate phase of the study, about 3-6 months after the quinine phase, this should not have

been too much of an issue since quinine has been used for decades in the DRC, and all health care providers were very familiar with it.

Interviews with patients / caregivers focused on the perception of the effectiveness and safety of injectable artesunate, especially with regard to adverse events. Here recall bias could have been more of an issue since patients were less familiar with quinine. In order to minimize this problem, one inclusion criterion for the interviews of patients/caretakers was a past experience with quinine treatment, either for themselves or for one member of the family.
Interviews were conducted in French, the official language in DR Congo or in Lingala and Kikongo, the national languages spoken in Kinshasa and Bas–Congo, respectively. Interviews typically lasted from a minimum of twenty to a maximum of thirty minutes. Multiple choice closed-ended questions were followed by open-ended questions to collect narratives responses. All answers were written down in French by the interviewers.

Ethics

The MATIAS study protocol was reviewed and approved by the ethics committee of the Kinshasa School of Public Health (University of Kinshasa) and by the ethics commission of both cantons of Basel, EKBB (Ethikkommission beider Basel) in Switzerland. Informed consent was obtained from health care providers, patients and care givers who participated in the study.

Data processing

Data were entered electronically using Epi data 3.1 (Epidata Association; Odense, Denmark). After standard quality control checks, data were transferred to Stata version 12 (Stata Corporation; College Station, Texas) for analysis. Qualitative data were summarized in emerging themes and presented as proportions of different variables. Some answers are

reported as narratives. Categorical variables were compared using pearson's Chi square test or fisher's exact test in case the expected value of any of the cells of the table was less than 5. A p-value ≤0.05 was considered as statistically significant.

7.4 Results

Health care providers

Key results of interviews with health care providers are summarized in Table 7.1. A total of 31 health care providers were interviewed, whereby medical doctors and nurses accounted for 22.6% (7/31) and 77.4% (24/31) of the interviewed personnel, respectively. The median number of providers interviewed per health facility was four, ranging from three to five per facility. The majority of the personnel interviewed (28/31, 90.3%) had more than three years of working experience, whilst three individuals (9.7%) had one to three years experience. None of the health care providers interviewed had used injectable artesunate before the beginning of the study.

Table 7-1: Summary of interviews with health care providers

Question / Parameter	Frequency	Percentage
Did you find more or less difficult to prepare artesunate compared to quinine (N=24)?		
More difficult	3	12.5
Same difficulty	3	12.5
Less difficult	18	75
Have you noticed any adverse effect that you think could be related to artesunate (N=31)?		
Yes	7	22.6
No	24	77.4
Do you think that the workload has reduced with artesunate compare to quinine (N=31)?		
The workload has diminished	26	83.9
The workload is the same	4	12.9
The workload has increased	1	3.2
What is your level of satisfaction with injectable artesunate (N=31)?		
Satisfied	12	38.7
Very satisfied	19	61.3

No answer was given for the category "dissatisfied".

Ease of use

Questions related to the handling of the drug were only asked to the 24 nurses who were responsible for the drug preparation and administration.

Compared to quinine, eighteen (75%) of all interviewed nurses reported to have spent less time to prepare and administer injectable artesunate, three (12.5%) spent more time and three (12.5%) said to have spent the same time. Eighteen (75%) found less difficult to prepare injectable artesunate compared to quinine, three (12.5%) found more difficult and three reported to have experienced the same difficulty. All those who found it more difficult to prepare injectable artesunate compared to quinine specified that too many steps were needed in artesunate preparation. For patients above 50 kg body weight, a minimum of 3 vials are needed for a single dose and obviously this increased the time spent in drug preparation since each vial must be opened and reconstituted separately.

All interviewed nurses involved in the administration of the treatment found it less difficult to administer artesunate compared to quinine. The most important reasons cited by the respondents were the rapid way of administration (48.4%), no accidents related to infusion (35.5%) and the reduced patient monitoring time (16.1%). None of the nurses interviewed encountered problems during drug preparation and drug administration.

All patients' files reviewed during supervision visits showed that health care providers handled and administered the drug according to recommendations in terms of dosing and time of administration.

Perceived effectiveness and safety

Regarding the time to observe the effects of injectable artesunate on their patients, twenty eight (93.3%) health workers reported that it took less time compared to quinine, one (3.3%) estimated it took the same time and one (3.3%) estimated it took more time. Thirty (96.7%)

health care providers reported to be very satisfied with the capacity of injectable artesunate to cure the symptoms of their patients compared to quinine, one (3.2%) experienced the same satisfaction level, and none found AS less satisfactory.

Seven (22.6%) health workers reported to have noticed adverse drug reactions: The most common ones mentioned were a decrease of hemoglobin rate (55.6%), shivering following the drug injection (33.3%) and loss of weight (11.1%). However, all the seven health care providers who reported adverse drug reactions answered that they were less frequent than those observed with quinine (data not shown).

Patient management

The majority (96.8%) of health care providers reported to have dedicated less time for patient monitoring after administration of artesunate compared to quinine. This proportion was not significantly different according to the type of health facility (hospitals vs. health centres; p=0.388 Fisher's exact test). Of all health care providers interviewed, twenty six (83.9%) reported that the personnel workload had diminished with the use of injectable artesunate, four (12.9%) reported the workload to be the same, while one (3.2%) reported that the workload had increased (Table 7.1). The most important reasons for reported workload reductions were reduced patient monitoring time (47.9%), gain of time by health personnel (43.8%) and shorter treatment duration (8.3%). A reason reported by one health care provider from Ngeba Health Centre for workload increase was increased patient monitoring time.

Care providers general satisfaction

When health care providers were asked about their level of satisfaction with injectable artesunate they were either satisfied (38.7%, 12/31) or very satisfied (61.3%, 19/31) with the new treatment, with nobody giving a negative feedback (Table 7.1). Reasons for being satisfied/very satisfied were lack of side effects (22.4%), rapid action of the drug (19.7%), the

easy way to prepare and administer the drug (11,8%) and injectable artesunate being more effective (11.8%) compared to quinine. The level of satisfaction towards injectable artesunate was not significantly different among type of health facility (hospitals vs. health centres; p=0.452 Fisher's exact test) and health care providers (medical doctors vs. nurses; p=0.384 Fisher's exact test).

A nurse said about Injectable artesunate: "… *I am very satisfied, it makes work easier, we have good time management, patient monitoring has been improved, there are no side effects, it has reduced mortality rate among children treated, the drug has attracted many patients to come to our health facility*".

A medical doctor stated: "*very satisfied ... it responds well, no side effects, but there's a risk of a high cost because it is so precise and easier to use that such a product can only be more expensive than quinine. .. Good outcome after treatment*"

Patients or caretakers

Results of interviews with patients/caretakers are summarized in Table 7.2. A total of 134 patients/care takers were interviewed (124 care takers and 10 patients aged 12 years or older). There were more female (73.3%, 96/134) than male (26.7%, 35/134) respondents (p-value<0.05). Of the 124 care takers interviewed, seventy six (61.3%) were mothers of patients, thirty three (26.6%) were fathers, fourteen (11.3%) were other members of the family and the remaining one (0.8%) was another member of the neighbourhood who accompanied a 2 years old female patient at Ceco Health Centre.

Effectiveness and safety

With regards to the time needed for injectable artesunate to cure the symptoms of the patients, the large majority of respondents (93.3%, N=125) felt that it took less time compared to quinine, while eight (5.9%) respondents said it took the same time and one (0.7%) more time. Forty six (34.6%) respondents reported to have noticed adverse events; asthenia (42.6%) and loss of appetite (10.3%) were the most common ones, while eighty seven (65.4%) did not report any complication. The proportion of care takers/patients reporting adverse events was not significantly different from that of care providers (p-value=0.2). Statistical analysis showed no significant difference in the occurrence of adverse events between patients under and above five years of age (p-value=0.687). Of those who reported to have noticed adverse events, twenty seven (65.9%) considered that they were less than those observed with quinine, while seven (17.1%) and one (2.4%) said respectively they are the same and more than those observed with quinine. Six (14.6%) did not know. Given the point made above on recall bias, these results should be interpreted with some caution.

Satisfaction

Regarding general satisfaction towards the ability of injectable artesunate to cure the symptoms that motivated the patients' consultation, the vast majority of patients/care takers (95.5%, N=128) reported to have been either satisfied or very satisfied. Six (4.5%) reported being less satisfied than with quinine, of whom three reported persistent fever as a main reason for their dissatisfaction, while two (33.3%) reported asthenia and dizziness. One respondent said he did not know what could be the long term side effects of this new drug. Patients/care takers level of satisfaction was not significantly different among type of health facility they consulted. (P-value=0.46, Fisher's exact test) and patient's age (p-value=0.77 Fisher's exact test). When asked if they would choose or recommend again injectable artesunate over quinine next time for themselves or a family member, the majority of respondents (97.7%) said they would choose injectable artesunate. The most important

reasons for choosing artesunate were rapid action (35.3%), no or less adverse events(33.4%), shorter treatment course and shorter hospital stay (19.9%).

A mother said: "*This is a short duration treatment, the symptoms disappear quickly. There is less manipulation compared to quinine, where you have to be in bed for 4 hours of infusion but with this treatment, just a few minutes of injection. This drug takes less time compared to quinine and there are no side effects. I think it is better suited to malaria treatment for children*"

A young mother said: "*Very satisfied - After the first injection, my child was doing fine already. The fever had dropped quickly. The treatment duration is very short. We stayed for a short time at the hospital*".

Table 7-2: Summary of interviews with patients/caretakers

Question / Parameter	Frequency	Percentage
Have you noticed any side effect that you think could be related to artesunate?		
Yes	46	34.3
No	88	65.7
If you had to make the choice in the future between quinine and artesunate, which one would you choose? (N=121)		
Quinine	4	3.3
Artesunate	117	96.7
Most important reasons for choosing injectable artesunate instead of injectable quinine (N=117)		
Rapid action	55	35.3
No side effects	38	24.4
Short treatment course	24	15.4
Less side effects	14	9.0
Rapid way of administration	13	8.3
Short hospital stay	7	4.5
More efficacious	5	3.2
What is your level of satisfaction towards injectable artesunate?		
Dissatisfied	6	4.5
Satisfied	66	49.2
Very satisfied	62	46.3

7.5 Discussion

This study was designed to assess the feasibility and acceptability of the implementation of IV/IM artesunate from the perspective of care providers, as well as its acceptability versus quinine treatment from the perspective of the patients / caretakers.

