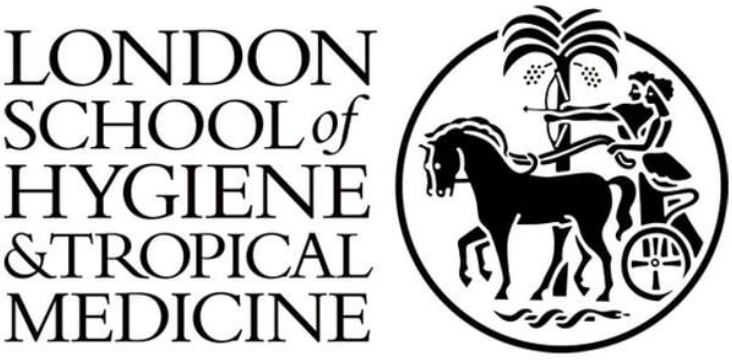

Recent overnight travel and the risk of malaria: case-control and prospective cohort studies in Uganda

EMMANUEL ARINAITWE

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Faculty of Infectious and Tropical Diseases

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

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Declaration by the candidate

I, Emmanuel Arinaitwe, declare that the work presented in this thesis is my own. I confirm that information derived from other sources has been well indicated in the thesis.

Signed:  Date: 23 September 2020

Abstract

Introduction: Travel is an underappreciated risk factor for malaria in residents of malaria-endemic countries. In Uganda, malaria transmission is heterogeneous, in part due to expansion of malaria control interventions and increased urbanization. As a result, individuals who travel may be at higher risk of malaria infection. However, our understanding of the association between travel and malaria infection in Uganda is limited.

Methods: This thesis aimed to address this evidence gap through: 1) case-control study in urban Kampala, 2) cohort study in three sites of varied malaria transmission, and 3) cohort study in rural Tororo, which is under intense malaria control with indoor residual spraying (IRS). For the case control study, 5 controls were selected for every 2 cases, matching on age. Data were collected in July and August 2019 on recent overnight travel out of Kampala (past 60 days), destination and duration of travel, and behavioural factors, including sleeping under a long-lasting insecticidal net (LLIN) during travel. For the cohort study at three sites in Uganda (PRISM 1), information on overnight travel was collected between 2015 and 2016 from children aged 0.5-10 years and one adult living in 266 randomly selected households. Malaria, defined as fever with parasites detected by microscopy, was measured using passive surveillance. For the cohort in rural Tororo (PRISM 2), data on overnight travel and behaviour during travel were collected from residents of 80 households between 2017 and 2019. Behaviour while at home was assessed using a similar questionnaire during two-weekly home visits.

Results: In the case-control study, 162 cases and 405 controls were enrolled. Overall, 158 (27.9%) participants reported recent overnight travel. Travellers were far more likely to be diagnosed with malaria than those who did not travel (80.4% vs 8.6%, odds ratio 58.9, 95%

confidence interval [CI] 23.1-150.1, p<0.001); travelling to a non-IRS district, not using LLINs during travel, and engaging in outdoor activities were associated with increased odds of malaria. In the PRISM 1 cohort study, at least one overnight trip was reported by 120 of 906 (13.3%) participants. Among individuals who travelled, the incidence of malaria was higher in the first 60 days after travelling, compared to periods without recent travel at all sites (overall 1.15 vs 0.33 episodes per person-year, incidence rate ratio 3.53, 95% CI 1.85-6.73, p<0.001). In the PRISM 2 cohort study, 527 participants were enrolled and 123 (23.2%) reported taking at least one overnight trip. Overall, participants were less likely to use LLINs when travelling than at home (41.0% vs. 56.2%, relative risk [RR] 0.73, 95% CI: 0.60-0.89, p=0.002). In the analysis adjusted for gender and age, significantly lower LLIN use during travel was found for female participants (38.8% when travelling vs 59.2% at home, risk ratio [RR] 0.66, 95% CI 0.52-0.83, p=0.001) but not for males (48.3% vs 46.6%, RR 0.96, 95% CI 0.67-1.40, p=0.85), and those > 15 years (33.9% travel vs 61.3% home, RR 0.55, 95% CI 0.41-0.74, p<0.001).

Conclusions: Residents of malaria endemic countries who travel are a high-risk group that should be targeted for malaria prevention. For these travellers, personal protection measures, including sleeping under LLINs when traveling, application of creams or sprays to prevent outdoor mosquito bites, and administration of chemoprophylaxis, should be advocated.

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Abbreviations

ACT	Artemisinin combination therapy
AL	Artemether-lumefantrine
CI	Confidence interval
DDT	Dichlorodiphenyltrichloroethane
EIR	Entomological inoculation rate
GCP	Good Clinical Practice
GMEP	Global Malaria Eradication Program
GPS	Global positioning system
Hb	Haemoglobin
HBR	Human biting rate
HRP-2	Histidine rich protein-2
IDRC	Infectious Diseases Research Collaboration
IPTi	Intermittent preventive treatment in infants
IPTp	Intermittent preventive treatment in pregnancy
IRR	Incidence rate ratio
IRS	Indoor residual spraying
ITN	Insecticide-treated net
LLIN	Long-lasting insecticidal net
MAP	Malaria Atlas Project
MIS	Malaria indicator survey
NMCP	National Malaria Control Programme
OR	Odds ratio
PMI	President's Malaria Initiative
PPY	Per person year at risk
PRISM	Programme for Resistance, Immunology, Surveillance and Modelling of malaria
RR	Rate ratio
RBM	Roll Back Malaria Partnership
RDT	Rapid diagnostic test
SSA	sub-Saharan Africa
SES	Socioeconomic status
UMSP	Uganda Malaria Surveillance Project
USAID	United States Agency for International Development
WHO	World Health Organization

Chapter 1: Introduction

1.1 Malaria burden and epidemiology in sub-Saharan Africa

Malaria remains a major public health challenge world-wide. Across Africa, proven malaria control measures have been scaled-up to an unprecedented coverage level, which has been associated with reductions in malaria burden.[1-3] However, recent evidence suggests that progress in malaria control has levelled off, and in 2018 alone, there were an estimated 228 million cases of malaria leading to 405 thousand deaths worldwide, of which 93% of cases and 94% of deaths were from Africa.[2] Despite the scaled up control interventions in sub-Saharan Africa, six countries contributed to more than 50% of all global malaria cases in 2018: Nigeria (25%), the Democratic Republic of Congo (12%), Uganda (5%), Cote d'Ivoire (4%), Mozambique (4%), and Niger (4%). The majority of malaria cases that were reported in 2018 were caused by *Plasmodium falciparum*, which is responsible for over 99% of cases in sub-Saharan Africa. Common malaria mosquito vectors are *Anopheles gambiae*, *Anopheles coluzzii* and *Anopheles funestus*.[4-6] The most common clinical presentation of P. falciparum malaria is fever, and other manifestations may include chills, headache, muscular pains, nausea and vomiting,[7] and the diagnosis is confirmed with microscopy or rapid diagnostic test.[8-11] Malaria transmission follows a seasonal pattern in most sub-Saharan countries with peaks occurring following rain seasons and low transmission during dry season.[12,13]

1.2 History of malaria control in Africa

Attempts made in 1950's and 1960's to control malaria using Dichloro-Diphenyl-Trichloro-Ethane (DDT) demonstrated that malaria elimination was possible.[14,15] However, gains in malaria control were not maintained and malaria elimination campaign collapsed in early 1970's. Factors that contributed to failure of the elimination campaign mainly in Africa, included inadequate funding, political instability, the emergence of antimalarial drug and

insecticide resistance, and human movement.[16] In the 1960's and 1970's, Prothero *et al.* described the contribution of human travel, also known as the 'human factor', to malaria transmission,[17,18] and presented evidence that when planning for malaria control and elimination, insufficient attention was given to travel by the WHO Global Malaria Eradication Program (GMEP).[16,17]

1.3 Current approach to malaria control in Africa

Integrated approach that involves mosquito vector control, malaria case management, and chemoprevention has been deployed.[19] Malaria control interventions currently being implemented in sub-Saharan Africa include; use of long-lasting insecticide treated nets (LLINs), and indoor residual spraying of insecticide (IRS) for vector control as recommended by WHO,[20] effective case management with artemisinin-based combination therapies (ACTs) for uncomplicated malaria and intravenous artesunate for complicated malaria, intermittent preventive treatment for the high-risk groups (pregnant women (IPTp), and children under 5 years (IPTi) in some countries), and seasonal malaria chemoprevention (SMC) in children under 5 years living in areas of the Sahel region with seasonal malaria transmission.[21] According to the 2019 World Malaria Report, scale-up of malaria control intervention has greatly improved. By 2018, 72% of households in sub-Saharan Africa had at least one LLIN and 60% of pregnant women received at least one dose of IPTp.[2]

1.4 Renewed interest in malaria elimination

Renewed efforts towards malaria control and elimination resumed in 2007 with a consensus that no single strategy could be applied everywhere and rather collective action was needed that included community involvement, integrated health systems and improved surveillance systems.[22] In the last two decades increase in funding for malaria control has led to a dramatic scale up of malaria control intervention,[23] and malaria control and elimination in sub-Saharan

Africa seems to be a possibility again. In support of the renewed efforts to control and eliminate malaria in Africa, there has been improved political will through the declaration of an Africa free from malaria by the African Union heads of state and governments.[24] In July 2018, a malaria-free Africa campaign with a slogan "Zero malaria starts with me" was launched by the African Union in partnership with Roll Back Malaria (RBM) to empower Africans to take a stand in the fight against malaria.[25] The campaign has since been adopted by several African countries and has contributed to mobilisation of resources to control and eliminate malaria Africa.[26]

1.5 Malaria burden and epidemiology in Uganda

Uganda is one of the high malaria burden countries; in 2018, it ranked 3rd in total number of malaria cases, and 7th in number of malaria deaths, globally.[2] According to the Uganda National Malaria Control Division (NMCD), malaria is responsible for 30-50% of outpatient visits, 15-20% of hospital admissions, and up to 20% of inpatient deaths.[27] Malaria is endemic in over 95% of the country, with higher transmission in lower altitudes (<1,200 m), low to medium transmission at higher altitudes (1,200 to 1,600 m) and low, unstable transmission in highland areas (1,800 m), which are prone to malaria epidemics.[28] Most malaria infections in Uganda are due to *Plasmodium falciparum (P. falciparum)* which is responsible for over 90% of infections.[29] Other malaria species that exist in the country include *P. malariae*, *P. vivax*, and *P. ovale*.[29,30] *P. falciparum* is responsible for most severe forms of malaria especially in children under five years and pregnant women.[31,32]

The most abundant malaria vector species in Uganda are *Anopheles gambiae* s.s., *An. arabiensis,* and *An. funestus,* which is responsible for malaria transmission in highland areas.[28,29,33] *An. gambiae* s.s. is the dominant species in most areas of Uganda, although some areas that have received several rounds of IRS have reported drastic reductions in *An.*

gambiae.[33,34] Malaria transmission in Uganda occurs throughout the year with peaks following two rainy seasons; the first rainy season being March to May, and the second one in August to October.[28,35,36]

1.6 Malaria control in Uganda

Current strategies for malaria control in Uganda are focused mainly on integrated vector control through targeted IRS and LLINs, prompt treatment with an ACT, and chemoprevention in pregnant women with sulfadoxine-pyrimethamine (SP). A combination of all the four control interventions in Uganda have demonstrated considerable gains towards malaria control as indicated by the drastic reduction in malaria transmission, infection and disease in some areas receiving IRS.[37-40] According to Uganda malaria indicator surveys, the country has registered progressive decrease in malaria parasite prevalence among children under 5 years of age of 45% in 2009,[41] 19% in 2014,[42] and 9% in 2018.[43]

1.6.1 History of IRS

IRS for malaria control has been used in Uganda since the 1950s.[14,44] The first pilot malaria elimination project was conducted in Kigezi region of south-western Uganda in 1959 where three rounds of IRS were administered using DDT.[44] The pilot led to reduction of malaria transmission to almost zero and was declared a success. However, a few years later, there were localised malaria outbreaks that could have been due to imported malaria from neighbouring areas where IRS was not administered.[14]

Following the success of experimental IRS in Kigezi region, large field trials of IRS with Malathion were carried out in Masaka in southern Uganda between 1963 and 1964.[45] However, there were no significant differences in parasite prevalence between non sprayed and sprayed areas which may have been due to migration in and out of the intervention areas

and high importation of malaria parasites.[45] Findings from this trial indicated that for malaria control to succeed, there are other factors to be considered including human travel between areas receiving malaria controls and those which are not.

With funding from United States Agency for International Development (USAID)/Presidents Malaria Initiative (PMI), IRS was resumed in 2006 (after nearly 40 years) in south-western Uganda using lambda-cyhalothrin (ICON™ 10% WP) and substantial reductions in the proportion of patients diagnosed with clinical malaria attending a health facility were achieved.[46] In 2007, IRS was expanded from south-western Uganda to 4 high transmission districts of northern Uganda (Apac, Pader, Gulu, and Kitgum) until 2009 when insecticide resistance to DDT and pyrethroids was reported.[47] In 2010, the insecticide was changed from DDT and pyrethroids to a carbamate (Bendiocarb), which was associated with significant declines in malaria burden,[48,49] and more recently with an organophosphate (Actellic).[50,51] IRS has since been scaled-up to 15 districts in north-eastern Uganda in a rotational manner resulting in significant reductions in malaria burden.[37,39,51] However, the IRS programme has been challenged by limited funding, and areas where malaria has been controlled have experienced upsurges of malaria when IRS has been stopped.[52,53]

1.6.2 Mass distribution of long-lasting insecticidal nets in Uganda

LLINs were used to prevent malaria on a small scale in Uganda until 2002 when Uganda was awarded a grant by Global Fund to Fight AIDS, Tuberculosis and Malaria, to support procurement of LLINs.[54] Several modalities of LLIN distribution by the government of Uganda have been carried out, initially through antenatal clinics to pregnant mothers,[55] followed by distribution through mass campaigns.[56-58] In compliance with WHO recommendations of universal coverage of LLINs, two national LLIN distribution campaigns have been conducted (in 2013-2014, and in 2017-2018), which has led to unprecedented increase in ownership of LLINs

in Uganda. According to the latest Uganda Malaria Indicator Survey conducted in 2018-2019 following the latest campaign, 83% of households owned at least one LLIN and 72% of households owned at least one LLIN for every two residents.[43,59]

1.6.3 Case management of malaria

Prompt treatment of malaria with ACTs in Uganda was adopted in 2004 using artemether-lumefantrine (AL) following evidence of increased resistance to chloroquine (CQ), sulfadoxine-pyrimethamine (SP), and a combination of SP+CQ.[22,60,61] Since then, the supply of AL at health facilities has greatly improved, and the proportion of children under five years of age treated with AL for malaria has increased from 39% in 2009 to 87% in 2014 and 88% in 2018/2019.[41-43] Although there has been no evidence of artemisinin resistance reported in Uganda, mutations associated with artemisinin resistance are widespread in the Greater Mekong subregion in south-eastern Asia, and have been detected in Guyana, Papua New Guinea and Rwanda.[62-67] Spread of artemisinin-resistant *P. falciparum* remains a major threat to malaria treatment globally.

1.6.4 Intermittent preventive treatment in pregnancy

Intermittent preventive treatment (IPTp) during pregnancy with SP was first adopted in Uganda by the malaria strategic plan (MSP) 2001-2005, but its uptake was limited by challenges of SP stock outs and knowledge gap among health care workers.[68-70] By 2009, only 31.7% of women attending ANC had received 2 doses of IPTp.[41] This was followed by restocking of SP, community and health care provider sensitisation, and by 2014, 64% of pregnant women were reported to have taken any SP (49% to have taken 2 doses) and 89% by 2018 (72% for two doses).[43]

1.7 Current status of malaria control in Uganda

Recent expansion of IRS and other standard malaria control measures such as LLINs and prompt treatment with ACT, has been associated with substantial reductions in malaria burden in some areas of Uganda (Figure 1.1).[38,46,48,49,51]

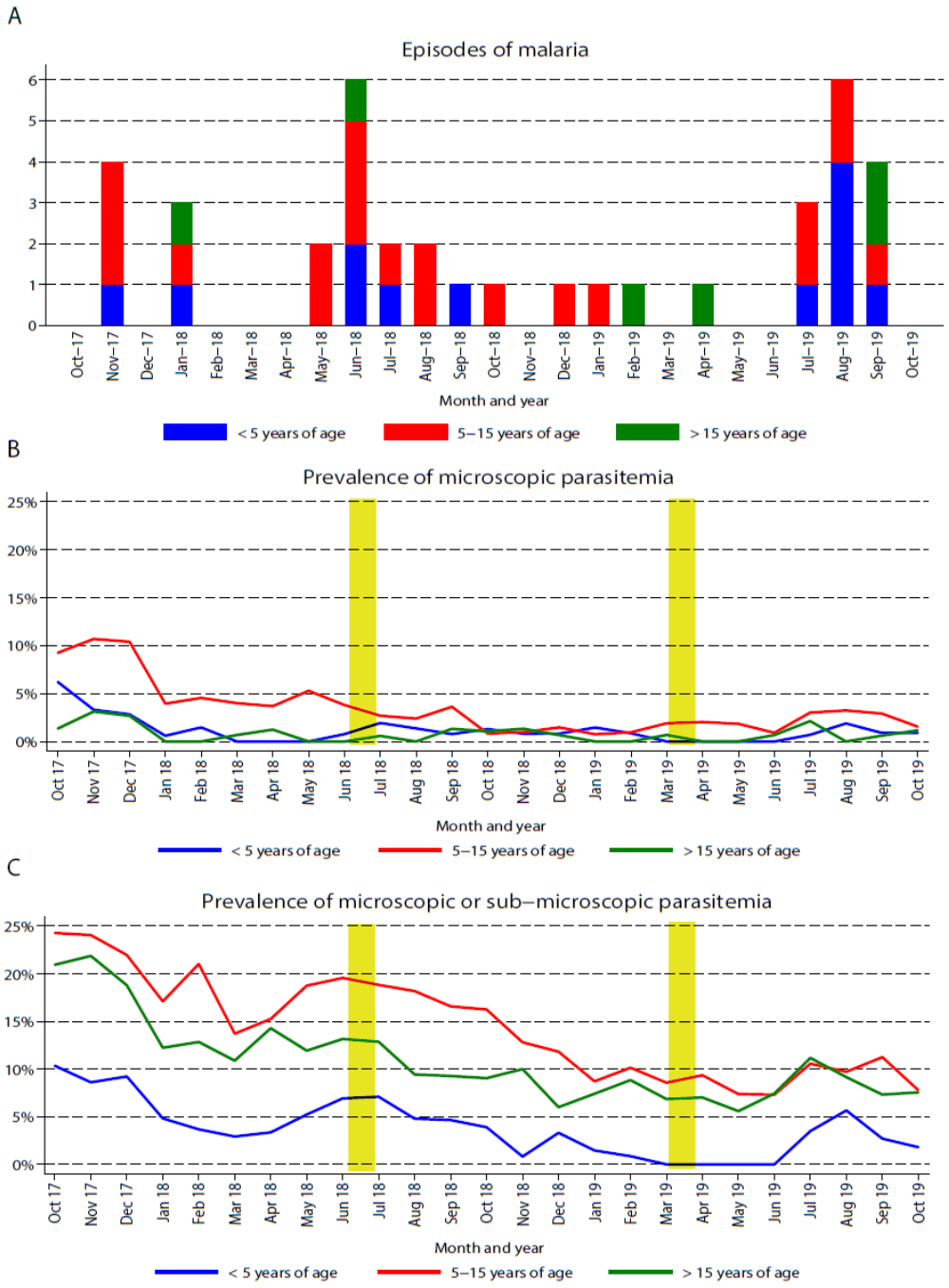

Figure 1.1. Declines in incidence of malaria and parasite prevalence following implementation of IRS in a cohort of 531 participants followed from October 2017 to October 2019 in Nagongera, Tororo district. (A) symptomatic malaria stratified by age-group. (B) microscopic

parasitaemia stratified by age-group. (C) microscopic and sub-microscopic parasitaemia stratified by age-group. Yellow vertical bar = round of IRS with Actellic.[38]

However, scale up of malaria control intervention in other areas of Uganda have not demonstrated similar reduction.[2,43,53] and this has contributed to increased heterogeneity in the prevalence of malaria in Uganda. According to the 2018 malaria indicator survey, parasite prevalence in children under 5 years of age in Kampala and some areas of south western Uganda was <1%, and as high as 34% in areas of the north eastern parts of the country (Figure 1.2).[43]

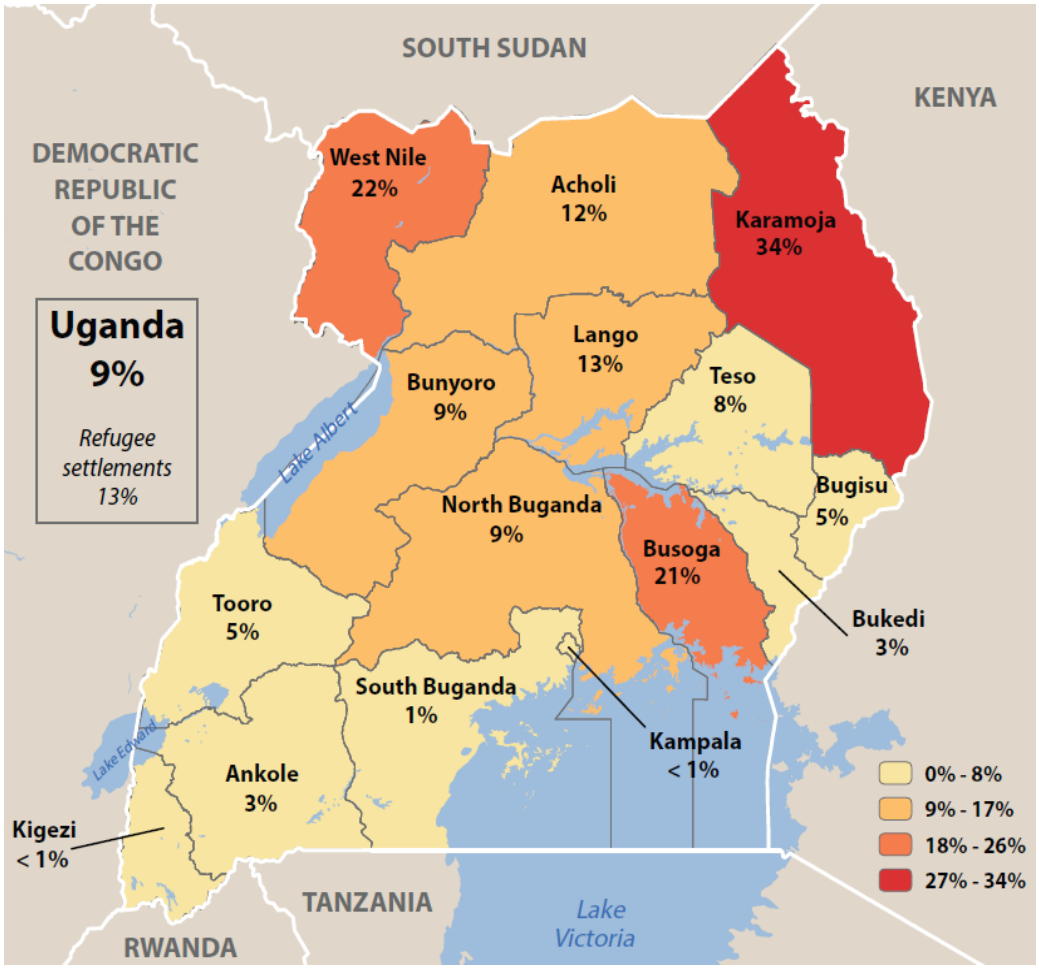

Figure 1.2. Varied malaria parasite prevalence in Uganda in 2018-2019 among children under 5 years.[43]

As malaria control interventions are scaled-up, some areas in Uganda have started reporting an age-shift in malaria burden from under-five to school aged children.[71-73] A study investigating the impact of age distribution of malaria cases using malaria routine surveillance data at 4 sites in Uganda, demonstrated that the proportion of confirmed malaria tests decreased in children under 5 years of age while it increased in those over 15 years of age between January 2006 and July2018.[71] This suggests that reduced exposure to malaria infection may have led to waning immunity in adolescents.[74,75]

1.8 Human travel and malaria

Human travel plays a crucial role in the spread of malaria and other infectious diseases.[76-78] Travel may be driven by pursuit of employment, business and educational opportunities, and to visit family and friends. Potential exposure to infections is influenced by the destination of travel, duration, and purpose of travel.[78,79] For instance, travelling from low to higher malaria transmission areas is more likely to lead to malaria exposure. Travelling for funeral services and social gatherings are likely to require shorter trips, farm workers and forest workers may take weeks away, while school children may stay away much longer when attending boarding schools.

Human travel has been shown to increase the risk of infection with malaria and other infectious diseases in various settings.[78,80-86] Stoddard *et al.* used the activity space model which represents travel associated with regular activity of individuals, to demonstrate that transmission of infectious diseases is highly influenced by human movement [78]. The authors further illustrated how length of stay varies with the kind of travel and determines the duration of contact, hence influencing the likelihood of infection (Figure 1.3). Remarkably, findings from this study showed that sites of potential high risk of malaria infection were not necessarily those of high mosquito vector abundance, suggesting that other factors such as

staying outdoors late and nature of sleeping places that increase exposure to vectors during overnight travel may play an important role in acquiring an infection. The authors concluded that human movement and subsequent variation in exposure was more important than vector density and hence played a bigger role in disease dynamics.

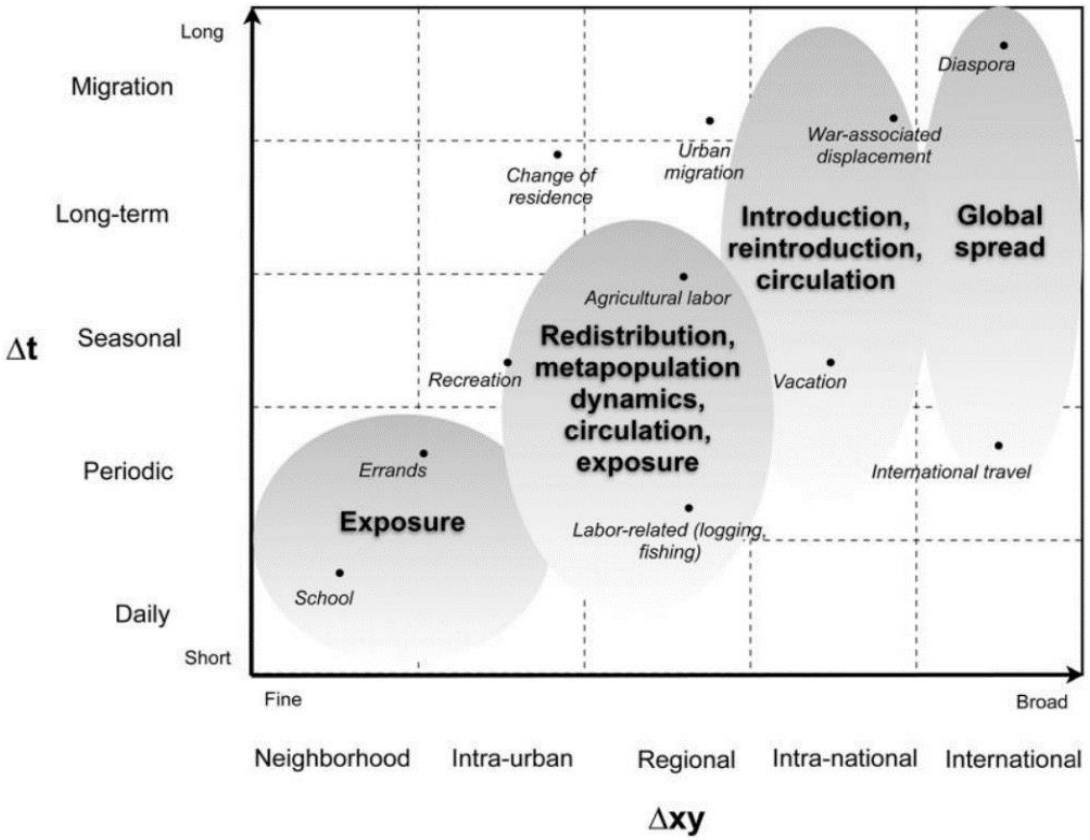

Figure 1.3. Relationship between destination (Δxy) and duration of travel/duration of stay (Δt).[78]

1.9 Human travel and malaria control

People living in areas of low malaria transmission, who travel to higher malaria transmission areas may be at an increased risk of infection with malaria parasites. This may complicate progress on malaria control as was seen in Zanzibar where parasites were reintroduced by residents travelling from mainland Tanzania [87]. Human travel from malaria endemic areas can introduce malaria parasites into susceptible populations as occurred in Venezuela between

1987 and 1997,[88] Thailand between 1980 and 2000,[89] and Colombia in 2001.[90] Furthermore, areas where malaria has been adequately controlled are at a risk of re-introduction of malaria parasites, if malaria control strategies are relaxed or discontinued,[52,53,91,92] or from residents travelling between areas of low and high malaria transmission intensity.[93,94] In Bioko Island of Equatorial Guinea and Zanzibar Island of Tanzania, malaria control interventions were scaled up, but malaria parasites were re-introduced by returning residents which frustrated malaria control efforts.[76,93] Similarly, in Swaziland and the regions of KwaZulu-Natal, Mpumalanga, and Limpopo in South Africa, malaria parasites were reintroduced from Mozambique following implementation of intensive malaria control.[92,95,96]

Some malaria control and elimination initiatives have used lessons learnt from the GMEP to control malaria but are challenged by the re-introduction of malaria parasites following travel when control interventions are relaxed. For example, the governments of southern Africa countries including Mozambique, Swaziland, and South Africa included a malaria component to the Lubombo Spatial Development Initiative (LSDI) in 1999, a joint development program.[96] The initiative resulted in substantial decreases in malaria transmission and malaria parasite prevalence among children in this region.[96] However, malaria rebounded when the Joint Malaria Control Initiative ended in 2011,[92,95,96] highlighting the significant role travel played in the re-introduction of malaria parasites mainly from neighbouring Mozambique.

1.10 Malaria exposure and immunity

Evidence dating from the 1960s indicates that repeated exposure to malaria infection results in slow acquisition of protective immunity against infection or progression of illness.[97,98] In malaria-endemic areas, antimalarial antibodies from mothers (maternal antibodies) transferred to the foetus via the placenta prenatally, provide protection to infants against malaria for the first year of life.[99-102] After protection from maternal antibodies in infants

wanes,[103-107] development of immunity against malaria infection and illness is acquired over time following repeated exposure to malaria parasites.[97,98,108] This may partly explain why in endemic settings older children and adults are at lower risk of infection and illness. This plays a very important role in clinical spectrum of malaria disease in different epidemiological settings.

Immunity against malaria can be classified into anti-disease immunity and anti-parasite immunity.[109,110] Anti-disease immunity provides protection against symptomatic malaria and develops within the first years of life if there is repeated exposure to malaria parasites.[111-115] The development of anti-disease immunity is associated with age and can lead to individuals with chronic asymptomatic parasitaemia.[116,117] These individuals develop ability to sustain relatively high parasite levels without developing a fever.[118,119] In high transmission settings, anti-parasite immunity develops gradually over time providing individuals, generally adolescents and adults, the ability to control infections and to maintain very low parasite densities that are sometimes undetectable by microscopy.[110,120-122] Although there are limited data on the duration of this type of immunity in the absence of continued exposure, anti-parasite immunity contribute significantly to reduced burden of malaria in adolescents and adults living in malaria-endemic areas.[123,124]

Both anti-disease and anti-parasite immunity seem to wane if exposure to malaria parasites reduces or ends.[125,126] Individuals who have acquired antimalarial immunity following repeated exposure, may partially lose this immunity as control interventions are scaled up and transmission falls. Similarly, the antimalarial immunity of residents may also wane when they move from rural to urban areas that are characterised by very low malaria transmission intensity. When these individuals travel to other parts of the country with higher transmission and are exposed again to infected mosquitoes, they are more likely to fall sick with malaria due to reduced antimalarial immunity and may be at a high risk of severe forms of malaria.

Likewise, when residents from rural areas carrying malaria parasites travel to urban areas, that are likely to infect urban mosquitoes that can effectively transmit malaria to less immune urban residents.

1.11 Urbanisation and malaria

There has been increased urbanicity in Africa since the 1980s,[127-129] and this has been associated with reduction in malaria transmission at a large spatial scale.[130,131] Urban areas generally have better infrastructure and have fewer mosquito breeding sites, and better access to malaria case management services.[132,133] In Uganda, higher urbanicity is associated with reduced malaria transmission and lower parasite prevalence.[134] According to Uganda malaria indicator survey of 2018-2019, urban areas reported lower parasite prevalence in children under 5 years of 3.3% compared to rural areas with a parasite prevalence of 10.8%.[43] Urban residents in Uganda are more likely to be exposed to malaria parasites and at risk of malaria disease when they travel to rural areas.

1.12 Thesis rationale

Historically, human travel has been shown to play an important role in transmission of infectious diseases which has been a major factor in failure of malaria control and elimination programs.[17,18] In Uganda, scale-up of malaria control interventions has led to increased heterogeneity in malaria transmission which puts certain Ugandan populations at increased risk of malaria when they travel from lower to higher malaria transmission areas.[42] The risk of malaria has become substantially lower in urban and highland areas as compared to rural, and lowland areas of Uganda. In addition, in areas of Uganda where IRS has been implemented, the burden of malaria has been markedly reduced.[37,46,48,49,135] This has resulted in a geographic difference in malaria risk, creating an environment where people living in low risk areas who are traveling to high-risk areas may be at increased risk of malaria infection. However, data on

overnight travel and malaria infection in Uganda are limited mainly to one study carried out in highland areas of western Uganda.[136] The study identified travel within the last 4 weeks, travel to an area of high transmission intensity, and longer duration of travel as risk factors for malaria infection. Currently, our understanding of how often people travel overnight in Uganda, particularly between areas of different epidemiological settings is limited and prospective cohort data on travel and risk of malaria infection are lacking. Further studies on overnight travel and the risk of malaria infection are needed.

In Kampala city, like in many other large cities in Africa, malaria transmission is low,[42] yet many cases of malaria are diagnosed at health facilities within Kampala.[137] It is not clear whether people are infected with malaria when travelling out of Kampala or whether Ugandans travelling out of Kampala city would embrace use of control interventions during overnight travel. If residents of Kampala city are more likely to be infected with malaria parasites while traveling, they may be more likely to infect local mosquito vectors and to transmit malaria parasites to their neighbours. Thus, it is important to understand risk factors of malaria infection during overnight travel out of an urban setting or areas in Uganda where malaria transmission has reduced almost to zero. This thesis explored these challenges and findings could inform the design of malaria control interventions targeted to travellers.

1.13 Study aims and objectives

1.13.1 Aim of the study

The overall aim of this project was to better understand the association between overnight travel and the risk of malaria in Uganda.

1.13.2 Specific objectives

1. To measure the association between recent overnight travel out of Kampala city and clinical malaria diagnosis among patients attending the outpatient department at Naguru General Hospital, Kampala.
2. To assess the association between recent overnight travel and the incidence of malaria among residents of 3 sites in Uganda of varied malaria transmission intensity
3. To evaluate the associations between overnight travel and behavioural factors that might modify the risk of malaria infection in Uganda.

Objective 1 was addressed using a matched case-control study design (Table 1.1). Recent overnight travel was defined as travel out of Kampala city (Nakawa, Makindye, Rubaga, Kawempe and Central divisions) within the last 60 days and spending at least one night away.

Objective 2 was addressed using the Program for Resistance, Immunology, Surveillance and Modelling for malaria (PRISM 1) cohort study at 3 sites in Uganda (Jinja, Kanungu, and Tororo) and objective 3 using PRISM 2 cohort study at a site (Tororo) that received IRS and malaria transmission drastically reduced almost to zero. PRISM was a malaria multi-project research program in Uganda funded by the United States National Institutes of Health (NIH) as part of the International Centre of Excellence in Malaria Research (ICEMR). Recent overnight travel was defined as travel out of the sub-county of residence within the previous 60 days and spending at least one night away.

Objective	Sample population	Exposed group	Outcomes with definition
1. To measure the association between recent overnight travel out of Kampala city and the risk of malaria	- Patients attending outpatient department with fever or/and history of fever at Naguru hospital in Kampala	- Participants with a history of recent overnight travel out of Kampala city	- Malaria diagnostic test results by RDT
2. To measure the associations between overnight travel and the incidence of malaria	- PRISM 1 cohort study participants with any overnight travel living in Jinja, Kanungu, and Tororo districts	- Travel within the last 1-60 days	- Incidence of malaria defined as number of new malaria episodes per person time of follow up
3. To evaluate behavioural factors that may modify the risk of malaria	- PRISM 2 cohort study participants with a history of travel living in Tororo district	- Reduced adherence to malaria prevention measures during travel	- Change in behavioural factors that may modify the risk of malaria

Table 1.1. A summary of objectives and outcome measures

1.14 Overview of research studies

This research project utilized 2 study designs; 1) a case-control study in Kampala city, that investigated the association between recent overnight travel out of an urban setting and diagnosis of malaria, and factors associated with malaria diagnosis following travel, and 2) PRISM 1, a prospective cohort study at 3 sites in Uganda of varied malaria transmission intensity, and PRISM 2 cohort at a site that have received several rounds of IRS.

The case-control part of the thesis identified important factors associated with acquiring malaria infection during travel. These data set a stage for discussions on malaria prevention during overnight travel in Uganda. The PRISM cohort studies provided an opportunity to compare the risk of malaria in the same study participants before overnight travel with the risk during the period after overnight travel. Nagongera site in Tororo district received LLINs as

part of the universal distribution in November 2013 and 6 rounds of IRS leading to a drastic reduction in vector density.[38,40] Similarly, malaria incidence in this area has greatly reduced leading to an island of low malaria transmission intensity surrounded by areas of high malaria transmission intensity. The state of this site provided a suitable environment to study overnight travel and malaria infection since malaria cases currently identified are fewer and likely to have been brought in by returning travellers.

1.15 Thesis structure

The thesis presents findings from the investigation of associations between recent overnight travel in Uganda and malaria, and is composed of chapters 1 to 9. Chapter 1 provides a background on malaria burden, epidemiology and control in Africa and specifically Uganda, the association between human movement and malaria, summarises the rationale for the thesis project, and outlines the aims and objectives of the study. Chapter 2 reviews the existing literature of recent overnight travel and the risk of malaria. Chapter 3 presents an overview of the study design and methods in detail to supplement methods sections of chapters 4, 5 and 6 which test specific hypotheses to answer individual objectives of the study.

Chapter 4 explores recent overnight travel out of an urban setting associated with malaria diagnosis in outpatient department of a hospital in Kampala using a case control study design. This chapter further presents factors associated with malaria diagnosis in individuals who travelled.

Chapter 5 presents findings from the evaluation between recent overnight travel and incidence of malaria using cohort study design at three sites of varied malaria transmission intensity. In this chapter, a cohort study design (PRISM 1) in Jinja, Kanungu and Tororo districts

was used to prospectively measure travel over time, and estimate malaria incidence as a gold standard measure of malaria burden.

Chapter 6 assesses changes in behaviours of participants that might modify the risk of malaria while travelling, using a cohort of participants (PRISM 2) living in Tororo district, a rural area that received several rounds of IRS and malaria incidence dramatically reduced.

The main findings are discussed in chapter 7 together with study limitations, and finally, conclusions and recommendations are presented in chapter 8.

Chapter 2: Literature review

2.1 Literature search strategy and selection

Previous studies evaluating the association between overnight travel and the risk of malaria have demonstrated mixed findings, and present several limitations. To better understand current evidence on the effect of overnight travel on the risk of malaria, and factors associated with malaria infection, a systematic review was conducted. Using electronic databases, including PubMed, EMBASE and MEDLINE, a literature review covering published research findings through May 31st 2020 was carried out. The search was performed using the following key words: 'travel' or 'overnight travel' or 'human movement' or 'human mobility' and 'malaria infection' or 'malaria transmission' or 'malaria risk'. Studies were included if they were published in English, assessed the association between travel within malaria endemic areas and the risk of malaria, and reported a malaria outcome.

Overall, 569 publications were identified that matched the search words. Abstracts for these publications were further reviewed for mention of travel (or movement or mobility) as the exposure, and malaria-related outcome (parasite prevalence or incidence or parasite rate) (Figure 2.1). Out of 569 publications, 39 reported on travel and malaria. Most of the articles excluded were not related to overnight travel and malaria (n=514), the rest referred to travel from malaria non-endemic countries to endemic regions (n=14) or were duplicates (n=2). Of the 39 publications selected for full review, 23 were excluded; 15 did not report on malaria-related outcomes, and 8 focused on malaria testing at borders points and drug administration to achieve elimination of malaria. Thus, 16 papers reported on overnight travel as the exposure of interest and malaria

outcomes, and were included in this review. The search outputs were imported into a reference manager (Endnote) and articles reviewed in full.

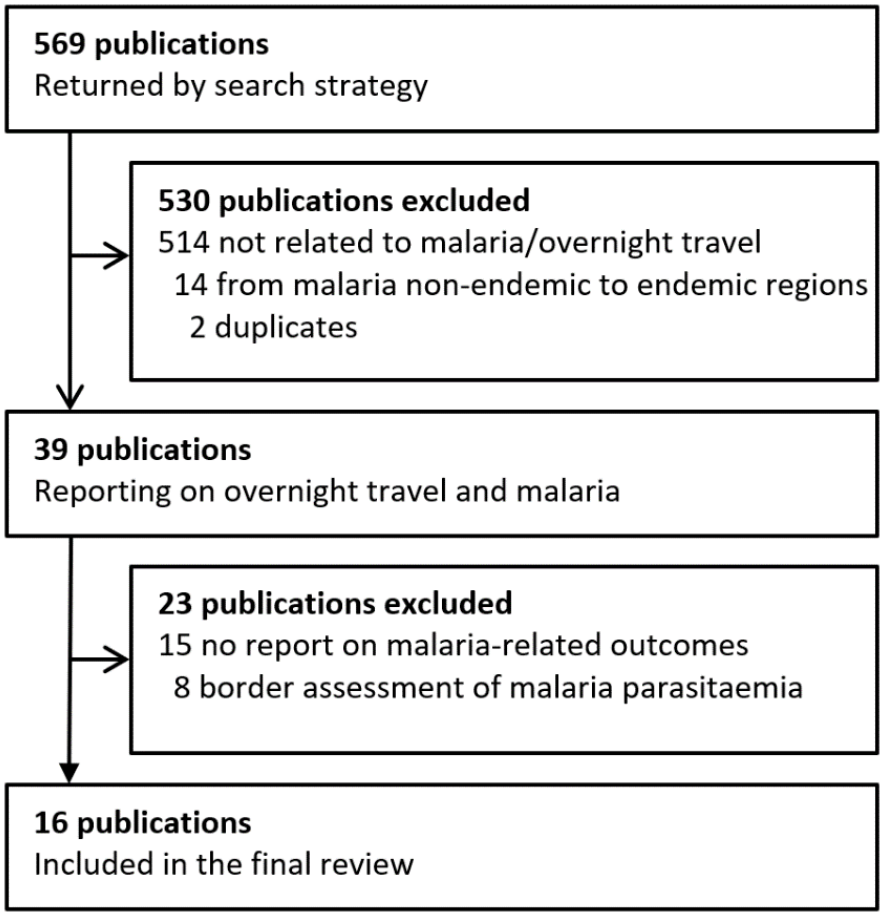

Figure 2.1. Literature review profile

2.2 Characteristics of studies included in this review

Of the 16 studies included in the final review (Table 2.1), 12 were conducted in sub-Saharan Africa, 3 in Asia, and one in South America. Most studies used cross-sectional and case-control study designs, and focused on malaria parasite prevalence and malaria diagnosis as the primary outcome. Ten studies directly evaluated the association between overnight travel and the risk of malaria, while 12 studies explored other factors associated with malaria risk following overnight travel. Initial studies evaluating overnight travel and malaria targeted migratory populations.[138] For example, a field study conducted on Rameswaram Island in India from 1983 to 1984 considered 3

groups of participants: fishermen, pilgrims and other people on transition from India to Sri Lanka,[138] who lived in camps on the island. A comprehensive assessment of the entire camp population found that prevalence of malaria parasites was higher in migrants compared to the general population on the island. It was concluded that participants from the 3 groups served as reservoirs for malaria parasites and were responsible for the persistence of malaria on the island.

	Author, year of publication	Study location, country	Design, target population	Measures of malaria burden
1	Rajagopalan, 1986[138]	Rameswaram (island), India	Cross-sectional surveys 6,355 participants surveyed	Malaria parasite prevalence
2	Osorio, 2004[90]	Quibdo, Colombia	Case-control 395 cases, and 534 controls	Clinical malaria diagnosis
3	Shanks, 2004[77]	Kericho tea estates, Kenya	Case-control 10,789 cases, 2,210 controls	Clinical malaria diagnosis
4	Wesolowski, 2012[139]	Country-wide, Kenya	Analysis of mobile phone data 15 million individuals over 1 year	Malaria parasite prevalence
5	Yukich, 2013[140]	Oromia, Ethiopia	Case-control 141 cases, 419 controls	Clinical malaria diagnosis
6	Chirebvu, 2014[141]	Tubu village, Botswana	Census questionnaire survey 483 participants	Clinical malaria diagnosis
7	Lynch, 2015[136]	Districts of Kabale and Rukungiri, Uganda	Case-control – health facility based, 272 cases and controls	Clinical malaria diagnosis
8	Xu, 2015[142]	Yunnan Province, China	Case-control 214 cases, 428 controls	Clinical malaria diagnosis
9	Mathanga, 2015[143]	Blantyre city, Malawi	Case-control study 187 cases, 286 controls	Clinical malaria diagnosis
10	Peeters Grietens, 2015[144]	Province of Ratanakiri, Cambodia	Mixed-methods 6,640 individuals from 113 villages	Malaria parasite prevalence
11	Njuguna, 2016[145]	Kibera slum of Nairobi, Kenya	Population-based surveillance 105,960 patient visits	Clinical malaria diagnosis
12	Martial, 2016[83]	Multi-country: Burkina Faso, Zambia and Tanzania	Cross-sectional survey 4,352 participants	Malaria parasite prevalence
13	Tejedor-Garavito, 2017[146]	Country-wide, Swaziland	Population-based surveillance 11,376 participants	Malaria parasite prevalence
14	Smith, 2017[147]	North central Namibia along border with Angola	Case-control 107 cases, 679 controls	Clinical malaria diagnosis
15	Haile, 2017[148]	Tahtay-Maychew district, Ethiopia	Matched case-control study 130 cases, 390 controls	Clinical malaria diagnosis
16	Lowa, 2018[149]	Lusaka district, Zambia	Cross-sectional study 260 participants	Malaria parasite prevalence

Table 2.1. Characteristics of studies included in the final review

2.3 Association between overnight travel and the risk of malaria

Ten studies conducted from various geographical zones assessed the association between overnight travel and a malaria-related outcome (Table 2.2).[77,90,136,140-145,148] Most studies (9) were based at health facilities and reported their outcome as clinical malaria diagnosis, with one study reporting on parasite prevalence. All studies presented raw data on the number and proportion of participants testing positive for malaria except for one study done in Botswana that used census data to identify travel outside of the study area as one of the risk factors of malaria diagnosis.[141] Due to the heterogeneity of study designs, it is intuitive to examine each study individually. Results from a case-control study conducted in Colombia in 2001 indicated that travel to a malaria endemic area 8 to 14 days before fever onset was a strong risk factor for *P. falciparum* malaria (OR 29.0, 95% CI: 14.0 – 60.3).[90] The study was carried out at both public and private health facilities in the town of Quibdo. History of travel was obtained by questionnaire and malaria diagnosis was made by microscopy. Other factors positively associated with malaria were age (highest in those aged 5-14 years), gender, and failure to use protection against mosquitoes during travel.