Results clearly show that utilization of injectable artesunate for the treatment of severe malaria in the context of the DRC is feasible and very well accepted. Patients/caretakers were very receptive to the new drug as it is perceived as being highly efficacious. Despite a few number of health providers reporting that several steps were needed in the preparation of artesunate, the handling of the drug was perceived to be easy. The vast majority of providers reported to have spent less time in this task. This is consistent with the results of quantitative measures of time and motion reported by Ferrari et al., which showed that the overall cumulative staff time dedicated to drug pre-administration tasks was 20 minutes for quinine compared to 13 minutes for artesunate. This difference is expected to improve in favour of the latter with health personnel gaining more experience (Ferrari et al., 2015).

Drug formulation had a significant impact on the duration of the preparation and administration. The drug used in the study was packaged in vials of 60 mg which, when reconstituted, was equivalent to 6 ml of solution for intravenous route. For an average 60 kg body weight adult, this equates to prepare 3 vials and repeating three times all steps of preparation, resulting in a longer preparation time. On the other hand, this drug formulation may cause significant drug wastage especially in small children who need small quantities. As the reconstituted solution is stable for only one hour, and that an opened vial cannot be reused, it is possible to lose up to more than half of the vial. In the context of limited resources, it is important that drug manufacturers develop adapted and easy-to-use forms of injectable artesunate.

The observations that health providers reported the use of artesunate to be easy and less time is required for patient management compared to quinine are in related to the characteristics of

the two drugs. Contrary to artesunate, the administration of quinine requires special precautions because of its potential toxicity, and close monitoring of the patient as the risk of incorrect dosage and side effects are common (AlKadi, 2007; W. R. Taylor & White, 2004; WHO, 2013b; Wolf et al., 1992).

The superior efficacy of injectable artesunate compared to intravenous quinine in the management of severe malaria has been demonstrated in clinical trials (A. Dondorp et al., 2005; Dondorp et al., 2010; Sinclair et al., 2012). Because of its small scale nature based on purposive sampling, this study cannot draw a conclusion on the effectiveness of injectable artesunate. However, both health care providers and patients/guardians perceive artesunate to be highly efficacious.

Findings of this study are consistent with what is known so far about the better short term safety of artesunate compared to quinine (Sam-Wobo et al., 2012; Sinclair et al., 2012), the most common adverse events reported by health workers was a decrease in haemoglobin, a fact supporting recent findings on the occurrence of delayed anaemia after parenteral artesunate for severe malaria (Ferrari et al., 2015; Rolling et al., 2014; Rolling et al., 2013). However, the training received by health workers before the implementation of artesunate had an emphasis on the monitoring of adverse events and especially a drop in hemoglobin, and this may have influenced the frequency of reporting.

The majority of interview questions were comparative between quinine and artesunate and since artesunate was implemented second, this could have led to a recall bias, especially for interviews with patients/care takers. The inclusion criterion of past experience with quinine treatment, either for themselves or for one member of the family was made to minimize this issue.

The design with a lack of concurrent controls and the relatively small scale nature of the study constitute a limitation to the generalizability of findings. Thus, the results of this study cannot be used to draw conclusion on the safety of intravenous artesunate, but rather only as supportive evidence to the acceptability of the new treatment.

One of the major challenges in switching from quinine to injectable artesunate is adherence of practitioners. Health workers may be reluctant to switch to a new treatment (MSF, 2011). In our study, the majority of health care providers were not aware of the latest evidence on safety and efficacy. It is important to promote the benefits of injectable artesunate among health workers and train them well in the use of the new treatment. The new treatment guidelines should be included in training curricula in medical and nursing schools and public awareness of the new drug should be raised through effective communication canals.

Our findings showed that the use of injectable artesunate for the treatment of severe malaria is feasible and acceptable in the context of the DRC and has the potential of improving the quality of patient management, and thus improving outcome of this life threatening condition.

8 General discussion and conclusions

The main purpose of the first part of this thesis was to update data on the prevalence of malaria among children aged six to 59 months in Kinshasa, the capital city of the Democratic Republic of the Congo (DRC), as well as to compile a comprehensive report on ongoing malaria control activities. Because Kinshasa has been expanding very rapidly over the past twenty years and the last epidemiological study dates back to 2000 (Kazadi et al., 2004), the updating of these data is urgently required. The first comprehensive map of malaria risk at the scale of the health area in greater Kinshasa is now available.

Generally, our findings, as described in Chapter 3, emphasise that the prevalence of malaria has decreased in Kinshasa over the last thirty years, and that the risk is higher in the peri-urban areas of recent occupation. At the same time, malaria control activities in Kinshasa have made significant progress in key malaria indicators compared to the DHS 2007 results (DHS-DRC, 2007). Nevertheless, ITN coverage is still too low in the less accessible areas on the periphery. Based on these results a number of suggestions for future research and interventions in urban malaria control were made.

In Chapter 4, a logistic regression analysis describes the contribution of various risk factors for *Plasmodium* infection in both children aged six to 59 months and individuals older than five years, and how these vary with varying prevalence in Kinshasa. In particular we observed that the effect of age and reported history of fever varied according to transmission setting. We observed a shift in the peak prevalence of malaria towards the older age groups, especially in the low transmission setting. For children under five years of age, the highest prevalence was observed in the 48 to 59 months group in both transmission settings but it increased more gradually for the lower transmission HZs ($p = 0.009$). In a separate analysis in children over five years in two selected HZs, the peak prevalence was in five to nine year-olds in the higher transmission setting and in 15 to 19 year-olds in the lower transmission setting.

Reported fever in the last two weeks increased the risk of having malaria in all age groups in all transmission settings. In addition, ITN usage was associated with a lower risk of malaria infection in children six to 59 months. Similar estimates were found in children over five years and the lower transmission HZ but the associations there were not significant. There was no evidence of a difference in these associations by strata. Finally, the risk of having anaemia decreased with increasing age, but was found to increase with malaria and reported fever. Socioeconomic status was also associated with the risk of having malaria and anaemia.

This thesis also aimed at proving the feasibility of using injectable artesunate for the treatment of severe malaria among health facilities in the DRC (Chapter 5), and to provide a cost estimate from the provider perspective at different levels of the health system. The DRC is one of the countries with the highest burden of severe cases of malaria and the rapid nationwide deployment of the new treatment is urgently required. We also assessed the perceived feasibility and acceptability of the new drug from both the health care and patient perspective. The results showed that injectable artesunate can be successfully offered by health care providers in hospitals and health centres in the DRC, with a reduced cost compared to quinine and with high acceptability by both patients and health-care providers. Although limited in design, the sub-study conducted in the frame of the MATIAS study described in Chapter 6, indicates a decrease in the hb levels during the seven to 21 days post-artesunate treatment.

8.1 Prospects for future research on risk map data

Continued routine malaria prevalence surveys are recommended to update the city malaria risk map in order to monitor progress in malaria control. Additional cross-sectional surveys carried out during the wet and dry seasons and covering Greater Kinshasa, done in a standardised way, could highlight differences in seasonal prevalence patterns. In the dry

season, malaria prevalence, although generally not very seasonal in areas of high transmission (Smith et al. 1993), can be much lower in areas of low transmission than in the wet season. Many surveys are carried out during the peak transmission season, potentially introducing a bias and this needs to be accounted for (Gemperli et al., 2006). Our risk map covers cross-sectional data from two different time periods and seasons and we have standardised malaria prevalence to allow a comparison and to reduce potential differences resulting from different seasons or years. We documented an increase in prevalence from the dry season in the year 2009 to the rainy season in the year 2011 in the HZ of Selembao (from 14.1 to 26.8%). However, we could not ascertain whether this reflected a true seasonal increase in the frequency of the disease or a between-year natural evolution of malaria transmission.

By using the Inverse Distance Weighting (IDW) spatial interpolation technique in ArcGIS 10.3 (Esri, Redlands CA), the distribution pattern of malaria prevalence was smoothed in the Kinshasa area. The IDW technique uses prevalence values in locations that are nearby to predict prevalence in unsampled locations, based on the assumption that, on average, neighbouring points have similar values (Messina et al., 2011). However, given the complexity of malaria transmission, additional environmental covariates could improve the accuracy of predicted risk maps. Hence, a possible extension for our analysis would be to develop a risk map including such environmental variables.

8.2 Prospects for malaria control in Kinshasa

The prevalence of malaria is highly dependent on the EIR (Beier et al., 1999), as well as other factors such as socioeconomic status, use of personal protective measures, the availability of effective treatment, urbanisation and age (Geissbuhler et al., 2007). Frequency, longevity of infection and disease outcomes are also interrelated (Smith et al., 2005). These factors could be important for explaining the heterogeneous infection patterns observed in the more central

urbanised areas of Kinshasa, based on experience obtained from other urban settings in Africa (Caldas de Castro et al., 2004; Keiser et al., 2004; Matthys et al., 2006; Staedke et al., 2003; Wang et al., 2005). Future malaria control measures will require adopting policies based on the specific context and the ecology of transmission to best target populations at higher risk.

In the DRC, existing distribution programmes are scaling up ITN with the support of many partners. The high coverage of ITNs (78.7%) seen in the 2009 survey has almost certainly contributed to a decrease in malaria transmission. In certain peripheral districts of Kinshasa, however, access to ITNs is still limited (Kikimi 37.0%; Byiela 34.4%; Kimbanseke 31.0%). The overall reduction of 27% in ITN ownership between the two surveys stresses the well-known issue of net coverage decay and calls for stronger programmes for routine ITN distribution in order to maintain coverage between campaigns. Our estimate is slightly above the expected 20% loss of nets in the second year post campaign, as assumed by the RBM Harmonisation Group. Currently, the NMCP recommends replacing nets every three years (PNLP, 2013a). In addition, complementary distribution measures (keep-up strategies) should be considered, in particular targeting the less accessible health areas (Grabowsky et al., 2007; Lengeler et al., 2007). It is evident that net attrition begins a few months after distribution campaigns, either because nets deteriorate or are given away, or because of population growth (Ochomo et al., 2013; Zhou et al., 2014). In addition, strengthening ITN distribution through antenatal care would likely increase the still too low utilisation rate among pregnant women.

Our findings indicate that history of fever has a low positive predictive value in identifying children with malaria in urban settings, consistent with results from other large cities in sub-Saharan Africa (Pond, 2013; Wang et al., 2006; Wang et al., 2005). This leads to overtreatment and a waste of resources and exposes patients to unnecessary side effects or to the risk of not being treated correctly for an alternative cause of fever (D'Acremont et al., 2010; Wang et al., 2006). In case of fever in Kinshasa, it is common practice by caregivers to

initially treat their child at home (54% of the cases) and private drug outlets represent the principal source of drugs (96.3% of all obtained drugs).