In Western Kenya, a case-control study conducted by Shanks *et al.* at a tea estate along the western rim of Rift Valley demonstrated that malaria case diagnosis was associated with travel out of the tea estate (OR 2.38, 95% CI: 2.17 – 2.60),[77] and was likely responsible for epidemics in that area. Cases were identified from the outpatient clinic, and controls were selected from the community around the tea estate. However, the study only included the community working at the tea estate limiting the generalisability of results. In addition, no measures to limit selection bias appear to have been taken.

Similarly, a case-control study conducted by *Yukich et al* in Ethiopia in 2011, collected travel history from patients attending a health facility and malaria test done by RDT. Overnight travel

away from home within the previous 30 days was associated with higher odds of malaria by microscopy (OR 1.76, 95% CI: (1.06 – 2.93), 0.03).

Author, year of publication	Study location, country	Exposure group (n)	Outcome	Proportion positive, n (%)	Measures of association, (95% CI), p-value
Osorio, 2004[90]	Quibdo, Colombia	No travel (n = 719)	Clinical	204 (28.4)	Reference
		Travelled (n = 103)	malaria cases	94 (91.3)	OR = 29.0 (14.0 – 60.3)
Shanks, 2004[77]	Kericho tea estates,	No travel (n = 6,455)	Clinical	1130 (17.5)	Reference
	Kenya	Travelled (n = 4,334)	malaria cases	1452 (33.5)	OR = 2.38 (2.17 – 2.60)
Yukich, 2013[140]	Oromia, Ethiopia	No travel (n = 360)	Clinical	49 (13.6)	Reference
		Travelled (n = 193)	malaria cases	36 (18.7)	OR = 1.76 (1.06 – 2.93), 0.03
Chirebvu, 2014[141]	Tubu village, Botswana	No travel (n = 29)	Clinical	-	Reference
		Travelled (n = 42)	malaria cases	-	OR = 2.70 (1.01 – 7.26)
Lynch, 2015[136]	Districts of Kabale and	No travel (n = 56)	Clinical	20 (35.7)	Reference
	Rukungiri, Uganda	Travelled (n = 48)	malaria cases	32 (66.7)	OR = 4.70 (1.40 – 16.3), 0.01
Xu, 2015[142]	Yunnan Province, China	No travel (n = 144)	Clinical	13 (9.02)	Reference
		Travelled (n = 275)	malaria cases	201 (73.1)	OR = 159.5 (75.1 – 338.9), <0.001
Mathanga, 2015[143]	Blantyre city, Malawi	No travel (n = 440)	Clinical	169 (38.4)	Reference
		Travelled (n = 33)	malaria cases	18 (54.5)	OR = 2.35 (1.04 – 5.30), 0.04
Peeters Grietens, 2015[144]	Province of Ratanakiri,	No travel (n = 2,238)	Positive	77 (3.44)	Reference
	Cambodia	Travelled (n = 2,727)	malaria tests	166 (6.10)	OR = 1.66 (1.21 – 2.28), 0.002
Njuguna, 2016[145]	Kibera slum of Nairobi,	No travel (n = 8,072)	Clinical	858 (10.6)	Reference
	Kenya	Travelled (n = 2,991)	malaria cases	1623 (54.3)	OR = 10.0 (9.00 – 11.0), <0.001
Haile, 2017[148]	Tahtay-Maychew	No travel (n = 300)	Clinical	23 (7.67)	Reference
	district, Ethiopia	Travelled (n = 220)	malaria cases	107 (48.6)	OR = 11.40 (6.91 – 18.82), <0.001

Table 2.2. Studies assessing the association between overnight travel and malaria

A study done in Botswana identified travel outside the study area measured using questionnaires as one of the risk factors of malaria (OR 2.70, 95% CI: 1.01 – 7.26).[141] This study obtained individual malaria case history by interviewing the respondents with a possibility of over/underreporting. The study included a small sample size and results could not be generalizable.

Another study carried out in a health facility setting in south-western Uganda found that travel from home and spending at least one night away within the last 4 weeks was associated with increased odds of malaria (OR 4.70, 95% CI: 1.40 – 16.3, 0.01).[136] The study lacked precision because of a small sample size and a recommendation was made for further research to be carried out.

A case-control study carried out in Yunnan, China by Xu *et al.* in 2012, enrolled 642 participants (214 cases and 428 controls). The findings of this study supported an association between overnight travel to a lowland area and increased odds of malaria diagnosis (OR 159.5, 95% CI: 75.1 – 338.9, <0.01).[142] Overnight travel history was obtained by questionnaire and malaria diagnosed by microscopy. The study provided important data on overnight travel and malaria infection in this area, but findings were limited by incomplete data on clinical outcomes which were obtained from the public health facility to identify cases. No history of recent treatment for malaria was available and travellers who could have cleared parasites after therapy were not identified.

In an urban and peri-urban setting of Malawi, Mathanga *et al.* conducted a case-control study and findings suggested that overnight travel increased the risk of infection with malaria parasites.[143] The risk of malaria case diagnosis was significantly higher in participants who reported travelling and spending a night away during the previous month (OR 2.35, 95% CI: 1.04 – 5.30, 0.04). Surprisingly, no association between reported use of a LLIN the previous night and risk of malaria were found suggesting that exposure to mosquito bites could have taken place during travel and not at home. Moreover, there was no report about the use of LLIN during travel and no associations with malaria prevention during travel could be inferred.

In Cambodia, a cross-sectional survey conducted by *Peeters et al*. carried out population surveys among residents of a remote province of Ratanakiri. Data on history of overnight stay at the farm hut and use of malaria preventive measures were collected using questionnaires, and a blood sample was taken for microscopy and molecular detection of malaria parasites. Participants who

travelled to the farm and stayed overnight had higher odds of a positive malaria test compared to those who did not travel (OR 1.66, 95% CI: 1.21 – 2.28, 0.002).[144]

A population-based survey carried out by Njuguna et al. in Kibera slum of Nairobi between 2007 and 2011 used structured questionnaires to collect data on demographics, history of travel and fever, and malaria diagnosis by microscopy.[145] Participants with a history of travel out of Nairobi within a month prior were more likely to be diagnosed with malaria (OR 10.0, 95% CI; 9.00 – 11.0 p <0.001. The majority of malaria cases diagnosed reported having travelled to just three counties.

In northern Ethiopia, a matched case-control study conducted by Haile *et al*. from August 1st to December 30th 2014, travel to low altitude villages within the previous month was associated with increased odds of malaria morbidity (OR 11.40, 95% CI: 6.91 – 18.82, <0.01). These findings indicate that travel to areas of relatively higher malaria transmission intensity increases the chances of malaria infection.

Although all studies found a positive association between recent travel and an increased risk of malaria, some studies had design limitations. These studies investigated the association between overnight travel and the risk of malaria using census survey data,[83,141,150-155] and case-control studies.[77,90,136,140,142,143,147] Findings were very informative but generally have uncertainties and caveats,[156] which include: 1) poor interpretation of migration questionnaire by respondents, 2) inability to fully capture data from migratory workers (always on the move), and 3) respondents are likely not to remember the exact day and time of travel. This calls for improved study designs and settings that can reduce design challenges.

2.4 Factors associated with increased risk of malaria among all study participants

Out of 16 studies included in this review, 12 studies assessed other factors that could be associated with malaria diagnosis (Table 2.3). Travelling to areas with high malaria prevalence was associated with increased risk of malaria diagnosis following travel,[77,83,136,142,147] as well as longer duration of travel,[136,149] and lack of malaria prevention measures during travel.[146,149] Increased age was associated with higher odds of malaria in Kenya,[145] while it was associated with lower odds on malaria in Ethiopia,[148] indicating other age-related factors including exposure and immunity may have played an important role in influencing malaria morbidity. On the other hand, higher socio-economic status of study participants was protective against malaria.[140,143]

In Kenya, travelling to areas of higher malaria endemicity was associated with increased odds of malaria diagnosis (OR 1.48, 95% CI: 1.30 – 1.69),[77] and similar trends were noticed in travelling from highland areas of Uganda (OR 3.2, 95% CI: 1.1 – 10.1, 0.04), Yunnan Province of China, and in Namibia along the border with Angola (OR 2.86, 95% CI: 1.17 – 6.97, 0.02).[147] These findings provide evidence that when individuals travel to areas of relatively higher malaria endemicity within the same country, they are at higher risk of malaria infection.

Other risk factors identified by a study conducted in Botswana using census data included engaging in late outdoor activities (OR 7.02, 95% CI: 1.80 – 27.56),[141] which was consistent with increased exposure to mosquito bites, and hence infection with malaria parasites. Another study evaluated special groups at higher risk of malaria during travel. A multi-centre study by Marshal *et al.* assessed human movement patterns depending on the malaria endemicity of the country studied.[83] This study was conducted in four countries all in sub-Saharan Africa: Mali, Burkina Faso, Zambia, and Tanzania. In each country, three to four survey sites were considered. Several factors were included during data collection and analysis. These included: malaria risk behaviour, children

accompanying travellers, and mobile phone usage. Women travelling with children were more likely to travel to areas of high malaria prevalence in all 4 countries and less likely to carry mobile phones in all sites except Tanzania. In Mali, youth between the ages of 16 and 29 years were more likely to travel to areas of high malaria prevalence predominantly for work-related reasons and were hence at risk of returning with malaria infection. It is important to note that results may apply to other study settings, but study environments and risk of malaria are different. Different groups/clusters have varying levels of exposure depending on the study site under consideration.

Author, year of publication	Risk factor		Exposure	Proportion positive, n (%)	Measures of association, (95% CI), p-value
Shanks, 2004[77]	Destination of travel		Epidemic prone areas	552 (28.7)	Reference
			Malaria endemic area	852 (37.4)	OR = 1.48 (1.30–1.69)
Yukich, 2013[140]	Socioeconomic status		More poor	-	Reference
			Less poor	-	OR = 0.36 (0.17–0.76), 0.01
Chirebvu, 2014[141]	Evening activities		No outdoor activities	-	Reference
			Late outdoor activities	-	OR = 7.02 (1.80–27.56)
Lynch, 2015[136]	Destination of travel		No travel	20 (35.7)	Reference
			Travel to areas of high risk	30 (76.9)	OR = 6.9 (1.4–33.1), 0.02
	Duration of travel		No travel	20 (35.7)	Reference
			> average time travelled	13 (72.2)	OR = 3.2 (1.1–10.1), 0.04
Xu, 2015[142]	Destination of travel		Upper part of a hill	57 (32.9)	Reference
			Lowland and foot of a hill	101 (33.9)	OR = 5.45 (2.52–11.80), <0.001
Mathanga, 2015[143]	Level of education		Below tertiary education	141 (44.1)	Reference
			Tertiary level of education	46 (30.1)	OR = 0.59 (0.36–0.96), 0.03
	Water source		No piped water	117 (44.7)	Reference
			Piped water in the house	70 (33.2)	OR = 0.80 (0.66–0.97), 0.02
Martial, 2016[83]	Malaria prevalence at destination of travel	Mali	Low malaria prevalence	165 (22.0)	Reference
			High malaria prevalence	227 (57.0)	OR = 4.46 (3.42–5.83), <0.001
		Burkina Faso	Low malaria prevalence	155 (24.9)	Reference
			High malaria prevalence	219 (34.3)	OR = 1.58 (1.23–1.58), <0.001
		Zambia	Low malaria prevalence	191 (24.8)	Reference
			High malaria prevalence	279 (36.2)	OR = 1.50 (1.20–1.89), 0.004
		Tanzania	Low malaria prevalence	87 (15.9)	Reference
			High malaria prevalence	188 (30.4)	OR = 2.28 (1.71–3.05), <0.001
Njuguna, 2016[145]	Age categories		< 6 months	44 (13.3)	Reference
			1 - 4 years	1,171 (22.8)	OR = 1.9 (1.4–2.7), <0.001
			5 - 14 years	777 (28.4)	OR = 2.6 (1.9–3.6), <0.001
			≥ 15 years	431 (18.6)	OR = 1.5 (1.1–2.1), 0.02
Tejedor-Garavito, 2017[146]	Malaria prevention		Any protection	9 (9.9)	Reference
			No prevention measures	197 (23.8)	OR = 2.84 (1.48–6.17), 0.004
Smith, 2017[147]	Destination of travel		>15km to Angola border	23 (7.2)	Reference
			< 15km to Angola border	75 (18.2)	OR = 2.86 (1.17–6.97), 0.02
	Age group		Under 5 years of age	9 (8.3)	Reference
			45 – 59 years of age	8 (17.4)	OR = 43.6 (2.12–896)
Haile, 2017[148]	Age group		>35 years old	18 (12.2)	Reference
			15 – 24 years old	84 (35.3)	OR = 3.20 (1.73–5.89), <0.001
	Level of education		Above primary level	33 (22.0)	Reference
			Primary level or less	97 (26.2)	OR = 2.21 (1.26–3.86), 0.02
Lowa, 2018[149]	Frequency of travel		One trip	-	Reference
			More than one trip	-	OR = 3.71 (1.27–10.84), 0.02
	Malaria prevention		No prophylaxis	-	Reference
			Prophylaxis used	-	OR = 0.22 (0.06–0.82), 0.02

Table 2.3. Assessment of factors associated with increased risk of malaria among all study participants

Two studies evaluated the risk of malaria when individuals travel within a country that has controlled malaria to destination near a neighbouring country that still has a high malaria burden. This may suggest that collaboration between neighbouring countries should be encouraged to control imported malaria cases. A study conducted by *Tejedor-Garavito et al*. in Swaziland examined malaria routine surveillance data recorded between January 2010 and June 2014 for malaria test results and history of travel.[146] Data on travel patterns among participants testing positive for malaria were compared to those found to test negative for malaria. The study found that the proportion of positive cases who reported history of travel was much higher compared to those with negative malaria test results. Travel to the border with Mozambique was strongly associated with positive malaria test. These findings demonstrated that for malaria control to be successful, travellers should be targeted for malaria prevention.

Another study conducted by, *Smith et al*. assessed the risk of malaria infection in north central Namibia.[147] Travel to the border with Angola was strongly associated with malaria in male participants. Male gender was also associated with a higher risk of malaria compared to females among non-travellers. These findings suggest that some population groups, young male travellers in this case, are more at risk and can be targeted for malaria control interventions.

To identify factors associated with malaria importation among residents of Lusaka district, Lowa et al. carried out a cross sectional study in Zambia between November 2015 and February 2016.[149] The study found that a high proportion of malaria cases were found to be imported from all ten provinces of Zambia into the urban area of Lusaka district. Travel history out of Lusaka district within 3 months prior to the study was associated with malaria. Factors associated with malaria importation during travel included age between 5 and 14 years (most affected group), duration of

stay with every increase in a week of stay associated with travellers more likely to import malaria infections, and frequency of travel.

Other factors identified by *Haile et al.* in northern Ethiopia in 2014, included lower level of education and engaging in non-agricultural occupation compared to farming, in addition to travel from the high altitude to low altitude villages within the previous month being associated with increased odds of malaria infection.[148] Findings from this study highlighted the need to strengthen malaria control interventions among travellers from highland areas of Ethiopia if malaria control and elimination are to be achieved.

Collectively, these studies identified important factors that influenced the risk of malaria in travellers, but left gaps due to design and scarcity of data and provided suggestions for further research. It is important to note that travel does not apply to just any movement of people. It has variations and hence changes in exposure. For example, travelling to a conference held in a 5-star hotel likely poses less risk to malaria exposure compared to a travel to a high malaria transmission area for a funeral rite function. These studies have been informative, but none have evaluated travel between urban and rural areas in Uganda and the risk of malaria, and none have followed up study participants over time using a cohort study design.

In conclusion, the available evidence on overnight travel and the risk of malaria suggest a strong association between travel and increased risk of malaria, but is inconclusive in ascertaining whether travel is or is not causally associated with malaria infection. Moreover, studies that have been carried out are insufficient to inform policy on malaria prevention during travel within malaria endemic countries Different approaches in terms of study design and settings are likely to

help provide additional evidence and contribute immensely towards our understanding of overnight travel and the risk of malaria.

Chapter 3. Overview of the study design and methodology

This thesis utilised 3 study settings to better represent malaria epidemiology in Uganda (Table 3.1); 1) a case-control study in Kampala, an urban setting, 2) a cohort study at 3 sites of varied malaria transmission in Uganda, and 3) a cohort study at a rural site in Uganda that had received several rounds of IRS.

Study	Dates	Procedures	Sample size
Case-control study	July 2019 through August 2019	- Questionnaire data collection on travel and factors associated with malaria infection during travel - Malaria diagnosis	567 Participants
PRISM 1 study	October 2015 through June 2016	- Questionnaire data collection on travel - Malaria diagnosis	906 Participants
PRISM 2 study	October 2017 through September 2019	- Detailed questionnaire data collection on travel and behavioural factors during travel, and while study participants were at their homes of residence	527 Participants

Table 3.1. A summary of phases of data collection

3.1 Study sites

3.1.1 Case-control study at Naguru General Hospital in Kampala

A matched case-control study was conducted to measure the associations between overnight travel out of Kampala city and the risk of malaria, and to determine factors associated with acquiring malaria during overnight travel out of Kampala. Kampala is the capital and largest city in Uganda with a population of approximately 1,650,800 people.[157,158] The city is located in central

part of Uganda and is divided into Kampala Central, Nakawa, Makindye, Rubaga, and Kawempe divisions.

Patients attending the outpatient department of Naguru General Hospital, a public hospital in Kampala, were eligible for recruitment. This public hospital serves the inhabitants of Kampala metropolitan area, mainly from the eastern part of the city. Naguru General Hospital provides outpatient services, inpatient services, diagnostic services, specialised curative care services. According to the Health Management Information System (HMIS), the outpatient department of Naguru General Hospital attended to an average of 13,000 patients per month between January 2019 and September 2019, out of which 1,000 patients were referred to the laboratory for malaria test, and on average 110 (33 (30%) under 5 years of age, 77 five years old and above) patients per month test positive for malaria.[137] Malaria is typically diagnosed using rapid diagnostic tests (RDTs), with microscopy as a backup in case of RDT stockouts.

3.1.2 PRISM studies

PRISM was a multi-project research program in Uganda established in 2010 as an International Centre of Excellence in Malaria Research (ICEMR) funded by the United States National Institutes of Health (NIH). The program was initially funded for 7 years (2010 to 2017) as PRISM 1, and the funding was renewed for another 7 years (2017 – 2024) as PRISM 2 which spanned the length of the PhD project. The cohort study part of the PhD research project was nested within PRISM and answered new objectives which were not being addressed by the primary PRISM research projects.

3.1.2.1 PRISM 1 Study

PRISM 1 included a cohort of participants in Walukuba sub-county in Jinja district, Kihihi sub-county in Kanungu district, and Nagongera sub-county in Tororo district (Figure 3.1). The main objectives included: 1) to optimize strategies for malaria surveillance and measure the impact of control interventions, 2) to assess malaria exposure and protection, and 3) surveillance and discovery of mediators of resistance to antimalarial drugs and insecticides. The three PRISM 1 sites were selected to represent different malaria transmission settings in Uganda (Figure 3.1): Walukuba sub-county in Jinja district is a peri-urban area with low malaria transmission intensity, Kihihi sub-county in Kanungu district is a rural area with relatively low malaria transmission intensity, and Nagongera sub-county in Tororo district is a rural area in Tororo district with a historically very high malaria transmission intensity of 310 infectious bites per person per year. [28] [35]

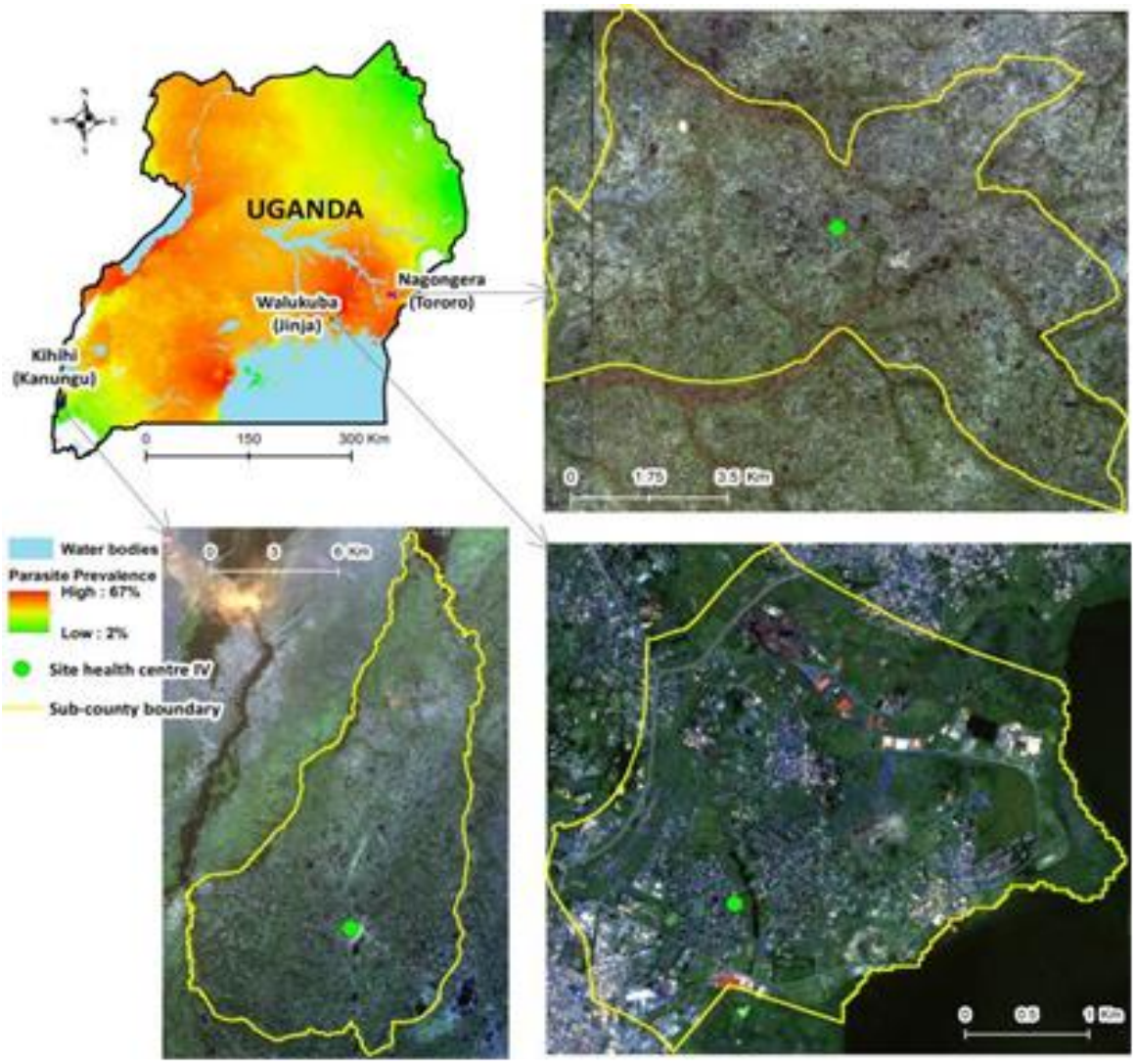

Figure 3.1. Three PRISM1 sites in Uganda with varied malaria transmission intensity; Walukuba sub-county in Jinja district, Kihihi sub-county in Kanungu district, and Nagongera sub-county in Tororo district [35]

3.1.2.2 PRISM 2 study

PRISM 2 included a cohort of participants in Nagongera sub-county in Tororo district. The objectives of the project included 1) to monitor trends in the burden of malaria, 2) to characterize the evolution of phenotypic and genotypic markers of drug and insecticide resistance, 3) to characterize factors that determine whether sporozoite inoculation results in the establishment of blood stage infection.

Because of the high malaria burden in Tororo district, it was selected to receive IRS (Table 3.1) in addition to LLINs delivered through the national distribution campaign (in November 2013).

IRS rounds	Months of spraying	Class of insecticide (insecticide used for IRS)
1st	December 2014-February 2015	Carbamate (bendiocard)
2nd	June 2015 to July 2015	Carbamate (bendiocard)
3rd	November 2015 to December 2015	Carbamate (bendiocard)
4th	June 2016 to July 2016	Organophosphate (pirimiphos-methyl)
5th	June 2017 to August 2017	Organophosphate (pirimiphos-methyl)
6th	June 2018 to July 2018	Organophosphate (pirimiphos-methyl)

Table 3.2. Dates for community-wide IRS campaign in Nagongera sub-county

Several rounds of IRS have led to drastic reduction in malaria vector density (Figure 3.2).[38] Similarly, malaria incidence in this area greatly reduced forming part of the area with low malaria transmission intensity neighbouring some areas of high malaria transmission intensity.[37,40] This site provided an attractive environment to study the association between overnight travel and malaria, given the reduction in transmission. The transition in epidemiology of malaria transmission in this area from very high transmission to very low malaria burden provided an opportunity to explore the possibility of increased risk of malaria associated with overnight travel.

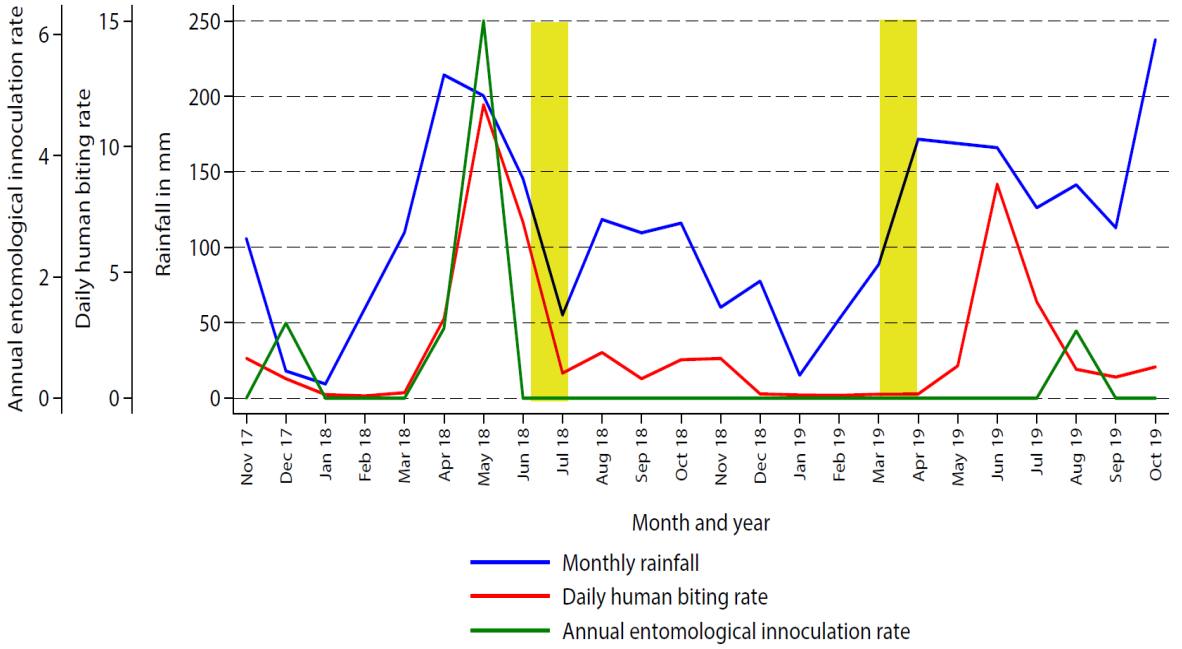

Figure 3.2. Changes in monthly estimates of daily human biting rate, annual entomological inoculation rates, and rainfall. Yellow bars indicate the timing of IRS with pirimiphos-methyl.[38]

Unlike previous studies of the associations between travel and malaria infection which employed cross-sectional or case-control designs, this prospective cohort follow up allowed for estimation of number of trips made over time and characterised each individual overnight travel, measured incidence of malaria, and point prevalence of malaria parasitaemia.

3.2 Study hypotheses

For objective 1, hypothesis that participants who present with fever without another obvious explanation are more likely to have malaria if they recently had an overnight travel, compared to those who did not travel was tested. For objective 2, the hypothesis tested was that the incidence of malaria within 60 days after returning from recent overnight travel was higher than the incidence of malaria during the preceding 60 days when there was no overnight travel. Objective 3 was addressed by testing the hypothesis that recent overnight travel was associated with

behavioural risk factors such as changes in measures taken to prevent malaria and going to bed late that may influence the risk of malaria infection.

The definition of overnight travel was based on the study site. During the case-control study in Kampala city, recent overnight travel was defined as travelling out of Kampala city (Nakawa, Makindye, Rubaga, Kawempe and Central divisions) within the last 60 days and spending at least one night away. During the cohort study in Tororo, recent overnight travel was defined as travelling out of a sub-county of residence (Nagongera sub-county) within previous 60 days and spending at least one night away.

To date, studies of the associations between travel and malaria infection have employed mainly cross-sectional or case-control designs. Part of this research project used a cohort study design with longitudinal follow up which allowed for estimation of number of trips made over time and characterised each individual overnight travel, measured the incidence of malaria, and assessed behavioural factors that may influence exposure to malaria infection. This thesis presents findings to provide evidence as to whether there are associations between overnight travel and the risk of malaria in Uganda or not, and whether there are factors that modify the risk of malaria during travel.

3.3 Case-control study at Naguru General Hospital in Kampala

A matched case-control study design was used for the following: 1) to measure the association between recent overnight travel and the risk of malaria, and 2) to determine risk factors of malaria during overnight travel out of Kampala. The exposure was recent overnight travel out of Kampala city and the destination of travel to the sub-county level, and the outcome was malaria test

results. The study population was patients attending outpatient department at Naguru General Hospital in Kampala, Uganda. Both children and adults were included in the study if they met the inclusion criteria (as detailed in sections 3.3.1 and 3.3.2). For every 2 cases, 5 hospital outpatient department controls were selected to increase the power of the study, matching on age categories (0.5 – 5 years, >5 – 15 years, and > 15 years).

3.3.1 Selection of cases

Cases were defined as patients of at least 6 months of age attending the outpatient department with documented axillary temperature of 37.5^0C or/and history of fever within the last 24 hours and a positive diagnostic test for malaria. Cases were identified according to the following eligibility criteria: 1) a positive diagnostic test for malaria, 2) resident of Kampala capital city for at least the last 3 months, 3) at least 0.5 year of age, 4) body weight of at least 4kg, 5) history of fever within the last 24 hours/ axillary temperature of 37.5^0 C or more, 6) able to speak English or Luganda (a local language spoken in Kampala), 7) willingness to provide informed consent in case of adults and parents or guardian, 8) Willingness to provide an assent in case of minors age 8 years to 17 years. Cases were excluded if: 1) they had answered the questionnaire before (prior participation in the study), 2) they had evidence of chronic illnesses that can explain the history of fever/documented temperature such as AIDS and sickle cell disease.

3.3.2 Selection of controls

Controls were defined as patients of at least 6 months in age attending outpatient department with documented axillary temperature of 37.5^0C or/and history of fever within the last 24 hours and a negative diagnostic test for malaria. The following eligibility criteria was used for enrolment of controls: 1) a negative diagnostic test for malaria, 2) resident of Kampala capital city for at least

the last 3 months, 3) at least 0.5 year of age, 4) body weight of at least 4kg, 5) history of fever within the last 24 hours/ axillary temperature of 37.5^0 C or higher, 6) able to speak English or Luganda (a local language spoken in Kampala), 7) willingness to provide informed consent/assent. Controls were excluded from the study if they had already participated in the study or there was evidence of chronic illnesses that can explain the history of fever/documented fever such as AIDS and sickle cell disease.

3.3.3 Study participant recruitment and data collection

Recruitment of study participants was conducted by the study staff at the laboratory where patients from outpatient department with suspected malaria (documented temperature >= 37.5 or/and history of fever within last 24hrs) were referred to for malaria tests. The outpatient department laboratory did not serve pregnant women (they were attended to at the antenatal clinics) and thus, they were not included in this study. Patients with a positive diagnostic test for malaria were assessed for eligibility to participate in the study as cases, and patients with a negative diagnostic test for malaria were assessed for enrolment as controls. For every case enrolled, a control was enrolled consecutively at a ratio 2 cases to 5 controls until 162 cases and 405 controls were enrolled.

For the patient who met the eligibility criteria, an informed consent was administered (assent for minors, and parents/caretaker consent for children). Participants who provided the informed consent were then asked questions about the history of travel and if they did a detailed questionnaire was administered. The detailed questionnaire captured data on destination of travel, length of the trip, and behavioural risk factors that are likely to influence exposure to mosquito bites such as staying outdoors late, going to bed late and use of LLINs during travel.

3.3.4 Diagnosis of malaria

Participants with documented temperature >= 37.5 or/and history of fever within the previous 24hrs and a positive laboratory test were diagnosed with malaria. All tests for malaria were done using RDTs (CareStart™) and carried out in the hospital laboratory by the hospital laboratory technicians with support from the study staff. The study staff made sure that all participants referred to the laboratory for a malaria test received malaria test results.

The RDT test kit comprises of a nitrocellulose strip encased in a plastic cassette, a capillary tube for transferring blood, and a clearing buffer solution.[159] Malaria tests were performed according to the manufacturer's guidelines as already described elsewhere.[160,161] Briefly, Blood was collected from the patient by finger prick. Finger pricks were done by first disinfecting the finger using an alcohol swab and allowing it to air dry, and then pricking the disinfected finger and wiping off the first drop of blood with dry cotton. Blood was then collected using a capillary tube, and transferred into the sample well on the RDT cassette. The clearing buffer solution was then introduced into the well on the RDT cassette and the RDT read after 20 minutes. The results were considered positive if both the control line and the test line were visible and negative if the control line was visible but not the test line.

3.3.5 Data collection and quality assurance

Participant data were entered into a computerised data base by study personnel and transferred to a password protected server maintained at the Infectious Diseases Research Collaboration (IDRC) data centre in Kampala. Data query programs were written into the database to limit the entry of incorrect data and ensure entry of data into required fields.

Records for this study were maintained and stored in compliance with the principles of good clinical practice (GCP) and regulatory and institutional requirements, and in compliance with the requirements for the protection of confidentiality of participants. All members of the study team were trained on the study protocol prior to the onset of the study. Study progress meetings were conducted regularly to review data collected, address any difficulties, and provide performance feedback to the members of the study group.

3.3.6 Exposure and outcome measures

The exposure was whether participants had a recent overnight travel out of Kampala city or not, and if they travelled, whether they travelled to a high or low (or district receiving IRS) malaria transmission district as indicated by the Uganda malaria risk map updated by LINK (an integral collaboration between the London School of Hygiene and Tropical Medicine, Information for Malaria Project (INFORM) and National Malaria Control Programmes (NMCPs) of African nations).[162] Recent overnight travel was defined as at least 7 days between when study participants first travelled overnight out of Kampala city and presentation to the hospital with fever/history of fever within the last 24 hours, and not more than 60 days from the day study participants returned from a trip out of Kampala city to presentation to the hospital with documented fever or history of fever within the last 24 hours. The outcome was malaria diagnostic test results.

3.3.7 Sample size estimation

The sample size for this study was primarily generated to answer whether participants travelled to areas of higher malaria transmission compared to Kampala. Assuming that among participants with recent overnight travel, 15% would have the exposure of interest (travel to a high

transmission area), 90 cases and 90 controls were needed to detect an odds ratio of 3.0 or greater given a two-sided type 1 error of 0.05 and power of 80%. Assuming that 56% of cases would have any recent overnight travel and 23% of controls would have any recent overnight travel (based on published data from a peri-urban area in Jinja, Uganda),[163] a total of 162 cases (90/0.56 = At least 161 cases) and 405 controls (90/0.23 = At least 392 controls) were enrolled. With respect to objective 1, our total sample size of 567 study participants (162 cases + 405 controls, for a ratio of 2:5) provided a 90% power (two-sided type 1 error of 0.05) to detect an odds ratio of 2.0 or greater.

3.3.8 Data analysis

The proportion of participants with positive diagnostic test for malaria following recent overnight travel were calculated and compared to proportion of participants with positive diagnostic test without recent overnight travel using chi square test. For the association between overnight travel out of Kampala city and the risk of malaria, odds of malaria in participants with a history of recent overnight travel were compared with odds of malaria in those without history of overnight travel using conditional logistic regression stratified by age categories (0.5 years to <15 years and >= 15 years). To determine the risk factors of malaria during overnight travel out of Kampala city, the destination of travel was dichotomised into high malaria transmission area and low malaria transmission area (IRS district, and highland areas). Comparisons of odds of malaria in participants who travelled to a high malaria transmission area and odds of malaria in participants who travelled to a low malaria transmission area were made using conditional logistic regression controlling for reported bed net use during travel.

3.4 PRISM 1 study

3.4.1 Study participants recruitment and follow up

PRISM 1 study participants were followed up for one year (October 2015 through September 2016). At each of the 3 sites, 100 households were randomly selected from the enumeration list and were enrolled if they had a resident 0.5-10 years of age. For the study purposes, a household was defined as a single permanent or semi-permanent dwelling structure acting as a primary residence of a person or a group of people that generally cook and eat together. Study participants were enrolled according to the following eligibility criteria: 1) age between 0.5 and 10 years, 2) fulltime resident of the selected household, 3) agreement to come to the study clinic whenever they fall sick, 4) agreement to avoid other medications administered outside the study, and 5) agreement to provide informed consent. At least one adult caregiver per household was enrolled as well to provide informed consent.

Study participants received all their medical care free of charge at a designated study clinic at each site open every day to avoid missing any malaria episode. The cohort was dynamic such that all newly eligible children born into households participating in the cohort were enrolled into the study, once they were > 6 months of age. Study participants were followed until they reached 11 years when they were withdrawn from the cohort. Additional criteria for withdrawal from the study included: 1) permanent movement out of the sub-county, 2) inability to be located for >4 months, 3) withdrawal of informed consent, 4) withdrawal of all children under their care in case of adults, and 5) inability to comply with the study schedule and procedures.

3.4.2 Study participant follow up

Follow up of study participants involved coming to a designated study clinic open 7 days a week, for routine visits once a month and when study participants fall sick. During each study clinic visit, data on demographics, history of fever, any treatment outside the study clinic (Appendix 1), and travel history (Appendix 2) were captured on case record forms, and double entered into a Microsoft access database. During routine visits, blood smears were taken from all participants for routine microscopy as well as filter paper sample for future evaluation of malaria parasites. Every 3 months, blood by venepuncture was collected in addition to blood smear for malaria parasite detection using microscopy. Participants found to have asymptomatic parasitaemia (microscopic and sub-microscopic) were not treated for malaria.

3.4.3 Diagnosis of malaria

Study participants who presented with a fever (tympanic temperature $\geq$ 38.0°C) or history of fever in the previous 24 hrs had blood obtained by finger-prick for a blood smear to be examined for malaria parasites and filter paper sample collection for future evaluation. If the thick blood smear was positive, the patient was diagnosed with malaria and managed according to WHO malaria treatment guidelines (Appendix 17).[164] Participants who did not have fever and were not suspected to have malaria received standard-of-care treatment.

3.4.4 Overnight travel follow up

Study participants were asked whether they spent a night outside their homes since the last clinic visit. If they did, a detailed questionnaire (Appendix 4) was administered to capture data on where they travelled to, the number of nights spent away, activities undertaken during travel, time spent outdoors, and malaria control measures used if any.

3.4.5 Blood smear reading by microscopy

Malaria parasites identification was by microscopy of blood smear slides. Thick blood smears were stained with 2% Giemsa, allowed to dry for 30 minutes and read by experienced laboratory technologists [165]. Parasite densities were calculated by counting the number of asexual parasites per 200 leukocytes or per 500 leukocytes if the count is less than 10 asexual parasites per 200 leukocytes, assuming a leukocyte count of 8,000 per microliter. A blood smear was considered negative if the examination of 100 high power fields did not reveal any asexual parasites

3.4.6 Power calculation considerations

The study sample size was determined by the parent study. Estimates for power calculations for Objective 2 were based on preliminary findings that 23% of participants reported overnight travel. The outcome measure was the incidence of malaria defined as the number of new episodes of malaria (fever or history of fever and a positive blood smear) per person-time of follow up. Data on travel histories were anticipated to be available from a total of 600 study participants, among which 138 (23%) reported any overnight travel. Among those with any reported overnight travel, a total of 257 person-years of observation were estimated of which 15% included observation time following recent travel (provisionally defined as overnight travel within the prior 60 days), 83% included observation time without recent travel (unexposed group), and 2% included observation time during overnight travel (which were not included in the analysis). The incidence of malaria was further estimated to be 0.20 episodes per person-year in the unexposed group (no recent overnight travel). Given these assumptions, there was 80% power (2-sided alpha = 0.05) to test the hypothesis for Objective 2 if the incidence of malaria in the exposed group (recent travel) was 0.44 or greater (which was equivalent to an incidence rate ratio of 2.2 or greater).

3.4.7 Data analysis

The exposure was recent overnight travel and the outcomes of interest was the incidence of malaria. To measure the association between overnight travel and the incidence of malaria, analyses were restricted to include only those participants with any overnight travel. Using questionnaire data, person-time of follow up was categorized according to the number of days since last overnight travel. Provisionally the exposure variable of interest was dichotomized into 1-60 days since recent overnight travel (exposed group) and more than 60 days since recent overnight travel or no prior overnight travel documented (unexposed group). Other categories of time since overnight travel were explored. Person-time during overnight travel was not included in the analyses. Comparisons between the incidence of malaria in the exposed and unexposed groups were made using generalized estimating equations with a negative binomial family and adjustment for repeated measures in the same participant (such that each participant serves as their own control).

3.5 PRISM 2 study

3.5.1 Study participants recruitment and follow up

The cohort at Nagongera study site in Tororo, Uganda was expanded from October 2017 through October 2019 to include all members of eligible households. Study participants recruitment procedures have been described elsewhere.[38] Briefly, a random sample of 80 households was selected from the list of 6,992 households enumerated in Nagongera sub-county. Households were enrolled according to the following eligibility criteria: 1) at least two members of the household aged 5 years or younger, 2) no more than 7 permanent residents currently residing in the household, 3) no plans for the household to move from Nagongera sub-county in the next 2 years, 4) willingness to respond to questionnaires administered at home. Households with at least

two members aged 5 years or younger and no more than 7 permanent residents currently residing in the household were enrolled to ensure that the cohort included a sufficient number of younger children, and that the number of household members did not exceed the capacity for participant follow-up.

All members of the enrolled household were approached for participation in the cohort study. Potential study participants were enrolled using the following selection criteria: 1) household considered their primary residence, 2) agreement to come to the study clinic for any febrile illness, 3) agreement to avoid antimalarial medications outside the study, 4) provision of written informed consent (for parent or guardian in case of children)

The cohort was dynamic in that any new permanent residents that joined the enrolled household over the course of the study, were enrolled into the study. Participants were asked to seek all medical care for themselves and their children at the study clinic open every day from 8.00 am to 5:00 pm. All participants were seen at the study clinic at least once every 4 weeks for up to two years. At each visit, a standardized evaluation was done and a standard case record form was completed.

3.5.2 Study participant follow up

Study participants were seen at least once a month and were asked whether they travelled or not since the last visit to the study clinic. If they had an overnight travel, a detailed questionnaire was administered (Appendix 1) to capture data on destination of travel, length of the trip, behavioural characteristics that are likely to influence exposure to mosquito bites such as staying outdoors late, sleeping late and use of LLINs during travel.

3.5.3 Overnight travel follow up at the study clinic

At the time of all visits to the study clinic, participants or their parents/guardians were asked whether they spent at least one night outside of their sub-county of residence since the last clinic visit. If they did, a detailed questionnaire (Appendix 1) was administered to capture data on where they travelled to, the number of nights they spent away, activities undertaken during travel, time spent outdoors, and malaria control interventions used, if any.

3.5.4 Travel data collection at participants homes of residence

Participants were visited at their homes by study staff every two weeks and a questionnaire administered on the presence and use of LLINs. Additional data were collected on behavioural risk factors such as staying outdoors late and leaving the bed early in the morning that may influence the risk of mosquito bites. Study staff also inspected sleeping places in participants household to see whether LLINs were hanging or not.

3.6 Data management

Clinical data for PRISM studies were collected and recorded onto the case record forms by the study clinicians, and reviewed for completeness and accuracy. Laboratory data were captured on laboratory report forms by laboratory staff and then reviewed and transferred to the case record form by the study clinicians. All clinical and laboratory data were entered into a Microsoft access database and data entry was double to verify accuracy of entry. Query programs were written into the database to limit entry of incorrect data and ensure entry of data into required fields.

3.7 Ethical consideration

The case control study in Kampala was approved by the 1) Mulago Hospital Research and Ethics Committee (reference number: 1592), 2) London School of Hygiene and Tropical Medicine Ethics Committee (reference number: 16625), and the 3) Uganda National Council of Science and Technology (reference number: SS-5012). PRISM studies were reviewed and approved by the IRBs of all the participating institutions including: 1) Makerere University, School of Medicine Research and Ethical Committee (reference number: 2017-099), 2) London School of Hygiene and Tropical Medicine Ethics Committee (reference number: 14266), as well as Uganda National Council of Science and Technology (reference number: HS-119ES).

3.8 Informed consent

Study participants' parents/guardians in case of children and participants in case of adults, provided written informed consent before enrolment into the study. Children 8 years and above provided written assent as per Uganda National Council for Science and Technology guidelines.[166] Both consents and assents were translated into local languages and back-translated into English to check for any loss of meaning. The local languages included Luganda in Kampala, and Japadhola and Swahili in Tororo. The study clinicians discussed the study procedures in the appropriate language to make sure the study is well understood by the participants' parent/guardian or adult participants.