The high proportion of asymptomatic infections, particularly in older children and adults in high-transmission areas, plays a fundamental role in ensuring transmission in these areas (Greenwood et al., 1987). In order to reduce further the risk of transmission and hence parasitaemia, additional control measures including environmental management and larviciding could be beneficial. Very little research has been done on larval ecology and control in Kinshasa, and the last entomological studies on vectors date back to the end of the 1980s (Coene, 1993). Larval control should be considered an integral part of vector control programmes to prevent remaining transmission, including outdoor biting, together with a good vector monitoring system (Sharp et al., 2007). Larviciding could be feasible particularly in those central urban areas where prevalence is low and breeding sites are easily detectable. Larviciding has proven to be cost-effective for urban malaria control (Maheu-Giroux & Castro, 2014) but it remains to be established whether this approach would be feasible in Kinshasa. Finally, there are studies reporting exophagic behaviour in *Anopheles* species in urban Africa (Geissbuhler et al., 2007; Oyewole & Awolola, 2006) and this has clear implications for personal protection through ITN use.

The current malaria information system in DRC is based on passive detection of clinical malaria cases; hence asymptomatic individuals are not detected and dealt with. More measures targeting parasites in asymptomatic populations could be effective in addition to early diagnosis and treatment. The identification of clusters of asymptomatic carriers could be particularly useful in areas of the city where transmission has decreased to the point where no further progress can be expected using existing measures (Bautista et al., 2006; Ernst et al., 2006; Gaudart et al., 2006). In such areas a small fraction of human hosts is likely to maintain transmission during the low transmission season (Clark et al., 2008; Mwangi et al., 2008;

Smith et al., 2005; Trape et al., 2002). Possible approaches to targeting parasite reservoirs include school surveys to identify possible hotspots in the community (Bousema et al., 2012). Furthermore, sensitive serological markers have been shown to be promising in defining small-scale variations in malaria exposure and in guiding targeted malaria control efforts (Bousema et al., 2010).

8.3 Prospects for the implementation of injectable artesunate in the DRC

There have been concerns that malaria control could lead to an increase in the number of cases of severe malaria in older age groups following a decline in transmission and the later acquisition of immunity (Carneiro et al., 2010; Ceesay et al., 2008; Griffin et al., 2015; Robert et al., 2003; Schellenberg et al., 2004). The observation of increased risk of malaria in older age groups in Kinshasa seems to point in this direction, and highlights the need for a more equal distribution of ITNs.

Our findings also point to increased attention being paid to severe malaria in older individuals and the fact that injectable artesunate has been clearly shown to be highly efficacious, acceptable and cost-effective in the treatment of this condition is a positive development. Operational research has helped to define the way in which an intervention of proven efficacy (Dondorp et al., 2010) can be implemented in settings of routine care (Bedelu et al., 2007; Culbert et al., 2007; Van Griensven et al., 2008; Zachariah et al., 2009).

8.4 Challenges to the introduction of injectable artesunate in the DRC setting

There may nevertheless be obstacles that could hinder the nationwide implementation of injectable artesunate. Firstly, the correct management of cases of severe malaria can be challenging in resource-limited settings (Achan et al., 2011; Losimba Likwela et al., 2012). In

DRC the percentage of children with severe malaria who are correctly managed is still very low (PNLP, 2013a). In Nigeria, the first country in Africa to officially adopt injectable artesunate in April 2011, low knowledge about severe malaria among health workers was a key challenge (unpublished information, Medicines for Malaria Venture [MMV]). Distinguishing severe malaria from other febrile infection can be difficult in the absence of laboratory confirmation, leading to under- or over-reporting of number of cases of severe malaria (Makani et al., 2003; Mudenda et al., 2011; Snow et al., 1992). Hence, the provision of an effective treatment will only have an impact on clinical outcome if it is accompanied by improvements in the management of severe malaria (Achan et al., 2011). This will be particularly relevant at the peripheral health care level, where nurses are mainly responsible of the provision of care. In addition, a large proportion of severe malaria illnesses and death occur in people's home, without coming to the attention of a formal health service (Greenwood et al., 1987; Mudenda et al., 2011; WHO, 2014). Re-training and updating the knowledge of clinical health workers on severe malaria diagnosis and case management will be critical to successful implementation, as will be the better engagement of caretakers of children and the community at large.

Improving laboratory capacity to either properly prepare thick blood smears or introduce reliable RDTs will increase the reliability of the results, which is likely to be low in the DRC setting (Mukadi et al., 2011). Children with delayed haemolysis seem to have in common higher parasitaemia. Hence, correctly measuring the level of parasitaemia could be helpful in dealing with the risk of delayed haemolysis following artesunate therapy (Jaureguiberry et al., 2014; Rolling, Agbenyega, et al., 2013; Rolling et al., 2012; Zoller et al., 2011).

Secondly, in endemic countries, post-discharge complications may remain undetected due to deficiencies in health care (Cramer et al., 2011; Rolling et al., 2014). This could explain why no cases of late onset anaemia/haemolysis have been reported from pharmacovigilance

systems in endemic areas so far (to the WHO centre in Uppsala, Sweden). A study done in Gabon and Ghana showed that delayed haemolysis occurred with a frequency of 7% among children treated with injectable artesunate, with clinically relevant consequences for one patient who required multiple blood transfusions (Rolling et al., 2014). MMV has proposed that haematological parameters and serological markers of haemolysis should be assessed weekly on days 0, 3, 7, 14, 21 and 28 to ascertain potential changes in hb levels (MMV Expert Meeting, Vienna). However, at present it is unclear how realistic this recommendation is, given the deficiencies in the laboratory systems at peripheral level. Our findings show that it can be feasible provided that patients receive transportation vouchers and other incentives to encourage them to make follow-up visits. Clearly, pharmacovigilance systems should be strengthened at country level with a strong involvement of the WHO Programme for International Drug Monitoring, to improve the detection of adverse events following the use of artesunate. In addition, further research is needed to understand the pathophysiological mechanism of delayed haemolysis and to define the frequency and magnitude of this event, and possibly be able to prevent it in the future.

Thirdly, in our study, injectable artesunate vials were donated by Guillin Pharmaceuticals and were consistently available; hence we could not investigate issues of drug stock out, which represent a major constraint in managing malaria and other diseases in the DRC. Strengthening the supply chain will therefore be critical to a successful implementation.

Fourthly, adherence of the health personnel to the new policy can also be challenging. For example, many factors are linked to the persistently low use of ACTs, despite a policy of general ACT use since 2005, with the consequence that chloroquine and oral quinine are still widely used (ACT Watch report). Reluctance to change among health staff was observed on the ground during preliminary visits to selected health centres for this study. Qualitative findings from Chapter 5 indicate a high level of acceptance of the new drug by health care

providers, following a carefully planned introduction of the new drug and consistent availability. Similarly, patients showed a high level of acceptance, and those who could recall previous treatment with injectable quinine perceived the new drug as a more effective treatment compared to quinine. Hence, promoting the benefits of injectable artesunate to clinicians and patients by providing additional arguments in situations where the health staff are reluctant to accept the change of treatment will be a key step in the dissemination of the new policy.

Fifthly, financial costs are a highly sensitive aspect for governments and international buyers. Findings from Chapter 3 provide a cost estimate for treating a case of severe malaria in real situations, and the fact that injectable artesunate is not more expensive when considering all costs should be useful and assist in the calculation of the national financial requirements required for its introduction. Training, communication supervision and monitoring should be included in the procurement costs (unpublished Stakeholders' Meeting Report).

Owing to the urgency to fit the results of the MATIAS study into the implementation plan of the Ministry of Health, the study was small in terms of the number of facilities surveyed. Consequently, the generalisability of the findings may be compromised as a result of the heterogeneity within of the facilities and whether these facilities are representative within and outside the DRC.

Despite these shortcomings, however, our results clearly support the need for the rapid adoption of injectable artesunate as a life-saving treatment by malaria endemic countries as recommended by the WHO. Currently, a large initiative supported by UNAID is supporting the procurement and introduction of injectable artesunate in the DRC, and hence the prospects are good for the improved treatment of severely ill children in the country.

9 References

Achan, J., Tibenderana, J., Kyabayinze, D., Mawejje, H., Mugizi, R., Mpeka, B., Talisuna, A., & D'Alessandro, U. (2011). Case management of severe malaria--a forgotten practice: experiences from health facilities in Uganda. *PLoS One, 6*(3), e17053.

Afrane, Y. A., Klinkenberg, E., Drechsel, P., Owusu-Daaku, K., Garms, R., & Kruppa, T. (2004). Does irrigated urban agriculture influence the transmission of malaria in the city of Kumasi, Ghana? *Acta Trop, 89*(2), 125-134.

Alirol, E., Getaz, L., Stoll, B., Chappuis, F., & Loutan, L. (2011). Urbanisation and infectious diseases in a globalised world. *Lancet Infectious Diseases, 11*(2), 131-141.

AlKadi, H. O. (2007). Antimalarial drug toxicity: a review. Chemotherapy, 53(6), 385-391.

Alves, F. P., Durlacher, R. R., Menezes, M. J., Krieger, H., Silva, L. H., Camargo, E. P (2002). High prevalence of asymptomatic Plasmodium vivax and Plasmodium falciparum infections in native Amazonian populations. *Am J Trop Med Hyg*, 66: 641–648.

Awolola, T. S., Oduola, A. O., Obansa, J. B., Chukwurar, N. J., & Unyimadu, J. P. (2007). Anopheles gambiae s.s. breeding in polluted water bodies in urban Lagos, southwestern Nigeria. *J Vector Borne Dis, 44*(4), 241-244.

Ayele, D. G., Zewotir, T. T., & Mwambi, H. G. (2012). Prevalence and risk factors of malaria in Ethiopia. *Malar J, 11*, 195.

Baird, J. K., Bangs, M. J., Maguire, J. D., & Barcus, M. J. (2002). Epidemiological measures of risk of malaria. *Methods Mol Med, 72*, 13-22.

Balarajan, Y., Ramakrishnan, U., Ozaltin, E., Shankar, A. H., & Subramanian, S. V. (2011). Anaemia in low-income and middle-income countries. *Lancet, 378*(9809), 2123-2135.

Barbazan, P., Baldet, T., Darriet, F., Escaffre, H., Djoda, D. H., & Hougard, J. M. (1998). Impact of treatments with Bacillus sphaericus on Anopheles populations and the transmission of malaria in Maroua, a large city in a savannah region of Cameroon. *J Am Mosq Control Assoc, 14*(1), 33-39.

Batra, C. P., Adak, T., Sharma, V. P., & Mittal, P. K. (2001). Impact of urbanization on bionomics of An. culicifacies and An. stephensi in Delhi. *Indian J Malariol, 38*(3-4), 61-75.

Bautista, C. T., Chan, A. S., Ryan, J. R., Calampa, C., Roper, M. H., Hightower, A. W., & Magill, A. J. (2006). Epidemiology and spatial analysis of malaria in the Northern Peruvian Amazon. *Am J Trop Med Hyg, 75*(6), 1216-1222.