3.9 Confidentiality

For all the studies, participant records were kept confidential and identified only by unique identification numbers. Maintaining study participants' confidentiality was part of the training offered to study staff before the study began in addition to study procedures. No participant

names were entered into the computerised database. In addition, no individual participant identity was used in any report published from the study. For the PRISM cohort study, all study records were kept in locked filing cabinets and participants identified by unique identification numbers.

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

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Student ID Number	1406078	**Title**	Dr
First Name(s)	Emmanuel		
Surname/Family Name	Arinaitwe		
Thesis Title	Association between overnight travel and the risk of malaria: case-control and prospective cohort studies in Uganda		
Primary Supervisor	Sarah Staedke		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

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SECTION E

Student Signature	[redacted]
Date	19 September 2020

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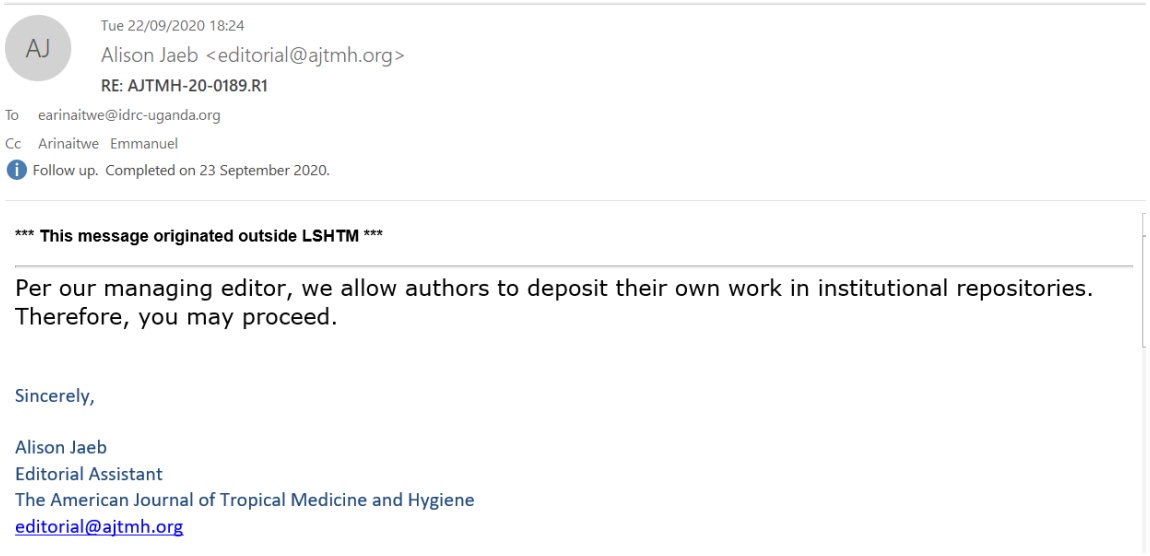
Date	21 Sept 2020



AJ

Tue 22/09/2020 18:24

Alison Jaeb <editorial@ajtmh.org>

RE: AJTMH-20-0189.R1

To earinaitwe@idrc-uganda.org

Cc Arinaitwe Emmanuel

Follow up. Completed on 23 September 2020.

***** This message originated outside LSHTM *****

Per our managing editor, we allow authors to deposit their own work in institutional repositories. Therefore, you may proceed.

Sincerely,

Alison Jaeb
Editorial Assistant
The American Journal of Tropical Medicine and Hygiene
editorial@ajtmh.org

Chapter 4. Malaria diagnosed in an urban setting strongly associated with recent overnight travel: a case-control study in Kampala, Uganda

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Abstract

Background. Malaria is frequently diagnosed in urban Kampala, despite low transmission intensity. To evaluate the association between recent travel out of Kampala and malaria, we conducted a matched case-control study.

Methods. Cases were febrile outpatients with a positive malaria test; controls were febrile outpatients with a negative test. For every 2 cases, 5 controls were selected, matching on age. Data were collected on recent overnight travel out of Kampala (past 60 days), destination and duration of travel, and behavioural factors, including sleeping under long-lasting insecticidal net (LLIN) during travel.

Results. From July to August 2019, 162 cases and 405 controls were enrolled. The locations of residence of cases and controls were similar. More controls were female (62.7% vs 46.3%, p<0.001). Overall, 158 (27.9%) participants reported recent overnight travel. Travellers were far more likely to be diagnosed with malaria than those who did not travel (80.4% vs 8.6%, OR 58.9, 95% CI 23.1-150.1, p<0.001). Among travellers, traveling to a district not receiving indoor residual spraying of insecticide (OR 35.0, 95% CI 4.80-254.9, p<0.001), no LLIN use (OR 30.1,

95% CI 6.37-142.7, p<0.001), engaging in outdoor activities (OR 22.0, 95% CI 3.42 - 141.8, p=0.001), and age <16 years (OR 8.36, 95% CI 2.22-56.2, p=0.03) were associated with increased odds of malaria.

Conclusions. Kampala residents who travelled overnight out of the city were at substantially higher risk of malaria than those who did not travel. For these travellers, personal protection measures, including sleeping under LLINs when traveling, should be advocated.

4.1 Background

Over the past twenty years, impressive reductions in malaria burden have been reported in across sub-Saharan Africa.[1] This progress has been attributed to high coverage of effective interventions including indoor residual spraying of insecticides (IRS), long-lasting insecticidal bed nets (LLINs), and prompt treatment with artemisinin-based combination therapy (ACT). Increasing urbanization of African populations has also contributed to malaria reduction.[130] Despite this success, malaria remains a global health challenge; recent evidence indicates that the number of malaria cases in many African countries has increased, suggesting that progress on malaria control has stalled.[2] In Uganda, expansion of malaria control measures has been associated with substantial decreases in malaria burden in some areas.[46,48,49] but not in all settings.[52,53] Consequently, malaria transmission in Uganda is heterogenous, with low risk of malaria in urban areas, highland areas and locations where IRS has been effectively implemented, but medium to high risk elsewhere in Uganda.[42]

In residents of malaria endemic areas, repeated exposure to malaria parasites results in acquisition of protective immunity against illness and infection.[108] However, this acquired immunity may wane if exposure to malaria parasites reduces, due to a non-endemic area or highly urbanized environment or in the setting of intense malaria control measures. In

Zanzibar, Tanzania and Bioko Island, Equatorial Guinea, travel between the island (low malaria risk following scale up of control measures) and the mainland (high malaria risk), was found to be associated with malaria infection and disease among returning residents.[76,87,93,167,168] In Malawi, among travellers between urban and rural areas, symptomatic malaria was higher in participants living in urban zones who reported spending at least one night in rural areas within a month before testing for malaria.[143] In Tororo District in Eastern Uganda, where an effective IRS campaign resulted in significant declines in malaria transmission intensity,[37] travel from Tororo to neighbouring high transmission settings was a risk factor for malaria.[163]

The World Health Organization recommends malaria chemoprophylaxis for those traveling from non-endemic regions to malaria endemic areas.[169] However, whether this policy or other preventative approaches would be applicable for travel within malaria-endemic countries is less clear. In Uganda, malaria transmission intensity appears to have decreased greatly in Kampala, the urban capital, but the number of malaria cases reported in Kampala fluctuates over time.[137] To investigate the association between overnight travel outside of Kampala and diagnosis of clinical malaria, we conducted a case-control study.

4.2 Methods

4.2.1 Study site

The study was conducted at Naguru General Hospital, a public hospital in Kampala. Kampala is a low malaria transmission setting, with a reported parasite prevalence rate in children aged 0-59 months of 3.7% by histidine-rich protein 2 (HRP-2) rapid diagnostic test (RDT) in 2014-2015.[42] The hospital serves the Kampala metropolitan area, and provides outpatient, inpatient, and specialized care services. Between January and September 2019, the outpatient department served an average of 13,000 patients per month, out of which 1,000 were referred to the laboratory for a malaria test, and an average of 110 tested positive for malaria per

month. Malaria was typically tested using RDT, with quantification of parasite density by microscopy for positive RDT tests. By policy, all patients with suspected malaria were referred to the laboratory for a malaria test before malaria treatment was prescribed.

4.2.2 Study design

This was a matched case-control study. Cases were defined as patients at least 6 months of age attending the outpatient department at Naguru General Hospital with documented axillary temperature of ≥ 37.5°C and/or history of fever within the last 24 hours with a positive RDT for malaria. Controls were patients attending the same outpatient department with documented axillary temperature of ≥ 37.5°C and/or history of fever within the last 24 hours, but with a negative malaria RDT.

4.2.3 Matching criteria

Controls were matched to cases by age and time of presentation (within one week of a case). For every 2 malaria positive cases, 5 malaria negative controls were enrolled. Age categories matched were 0.5 – 5 years, > 5 – 15 years, and > 15 years.

4.2.4 Study participant enrolment and data collection

All patients referred to the outpatient department laboratory for suspected malaria were assessed for eligibility to participate in the study. Study participants were enrolled if they 1) resided in Kampala (Kampala Central, Kawempe, Makindye, Nakawa, Rubaga divisions) for at least the last 3 months, 2) were at least 0.5 years of age, 3) weighed at least 4 kg, 4) had axillary temperature of at least 37.5°C and/or a history of fever within the last 24 hours, 5) were able to speak English or Luganda, and 6) provided written informed consent (from parents or guardians in children, with assent for ages 8 to 17 years). Patients with a positive

malaria RDT result were screened as cases, and patients with a negative malaria RDT result were screened as controls until 162 cases and 405 controls were enrolled.

For patients who met all the eligibility criteria and provided informed consent, a questionnaire was administered on whether they had travelled out of Kampala within the last 60 days. If they had travelled, a detailed travel questionnaire was administered, to capture data on destination of travel, length of the trip, and behavioural risk factors likely to influence exposure to mosquito bites such as staying outdoors late, time to bed, and use of LLINs during travel.

4.2.5 Laboratory procedures

Malaria was diagnosed at Naguru General Hospital by experienced laboratory technicians with the CareStart™ RDT, which assesses HRP-2, according to the manufacturer instructions.

4.2.6 Sample size

We aimed to detect an association between recent (within 60 days) overnight travel out of Kampala and diagnosis of malaria. To do this, a sample size of 567 participants was required to detect an odds ratio of at least 3.0 for the association between overnight travel and malaria diagnosis, given a two-sided type 1 error of 0.05 and power of 80%. We predicted that 56% of cases and 23% of controls would report recent overnight travel based on data from a peri-urban area in Jinja, Uganda,[163] and thus 162 cases and 405 controls (ratio of 2:5) were required to evaluate the association between risk factors for malaria and overnight travel.

4.2.7 Ethical considerations

Ethical approvals were obtained from Mulago Hospital Research and Ethics Committee (MHREC; ref. 1592), the London School of Hygiene and Tropical Medicine Ethics Committee

(LSHTM; ref. 16625), and the Uganda National Council of Science and Technology (UNCST; ref. SS 5012). Informed consents were obtained from all study participants before data collection. All study staff were trained on procedures for maintaining confidentiality before study activities commenced.

4.2.8 Statistical analysis

All data were collected by trained study staff using a hand-held computer and transferred weekly to a secure server. Data analyses were performed using Stata 14 software (StataCorp, College Station, TX, USA). Recent overnight travel was defined as any travel outside the geographical confines of Kampala, spending at least one night away, within the previous 60 days. For study participants with a history of recent overnight travel, destination of travel was dichotomized into districts receiving or not receiving IRS according to United States President's Malaria Initiative VectorLink, Uganda IRS project.[50] Other risk factors that were evaluated included duration of stay at destination of travel, reported LLIN use, time to bed, place of stay, and activities carried out during travel. Malaria cases diagnosed within 7 days from the start of a trip were not included in the analysis, because the incubation period of *Plasmodium falciparum* malaria typically ranges from 7-14 days depending on the immune status of the individual.[170-172] Characteristics of study participants and individual trips were reported as proportions for categorical variables and medians with interquartile ranges for continuous variables.

The primary exposure of interest was recent overnight travel. Conditional logistic regression analysis was used to estimate associations (as odds ratios) between recent overnight travel out of Kampala and diagnosis of malaria. Since the analysis was matched on age, association between age and the odds of malaria was not reported. For study participants with a history of recent overnight travel, unmatched analysis of risk factors associated with malaria during

travel out of Kampala was performed using logistic regression, with associations reported as odds ratios. Reasons why participants travelled were not included in the final model analysis because of collinearity of some reasons, e.g. accompanying parents or guardians with age categories. All comparisons were considered statistically significant at $P < 0.05$.

4.3 Results

4.3.1 Characteristics of study participants

From July through August 2019, 186 cases and 524 controls were screened, and 162 cases and 405 controls were enrolled (Figure 4.1). All cases and most controls were excluded because they resided outside of Kampala. Cases and controls were similar in age and location of residence, but not gender (Table 4.1); more controls than cases were female (62.7% vs 46.3%, $p<0.001$). Overall, 158 participants reported recent overnight travel, including 128 (78.4%) cases and 31 (7.7%) controls.

Characteristic	Cases *(Positive malaria test)*	Controls *(Negative malaria test)*
Total number of study participants (N=567)	162	405
Mean age, (SD)	17.7 (16.3)	18.6 (17.3)
Age categories, n (%)		
Below 5 years	56 (34.6)	140 (34.6)
5 to 15 years	26 (16.1)	65 (16.1)
16 years or older	80 (49.4)	200 (49.4)
Female gender, n (%)	75 (46.3)	254 (62.7)
Division of residence in Kampala, n (%)		
Kampala central	11 (6.8)	37 (9.1)
Kawempe	15 (9.3)	21 (5.2)
Makindye	20 (12.4)	41 (10.1)
Nakawa	114 (70.4)	295 (72.8)
Rubaga	2 (1.2)	11 (2.7)
Participants with history of overnight travel, n (%)	127 (78.4)	31 (7.7)

Table 4.1. Characteristics of study participants

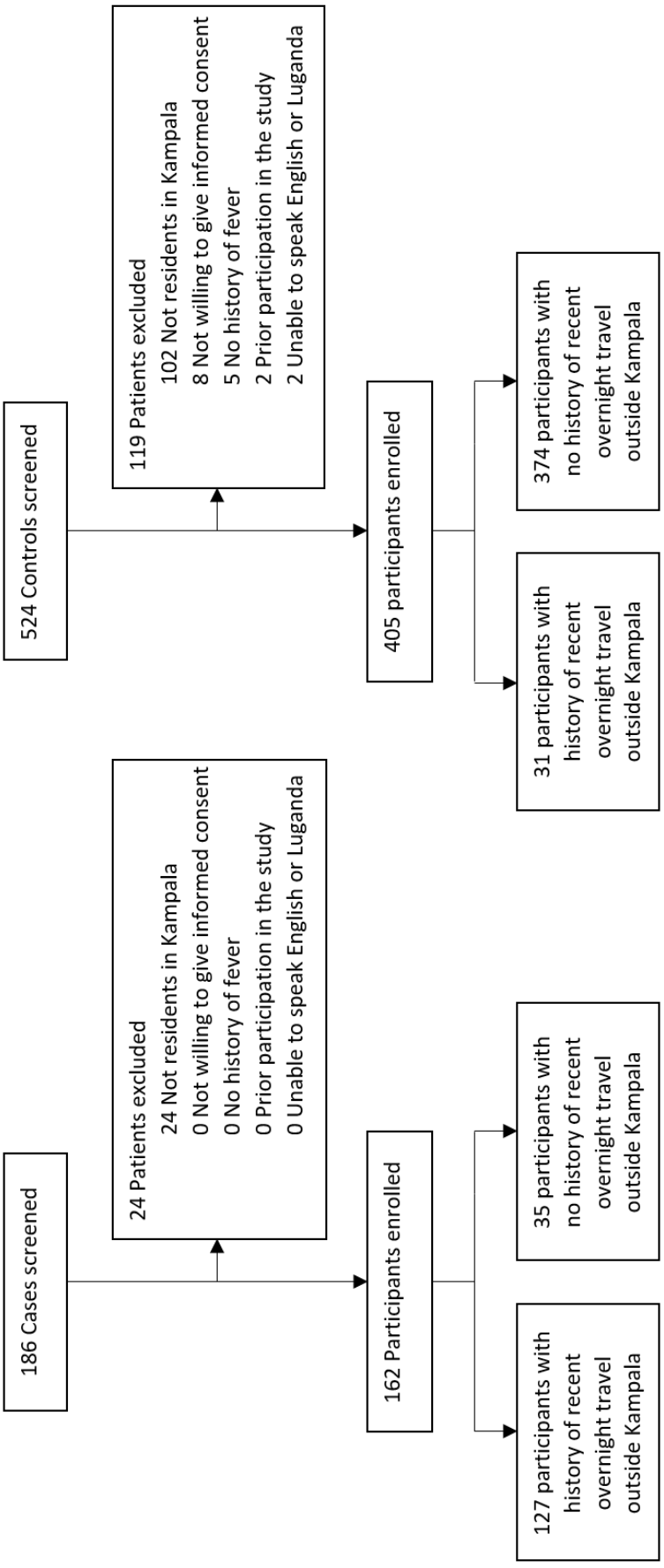

Figure 4.1. Study profile

Characteristic	Cases *(Positive malaria test)*	Controls *(Negative malaria test)*
Age categories, n (%)		
Below 5 years	42 (33.1)	3 (9.7)
5 to 15 years	18 (14.2)	0
16 years or older	67 (52.8)	28 (90.3)
Female gender, n (%)	60 (47.2)	19 (61.3)
Duration of stay, median (IQR), d	3 (2 – 9)	3 (2 – 6)
Travel to Non-IRS district*, n (%)	124 (97.6)	21 (67.7)
Primary reason for overnight travel, n (%)		
Visiting relatives	58 (45.7)	19 (61.3)
Accompanying parents	33 (26.0)	1 (3.2)
Funeral rite	18 (14.2)	3 (9.7)
Work	5 (3.9)	6 (19.4)
Attending school	5 (3.9)	0
Conference or workshop	5 (3.9)	0
Party or cultural gathering	3 (2.4)	2 (6.5)
Primary place for accommodation, n (%)		
Friend's or relative's home	108 (85.0)	26 (83.9)
Camp	10 (7.9)	2 (6.5)
School	5 (3.9)	0
Hotel	2 (1.6)	3 (9.7)
Hospital	1 (0.8)	0
Church	1 (0.8)	0
Primary activity in the evening, n (%)		
Went to bed right away	42 (33.1)	6 (19.4)
Watched TV/listened to the radio	36 (28.4)	17 (54.8)
Sat in the gardens	30 (23.6)	2 (6.3)
Stayed at work	9 (7.1)	0
Had a drink with friends	9 (7.1)	0
Helped with house chores	1 (0.8)	6 (19.4)
Went to bed after 9pm, n (%)	84 (66.1)	25 (80.7)
Slept under LLIN, n (%)	20 (15.8)	20 (64.5)

*District that has not received IRS within 12 months before travel

Table 4.2. Characteristics of participants with a history of recent overnight travel

4.3.2 Characteristics of participants who travelled

Of those participants who travelled (Table 4.2), more cases than controls were aged < 5 years (33.1% vs 9.7%, p<0.001). Participants travelled to all regions across the country, although they travelled to districts neighbouring Kampala more frequently (Figure 4.2).

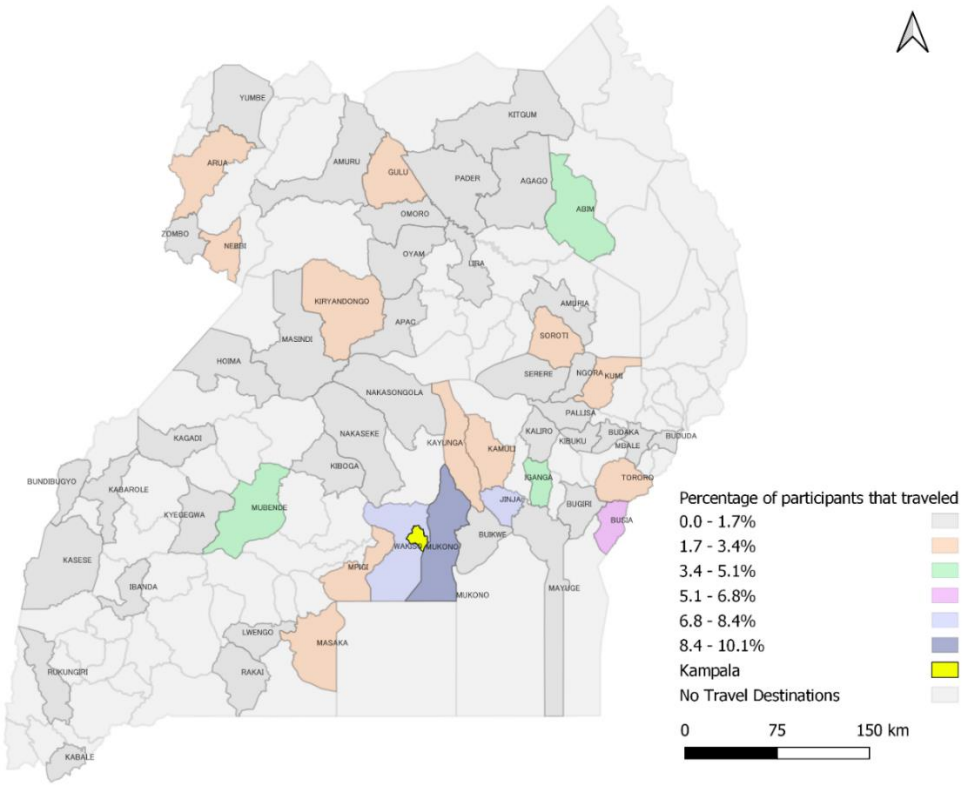

Figure 4.2. Map of Uganda showing destination of travel for study participants with a history of recent overnight travel out of Kampala

For both groups, the median duration of stay when traveling was 3 days. Compared to controls, cases were more likely to have travelled to a district without an ongoing IRS program (97.6% vs 67.7%, p<0.001). Both cases and controls commonly travelled to visit relatives and for funerals, but controls were more likely to travel for work (19.4% vs 3.9%, p=0.02). Travellers in both groups commonly stayed in the home of friends or relatives and engaged in similar evening activities, although cases were more likely to sit outside in the garden (23.6% vs 6.3%, p<0.001). Controls were far more likely than cases to have slept under LLINs while traveling (64.5% vs 15.8%, p<0.001).

4.3.3 Association between recent overnight travel outside Kampala and malaria

In an analysis adjusted for gender and location of residence (Table 4.3), recent overnight travel was strongly associated with malaria; participants who travelled within the last 60 days had

significantly higher odds of a positive malaria test than those without recent travel (80.4% vs 8.6%, adjusted odds ratio [aOR] 58.9, 95% CI: 23.1-150.1, p<0.001). The odds of malaria was also higher in males than in females (36.6% vs 22.8%, aOR 2.0, 95% CI: 1.02-3.91, p=0.04). No association between location of residence and malaria was found (Table 4.3).

4.3.4 Factors associated with malaria among participants who travelled

In an analysis restricted to participants who travelled, older age, travel to a non-IRS district, duration of travel for 10 days or more, not using LLINs during travel, staying with friends and relatives, and engaging in outdoor evening activities were all associated with malaria (Table 4.4).

Participants who travelled to a non-IRS district were at much higher odds of malaria than those who travelled to a district covered by IRS (85.5% vs 23.1%, OR 35.0, 95% CI: 4.8-254.9, p<0.001) as were participants who did not use LLINs when traveling, compared to those who did (90.7% vs 50.0%, OR 30.1, 95% CI: 6.4-142.7, p<0.001).