Bedelu, M., Ford, N., Hilderbrand, K., & Reuter, H. (2007). Implementing antiretroviral therapy in rural communities: the Lusikisiki model of decentralized HIV/AIDS care. *J Infect Dis, 196 Suppl 3*, S464-468.

Beier, J. C., Killeen, G. F., & Githure, J. I. (1999). Short report: entomologic inoculation rates and Plasmodium falciparum malaria prevalence in Africa. *Am J Trop Med Hyg, 61*(1), 109-113.

Biemba, G., Dolmans, D., Thuma, P. E., Weiss, G., & Gordeuk, V. R. (2000). Severe anaemia in Zambian children with Plasmodium falciparum malaria. *Trop Med Int Health, 5*(1), 9-16.

Bousema, T., Drakeley, C., Gesase, S., Hashim, R., Magesa, S., Mosha, F., Otieno, S., Carneiro, I., Cox, J., Msuya, E., Kleinschmidt, I., Maxwell, C., Greenwood, B., Riley, E., Sauerwein, R., Chandramohan, D., & Gosling, R. (2010). Identification of hot spots of malaria transmission for targeted malaria control. *J Infect Dis, 201*(11), 1764-1774.

Bousema, T., Griffin, J. T., Sauerwein, R. W., Smith, D. L., Churcher, T. S., Takken, W., Ghani, A., Drakeley, C., & Gosling, R. (2012). Hitting hotspots: spatial targeting of malaria for control and elimination. *PLoS Med, 9*(1), e1001165.

Brabin, B. J., Premji, Z., & Verhoeff, F. (2001). An analysis of anaemia and child mortality. *J Nutr, 131*(2S-2), 636S-645S; discussion 646S-648S.

Brooker, S., Akhwale, W., Pullan, R., Estambale, B., Clarke, S. E., Snow, R. W., & Hotez, P. J. (2007). Epidemiology of plasmodium-helminth co-infection in Africa: populations at risk, potential impact on anaemia, and prospects for combining control. *Am J Trop Med Hyg, 77*(6 Suppl), 88-98.

Burri, C., Ferrari, G., Ntuku, H. M., Kitoto, A. T., Duparc, S., Hugo, P., Mitembo, D. K., & Lengeler, C. (2014). Delayed anaemia after treatment with injectable artesunate in the democratic republic of the congo: a manageable issue. *Am J Trop Med Hyg, 91*(4), 821-823.

Byrne, N. (2007). Urban malaria risk in sub-Saharan Africa: where is the evidence? *Travel Med Infect Dis, 5*(2), 135-137.

Caldas de Castro, M., Yamagata, Y., Mtasiwa, D., Tanner, M., Utzinger, J., Keiser, J., & Singer, B. H. (2004). Integrated urban malaria control: a case study in dar es salaam, Tanzania. *Am J Trop Med Hyg, 71*(2 Suppl), 103-117.

Carneiro, I., Roca-Feltrer, A., Griffin, J. T., Smith, L., Tanner, M., Schellenberg, J. A., Greenwood, B., & Schellenberg, D. (2010). Age-patterns of malaria vary with severity, transmission intensity and seasonality in sub-Saharan Africa: a systematic review and pooled analysis. *PLoS One, 5*(2), e8988.

CDC. (2013). Published reports of delayed hemolytic anaemia after treatment with artesunate for severe malaria--worldwide, 2010-2012. *MMWR Morbidity Mortality Weekly Report, 62*(1), 5-8.

Ceesay, S. J., Casals-Pascual, C., Erskine, J., Anya, S. E., Duah, N. O., Fulford, A. J., Sesay, S. S., Abubakar, I., Dunyo, S., Sey, O., Palmer, A., Fofana, M., Corrah, T., Bojang, K. A., Whittle, H. C., Greenwood, B. M., & Conway, D. J. (2008). Changes in malaria indices between 1999 and 2007 in The Gambia: a retrospective analysis. *Lancet, 372*(9649), 1545-1554.

Clark, T. D., Greenhouse, B., Njama-Meya, D., Nzarubara, B., Maiteki-Sebuguzi, C., Staedke, S. G., Seto, E., Kamya, M. R., Rosenthal, P. J., & Dorsey, G. (2008). Factors determining the heterogeneity of malaria incidence in children in Kampala, Uganda. *J Infect Dis, 198*(3), 393-400.

Coene, J. (1993). Malaria in urban and rural Kinshasa: the entomological input. *Med Vet Entomol, 7*(2), 127-137.

Cramer, J. P., Lopez-Velez, R., Burchard, G. D., Grobusch, M. P., & de Vries, P. J. (2011). Treatment of imported severe malaria with artesunate instead of quinine--more evidence needed? *Malar J, 10*, 256.

Culbert, H., Tu, D., O'Brien, D. P., Ellman, T., Mills, C., Ford, N., Amisi, T., Chan, K., & Venis, S. (2007). HIV treatment in a conflict setting: outcomes and experiences from Bukavu, Democratic Republic of the Congo. *PLoS Med, 4*(5), e129.

D'Acremont, V., Lengeler, C., & Genton, B. (2010). Reduction in the proportion of fevers associated with Plasmodium falciparum parasitaemia in Africa: a systematic review. *Malar J, 9*, 240.

D'Ascenzo, F. (2010). La territorialità urbana a Kinshasa. *Università degli Studi di Padova*.

De Beaudrap, P., Nabasumba, C., Grandesso, F., Turyakira, E., Schramm, B., Boum, Y., 2nd, & Etard, J. F. (2011). Heterogeneous decrease in malaria prevalence in children over a six-year period in south-western Uganda. *Malar J, 10*, 132.

de Benoist, B., Fontaine, O., Lynch, S., & Allen, L. (2007). Report of the world health organization technical consultation on prevention and control of iron deficiency in infants and young children in malaria-endemic areas - Foreword. *Food and Nutrition Bulletin, 28*(4), S489-S490.

de Maximy, R., 1975, Site général, in: J. Flouriot, R. de Maximy, M. Pain, Atlas de Kinshasa, Kinshasa, Institut Géographique du Zarïe (IGZ), Bureau d'Études d'Aménagement du territoire. (1975).

Demographia (2014). World Urban Areas. *10th Ed. May 2014 Revision*.

Dempster TE (1848). Notes on the application of the test of organic disease of the spleen, as an easy and certain method of detecting malarious localities in hot climates, Agra. *(reprint in Rec Malar Surv India; 1930; 1: 69).*

Desai, M. R., Terlouw, D. J., Kwena, A. M., Phillips-Howard, P. A., Kariuki, S. K., Wannemuehler, K. A., Odhacha, A., Hawley, W. A., Shi, Y. P., Nahlen, B. L., & Ter Kuile, F. O. (2005). Factors associated with hemoglobin concentrations in pre-school children in Western Kenya: cross-sectional studies. *Am J Trop Med Hyg, 72*(1), 47-59.

DHS-DRC (2007). Demographic and Health Survey *Ministère du plan, Measure DHS*.

DHS-DRC (2013). Demographic and Health Survey. *Ministère du plan, Measure DHS*.

Dondorp, A., Nosten, F., Stepniewska, K., Day, N., & White, N. (2005). Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial. *Lancet, 366*(9487), 717-725.

Dondorp, A. M., Fanello, C. I., Hendriksen, I. C., Gomes, E., Seni, A., Chhaganlal, K. D., Bojang, K., Olaosebikan, R., Anunobi, N., Maitland, K., Kivaya, E., Agbenyega, T., Nguah, S. B., Evans, J., Gesase, S., Kahabuka, C., Mtove, G., Nadjm, B., Deen, J., Mwanga-Amumpaire, J., Nansumba, M., Karema, C., Umulisa, N., Uwimana, A., Mokuolu, O. A., Adedoyin, O. T., Johnson, W. B., Tshefu, A. K., Onyamboko, M. A., Sakulthaew, T., Ngum, W. P., Silamut, K., Stepniewska, K., Woodrow, C. J., Bethell, D., Wills, B., Oneko, M., Peto, T. E., von Seidlein, L., Day, N. P., & White, N. J. (2010). Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): an open-label, randomised trial. *Lancet, 376*(9753), 1647-1657.

Ekvall, H. (2003). Malaria and anaemia. *Curr Opin Hematol, 10*(2), 108-114.

Ernst, K. C., Adoka, S. O., Kowuor, D. O., Wilson, M. L., & John, C. C. (2006). Malaria hotspot areas in a highland Kenya site are consistent in epidemic and non-epidemic years and are associated with ecological factors. *Malar J, 5*, 78.

Ferrari, G., Ntuku, H. M., Burri, C., Tshefu, A. K., Duparc, S., Hugo, P., Mitembo, D. K., Ross, A., Ngwala, P. L., Luwawu, J. N., Musafiri, P. N., Ngoie, S. E., & Lengeler, C. (2015). An operational comparative study of quinine and artesunate for the treatment of severe malaria in hospitals and health centres in the Democratic Republic of Congo: the MATIAS study. *Malar J, 14*(1), 226.

Filmer, D., & Pritchett, L. H. (2001). Estimating wealth effects without expenditure data--or tears: an application to educational enrollments in states of India. *Demography, 38*(1), 115-132.

Gahutu, J. B., Steininger, C., Shyirambere, C., Zeile, I., Cwinya-Ay, N., Danquah, I., Larsen, C. H., Eggelte, T. A., Uwimana, A., Karema, C., Musemakweri, A., Harms, G., & Mockenhaupt, F. P. (2011). Prevalence and risk factors of malaria among children in southern highland Rwanda. *Malar J, 10*, 134.

Gaudart, J., Poudiougou, B., Dicko, A., Ranque, S., Toure, O., Sagara, I., Diallo, M., Diawara, S., Ouattara, A., Diakite, M., & Doumbo, O. K. (2006). Space-time clustering of childhood malaria at the household level: a dynamic cohort in a Mali village. *BMC Public Health, 6*, 286.

Geissbuhler, Y., Chaki, P., Emidi, B., Govella, N. J., Shirima, R., Mayagaya, V., Mtasiwa, D., Mshinda, H., Fillinger, U., Lindsay, S. W., Kannady, K., de Castro, M. C., Tanner, M., & Killeen, G. F. (2007). Interdependence of domestic malaria prevention measures and mosquito-human interactions in urban Dar es Salaam, Tanzania. *Malar J, 6*, 126.

Gemperli, A., Sogoba, N., Fondjo, E., Mabaso, M., Bagayoko, M., Briet, O. J., Anderegg, D., Liebe, J., Smith, T., & Vounatsou, P. (2006). Mapping malaria transmission in West and Central Africa. *Trop Med Int Health, 11*(7), 1032-1046.

Gillies MT, & de Meillon B. (1968). The Anophelinae of Africa South of the Sahara. P*ublications of the South African Institute forMedical Research, no. 54, Johannesburg, South Africa*.

Grabowsky, M., Nobiya, T., & Selanikio, J. (2007). Sustained high coverage of insecticide-treated bednets through combined Catch-up and Keep-up strategies. *Trop Med Int Health, 12*(7), 815-822.

Greenberg, A. E., Ntumbanzondo, M., Ntula, N., Mawa, L., Howell, J., & Davachi, F. (1989). Hospital-based surveillance of malaria-related paediatric morbidity and mortality in Kinshasa, Zaire. *Bull World Health Organ, 67*(2), 189-196.

Greenwood, B. M., Bradley, A. K., Greenwood, A. M., Byass, P., Jammeh, K., Marsh, K., Tulloch, S., Oldfield, F. S., & Hayes, R. (1987). Mortality and morbidity from malaria

among children in a rural area of The Gambia, West Africa. *Trans R Soc Trop Med Hyg, 81*(3), 478-486.

Griffin, J. T., Hollingsworth, T. D., Reyburn, H., Drakeley, C. J., Riley, E. M., & Ghani, A. C. (2015). Gradual acquisition of immunity to severe malaria with increasing exposure. *Proc Biol Sci, 282*(1801).

Guerra, C. A., Gikandi, P. W., Tatem, A. J., Noor, A. M., Smith, D. L., Hay, S. I., & Snow, R. W. (2008). The limits and intensity of Plasmodium falciparum transmission: implications for malaria control and elimination worldwide. *PLoS Med, 5*(2), e38.

Guerra, C. A., Hay, S. I., Lucioparedes, L. S., Gikandi, P. W., Tatem, A. J., Noor, A. M., & Snow, R. W. (2007). Assembling a global database of malaria parasite prevalence for the Malaria Atlas Project. *Malar J, 6*, 17.

Hall, A., Latham, M. C., Crompton, D. W., Stephenson, L. S., & Wolgemuth, J. C. (1982). Intestinal parasitic infections of men in four regions of rural Kenya. *Trans R Soc Trop Med Hyg, 76*(6), 728-733.

Harris I, Sharrock, W. W., Bain, L. M., Gray, K. A., Bobogare, A., Boaz, L., Lilley, K., Krause, D., Vallely, A., Johnson, M. L., Gatton, M. L., Shanks, G. D., Cheng, Q. (2010). A large proportion of asymptomatic Plasmodium infections with low and sub-microscopic parasite densities in the low transmission setting of Temotu Province, Solomon Islands: challenges for malaria diagnostics in an elimination setting. *Malar J, 9*, 254.

Hay, S. I., Guerra, C. A., Tatem, A. J., Atkinson, P. M., & Snow, R. W. (2005). Urbanization, malaria transmission and disease burden in Africa. *Nat Rev Microbiol, 3*(1), 81-90.

Hay, S. I., Rogers, D. J., Toomer, J. F., & Snow, R. W. (2000). Annual Plasmodium falciparum entomological inoculation rates (EIR) across Africa: literature survey, Internet access and review. *Trans R Soc Trop Med Hyg, 94*(2), 113-127.

Hotez, P. J., & Molyneux, D. H. (2008). Tropical anaemia: one of Africa's great killers and a rationale for linking malaria and neglected tropical disease control to achieve a common goal. *PLoS Negl Trop Dis, 2*(7), e270.

Idro, R., Aloyo, J., Mayende, L., Bitarakwate, E., John, C. C., & Kivumbi, G. W. (2006). Severe malaria in children in areas with low, moderate and high transmission intensity in Uganda. *Trop Med Int Health, 11*(1), 115-124.

Jaureguiberry, S., Ndour, P. A., Roussel, C., Ader, F., Safeukui, I., Nguyen, M., Biligui, S., Ciceron, L., Mouri, O., Kendjo, E., Bricaire, F., Vray, M., Angoulvant, A., Mayaux, J., Haldar, K., Mazier, D., Danis, M., Caumes, E., Thellier, M., & Buffet, P. (2014).

Postartesunate delayed hemolysis is a predictable event related to the lifesaving effect of artemisinins. *Blood, 124*(2), 167-175.

Karch, S., Asidi, N., Manzambi, Z. M., & Salaun, J. J. (1992). [The Anopheles fauna and the transmission of human malaria in Kinshasa (Zaire)]. *Bull Soc Pathol Exot, 85*(4), 304-309.

Kazadi, W., Sexton, J. D., Bigonsa, M., W'Okanga, B., & Way, M. (2004). Malaria in primary school children and infants in kinshasa, democratic republic of the congo: surveys from the 1980s and 2000. *Am J Trop Med Hyg, 71*(2 Suppl), 97-102.

Keiser, J., Utzinger, J., Caldas de Castro, M., Smith, T. A., Tanner, M., & Singer, B. H. (2004). Urbanization in sub-saharan Africa and implication for malaria control. *Am J Trop Med Hyg, 71*(2 Suppl), 118-127.

Kinshasa, Ville de Kinshasa http://www.kinshasa.cd/index.php?option=com_content&view=article&id=300&Itemid=146].

Klinkenberg, E., McCall, P., Wilson, M. D., Amerasinghe, F. P., & Donnelly, M. J. (2008). Impact of urban agriculture on malaria vectors in Accra, Ghana. *Malar J, 7*, 151.

Knudsen, A. B., & Slooff, R. (1992). Vector-borne disease problems in rapid urbanization: new approaches to vector control. *Bull World Health Organ, 70*(1), 1-6.

Korenromp, E. L., Armstrong-Schellenberg, J. R., Williams, B. G., Nahlen, B. L., & Snow, R. W. (2004). Impact of malaria control on childhood anaemia in Africa - a quantitative review. Trop Med Int Health, 9(10), 1050-1065.

Kottek, M., Greiser, J., Beck, C., Rudolf, B., Rubel, F. (2006): World Map of the Köppen-Geiger climate classification updated. *Meteorol Z, 15*:259-263

Kyaw, S. S., Drake, T., Ruangveerayuth, R., Chierakul, W., White, N. J., Newton, P. N., & Lubell, Y. (2014). Cost of treating inpatient falciparum malaria on the Thai-Myanmar border. Malar *J, 13*, 416.

Ladhani, S., Patel, V. S., El Bashir, H., & Shingadia, D. (2005). Changes in laboratory features of 192 children with imported falciparum malaria treated with quinine. *Pediatr Infect Dis J, 24*(11), 1017-1020.

Lengeler, C. (2004). Insecticide-treated bed nets and curtains for preventing malaria. *Cochrane Database Syst Rev*(2), CD000363.

Lengeler, C., Grabowsky, M., McGuire, D., & deSavigny, D. (2007). Quick wins versus sustainability: options for the upscaling of insecticide-treated nets. *Am J Trop Med Hyg, 77*(6 Suppl), 222-226.

Lindsay, S. W., & Martens, W. J. (1998). Malaria in the African highlands: past, present and future. *Bull World Health Organ, 76*(1), 33-45.

Losimba Likwela, J., D'Alessandro, U., Donnen, P., & Wilmet Dramaix, M. (2012). Clinical aspects and outcome of suspected severe pediatric malaria. *Med Mal Infect, 42*(7), 315-320.

Lubell, Y., Mills, A. J., Whitty, C. J., & Staedke, S. G. (2010). An economic evaluation of home management of malaria in Uganda: an interactive Markov model. *PLoS One, 5*(8), e12439.

Lubell, Y., Riewpaiboon, A., Dondorp, A. M., von Seidlein, L., Mokuolu, O. A., Nansumba, M., Gesase, S., Kent, A., Mtove, G., Olaosebikan, R., Ngum, W. P., Fanello, C. I., Hendriksen, I., Day, N. P., White, N. J., & Yeung, S. (2011). Cost-effectiveness of parenteral artesunate for treating children with severe malaria in sub-Saharan Africa. *Bull World Health Organ, 89*(7), 504-512.

Lubell, Y., Yeung, S., Dondorp, A. M., Day, N. P., Nosten, F., Tjitra, E., Abul Faiz, M., Yunus, E. B., Anstey, N. M., Mishra, S. K., Mohanty, S., White, N. J., & Mills, A. J. (2009). Cost-effectiveness of artesunate for the treatment of severe malaria. *Trop Med Int Health, 14*(3), 332-337.

Macdonald, G. (1957). The epidemiology and control of malaria. London. *Oxford University Press, 201*.

Machault, V., Gadiaga, L., Vignolles, C., Jarjaval, F., Bouzid, S., Sokhna, C., Lacaux, J. P., Trape, J. F., Rogier, C., & Pages, F. (2009). Highly focused anopheline breeding sites and malaria transmission in Dakar. *Malar J, 8*, 138.

Maheu-Giroux, M., & Castro, M. C. (2014). Cost-effectiveness of larviciding for urban malaria control in Tanzania. *Malar J, 13*, 477.

Makani, J., Matuja, W., Liyombo, E., Snow, R. W., Marsh, K., & Warrell, D. A. (2003). Admission diagnosis of cerebral malaria in adults in an endemic area of Tanzania: implications and clinical description. *QJM, 96*(5), 355-362.

Malaria Atlas Project. (2010). Democratic Republic of Congo, Malaria risk. 2010.

MARA/ARMA (1998). Towards an Atlas of Malaria Risk in Africa. First technical report of the MARA/ARMA collaboration (www.mara.org.za) South Africa.

Marsh, K., Forster, D., Waruiru, C., Mwangi, I., Winstanley, M., Marsh, V., Newton, C., Winstanley, P., Warn, P., Peshu, N., & et al. (1995). Indicators of life-threatening malaria in African children. *N Engl J Med, 332*(21), 1399-1404.

Martens, P., & Hall, L. (2000). Malaria on the move: human population movement and malaria transmission. *Emerg Infect Dis, 6*(2), 103-109.

Matangila, J. R., Doua, J. Y., Linsuke, S., Madinga, J., Inocencio da Luz, R., Van Geertruyden, J. P., & Lutumba, P. (2014). Malaria, schistosomiasis and soil transmitted helminth burden and their correlation with anaemia in children attending primary schools in Kinshasa, Democratic Republic of Congo. *PLoS One, 9*(11), e110789.

Mathanga, D. P., Campbell, C. H., Jr., Vanden Eng, J., Wolkon, A., Bronzan, R. N., Malenga, G. J., Ali, D., & Desai, M. (2010). Comparison of anaemia and parasitaemia as indicators of malaria control in household and EPI-health facility surveys in Malawi. *Malar J, 9*, 107.

Matthys, B., Vounatsou, P., Raso, G., Tschannen, A. B., Becket, E. G., Gosoniu, L., Cisse, G., Tanner, M., N'Goran E, K., & Utzinger, J. (2006). Urban farming and malaria risk factors in a medium-sized town in Cote d'Ivoire. *Am J Trop Med Hyg, 75*(6), 1223-1231.