Risk factor	Proportion testing positive for malaria, n/N (%)	Univariate analysis		Multivariate analysis	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Gender					
—Female	75/329 (22.8)	Reference		Reference	
—Male	87/238 (36.6)	1.95 (1.33 – 2.87)	0.001	2.0 (1.02 – 3.91)	0.04
Place of residence within Kampala					
—Kampala central	11/48 (22.9)	Reference		Reference	-
—Kawempe division	15/36 (41.7)	2.40 (0.94 – 6.15)	0.07	3.12 (0.64 – 15.2)	0.16
—Makindye division	20/61 (32.8)	1.43 (0.61 – 3.38)	0.41	2.14 (0.47 – 9.81)	0.33
—Nakawa division	114/409 (27.9)	1.23 (0.61 – 2.47)	0.57	1.59 (0.51 – 4.93)	0.42
—Rubaga division	2/13 (15.4)	0.59 (0.11 – 3.06)	0.53	0.46 (0.03 – 6.26)	0.56
Recent overnight travel out of Kampala					
—No history of travel within 60 days	35/409 (8.6)	Reference		Reference	
—History of travel within 60 days	127/158 (80.4)	55.4 (22.6 – 135.8)	<0.001	58.9 (23.1 – 150.1)	<0.001

Table 4.3. Recent overnight travel out of Kampala and the risk of malaria

Risk factor	Proportion testing positive for malaria, n/N (%)	Univariate analysis		Multivariate analysis	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Age category					
16 years or older	67/95 (70.5)	reference		reference	
Below 16 years	60/63 (95.2)	8.36 (2.42 – 28.9)	0.001	11.2 (2.22 – 56.2)	0.03
Gender					
Female	60/79 (76.0)	reference		reference	
Male	67/79 (84.8)	1.77 (0.79 – 3.94)	0.16	1.86 (0.54 – 6.46)	0.33
Destination of travel					
IRS district	3/13 (23.1)	reference		reference	
Non-IRS district	124/145 (85.5)	19.7 (5.00 – 77.5)	<0.001	35.0 (4.80 – 254.9)	<0.001
Duration of stay during travel					
Less than 10 days	97/125 (77.6)	reference		reference	
10 days or more	30/33 (90.9)	2.89 (0.82 – 10.17)	0.10	6.59 (1.10 – 39.6)	0.04
Reported LLIN use during travel					
Used LLINs	20/40 (50.0)	reference		reference	
Did not use LLINs	107/118 (90.7)	9.73 (4.05 – 23.4)	<0.001	30.1 (6.37 – 142.7)	<0.001
Time of sleep during travel					
After 9 pm	84/109 (77.1)	reference		reference	
9 pm or earlier	43/49 (87.8)	2.13 (0.81 – 5.59)	0.12	0.91 (0.18 – 4.68)	0.91
Place for accommodation during travel					
Other places	16/21 (76.2)	reference		reference	
Relative's place	111/137 (81.0)	1.33 (0.45 – 3.97)	0.61	9.55 (1.32 – 69.1)	0.03
Activity carried out in the evening					
Indoor activities	91/120 (75.8)	reference		reference	
Outdoor activities	36/38 (94.7)	5.74 (1.30 – 25.3)	0.02	22.0 (3.42 – 141.8)	0.001

Table 4.4. Risk factors for malaria in participants with a history of recent overnight travel out of Kampala

4.4 Discussion

Our findings from a matched case-control study indicate that travel out of Kampala was strongly associated with increased odds of clinical malaria for Kampala residents presenting with fever. Among travellers, failure to sleep under LLINs during travel and travel to districts that had not received IRS were strongly associated with increased risk of malaria. Other factors associated with increased odds of malaria in travellers included engaging in outdoor activities, age below 16 years, travel for ≥ 10 days, and staying at a relative's home. These findings suggest that Kampala residents who are diagnosed with malaria are likely to have acquired their infection due to recent overnight travel outside of the city, and personal protection measures such as sleeping under LLINs should be emphasized to prevent malaria infection during travel.

We found that many study participants (27.9%), all residents of Kampala presenting to Naguru General Hospital with fever, had a history of recent overnight travel. Our findings were consistent with a prior study conducted at three sites of varied malaria transmission intensity in Uganda, in which 23.3% of participants living in a peri-urban setting had travelled out of their sub-county of residence, with a greater than 3-fold increase in the incidence of malaria following overnight travel.[163] Our findings are also consistent with those from additional studies from other African countries.[143,173] A case-control study conducted in urban and peri-urban Malawi suggested that overnight travel increased the risk of malaria.[143] Additional studies conducted in Africa showed that returning residents, defined as residents of a low-risk area returning from a trip away were at a high risk of malaria.[76,92,93] In Zanzibar and Bioko Island, parasites were introduced by returning residents, which frustrated malaria control efforts.[76,93] Similarly, in Swaziland and the regions of KwaZulu-Natal, Mpumalanga, and Limpopo in South Africa, malaria parasites were reintroduced from Mozambique following

implementation of intensive malaria control.[92,95] These findings from malaria endemic areas in Africa highlight that traveling between low and high malaria transmission areas is common, and that returning residents are at increased risk of malaria.

In study participants who travelled, we found that LLIN use was uncommon and strongly associated with an increased risk of malaria. Previous studies have also suggested that LLIN use was reduced during overnight travel.[79,163] Reasons for infrequent use of LLINs included having no place to hang the LLIN, limited availability of LLINs, and social barriers, such as fear of appearing disrespectful to others.[79] As malaria control interventions are scaled-up, it is important to encourage use of LLINs during travel to areas of high malaria risk. Policy makers should also consider increasing LLIN availability so that those at risk, whether travellers or residents, are protected.

We also found that, among travellers, engaging in outdoor activities was strongly associated with increased risk of malaria. Outdoor activities may increase exposure to mosquito bites and subsequent malaria,[141,174-177] suggesting that use of personal protection measures to avoid outdoor exposure to malaria vectors during travel, including mosquito repellent creams and sprays, should be explored. Mosquito repellents have been used primarily for malaria prevention by travellers from malaria non-endemic countries when traveling to malaria endemic areas.[178,179] Commonly used repellents, including N,N-diethyl-3-methylbenzamide (DEET), have good safety profiles.[180] When used properly, repellents can provide protection for up to 6 hours.[181] However, studies evaluating mass distribution of topical mosquito repellents have not shown an impact on malaria parasite prevalence due to poor compliance with daily use.[182]

Use of chemoprophylaxis, that is regular administration of a drug to prevent malaria infection, is a key component of malaria prevention in travellers.[183,184] Chemoprophylaxis is recommended for travellers from malaria non-endemic countries to malaria-endemic regions,[169] but the same practice might be beneficial for travellers to high transmission areas in Africa. Standard recommendations for malaria chemoprophylaxis are daily atovaquone-proguanil, daily doxycycline, or weekly mefloquine; primaquine and tafenoquine are also effective, but require confirmation of normal glucose-6-phosphate dehydrogenase levels before use.[183] Chemoprophylaxis should be initiated shortly before the trip begins and continued for 1 - 4 weeks after completion of travel, depending on the drug used. As with travellers from non-endemic countries, the use of chemoprophylaxis in addition to specific mosquito control measures is likely to provide substantial protection against malaria infection in travellers to high risk areas of Africa.

Our study had some limitations. First, like all case-control studies, the study was prone to selection bias which can lead to mistaken inference. However, selection bias was minimized by selecting both cases and controls from the same outpatient department and using the same diagnostic algorithm for both groups. Second, we could not rule out recall bias concerning travel histories, especially because history of travel was defined over a 60-day period. It is possible that participants with malaria might have been more likely to recall or report travel as compared to those without malaria. Third, the case-control study design only allowed assessment of the association between overnight travel and malaria as odds ratios, and it was not possible to estimate the actual risk of malaria among travellers. Lastly, the study was conducted over a period of 2 months and in only one hospital in Kampala, and results cannot be considered representative of risks at other times of the year, or for other locations. Nonetheless, the very high odds for malaria after travel strongly suggests that travel from low-

endemicity cities to higher transmission settings in Africa may be responsible for the majority of malaria diagnosed in urban settings.

In summary, malaria among Kampala residents was strongly associated with overnight travel, especially travel to high risk districts of Uganda that were not receiving IRS. Among those who travelled, not using LLINs during travel, engaging in outdoor activities, staying with friends and relatives, and duration of travel for 10 days or more, were associated with increased odds of malaria. Our findings suggest that personal malaria prevention measures, including use of LLINs, application of creams or sprays to prevent outdoor mosquito bites, and administration of chemoprophylaxis, should be emphasized to protect individuals traveling overnight from low to high transmission regions of Africa.

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London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

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First Name(s)	Emmanuel		
Surname/Family Name	Arinaitwe		
Thesis Title	Association between overnight travel and the risk of malaria: case-control and prospective cohort studies in Uganda		
Primary Supervisor	Sarah Staedke		

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Chapter 5. Association between recent overnight travel and the incidence of malaria: a prospective cohort study at 3 sites in Uganda

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ABSTRACT

Background. Human movement can undermine malaria control efforts. However, understanding of the association between travel and malaria infection in Africa is limited. We evaluated the association between recent overnight travel and malaria incidence in Uganda.

Methods. All children aged 0.5-10 years and one adult living in 266 randomly selected households within 3 different regions of Uganda were followed prospectively. Information on overnight travel was collected in 2015 - 2016. Malaria, defined as fever with parasites detected by microscopy, was measured using passive surveillance.

Results. At least one overnight trip was reported by 64 of 275 (23.3%) participants in Walukuba, 37 of 317 (11.7%) in Nagongera, and 19 of 314 (6.1%) in Kihihi. Among individuals who travelled, the incidence of malaria was higher in the first 60 days after traveling, compared to periods without recent travel at all 3 sites (overall 1.15 vs 0.33 episodes per person-year, incidence rate ratio 3.53, 95% confidence interval [CI] 1.85-6.73, p<0.001). Risk factors for malaria within 60 days following overnight travel included young age (19.5% in children vs 4.9% in adults, odds ratio [OR]

5.29, 95% CI 1.34-21.0, p=0.02) and not using long-lasting insecticidal net (LLIN) during travel (18.0% for no use vs 4.1% for any use, OR 5.10, 95% CI 1.07-24.5, p=0.04).

Conclusions. Recent overnight travel was associated with a higher incidence of malaria. Individuals who travel may represent a high-risk group that could be targeted for malaria control interventions, particularly use of LLINs.

5.1. Background

Over the past decade, substantial reductions in the burden of malaria have been documented worldwide, following heavy investment in control interventions.[1] Despite this success, malaria control remains a major challenge, and recent evidence suggests that progress has stalled or reversed in some regions.[185] In Uganda, the scale-up of long-lasting insecticidal nets (LLINs), indoor residual spraying of insecticides (IRS), and treatment of symptomatic malaria cases with artemisinin-based combination therapies (ACTs) has been associated with reduced malaria incidence and prevalence in some areas.[39,135,186] However, malaria control gains have been greatest in areas receiving IRS, and a dramatic resurgence of malaria occurred in Northern Uganda following the withdrawal of IRS.[52,53,187] To ensure that recent gains are not lost, strategic deployment of malaria control interventions is needed. In addition to widescale implementation of malaria control interventions, targeting individuals with major contributions to the infectious reservoir of parasites may be a valuable approach.[188]

Human movement is an underappreciated risk factor for malaria transmission.[90,138,189] Individuals living in low-transmission areas, or in areas where malaria has been controlled, may be at increased risk of infection when traveling to areas of higher transmission intensity.[77,93,143] Infected travellers may return home with symptomatic malaria, or asymptomatic parasitaemia,

contributing to the burden of malaria cases, and serve as a reservoir of parasites for onward transmission.[93,138] Furthermore, returning travellers may re-introduce parasites in areas where malaria has previously been eliminated, presenting a major challenge to control efforts. Although travel to endemic countries has been recognized as a risk factor for malaria,[190-192] evidence on the risk posed by travel within endemic areas is less robust. Several studies from Africa, [77,83,93,136,141,143,189] and elsewhere,[90,138,142,144] suggest that recent travel (generally within the last month) is associated with an increased risk of malaria. However, our understanding of the causal association between travel and malaria infection, particularly in Africa, is limited by the design of prior studies, which have been either cross-sectional [83,93,141] or case-control studies,[77,136,143,189] and by heterogeneity in methods used to determine malaria outcomes.[141,193]

In Uganda, little information on the association between overnight travel and the risk of malaria exists. One case-control study conducted among children presenting to health facilities in western Uganda found that travel from highland areas with low-level malaria transmission to higher transmission areas was strongly associated with risk of malaria.[136] To further investigate travel as a risk factor for malaria in Uganda, we analysed prospective data from cohorts in 3 different regions to evaluate the association between recent overnight travel and the incidence of malaria.

5.2. Methods

5.2.1. Study area and site characteristics

The study was conducted at 3 sub-counties with varied malaria epidemiology (Figure 5.1). Walukuba sub-county in Jinja district is a peri-urban area in the south-central part of Uganda near Lake Victoria with relatively low malaria transmission intensity.[194] Nagongera sub-county in Tororo district is a rural area in south-eastern Uganda bordering Kenya. Prior to the introduction of indoor

residual spraying (IRS), malaria transmission in Nagongera was intense, but following three rounds of IRS with bendiocarb at 6 months intervals, initiated in December 2014, malaria transmission reduced considerably.[135] A fourth round of IRS with pirimiphos-methyl was conducted in June-July 2016. Kihihi sub-county in Kanungu district is a rural area in the south-west part of the country bordering a national park, where malaria transmission intensity is classified as moderate (10 – 100 infectious bites per person per year). All study sites received LLINs between 2013 and 2014 as part of a national LLIN distribution campaign.[186]

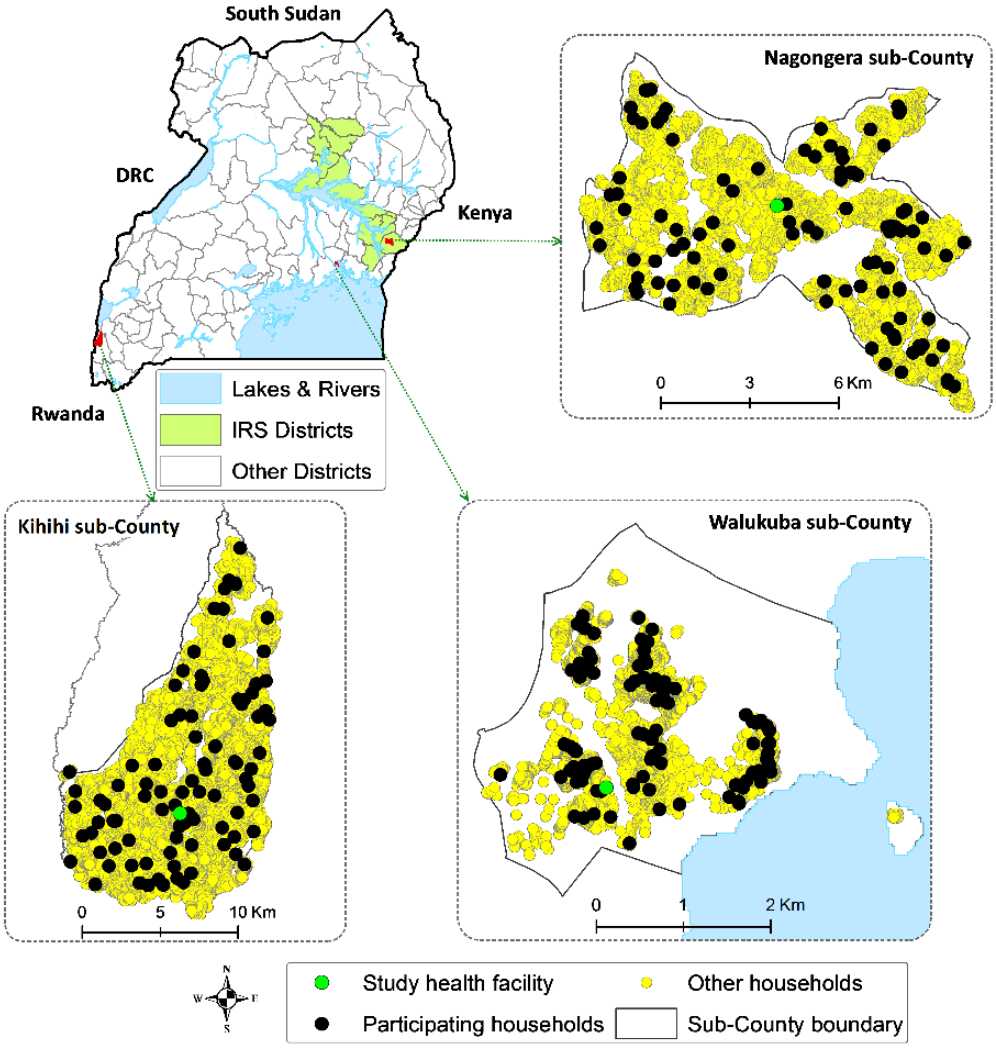

Figure 5.1. Map of Uganda showing study sites (red): Walukuba sub-county located in south central part of Uganda, Kihihi in south-western part, and Nagongera in South-east Uganda.

5.2.2. Enrolment and follow up of study participants

The methods are described in detail elsewhere.[35] Briefly, all children aged 0.5-10 years and one adult from 100 randomly selected households per site were enrolled into the cohorts. Study participants were included if they 1) were full time residents of the selected household, 2) had no intention to move outside the sub-county for the next two years, 3) agreed to come to a dedicated study clinic located within the sub-county for any febrile illness, 4) agreed to avoid antimalarial medications administered outside the study, and 5) provided written informed consent, or consent was obtained from parents or guardians for children.

All participants were given LLINs (PermaNet®, Vestergaard Frandsen, Switzerland) at enrolment and were followed for all their healthcare needs at the study clinic, which was open 7 days a week. Participants were provided free health care, clinic travel expenses and LLINs, but received no other incentives to participate. Episodes of malaria were diagnosed by passive case detection and defined as a history of fever within the past 24 hours or an elevated temperature ($\geq$ 38.0° C tympanic) with a positive malaria blood smear. Episodes of malaria were treated with artemether-lumefantrine (uncomplicated malaria) or quinine (complicated malaria). In addition, participants were invited to make a routine visit to the study clinic every 3 months. At each of these visits, a thick blood smear was taken to assess for parasitaemia. LLIN use, defined as whether the participant reported sleeping under LLINs the previous night, was measured at the time of routine clinic visits. The cohorts were dynamic, such that all newly eligible children were enrolled, and participants were withdrawn when they reached 11 years of age. Additional criteria for withdrawal from the study included 1) permanent movement out of the sub-county, 2) inability to be located for >4 months, 3) withdrawal of informed consent, 4) withdrawal of all children under their care in the case of adults, and 5) inability to comply with the study schedule and procedures.

5.2.3. Recent overnight travel follow-up

As part of the scheduled 3-month visit assessment, study participants were asked about their travel history from July 2015 through June 2016 at every visit to the study clinic. Overnight travel was defined as spending at least one night away from the sub-county of residence. For study participants who reported any overnight travel, data on dates of travel, destination of travel, LLIN use, and the reasons for travel were collected.

5.2.4. Estimation of entomological inoculation rates

Entomological inoculation rates were estimated using data from entomologic surveys carried out concurrently with the cohort study. Details on surveys, processing of mosquito specimens, and identification of sporozoites have been described elsewhere.[194] Briefly, one CDC light trap collection was carried out monthly in the main sleeping room of each house. Light traps were positioned with the light 1.5 m from the floor near the foot of the bed and were left hanging to collect mosquitoes between 19.00 h and 07.00 h the following morning.

5.2.5. Laboratory procedures

Thick blood smears were stained with 2% Giemsa, allowed to dry for 30 minutes, and read by experienced laboratory technologists. Parasite densities were calculated by counting the number of asexual parasites per 200 leukocytes or per 500 leukocytes if the count was less than 10 asexual parasites per 200 leukocytes, assuming a leukocyte count of 8,000 per microliter. A blood smear was considered negative if the examination of 100 high power fields did not reveal any asexual parasites. For quality control, blood smears were read by a second microscopist, and discrepancies in malaria parasites detection or parasite density readings of $\geq$ 25% were resolved by a third

microscopist. The third reading was assessed, and the final reading results selected according to whether they agreed with first or second reading. Mosquito specimens were sorted to species level and counted. Sporozoites were identified using an enzyme-linked immunosorbent assay, as previously described.[194]

5.2.6. Statistical analysis

All data were recorded onto standardised case record forms, double-entered into Microsoft Access (Microsoft Corporation, Redmond, Washington, USA), and analysed using Stata 14 (STATA Corp., College Station, TX, USA). The observation period for this project covered July 1st 2015 through June 30th 2016, during which time data on travel were collected. For each cohort participant, person-time of follow-up was categorized according to the number of days since last overnight travel, dichotomized into ≤60 days or > 60 days since overnight travel. A cut-off of 60 days was determined after exploring thresholds of 14, 30, 60, and 120 days following overnight travel. Person-time of follow-up while traveling was not included in the analyses since it was not possible to diagnose malaria while study participants were away. The outcome of interest was the incidence of malaria, defined as the number of new episodes of malaria per person time of follow up. Comparisons between the incidence of malaria during exposed and unexposed periods included only individuals with at least one overnight trip, such that each participant served as their own control. Associations between recent overnight travel and malaria incidence were expressed as incidence rate ratios (IRR) and estimated using generalized estimating equations with a Poisson family adjusting for seasonality and repeated measures in the same study participant. To determine seasonality, the follow-up period was stratified into January to February and May to June (dry seasons), and March to April and July to December (rainy seasons). Associations between risk factors and whether or not a person was diagnosed with malaria in the 60 days

following each individual trip were expressed as odds ratios (OR) and estimated using generalized estimating equations with a binomial family adjusting for repeated measures in the same participant. A p-value < 0.05 was considered statistically significant.

5.2.7. Ethics considerations

The study obtained ethical approvals from the Makerere University School of Medicine Research and Ethics Committee, the Uganda National Council of Science and Technology, the London School of Hygiene and Tropical Medicine Ethics Committee, Durham University School of Biological and Biomedical Sciences Ethics Committee and the University of California, San Francisco Committee on Human Research.

5.3. Results

5.3.1. Characteristics of the study sites, participants and travel histories

From July 2015 to June 2016, travel histories were taken from 906 participants living in 266 households across the 3 study sites (Table 5.1). Of these, 120 (13.3%) participants reported at least one episode of recent overnight travel, resulting in a total of 138 individual trips. Most participants (86.7%) who travelled reported taking only one trip. The proportion of participants reporting any recent overnight travel, and the total number of trips taken, were highest in Walukuba, followed by Nagongera, and Kihihi. Overall, the median duration of each trip was 7 nights. Most participants reported traveling for pleasure or to attend a funeral; very few travelled for business. Reported use of LLINs during recent overnight trips was much lower than that reported at scheduled 3-monthly visits (35.5% vs 99.8%, p<0.001).

5.3.2. Association between recent overnight travel and risk of malaria

Among individuals who travelled, the incidence of malaria was over 3 times higher in the 1-60 days after traveling, as compared to periods without travel in the previous 60 days (1.15 vs. 0.33 episodes of malaria PPY, IRR=3.53, 95% CI 1.85-6.73, $p<0.001$) after adjustment for seasonality (Table 5.2). When the analysis was stratified by age, this finding was statistically significant only in children. Recent overnight travel was associated with a higher risk of malaria incidence in all 3 study sites, most notably in Nagongera, where the incidence of malaria was over 6-fold higher during the post-travel period.

Characteristic	Study site			
	All sites	Walukuba	Nagongera	Kihihi
Entomological inoculation rate*	N/A	2.4	4.5	11.2
Total number assessed for overnight travel	906	275	317	314
Total number of children, n (% total)	687 (75.8%)	205 (74.6%)	242 (76.3%)	240 (76.4%)
Female children, n (% children)	339 (49.3%)	101 (49.3%)	116 (47.9%)	122 (50.8%)
Total number of adults, n (% total)	219 (24.2%)	70 (25.5%)	75 (23.7%)	74 (23.6%)
Female adults, n (% adults)	205 (93.6%)	66 (94.3%)	68 (90.7%)	71 (96.0%)
Participants reporting any overnight travel, n (% total)	120 (13.3%)	64 (23.3%)	37 (11.7%)	19 (6.1%)
Characteristics of participants reporting any recent overnight travel				
Total number of children, n (% total)	69 (57.5%)	38 (59.4%)	25 (67.6%)	6 (31.6%)
Female children, n (% children)	27(39.1%)	13(34.2%)	9 (36.0%)	5 (83.3%)
Total number of adults, n (% total)	51 (42.5%)	26 (40.6%)	12 (32.4%)	13 (68.4%)
Female adults, n (% adults)	48 (94.1%)	24 (92.3%)	11 (91.7%)	13 (100%)
Total duration of observation in days, median (IQR)	266 (223-280)	239 (208-267)	280 (263-284)	268 (260-283)
Total duration of overnight travel in days, median (IQR)	8 (4-19)	10 (6-21)	7 (3-13)	4 (2-16)
Number of overnight trips reported, n (%)				
1	104 (86.7%)	55 (85.9%)	31 (83.8%)	18 (94.7%)
2	14 (11.7%)	8 (12.5%)	5 (13.5%)	1 (5.3%)
3	2 (1.7%)	1 (1.6%)	1 (2.7%)	0
Characteristics of individual recent overnight trips taken by children				
Total number of overnight trips	77	42	29	6
Duration of each trip, median (range)	9 (1 – 39)	10 (1 – 39)	7 (2 – 33)	3 (2 – 16)
Reasons for travel, n (%)				
Pleasure / visiting relatives	60 (77.9%)	34 (81.0%)	21 (72.4%)	5 (83.3%)
Attending funeral	11 (14.3%)	7 (16.7%)	4 (13.8%)	0
Caring for sick relative	4 (5.2%)	0	4 (13.8%)	0
Business	0	0	0	0
Seeking medical care	1 (1.3%)	0	0	1 (16.7%)
Not specified	1 (1.3%)	1 (2.4%)	0	0
Any reported LLIN use during travel, n (%)	26 (33.8%)	10 (23.8%)	14 (48.3%)	2 (33.3%)
Characteristics of individual recent overnight trips taken by adults				
Total number of overnight trips	61	32	15	14
Duration of each trip, median (range)	6 (1 - 79)	6 (1 - 34)	5 (1 - 33)	5 (1 - 79)
Reasons for travel, n (%)				
Pleasure / visiting relatives	28 (45.9%)	15 (46.9%)	9 (60.0%)	4 (28.6%)
Attending funeral	19 (31.2%)	14 (43.8%)	3 (20.0%)	2 (14.3%)
Caring for sick relative	9 (14.8%)	1 (3.1%)	3 (20.0%)	5 (35.7%)
Business	3 (4.9%)	2 (6.3%)	0	1 (7.1%)
Seeking medical care	2 (3.3%)	0	0	2 (14.3%)
Not specified	0	0	0	0
Any reported LLIN use during travel, n (%)	23 (37.7%)	9 (28.1%)	9 (60.0%)	5 (35.7%)

*infective bites per person per year July 2015 – June 2016

Table 5.1. Characteristics of study sites, participants, and travel history by study site

Age group	Study site	Time in relationship to overnight travel	Episodes of malaria	Person years of observation	Incidence of malaria[¥]	Unadjusted		Adjusted	
						IRR (95% CI)	p-value	aIRR[©] (95% CI)	p-value
All	All	No overnight travel in previous 60 days	20	60.1	0.33	reference		reference	
		1-60 days since overnight travel	21	18.3	1.15	3.61 (1.84-7.11)	<0.001	3.53 (1.85-6.73)	<0.001
Stratified by age group									
Children	All	No overnight travel in previous 60 days	16	32.9	0.49	reference		reference	
		1-60 days since overnight travel	18	10.1	1.78	3.93 (1.82-8.49)	<0.001	3.67 (1.77-7.61)	<0.001
Adults	All	No overnight travel in previous 60 days	4	27.2	0.15	reference		reference	
		1-60 days since overnight travel	3	8.2	0.37	2.51 (0.47-13.6)	0.28	2.28 (0.48-10.8)	0.30
Stratified by study site									
All	Walukuba	No overnight travel in previous 60 days	7	28.5	0.25	reference		reference	
		1-60 days since overnight travel	9	10.0	0.90	3.73 (1.26-11.0)	0.02	3.26 (1.12-9.48)	0.03
All	Nagongera	No overnight travel in previous 60 days	3	20.9	0.14	reference		reference	
		1-60 days since overnight travel	6	5.2	1.16	7.95 (1.78-35.5)	0.007	6.54 (1.65-26.0)	0.008
All	Kihihi	No overnight travel in previous 60 days	10	10.8	0.93	reference		reference	
		1-60 days since overnight travel	6	3.1	1.91	2.02 (0.93-4.35)	0.07	2.84 (1.32-6.13)	0.008

[¥]per person years
[©]adjusted for seasonality

Table 5.2. Associations between recent overnight travel and incidence of malaria among participants with any overnight travel

5.3.3. Risk factors of any malaria following recent overnight travel

In an analysis adjusted for repeated measures in the same study participant, being a child less than 11 years of age, and not using an LLIN during travel, were associated with an increased odds of being diagnosed with malaria within 60 days of return from overnight travel. The odds of malaria following travel was over 5 times greater in children than in adults. Similarly, the odds of malaria following travel in participants who did not use LLINs during travel was 5 times that of those who reported any LLIN use (Table 5.3). Traveling during the rainy season and traveling for shorter durations were associated with an increased odds of being diagnosed with malaria, but these associations did not reach statistical significance in multivariate analyses (Table 5.3).

5.3.4. Blood smear results before and after recent overnight travel

Of the 138 overnight trips, 133 (96.4%) had at least one routine blood smear result available before and after travel (Figure 5.2). In most cases (93.2%), the pre-travel blood smear was negative. Of the 9 trips in which the pre-travel blood smear was positive, only 2 had symptomatic malaria; both cases were treated and had a negative blood smear after travel. Of the 7 cases of asymptomatic parasitaemia before travel, only one had symptomatic malaria diagnosed after travel, 2 had asymptomatic parasitaemia after travel, and 4 had a negative blood smear after travel. Of the 124 trips that were blood smear negative before travel, 17 (13.7%) were diagnosed with symptomatic malaria after travel, 6 (4.8%) had asymptomatic parasitaemia after travel, and 101 (81.5%) had a negative blood smear after travel. Thus, of the 18 trips in which symptomatic malaria was diagnosed after travel, and for which a pre-travel blood smear result was available, 17 (94.4%) had a negative blood smear before traveling, suggesting that the infection was acquired during travel

Risk factor	Categories	Proportion of participants diagnosed with malaria 1-60 days following overnight travel	Univariate*		Multivariate*	
			OR (95% CI)	p-value	OR (95% CI)	p-value
Age group	Adult	3/61 (4.9%)	reference		reference	
	Child	15/77 (19.5%)	4.62 (1.27-16.8)	0.02	5.29 (1.34-21.0)	0.02
Report LLIN use during travel	Any	2/49 (4.1%)	reference		reference	
	None	16/89 (18.0%)	5.06 (1.12-22.7)	0.04	5.10 (1.07-24.5)	0.04
Season when traveling	Dry season[¥]	6/80 (7.5%)	reference		reference	
	Rainy season	12/58 (20.7%)	3.19 (1.13-9.04)	0.03	2.94 (0.97-8.92)	0.06
Duration of travel	9-79 days	6/60 (10.0%)	reference		reference	
	< 9 days	12/78 (15.4%)	1.61 (0.58-4.50)	0.36	2.30 (0.72-7.33)	0.16

*adjusted for repeated measures in the same participant
[¥]January to February and May to June

Table 5.3. Risk factors for malaria following recent overnight travel

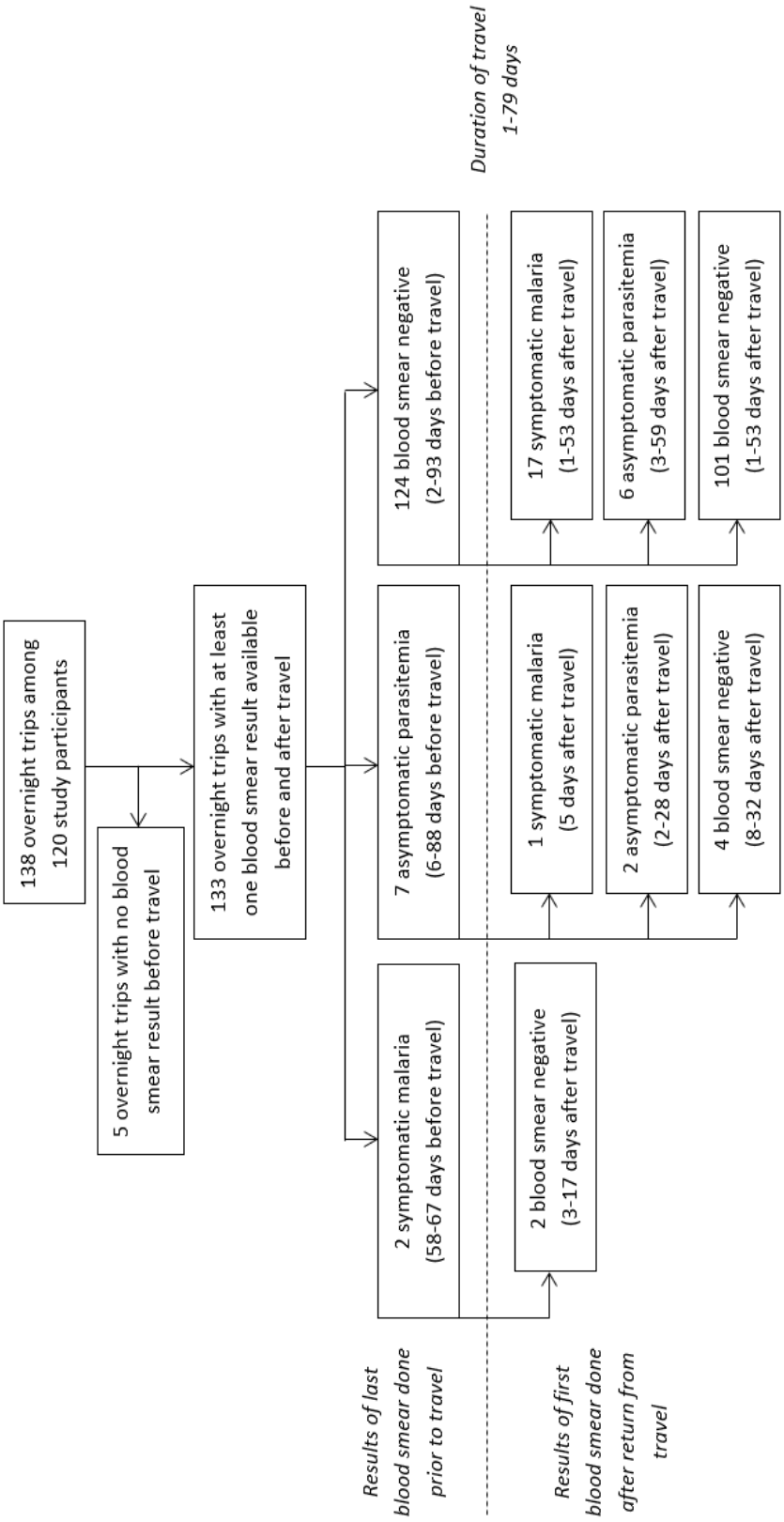

Figure 5.2. Blood smear microscopy results before and after overnight travel

5.3. Discussion

Human movement plays an important role in the spread of malaria and other infectious diseases.[76,77,195] However, gaps remain in our understanding of associations between travel and malaria incidence in malaria endemic areas. To further investigate travel as a risk factor for malaria in Uganda, we analysed data from cohorts in 3 different epidemiological settings. Among individuals who travelled, the incidence of malaria was significantly higher in the first 2 months after traveling compared to periods without recent travel. Residents who travelled from Nagongera, a rural site where IRS has been successfully deployed, were at particularly high-risk following travel, as were children and those participants who did not sleep under LLINs when traveling. These results suggest that individuals who travel within Uganda constitute a high-risk group that could be targeted for malaria control interventions.

Human movement has been shown to contribute to the rebound of malaria when programs fail, or control efforts are discontinued. In the 1960s, human mobility contributed to the resurgence of malaria in Africa after the World Health Organisation's Global Malaria Eradication Program collapsed and has been highlighted as a factor that received insufficient attention.[16,18] A similar resurgence of malaria occurred more recently in southern Africa when the Joint Malaria Control Initiative ended due to lack of funding, fuelled by the reintroduction of parasites into South Africa and Swaziland from travellers from Mozambique.[95,96] In another recent study from Zanzibar, individuals traveling to malaria endemic areas were found to be the most important source of imported infection, contributing up to 15 times more malaria cases than non-residents visiting the island.[76] In Equatorial Guinea, travel between Bioko Island and the mainland within the previous eight weeks was associated with an increased risk malaria infection; parasite prevalence was substantially higher in passengers arriving on Bioko Island than those departing.[93] Thus, evidence

from across Africa highlights that human movement is an important but often underappreciated challenge for malaria control.

Our findings support those of prior studies that showed travel to be a risk factor for malaria in Africa and help to clarify the causal association between travel and malaria risk. Previous studies included cross-sectional surveys,[83,93,141] which are limited to observations at a single point in time, and case-control studies,[77,136,143,189] which are susceptible to biases. Additional attempts to evaluate recent travel as a risk factor for malaria have been made using census data,[141,152,155] which is limited by the potential for recall bias, and inability to assess causal associations.[83,141] In our study, participants were followed prospectively, and data on parasitaemia and clinical symptoms were collected longitudinally, before and after travel. This robust study design allowed us to capture incident cases, and to track changes in parasitaemia within individual travellers over time. However, most of the adults included in the cohort were females (93.6%), which limits our ability to generalize our findings to other populations at risk, including young male workers.

Our study had several limitations. First, we relied on microscopy for identification of parasitaemia. By relying on microscopy for malaria diagnosis, which has limited sensitivity, we likely underestimated the number of malaria infections in our study. However, because our primary outcome was clinical incidence, which is typically associated with higher parasite densities within the level of detection by microscopy, this is unlikely to have impacted on our results. Second, the numbers of participants who travelled in our cohort study was small, limiting our ability to evaluate behavioural risk factors and activities associated with travel. In addition, these data were too sparse to make comparisons of the risk of malaria infection between adults and children who travelled together on the same trip. Third, we could not account for all potential risk factors for

malaria infection in cohort members. However, the analysis was constructed such that each individual served as their own control, allowing us to adjust for potential unmeasured confounders. Finally, the destination of travel and level of malaria transmission relative to that where people were traveling from, was not considered in our analysis, limiting our ability to evaluate interactions between transmission intensity and seasonality of home compared to destination of travel.

Malaria control in Africa relies heavily on vector control applied at the population level. However, there is increasing awareness of the roles of high-risk individuals in transmission of malaria. Our results showed that recent overnight travel was a significant risk factor for malaria. If travellers contribute significantly to the burden of malaria and to the infectious reservoir, they can be targeted for specific actions, including education on the risks of travel, emphasis on using LLINs while traveling, and possibly use of chemoprevention, as is routine for travellers from non-endemic countries. Future research should further explore travel-related behaviours to better identify individuals at greatest risk of malaria. To successfully control and eventually eliminate malaria in Africa, innovative methods directed at high risk individuals will be a valuable addition to complement population-level vector control

Acknowledgement

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LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	1406078	**Title**	Dr
First Name(s)	Emmanuel		
Surname/Family Name	Arinaitwe		
Thesis Title	Association between overnight travel and the risk of malaria: case-control and prospective cohort studies in Uganda		
Primary Supervisor	Sarah Staedke		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	N/A		
When was the work published?	N/A		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	N/A		
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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	Malaria Journal
Please list the paper's authors in the intended authorship order:	Emmanuel Arinaitwe, Joaniter I. Nankabirwa, Paul Krezanoski, John Rek, Victor Kamya, Adrienne Epstein, Philip J. Rosenthal, Chris Drakeley, Moses R. Kamya, Grant Dorsey, Sarah G. Staedke.
Stage of publication	**Submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I designed the study and participated in the data collection together with other co-authors, analysed data and wrote the first draft of the manuscript under my supervisor's guidance

SECTION E

Student Signature	[redacted]
Date	19 September 2020

Supervisor Signature	[redacted]
Date	21 Sept 2020

Chapter 6. Association between recent overnight travel and the use of long-lasting insecticidal nets in rural Uganda: a prospective cohort study in Tororo

Submitted as: Emmanuel Arinaitwe, Joaniter I. Nankabirwa, Paul Krezanoski, John Rek, Victor Kamya, Adrienne Epstein, Philip J. Rosenthal, Chris Drakeley, Moses R. Kamya, Grant Dorsey, Sarah G. Staedke. Association between recent overnight travel and the use of long-lasting insecticidal nets in rural Uganda: a prospective cohort study in Tororo.

ABSTRACT

Background: The burden of malaria in Uganda remains high, but has become increasingly heterogenous following intensified malaria control. Travel within Uganda is recognised as a risk factor for malaria, but behaviours associated with travel are not well-understood. To address this knowledge gap, malaria-relevant behaviours of cohort participants were assessed during travel and at home in Uganda.

Methods: Residents from 80 randomly selected households in Nagongera sub-county, Tororo district were enrolled into a cohort to study malaria in rural Uganda. All participants were given long-lasting insecticidal nets (LLINs) at enrolment and were evaluated every 4 weeks at the study clinic. Participants were asked if they had travelled overnight from their home, and if so, a questionnaire was administered to capture information on travel details and behaviours. Behaviour while at home was assessed using a similar questionnaire during two-weekly home visits. Behaviours while travelling vs at home were compared using generalized estimating equations adjusting for repeated measures in the same individual.

Results: Between October 2017 and October 2019, 527 participants were enrolled and assessed for travel. Of these, 123 (23.2%) reported taking 211 overnight trips; 149 (70.6%)

trips were within Tororo. Participants were less likely to use LLINs when travelling than when at home (41.0% vs. 56.2%, relative risk [RR] 0.73, 95% CI: 0.60-0.89, p=0.002); this difference was noted for women (38.8% vs 59.2%, RR 0.66, 95% CI 0.52-0.83, p=0.001) but not men (48.3% vs 46.6%, RR 0.96, 95% CI 0.67-1.40, p=0.85). In an adjusted analysis, factors associated with LLIN use when travelling included destination (travelling to districts not receiving indoor residual spraying [IRS] 65.8% vs Tororo district 32.2%, RR 1.80, 95% CI 1.31-2.46, p<0.001) and duration of travel (>7 nights 60.3% vs one night 24.4%, RR 1.97, 95% CI 1.07-3.64, p=0.03).

Conclusions: Travellers, particularly women, were less likely to use LLINs when travelling than when at home. LLIN adherence was higher among those who travelled to non-IRS districts and for more than one week, suggesting that perceived malaria risk influences LLIN use. Strategies are needed to raise awareness of the importance of using LLINs while travelling.

6.1 Introduction

Malaria control interventions have been scaled-up globally, resulting in significant declines in malaria burden.[1-3] Despite these achievements, malaria morbidity remains high worldwide; in 2018, 228 million malaria cases, 93% from Africa, were reported.[2] In Uganda, key malaria control strategies include prompt treatment with artemisinin-based combination therapy (ACTs), universal distribution of long-lasting insecticidal nets (LLINs), and targeted indoor residual spraying of insecticides (IRS).[1,37,39,42] Although intervention coverage has expanded remarkably in Uganda over the past decade, progress on malaria control has been uneven.[43] According to the 2018-19 Malaria Indicator Survey, parasite prevalence, measured by microscopy in children under-five, ranged from 0.2% in Kampala to 34.3% in the Karamoja region.[43] Factors contributing to the heterogeneity of malaria in Uganda include geographical variation in transmission intensity, increasing urbanisation, and delivery of IRS to a limited number of districts.[50,134]

Travel is a well-recognised risk factor for malaria.[76,77,83,140,149,196] Studies from Uganda and elsewhere in Africa have shown that overnight travel is associated with an increased risk of malaria, especially when individuals travel from areas of lower transmission intensity to higher risk areas.[136,163] Although travellers may be at increased risk of malaria due to exposure to higher malaria transmission,[147,197] changes in behaviour while away from home may also contribute. Some studies have suggested that individuals who travel within malaria-endemic areas may take part in outdoor activities, go to bed late, and be less likely to use LLINs, all behaviours that increase exposure to mosquitoes and risk of malaria infection.[79,176,198,199] To further explore associations between overnight travel and behaviours that might modify the risk of malaria infection, data collected over a two-year period from a cohort of individuals living in Tororo, Uganda, were analysed.

6.2 Methods

6.2.1 Study site

The study was conducted in Nagongera sub-county in Tororo district, Uganda. Details about the site have been described elsewhere.[35,72,163] Briefly, Nagongera is a rural area with very high malaria transmission, which is now under intensive malaria control. In 2012, the entomological inoculation rate (EIR) in Tororo was 310 infectious bites per person per year.[194] Because of its high malaria burden, Tororo was selected to receive IRS starting in 2015, and to date the district has received seven rounds of IRS (three rounds of the carbamate Bendiocarb, followed by four rounds of the organophosphate pirimiphos-methyl [Actellic]). In addition to IRS, LLINs were distributed to all households in Tororo through national campaigns in 2013 and 2017. The interventions have been associated with a drastic reduction in key malaria indicators, including malaria incidence, parasite prevalence and EIR which was <1% in 2018.[34,37,135]

6.2.2 Study design and participant enrolment

The cohort study has been described in detail elsewhere.[200] Briefly, all households in the study area were enumerated, and 80 households were randomly selected for participation. Households were included if they met the following selection criteria: 1) at least two household members under 5 years of age, 2) no more than 7 permanent residents, 3) no intention for the household to move from Nagongera sub-county during the study period, and 4) willingness to participate in study follow up activities.

All members of the enrolled households were screened and enrolled in the cohort study if they met the following selection criteria: 1) full-time resident of the selected household, 2) agreement to come to the study clinic for any illness and scheduled follow up, and 3) provision of written informed consent. Participants were followed up for 2 years, and the cohort was dynamic; any residents that were born into or joined the household were screened for enrolment during the course of the study. Participants were withdrawn from the study if they met the following criteria: 1) permanent movement out of Nagongera sub-county, 2) unable to be located for > 120 days, 3) withdrawal of informed consent, or 4) unable to comply with the study schedule and procedures. All enrolled participants were given LLINs at enrolment and were encouraged to come to the study clinic for all of their medical care.

6.2.3 Study participant follow up and data collection

Participants were seen at the study clinic monthly for routine follow up. At these visits, participants were asked whether they had travelled overnight since the last visit. A detailed questionnaire was administered to those who travelled to capture data on destination and duration of travel, behavioural factors such as time spent outdoors in the evening, time to bed, and use of LLINs during travel. Every two weeks, participants were visited at home and the same questionnaire was administered to collect data on behavioural factors while at home.