Messina, J. P., Taylor, S. M., Meshnick, S. R., Linke, A. M., Tshefu, A. K., Atua, B., Mwandagalirwa, K., & Emch, M. (2011). Population, behavioural and environmental drivers of malaria prevalence in the Democratic Republic of Congo. *Malar J, 10*, 161.

Metselaar, D., & Van Thiel, P. (1959). Classification of malaria. *Trop Geogr Med, 11*, 157–161.

MMV (2013) Expert group meeting on the safety profile of injectable artesunate. Medicines for Malaria Venture; Vienna; 2013.

MMV Medicines for Malaria Venture. Available at http://www.mmv.org/access/injectable-artesunate-tool-kit. Retrieved 04 September 2014

MMV (2012). Saving more lives with artesunate injectable. Injectable Artesunate Stakeholders' Meeting Report. Geneva. Medicines for Malaria Venture. Available at: http://www.mmv.org/sites/default/files/uploads/docs/publications/Injectable%20Artesunate%20Stakeholders%20Meeting%20Report.pdf [accessed 03.06.15]).

Modiano, D., Sirima, B. S., Sawadogo, A., Sanou, I., Pare, J., Konate, A., & Pagnoni, F. (1998). Severe malaria in Burkina Faso: influence of age and transmission level on clinical presentation. *Am J Trop Med Hyg, 59*(4), 539-542.

Modiano, D., Sirima, B. S., Sawadogo, A., Sanou, I., Pare, J., Konate, A., & Pagnoni, F. (1999). Severe malaria in Burkina Faso: urban and rural environment. *Parassitologia, 41*(1-3), 251-254.

MSF (2011). Making the Switch. Ensuring access to improved treatment for severe malaria in Africa. Médecins Sans Frontières. Geneva; 2011.

MSH (2014). International Drug Price Indicator Guide, 2013 Edition. Medford: Mass: Management Science for Health; 2014. Updated annually.

Mudenda, S. S., Kamocha, S., Mswia, R., Conkling, M., Sikanyiti, P., Potter, D., Mayaka, W. C., & Marx, M. A. (2011). Feasibility of using a World Health Organization-standard methodology for Sample Vital Registration with Verbal Autopsy (SAVVY) to report leading causes of death in Zambia: results of a pilot in four provinces, 2010. *Popul Health Metr, 9*, 40.

Mukadi, P., Gillet, P., Lukuka, A., Atua, B., Kahodi, S., Lokombe, J., Muyembe, J. J., & Jacobs, J. (2011). External quality assessment of malaria microscopy in the Democratic Republic of the Congo. *Malar J, 10*, 308.

Mulumba, M. P., Wery, M., Ngimbi, N. N., Paluku, K., Van der Stuyft, P., & De Muynck, A. (1990). [Childhood malaria in Kinshasa (Zaire). Influence of seasons, age, environment, and family social conditions]. *Med Trop (Mars), 50*(1), 53-64.

Mwangi, T. W., Fegan, G., Williams, T. N., Kinyanjui, S. M., Snow, R. W., & Marsh, K. (2008). Evidence for over-dispersion in the distribution of clinical malaria episodes in children. *PLoS One, 3*(5), e2196.

Nations, U. World Urbanization Prospects, the 2014 revision. *Department of Economic and Social Affairs, Population Division*.

Ngimbi, N. P., Beckers, A., & Wery, M. (1982). [Survey of the epidemiological status of malaria in Kinshasa (Republic of Zaire) in 1980]. *Ann Soc Belg Med Trop, 62*(2), 121-137.

Nguyen-Dinh P, Schwartz IK, Sexton JD, Bomboto E, Botomwito B, Kalisa R, Ngimbi NP, & Wery M. (1985). In vivo and in vitro susceptibility to chloroquine of Plasmodium falciparum in Kinshasa and Mbuji-Mayi, Zaire. *Bull World Health Organ, 63*, 325–330.

Nicastri, E., Bevilacqua, N., Sane Schepisi, M., Paglia, M. G., Meschi, S., Ame, S. M., Mohamed, J. A., Mangi, S., Fumakule, R., Di Caro, A., Capobianchi, M. R., Kitua, A., Molteni, F., Racalbuto, V., Ippolito, G. (2009). Accuracy of malaria diagnosis by microscopy, rapid diagnostic test, and PCR methods and evidence of antimalarial overprescription in nonsevere febrile patients in two Tanzanian hospitals. *Am J Trop Med Hyg, 80*, 712–717.

OANDA. Currency Converter. http://www.oanda.com [accessed 07 August 2014].

Ochomo, E. O., Bayoh, N. M., Walker, E. D., Abongo, B. O., Ombok, M. O., Ouma, C., Githeko, A. K., Vulule, J., Yan, G., & Gimnig, J. E. (2013). The efficacy of long-lasting nets with declining physical integrity may be compromised in areas with high levels of pyrethroid resistance. *Malar J, 12*, 368.

Okiro, E. A., Kazembe, L. N., Kabaria, C. W., Ligomeka, J., Noor, A. M., Ali, D., & Snow, R. W. (2013). Childhood malaria admission rates to four hospitals in Malawi between 2000 and 2010. *PLoS One, 8*(4), e62214.

Omumbo, J. A., Guerra, C. A., Hay, S. I., & Snow, R. W. (2005). The influence of urbanisation on measures of Plasmodium falciparum infection prevalence in East Africa. *Acta Trop, 93*(1), 11-21.

Ong'echa, J. M., Keller, C. C., Were, T., Ouma, C., Otieno, R. O., Landis-Lewis, Z., Ochiel, D., Slingluff, J. L., Mogere, S., Ogonji, G. A., Orago, A. S., Vulule, J. M., Kaplan, S. S., Day, R. D., & Perkins, D. J. (2006). Parasiteamia, anaemia, and malarial anaemia in infants and young children in a rural holoendemic Plasmodium falciparum transmission area. *Am J Trop Med Hyg, 74*(3), 376-385.

Owusu-Agyei, S., Fryauff, D. J., Chandramohan, D., Koram, K. A., Binka, F. N., Nkrumah, F. K., Utz, G. C., & Hoffman, S. L. (2002). Characteristics of severe anaemia and its association with malaria in young children of the Kassena-Nankana District of northern Ghana. *Am J Trop Med Hyg, 67*(4), 371-377.

Oyewole, I. O., & Awolola, T. S. (2006). Impact of urbanisation on bionomics and distribution of malaria vectors in Lagos, southwestern Nigeria. *J Vector Borne Dis, 43*(4), 173-178.

Peel E, & van Hoof L. (1948). Le paludisme a la maternite indigene de Leopoldville. *Ann Soc Belg Med Trop*(18), 413–420.

PMI (2015). Malaria Operational Plan FY 2015, Democratic Republic of the Congo. President's Malaria Initiative.

PNLP: Guide technique de formation en prevention et prise en charge du paludisme (Hopital General de reference), RDC.

PNLP (2012). Directives nationales de traitement du paludisme en République Démocratique du Congo (Draft). *Kinshasa, Ministère de la Santé*.

PNLP (2013a). Plan Stratégique National de Lutte contre le Paludisme 2013-2015. Kinshasa; 2013.

PNLP (2013b). Rapport annuel d'activités 2012.

Pond, B. S. (2013). Malaria indicator surveys demonstrate a markedly lower prevalence of malaria in large cities of sub-Saharan Africa. *Malar J, 12*, 313.

Premji, Z., Hamisi, Y., Shiff, C., Minjas, J., Lubega, P., & Makwaya, C. (1995). Anaemia and Plasmodium falciparum infections among young children in an holoendemic area, Bagamoyo, Tanzania. *Acta Trop, 59*(1), 55-64.

Premji, Z., Lubega, P., Hamisi, Y., McHopa, E., Minjas, J., Checkley, W., & Shiff, C. (1995). Changes in malaria associated morbidity in children using insecticide treated mosquito nets in the Bagamoyo district of coastal Tanzania. *Trop Med Parasitol, 46*(3), 147-153.

Reyburn, H., Mbatia, R., Drakeley, C., Bruce, J., Carneiro, I., Olomi, R., Cox, J., Nkya, W. M., Lemnge, M., Greenwood, B. M., & Riley, E. M. (2005). Association of transmission intensity and age with clinical manifestations and case fatality of severe Plasmodium falciparum malaria. *JAMA, 293*(12), 1461-1470.

Reyburn, H., Mbatia, R., Drakeley, C., Carneiro, I., Mwakasungula, E., Mwerinde, O., Saganda, K., Shao, J., Kitua, A., Olomi, R., Greenwood, B. M., & Whitty, C. J. (2004). Overdiagnosis of malaria in patients with severe febrile illness in Tanzania: a prospective study. *BMJ, 329*(7476), 1212.

Robert, V., Macintyre, K., Keating, J., Trape, J. F., Duchemin, J. B., Warren, M., & Beier, J. C. (2003). Malaria transmission in urban sub-Saharan Africa. *Am J Trop Med Hyg, 68*(2), 169-176.

Roll Back Malaria. (2014). Malaria situation and response, Democratic Republic of the Congo. *2014*(5 Dec).

Roll Back Malaria Partnership. (2011). Refined / Updated GMAP Objectives , Targets , Milestones and Priorities Beyond 2011. Geneva; 2011.

Rolling, T., Agbenyega, T., Issifou, S., Adegnika, A. A., Sylverken, J., Spahlinger, D., Ansong, D., Lohr, S. J., Burchard, G. D., May, J., Mordmuller, B., Krishna, S., Kremsner, P. G., & Cramer, J. P. (2013). Delayed hemolysis after treatment with parenteral artesunate in African children with severe malaria - a double-center prospective study. *J Infect Dis*.

Rolling, T., Agbenyega, T., Issifou, S., Adegnika, A. A., Sylverken, J., Spahlinger, D., Ansong, D., Lohr, S. J., Burchard, G. D., May, J., Mordmuller, B., Krishna, S., Kremsner, P. G., & Cramer, J. P. (2014). Delayed Hemolysis After Treatment With Parenteral Artesunate in African Children With Severe Malaria--A Double-center Prospective Study. *J Infect Dis*.

Rolling, T., Schmiedel, S., Wichmann, D., Wittkopf, D., Burchard, G. D., & Cramer, J. P. (2012). Post-treatment haemolysis in severe imported malaria after intravenous artesunate: case report of three patients with hyperparasitaemia. *Malar J, 11*, 169.

Rolling, T., Wichmann, D., Schmiedel, S., Burchard, G. D., Kluge, S., & Cramer, J. P. (2013). Artesunate versus quinine in the treatment of severe imported malaria: comparative analysis of adverse events focussing on delayed haemolysis. *Malar J, 12*, 241.

Sam-Wobo, S. O., Agbeyangi, O. A., Ekpo, U. F., Akinloye, O. A., Mafiana, C. F., & Adeleke, M. A. (2012). Rectal artesunates, their utilization, and parental perception in the management of malaria in children from Abeokuta, southwestern Nigeria. *Vector Borne Zoonotic Dis, 12*(2), 151-155.

Sattler, M. A., Mtasiwa, D., Kiama, M., Premji, Z., Tanner, M., Killeen, G. F., & Lengeler, C. (2005). Habitat characterization and spatial distribution of Anopheles sp. mosquito larvae in Dar es Salaam (Tanzania) during an extended dry period. *Malar J, 4*, 4.

Schellenberg, D., Menendez, C., Aponte, J., Guinovart, C., Mshinda, H., Tanner, M., & Alonso, P. (2004). The changing epidemiology of malaria in Ifakara Town, southern Tanzania. *Trop Med Int Health, 9*(1), 68-76.

Sexton, J. D., Bigonsa, M., Okanga, B., & W. & Waty, M. (Baltimore, December 2-6 1984, 99). The prevalence of malaria in primariy schoolchildren in Kinshasa, Zaire *Proceeding of the Joint Meetings of the Royal and American Societies of Tropical Medicine and Hygiene*.

Sharp, B. L., Kleinschmidt, I., Streat, E., Maharaj, R., Barnes, K. I., Durrheim, D. N., Ridl, F. C., Morris, N., Seocharan, I., Kunene, S., JJ, L. A. G., Mthembu, J. D., Maartens, F., Martin, C. L., & Barreto, A. (2007). Seven years of regional malaria control collaboration--Mozambique, South Africa, and Swaziland. *Am J Trop Med Hyg, 76*(1), 42-47.

Shaukat, A. M., Breman, J. G., & McKenzie, F. E. (2010). Using the entomological inoculation rate to assess the impact of vector control on malaria parasite transmission and elimination. *Malar J, 9*, 122.

Sinclair, D., Donegan, S., Isba, R., & Lalloo, D. G. (2012). Artesunate versus quinine for treating severe malaria. *Cochrane Database Syst Rev, 6*, CD005967.

Slutsker, L., Taylor, T. E., Wirima, J. J., & Steketee, R. W. (1994). In-hospital morbidity and mortality due to malaria-associated severe anaemia in two areas of Malawi with different patterns of malaria infection. *Trans R Soc Trop Med Hyg, 88*(5), 548-551.

Smith, D. L., Dushoff, J., Snow, R. W., & Hay, S. I. (2005). The entomological inoculation rate and Plasmodium falciparum infection in African children. *Nature, 438*(7067), 492-495.

Smith, T., Hii, J. L., Genton, B., Muller, I., Booth, M., Gibson, N., Narara, A., & Alpers, M. P. (2001). Associations of peak shifts in age--prevalence for human malarias with bednet coverage. *Trans R Soc Trop Med Hyg, 95*(1), 1-6.

Snow, R. W., Armstrong, J. R., Forster, D., Winstanley, M. T., Marsh, V. M., Newton, C. R., Waruiru, C., Mwangi, I., Winstanley, P. A., & Marsh, K. (1992). Childhood deaths in Africa: uses and limitations of verbal autopsies. *Lancet, 340*(8815), 351-355.

Snow, R. W., Bastos de Azevedo, I., Lowe, B. S., Kabiru, E. W., Nevill, C. G., Mwankusye, S., Kassiga, G., Marsh, K., & Teuscher, T. (1994). Severe childhood malaria in two areas of markedly different falciparum transmission in east Africa. *Acta Trop, 57*(4), 289-300.

Snow, R. W., & Marsh, K. (2002). The consequences of reducing transmission of Plasmodium falciparum in Africa. *Adv Parasitol, 52*, 235-264.

Snow, R. W., Omumbo, J. A., Lowe, B., Molyneux, C. S., Obiero, J. O., Palmer, A., Weber, M. W., Pinder, M., Nahlen, B., Obonyo, C., Newbold, C., Gupta, S., & Marsh, K. (1997). Relation between severe malaria morbidity in children and level of Plasmodium falciparum transmission in Africa. *Lancet, 349*(9066), 1650-1654.

Soares Magalhaes, R. J., & Clements, A. C. (2011). Spatial heterogeneity of haemoglobin concentration in preschool-age children in sub-Saharan Africa. *Bull World Health Organ, 89*(6), 459-468.

Staedke, S. G., Nottingham, E. W., Cox, J., Kamya, M. R., Rosenthal, P. J., & Dorsey, G. (2003). Short report: proximity to mosquito breeding sites as a risk factor for clinical malaria episodes in an urban cohort of Ugandan children. *Am J Trop Med Hyg, 69*(3), 244-246.

Stoler, J., Weeks, J. R., Getis, A., & Hill, A. G. (2009). Distance threshold for the effect of urban agriculture on elevated self-reported malaria prevalence in Accra, Ghana. *Am J Trop Med Hyg, 80*(4), 547-554.

Taylor, S. M., Messina, J. P., Hand, C. C., Juliano, J. J., Muwonga, J., Tshefu, A. K., Atua, B., Emch, M., & Meshnick, S. R. (2011). Molecular malaria epidemiology: mapping and burden estimates for the Democratic Republic of the Congo, 2007. *PLoS One, 6*(1), e16420.

Taylor, T. E., Fu, W. J., Carr, R. A., Whitten, R. O., Mueller, J. S., Fosiko, N. G., Lewallen, S., Liomba, N. G., & Molyneux, M. E. (2004). Differentiating the pathologies of cerebral malaria by postmortem parasite counts. *Nat Med, 10*(2), 143-145.

Taylor, W. R., & White, N. J. (2004). Antimalarial drug toxicity: a review. *Drug Saf, 27*(1), 25-61.

ter Kuile, F. O., Terlouw, D. J., Phillips-Howard, P. A., Hawley, W. A., Friedman, J. F., Kolczak, M. S., Kariuki, S. K., Shi, Y. P., Kwena, A. M., Vulule, J. M., & Nahlen, B. L. (2003). Impact of permethrin-treated bed nets on malaria and all-cause morbidity in young children in an area of intense perennial malaria transmission in western Kenya: cross-sectional survey. *Am J Trop Med Hyg, 68*(4 Suppl), 100-107.

Trape, J. F. (1987). Malaria and urbanization in central Africa: the example of Brazzaville. Part IV. Parasitological and serological surveys in urban and surrounding rural areas. *Trans R Soc Trop Med Hyg, 81 Suppl 2*, 26-33.

Trape, J. F., Lefebvre-Zante, E., Legros, F., Ndiaye, G., Bouganali, H., Druilhe, P., & Salem, G. (1992). Vector density gradients and the epidemiology of urban malaria in Dakar, Senegal. *Am J Trop Med Hyg, 47*(2), 181-189.

Trape, J. F., Pison, G., Spiegel, A., Enel, C., & Rogier, C. (2002). Combating malaria in Africa. *Trends Parasitol, 18*(5), 224-230.

Trape, J. F., & Zoulani, A. (1987). Malaria and urbanization in central Africa: the example of Brazzaville. Part II: Results of entomological surveys and epidemiological analysis. *Trans R Soc Trop Med Hyg, 81 Suppl 2*, 10-18.

Tshilolo, L., Aissi, L. M., Lukusa, D., Kinsiama, C., Wembonyama, S., Gulbis, B., & Vertongen, F. (2009). Neonatal screening for sickle cell anaemia in the Democratic Republic of the Congo: experience from a pioneer project on 31 204 newborns. *J Clin Pathol, 62*(1), 35-38.

UN-HABITAT. (2003). The Challenge of Slums. Global Report on Human Settlements. United Nations Human Settlements Prgramme.

UN (2014a). The State of African Cities. United Nations Human Settlements Programme. UN-Habitat 2014.

UN (2014c). World Urbanization Prospects. The 2014 Revision. United Nations 2014.

UNDP (2014). Human Development Report 2014 Sustaining Human Progress: Reducing Vulnerabilities and Building Resilience. United Nations Development Programme.

UNICEF (2010). Multiple Indicator Cluster Survey (MICS), Democratic Republic of Congo, Preliminary Findings.

van Griensven, J., De Naeyer, L., Uwera, J., Asiimwe, A., Gazille, C., & Reid, T. (2008). Success with antiretroviral treatment for children in Kigali, Rwanda: experience with health center/nurse-based care. *BMC Pediatr, 8*, 39.

Wang, S. J., Lengeler, C., Smith, T. A., Vounatsou, P., Akogbeto, M., & Tanner, M. (2006). Rapid Urban Malaria Appraisal (RUMA) IV: epidemiology of urban malaria in Cotonou (Benin). *Malar J, 5*, 45.

Wang, S. J., Lengeler, C., Smith, T. A., Vounatsou, P., Cisse, G., Diallo, D. A., Akogbeto, M., Mtasiwa, D., Teklehaimanot, A., & Tanner, M. (2005). Rapid urban malaria appraisal (RUMA) in sub-Saharan Africa. *Malar J, 4*, 40.

Ward RA. (1977). Recent changes in the epidemiology of malaria relating to human ecology. *Proceedings of the 15th International Congress of Entomology, Washington, DC, August 19−22, 1976. New York: Plenum Press*, 523−529.

Watts, T. E., Wray, J. R., Ng'andu, N. H., & Draper, C. C. (1990). Malaria in an urban and a rural area of Zambia. *Trans R Soc Trop Med Hyg, 84*(2), 196-200.

WHO (2006). Guidelines for the Treatment of Malaria. Geneva: World Health Organization. 2006.

WHO (2007). Malaria elimination: a field manual for low and moderate endemic countries. Geneva: World Health Organization; 2007.

WHO (2010). Guidelines for the Treatment of Malaria. 2nd edition. Geneva: World Health Organization; 2010.

WHO (2011a). Guidelines for the Treatment of Malaria. 2nd edition. Geneva: World Health Organization; 2011.

WHO (2011c). World Malaria Report. Geneva: World Health Organization; 2011.

WHO (2012a). World Health Organization. Disease Surveillance for Malaria Control: an operational manual. Geneva 2012. http://www.who.int/malaria/surveillance_monitoring/operationalmanuals/en, accessed 4 June 2015.

WHO (2012b). World Malaria Report. Geneva: World Health Organization. 2012.

WHO (2013a). Information Note on Delayed Haemolytic Anaemia following Treatment with Artesunate. http://www.who.int/malaria/publications/atoz/who_note_delayed_haemolytic_anaemia_oct13.pdf Accessed 16 February 2014.

WHO (2013b). Management of severe malaria – A practical handbook. 3rd edition, Geneva, World Health Organization; 2013.

WHO (2014). World Malaria Report. Geneva: World Health Organization; 2014.

WHOPARs. World Health Organization Public Assessment Reports. http://apps.who.int/prequal/whopar/whoparproducts/MA051part6v1.pdf

Winskill, P., Rowland, M., Mtove, G., Malima, R. C., & Kirby, M. J. (2011). Malaria risk factors in north-east Tanzania. *Malar J, 10*, 98.

Wolf, L. R., Otten, E. J., & Spadafora, M. P. (1992). Cinchonism: two case reports and review of acute quinine toxicity and treatment. *J Emerg Med, 10*(3), 295-301.

Woolhouse, M. E. (1998). Patterns in parasite epidemiology: the peak shift. *Parasitol Today, 14*(10), 428-434.

World Bank. (2013). http://data.worldbank.org/country/congo-dem-rep Accessed 04.06.2015.

Wu, L., van den Hoogen, L. L., Slater, H., Walker, P. G., Ghani, A. C., Drakeley, C. J., Okell, L. C. (2015). Comparison of diagnostics for the detection of asymptomatic *Plasmodium falciparum* infections to inform control and elimination strategies. *Nature, 528*: S86–93.

Yadouleton, A., N'Guessan, R., Allagbe, H., Asidi, A., Boko, M., Osse, R., Padonou, G., Kinde, G., & Akogbeto, M. (2010). The impact of the expansion of urban vegetable farming on malaria transmission in major cities of Benin. *Parasit Vectors, 3*, 118.

Zachariah, R., Harries, A. D., Ishikawa, N., Rieder, H. L., Bissell, K., Laserson, K., Massaquoi, M., Van Herp, M., & Reid, T. (2009). Operational research in low-income countries: what, why, and how? *Lancet Infectious Diseases, 9*(11), 711-717.

Zhou, G., Li, J. S., Ototo, E. N., Atieli, H. E., Githeko, A. K., & Yan, G. (2014). Evaluation of universal coverage of insecticide-treated nets in western Kenya: field surveys. *Malar J, 13*, 351.

Zoller, T., Junghanss, T., Kapaun, A., Gjorup, I., Richter, J., Hugo-Persson, M., Morch, K., Foroutan, B., Suttorp, N., Yurek, S., & Flick, H. (2011). Intravenous artesunate for severe malaria in travelers, Europe. *Emerg Infect Dis, 17*(5), 771-777.

10 Curriculum vitae

Giovanfrancesco Ferrari

Personal Information

Employer	Swiss Tropical and Public Health Institute (Swiss TPH)
Profession	Biologist – PhD student
Expertise	Molecular Biology, Biomedical Research
Nationality	Italian
With Swiss TPH	Since September 2009

Address

Swiss Tropical and Public Health Institute (Swiss TPH)
Health Interventions Unit
Department of Epidemiology and Public Health
P.O. Box
4002 Basel/Switzerland
Tel. business: (41) 61 284 8613
Tel. private: (39) 338 1986691
Email: giovanfrancesco.ferrari@unibas.ch

Education/ Continued Education/ Degree

2011 - 2015	PhD candidate in Public Health and Epidemiology (Supervisor: Christian Lengeler, PhD)
2010	The price of Globalization: drug trials in resource limited settings, seminar (European Centre of Pharmaceutical Medicine / Swiss TPH)
2010	Quality Assurance in Clinical Research, Kinshasa, RD Congo
2009	United Nations Department of Safety and Security (E-courses): Basic and Advanced Security in the Field
2006-2007	Postgraduate in Tropical Medicine and International Health. Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium
2000	University degree in Biological Sciences, Molecular Biology Major, *Università degli Studi di Milano*, 105/110
1992	Classical High School Degree, from *Liceo Classico* "Alessandro Volta", Como

Professional/ Work Experience

2009-2011	Pharmaceutical Medicine Unit, Department of Medicines Research, Swiss TPH Research Assistant based at Swiss TPH office in Kinshasa Assisted in the development of the research centres of two Swiss TPH partners in Kinshasa, in the framework of the Alliance for Clinical Research and Clinical Epidemiology ARCEAU-RDC
2009	University of Milan, Department of Clinical Sciences "L. Sacco", Infectious disease Unit, Italy Research Assistant

	Contributed to the drafting of epidemiological research projects in view of the Universal Exposition, EXPO' 2015, in Milan, Italy
	Contributed to the analysis of genetic polymorphisms and lipodystrophy associated with antiretroviral therapy
2008	*Médecins Sans Frontières*, Holland, Wardher Project, Somali region, Ethiopia.
	Lab technician supervisor
	Organised and implemented tuberculosis laboratory activities, parasitology and blood transfusion unit
2007–2008	*Médecins Sans Frontières*, Belgium, Gondama Referral Center, Bo, Sierra Leone.
	Lab technician supervisor
	Assisted in the organization and supervision of the project: Efficacy of Artesunate plus Amodiaquine for uncomplicated *Plasmodium falciparum* Malaria. Prospective study of children under five years in holoendemic Area for Malaria in Bo, Sierra Leone
	Set up of transfusion unit and the parasitology area
2002-2006	National Neurological Institute "*C. Besta*", Molecular Neurogenetics Unit, Milan, Italy.
	Research Assistant
	Identified and characterized nuclear disease genes responsible for mitochondrial disorders
2001	University of New South Wales, Sydney, Australia. School of Biological Sciences.
	Research Assistant
	Conservation genetics of the Green and Golden Bell frog (*Litoria aurea*). Identified level of genetic diversity using molecular markers to develop more effectual strategies to preserve biodiversity
1999-2001	Department of Physiology and General Biochemistry, Comparative Biochemistry, *Università degli Studi di Milano*
	Undergraduate student
	Dissertation project: "The effects of the deletion of the gene triosofosfate isomerase (TPI1) on the carbon metabolism of *Kluyveromices lactis*

Language Proficiency

Italian	Mother tongue
English	Fluent in spoken and written
German	Good in spoken, basic written
French	Good in spoken and written
Spanish	Basic knowledge

Publications

Peer Reviewed

- A comprehensive malaria risk map in Kinshasa, Democratic Republic of Congo. **Ferrari G**, Ntuku HM, Schmidlin S, Diboulo E, Kitoto AT, Lengeler C, Malar J. 2016 Jan 13;15:27

- Identifying risk factors for Plasmodium infection and anaemia in Kinshasa, Democratic Republic of Congo. **Ferrari G**, Ntuku HM, Ross A, Schmidlin S, Kitoto AT, Lengeler C, Malar J. 2016 Jul 15;15:362
- Feasibility and acceptability of injectable artesunate for the treatment of severe malaria in the Democratic Republic of Congo. Ntuku HM, **Ferrari G**, Burri C, Kitoto AT, Mitembo DK, Lengeler C, Malar J. 2016 Jan 8;15:18
- An operational comparative study of quinine and artesunate for the treatment of severe malaria in hospitals and health centres in the Democratic Republic of Congo: the MATIAS study. **Ferrari G**, Ntuku HM, Burri C, Kitoto AT, Duparc S, Hugo P, Mitembo DK, Ross A, Ngwala PL, Luwawu JN, Musafiri PN, Ngoie SE, Lengeler C. Malar J. 2015 May 30;14 (1):226.
- Delayed anaemia after treatment with injectable artesunate in the Democratic Republic of the Congo: a manageable issue. Burri C, **Ferrari G**, Ntuku HM, Kitoto AT, Duparc S, Hugo P, Mitembo DK, Lengeler C. Am J Trop Med Hyg 2014, 91:821-823
- The A467Tand W748S POLG substitutions are a rare cause of adult-onset ataxia in Europe. Kate Craig, **G. Ferrari**, W. Tiangyou, G. Hudson, C. Gellera, M. Zeviani and P. F. Chinnery. Brain 2007, Apr; 130POLG1 in idiopathic Parkinson disease. Tiangyou W, Hudson G, Ghezzi D, **Ferrari G**, Zeviani M, Burn DJ, Chinnery PF. Neurology. 2006 Nov 14;67 :1698-700.
- The spectrum of clinical disease caused by the A467T and the W748S POLG mutations: a study of 26 cases. Tzoulis C, Engelsen BA, Telstad W, Aasly J, Zeviani M, Winterthun S, **Ferrari G**, Aarseth J H Bindoff LA. Brain 2006, Jul; 129: 1685-1692
- Phenotypic spectrum associated with mutations of the mitochondrial polymerase gamma gene. Horvath R, Hudson G, **Ferrari G**, Futterer N, Ahola S, Lamantea E, Prokisch H, Lochmuller H, McFarland R, Ramesh V, Klopstock T, Freisinger P, Salvi F, Mayr JA, Santer R, Tesarova M, Zeman J, Udd B, Taylor RW, Turnbull D, Suomalainen A, Zeviani M, Chinnery PF. Brain 2006 Jul; 129: 1674-1684
- Infantile hepatocerebral syndromes associated with mutations in the mitochondrial DNA polymerase-γA. **Ferrari G**, Lamantea E, Donati A, Filosto M, Briem E, Carrara F, Parini R, Simonati A, Santer R, Zeviani M. Brain. 2005 Apr;128: 723-31
- The polymorphic polyglutamine repeat in the mitochondrial DNA polymerase γ gene (POLG1) is not associated with oligozoospermia. Brusco A, Michielotto C, Gatta V, Foresta C, Matullo G, Zeviani M, **Ferrari G**, Dragone E, Calabrese G, Rossato M, Stuppia L, Migone N. J Endocrinol Invest. 2006 Jan;29:1-4.
- Autosomal recessive mitochondrial ataxic syndromes due to mitochondrial polymerase γ mutations. Winterthun S, **Ferrari G**, He L, Taylor RW, Zeviani M, Turnbull DM, Engelsen BA, Moen G, Bindoff LA Neurology. 2005 Apr 12;64:1204-8
- Ethylmalonic encephalopathy is caused by mutations in ETHE1, a gene encoding a mitochondrial matrix protein. Tiranti V, D'Adamo P, Briem E, **Ferrari G**, Mineri R, Lamantea E, Mandel H, Balestri P, Garcia-Silva MT, Vollmer B, Rinaldo P, Hahn SH, Leonard J, Rahman S, Dionisi-Vici C, Garavaglia B, Gasparini P, Zeviani M. Am J Hum Genet. 2004 Feb;74:239-52.
- Mutations of ANT1, Twinkle, and POLG1 in sporadic progressive external ophthalmoplegia (PEO). Agoatino A, Valletta L, Chinnery PF, **Ferrari G**,

Carrara F, Taylor RW, Schaefer AM, Turnbull DM, Tiranti V, Zeviani M. Neurology. 2003 Apr 22; 60:1354-6

- Microsatellite loci for the green and golden bell frog (*Litoria aurea*). Conservation Genetics. Burns, E.L., **Ferrari, G**. 2003.