6.2.4 Statistical analysis

Data were collected by trained study staff using standardised case record forms and double-entered using Microsoft Access (Microsoft Corporation, Redmond, Washington, USA). All the analyses were performed using Stata, version 14 (Stata Corporation, College Station, Texas, USA). This analysis included data on any overnight travel and behavioural factors collected between October 2017 and October 2019. Overnight travel was defined as travel out of the sub-county of residence and spending at least one night away.

Behavioural factors including adherence to reported LLIN usage the prior night and time to bed were evaluated when study participants were at home and during overnight travel. LLINs use during travel was dichotomised into use most of the time during travel and no use, and time to bed was dichotomised into going to bed before 9pm most of the time and going to bed at 9pm or later mostly. Each overnight trip was paired with the most recent assessment at home as a comparison. For each pair, comparisons between behavioural factors during travel and while at home were made using generalised estimating equations adjusting for repeated measures in the same individual, and estimates were reported as relative risks (RR). In addition, an analysis of factors associated with LLIN adherence, such as destination and duration of travel, time to bed during travel, gender and age at time of travel, were also assessed using generalised estimating equations and expressed as relative risks. A p-value <0.05 was considered statistically significant.

6.3 Results

6.3.1 Characteristics of study participants

From October 2017 through October 2019, 531 study participants from 80 randomly selected households were enrolled (Figure 6.1). Of these, 527 were assessed for overnight travel. Overall, 123 (23.2%) participants reported at least one overnight trip and were included in the

analysis (Table 6.1). Of these participants who travelled, 65.9% were female. Adults were more likely to travel than children, but school-aged children (5-15 years) were more likely to take longer trips (17 days) than younger children (7 days) or adults (3 days). Most participants travelled short distances (<30km) and generally stayed within Tororo district (70.6%). Travel destinations outside Tororo included Bugiri (65km away) and Butaleja (25km away), both districts receiving IRS (Figure 6.2)

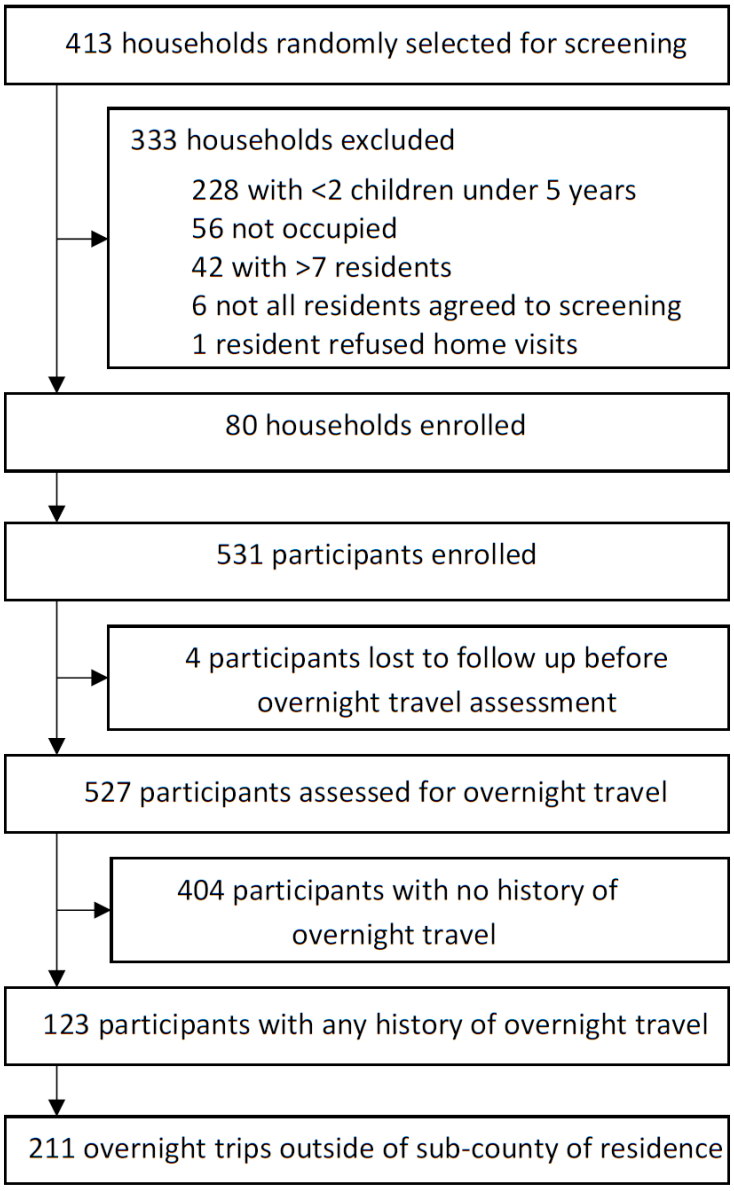

Figure 6.1. Study profile.

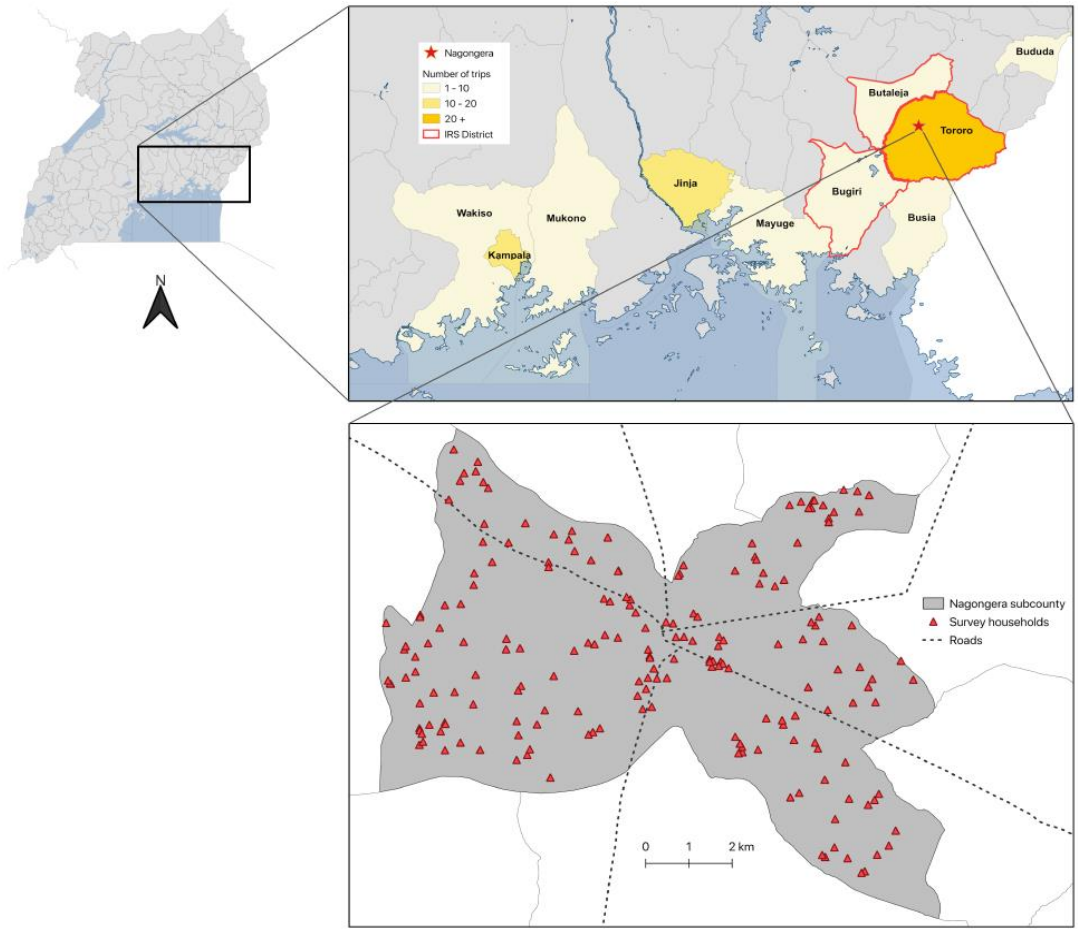

Figure 6.2. Map of Uganda showing travel destination of study participants to the districts level.

Other participants travelled to non-IRS districts (18.0%), and to Kampala (8.1%). The main reason older children and adults travelled was to visit relatives, while children under-five mainly accompanied their parents or guardians. Travellers most commonly stayed with their relatives while away.

6.3.2 Differences in behaviour during overnight travel versus at home

Overall, LLIN use among cohort participants who travelled was low both at home and while travelling. Participants were significantly less likely to use LLINs when they travelled than when at home (41.0% travel vs. 56.2% home, relative risk (RR) 0.73, 95% CI: 0.60-0.89, p=0.002) (Table 6.2). However, this difference was modified by gender and age. Women were less likely

to use LLINs when they travelled than when at home (38.8% vs 59.2%; RR 0.66, 95% CI: 0.52-0.83, p=0.001) but no difference in LLIN use was observed for men (46.6% vs 48.3%; RR 0.96, 95% CI: 0.67-1.40, p=0.85). Stratifying by age, no differences in LLIN use during travel were observed for younger or school-aged children. However, older participants (> 15 years) were significantly less likely to use LLINs when travelling than at home (33.9% vs 61.3%; RR 0.55, 95% CI: 0.41-0.74, p<0.001). Overall, there were no differences in the proportion of participants who went to bed after 9pm when travelling versus at home.

Characteristic	Age categories			
	All ages	< 5 years	5–15 years	> 15 years
Characteristics of study participants*				
All participants	531	177	193	161
Female gender, n (% among all participants)	278 (52.4%)	97 (54.8%)	85 (44.0%)	96 (59.6%)
Participants with any overnight trip, n (% total participants)	123 (23.2%)	39 (22.0%)	21 (11.1%)	63 (39.1%)
Female gender, n (% among participants with any travel)	81 (65.9%)	20 (51.3%)	13 (61.9%)	48 (76.2%)
Proportion of travellers from least poor households	43 (35.0%)	10 (25.6%)	7 (33.3%)	26 (41.3%)
Proportion of non-travellers from least poor households	137 (33.6%)	50 (36.2%)	56 (32.6%)	31 (31.6%)
Characteristics of individual overnight trips**				
Number of overnight trips	210	53	33	124
Number of trips made by individual participants, n				
1 trip	79	25	17	37
2 trips	18	5	1	12
3 trips	5	2	2	1
4 or more trips	20	3	2	15
Duration of each trip in nights away, median (range)	5 (1-115)	7(1-53)	17 (1-53)	3 (1-115)
Duration of travel categories, n (% total trips)				
1 night	41 (19.5%)	7 (13.2%)	1 (3.0%)	33 (26.6%)
2-3 nights	49 (23.3%)	9 (17.0%)	4 (12.1%)	36 (29.0%)
4-7 nights	43 (20.5%)	11 (20.8%)	6 (18.2%)	26 (21.0%)
More than 7 nights	77 (36.7%)	26 (49.1%)	22 (66.7%)	29 (23.4%)
Destination of travel, n (% total trips)				
Tororo (IRS district)	148 (70.5%)	37 (69.8%)	21 (63.6%)	90 (72.6%)
Other IRS districts	7 (3.3%)	1 (1.9%)	1 (3.0%)	5 (4.0%)
Kampala (no IRS)	17 (8.1%)	5 (9.4%)	7 (21.2%)	5 (4.0%)
Other non-IRS districts	38 (18.1%)	10 (18.9%)	4 (12.1%)	24 (19.4%)
Reason for travel, n (% total trips)				
Visiting relatives	105 (50.0%)	20 (37.7%)	26 (78.8%)	59 (47.6%)
Funeral rite	45 (21.4%)	1 (1.9%)	0	44 (35.5%)
Accompanying parents	39 (18.6%)	32 (60.4%)	7 (21.2%)	0
Caring for the sick	10 (4.8%)	0	0	10 (8.1%)
Business	8 (3.8%)	0	0	8 (6.5%)
Pleasure	2 (1.0%)	0	0	2 (1.6%)
Attending school	1 (0.5%)	0	0	1 (0.8%)
Where participant stayed, n (% total trips)				
Friend/relative's home	175 (83.3%)	49 (92.5%)	32 (97.0%)	94 (75.8%)
Hospital	13 (6.2%)	2 (3.8%)	1 (3.0%)	10 (8.1%)
Camp or Gardens	22 (10.5%)	2 (3.8%)	0	20 (16.1%)

* based on age at the time of study enrolment

** based on age at the time of travel

Table 6.1. Characteristics of study participants and individual overnight trips

Behavioural factor	Groups		Number of paired observations	Number with a reported behavioural factor (%)		RR (95% CI)	p-value
				At home of residence	During overnight travel		
Sleeping under an LLIN	All		210	118 (56.2%)	86 (41.0%)	0.73 (0.60-0.89)	0.002
	Gender	Male	58	28 (48.3%)	27 (46.6%)	0.96 (0.67-1.40)	0.85
		Female	152	90 (59.2%)	59 (38.8%)	0.66 (0.52-0.83)	0.001
	Age	< 5 years	53	26 (49.1%)	24 (45.3%)	0.92 (0.62-1.38)	0.70
		5-15 years	33	16 (48.5%)	20 (60.6%)	1.25 (0.86-1.82)	0.25
		> 15 years	124	76 (61.3%)	42 (33.9%)	0.55 (0.41-0.74)	<0.001
Going to bed after 9pm	All		210	147 (70.0%)	149 (71.0%)	1.01 (0.91-1.13)	0.80
	Gender	Male	58	27 (46.6%)	31 (53.5%)	1.15 (0.86-1.53)	0.35
		Female	152	120 (79.0%)	118 (77.6%)	0.98 (0.88-1.10)	0.77
	Age	< 5 years	53	15 (28.3%)	20 (37.7%)	1.33 (0.77-2.31)	0.30
		5-15 years	33	23 (69.7%)	19 (57.6%)	0.83 (0.58-1.17)	0.28
		> 15 years	124	109 (87.9%)	110 (88.7%)	1.01 (0.92-1.11)	0.85

Table 6.2. Comparison of behavioural factors at home of residence and during overnight travel

6.3.3 Factors associated with LLIN adherence during overnight travel

Participants who travelled to districts without an IRS program were more likely to sleep under LLINs than those who travelled within Tororo district (65.8% vs 32.2%; RR 1.80, 95% CI: 1.31-2.46, p<0.001) (Table 6.3). There were no differences in LLIN use when travelling to other destinations (Kampala and other IRS districts). Participants who travelled for more than 7 nights were significantly more likely to use LLINs while travelling than those who travelled for only one night (60.3% vs 24.4%; RR 1.97, 95% CI: 1.07-3.64, p=0.03). Other factors that were not associated with LLIN use during travel included time to bed, gender of the participant, and age at the time of travel.

Factors	Categories	Proportion of trips adherent to LLINs (%)	Univariable analysis		Multivariable analysis	
			RR (95% CI)	p-value	RR (95% CI)	p-value
Destination of travel	Tororo district (IRS)	48/149 (32.2%)	Reference	-	Reference	-
	Other IRS districts	1/7 (14.3%)	0.44 (0.07-2.77)	0.39	0.39 (0.05-2.88)	0.35
	Kampala district	12/17 (70.6%)	2.19 (1.49-3.22)	<0.001	1.49 (0.93-2.40)	0.10
	Other non-IRS districts	25/38 (65.8%)	2.04 (1.47-2.83)	<0.001	1.80 (1.31-2.46)	<0.001
Duration of travel	1 night	10/41 (24.4%)	Reference	-	Reference	-
	2 – 3 nights	11/49 (22.5%)	0.92 (0.43-1.95)	0.83	0.90 (0.43-1.90)	0.79
	4 – 7 nights	18/43 (41.9%)	1.72 (0.90-3.27)	0.10	1.44 (0.75-2.80)	0.27
	More than 7 nights	47/78 (60.3%)	2.47 (1.40-4.37)	0.002	1.97 (1.07-3.64)	0.03
Time to bed during travel	Before 9pm	28/62 (45.2%)	Reference	-	Reference	-
	9pm or later	58/149 (38.9%)	0.86 (0.61-1.21)	0.39	1.08 (0.72-1.62)	0.73
Gender	Male	27/58 (46.6%)	Reference	-	Reference	-
	Female	59/153 (38.6%)	0.83 (0.59-1.17)	0.28	1.00 (0.70-1.44)	0.99
Age at time of travel	< 5 years	24/53 (45.3%)	Reference	-	Reference	-
	5-15 years	20/34 (58.8%)	1.30 (0.86-1.96)	0.21	1.16 (0.78-1.73)	0.47
	> 15 years	42/124 (33.9%)	0.75 (0.51-1.10)	0.14	0.87 (0.56-1.37)	0.55

Table 6.3. Factors associated with LLIN adherence during overnight travel

6.4 Discussion

To better understand behavioural factors that might modify the risk of malaria during travel, a cohort of individuals living in Tororo under highly effective malaria control were assessed. Overall, LLIN use in travellers was low, and participants were less likely to use LLINs when they travelled than when at home. However, this finding was true only for women, and adults. Factors associated with higher LLIN use while travelling included travel to non-IRS districts, and travelling for more than one week, suggesting that perceived risk of malaria may influence the decision to sleep under an LLIN while away from home.

Travel has been identified as a risk factor for malaria across Africa.[83,93,148,149,197] In Uganda, a study conducted at three sites of varied malaria transmission demonstrated that the incidence of malaria in travellers was over three times higher in the 60 days after overnight travel compared to the 60 days before travelling.[163] Another study in western Uganda found that travelling within the previous 4 weeks from highland areas with low malaria transmission to higher transmission areas was strongly associated with increased malaria risk.[136] Two studies conducted on Bioko Island, Equatorial Guinea, demonstrated that island residents who travelled to the mainland were at increased risk of malaria infection.[93,197] A survey of Bioko island travellers found that malaria prevalence was significantly higher in passengers returning to the island from the mainland compared to those departing the island.[93] The odds of malaria among Bioko Island residents who travelled was significantly higher than in non-travellers, suggesting that imported malaria cases contributed to the sustained transmission of malaria on the island.[197] Similarly, a study in northern Ethiopia found that travel from high-altitude (low transmission) villages to other areas within the previous month was associated with increased odds of malaria.[148] This evidence suggests that travel within Africa is a risk factor for malaria infection. However, behavioural factors associated with travel that might increase exposure to mosquito vectors, and thus malaria infection, have been less well-explored.

There are several potential reasons why people may be at increased risk of malaria during travel. In this study, gender differences in LLIN use while travelling were observed. Women were less likely to use LLINs when travelling than at home, but this was not true for men. Interestingly, women reported using LLINs more often than men when at home; however, when travelling, the opposite was true. This suggests that at home, women may be more aware of the importance of sleeping under LLINs to protect against malaria, perhaps reflecting routine distribution of LLINs at antenatal clinics and targeted campaigns to increase LLIN use among pregnant women.[201-203] When travelling, women may either lack LLINs or the agency to use them, particularly when visiting the home of a friend or relative.

Individuals who travelled to non-IRS districts and those who travelled for more than 7 days were more likely to use LLINs. These findings suggest that the decision to use LLINs may be influenced by destination or duration of travel and the individuals' perceptions of malaria risk. Indeed, perceptions of malaria risk have been shown to influence the use of LLINs when people travel.[176] In south-eastern Tanzania, in-depth interviews were used to assess perceptions of malaria risk during outdoor and indoor activities. In this study, participants believed that outdoor activities such as fishing in the river late at night, travelling to farms overnight, and attending parties and funerals held at night, all increased their risk of malaria infection. For situations where use of LLINs was not feasible, participants believed that alternative malaria prevention approaches, including use of mosquito repellents and chemoprophylaxis, were needed.

LLINs are known to reduce malaria morbidity and mortality and are widely used for vector control in Africa,[204] but achieving high adherence to LLINs, even at home, is challenging. In this study, just over half of cohort participants who travelled slept under LLINs when at home, despite universal access. Many barriers to LLIN use have been described, including many

household members,[56,205] lack of space to hang LLINs,[206] lower socioeconomic status, and time since the last LLINs distribution.[59] In this study setting, where malaria transmission dropped substantially, individuals may have felt that it was no longer necessary to use their LLINs.[34,37] During travel, a possible barrier to LLIN adherence is limited availability of LLINs to use away from home. Mass distribution of LLINs in Uganda follows WHO guidelines, which recommend distributing one LLIN for every two household residents.[207] This may leave no spare LLINs for visitors, or for carrying during travel. In this study, other factors that may have contributed to limited use of LLINs during travel include social barriers, such as attending a funeral or wedding where individuals are expected to stay outdoors all night, or fear of appearing rude or disrespectful during communal gatherings.[79] These factors should be considered when designing strategies to increase LLIN adherence in travellers.

A strength of this study is that behaviours at home and during travel within the same individuals were prospectively compared, minimizing the potential for confounding. Similar studies have only assessed malaria-relevant behaviours while travelling, or at home, but not both.[175,199,208] A study conducted in south-eastern Tanzania evaluated human behaviour of participants at home.[176] The study found that a high proportion of participants (75%) stayed outdoors in the evenings (between 6pm and 9pm), resulting in exposure to malaria vectors before going to bed. Another study carried out in the Kilombero Valley of Tanzania from November 2015 to March 2016, assessed patterns of behaviour only when travelling, and demonstrated that when individuals travelled for religious, cultural and social gatherings, they stayed outdoors at night till dawn.[175] Previous studies in Uganda that have assessed travel and malaria risk also examined behavioural factors during travel, such as use of LLINs.[136,163] However, differences in behaviour while travelling versus at home were not explored. The findings from this study suggest that a better understanding of circumstances leading to lower use of LLINs when travelling may be important in guiding malaria prevention measures.

This study had several limitations. First, data on behavioural factors during travel could have been subject to recall bias. However, questionnaires were administered within 4 weeks following travel, and adherence to LLINs at home was assessed every two weeks by home visits, to closely evaluate the relationship between behaviours at home and when travelling. Second, the study was conducted in rural Tororo, and few individuals travelled outside of the district. Thus, results may not be generalisable to other settings. Lastly, intensive malaria control with IRS and LLINs resulted in few malaria cases in Tororo. Thus, it was not possible to directly measure the association between behaviours and malaria risk.

Conclusion

Travel is an important individual risk factor for malaria, and individuals who travel may also threaten malaria control gains, especially in areas on a pathway to elimination. Results from this study suggest that individuals were less likely to use LLINs when travelling. Strategies to increase awareness about the importance of LLIN adherence, particularly in travellers, should be developed and deployed by the National Malaria Control Division of the Ministry of Health, or other stakeholders. Use of LLIN during travel, especially during the holiday season when most people are likely to visit family and friends, should be emphasised. Travellers should be encouraged to carry an extra LLIN when travelling, especially when visiting rural areas or those without ongoing IRS. Further research on innovative approaches to prevent malaria in travellers including portable LLINs, effective ways to influence behaviour and increase LLIN use, and acceptability of other malaria prevention measures such as mosquito repellents and chemoprophylaxis, should be encouraged.

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Chapter 7. Discussion

7.1 Chapter introduction

Travel is common and is known to be associated with the increased spread of infectious diseases.[78,85,86,209] However, there are evidence gaps in the association between overnight travel and the risk of malaria in residents of malaria-endemic countries. This chapter provides a summary of key findings on the association between travel and the risk of malaria, and discusses the implications of the findings presented in this thesis. Section 7.2 summarises key results, section 7.3 provides more details about the effect of increased urbanisation on the risk of malaria and reduced exposure to malaria parasites, section 7.4 discusses the threat of malaria control by travellers mainly through reintroduction of malaria parasites in areas where malaria transmission has decreased over time, and section 7.5 provides a discussion of challenges with use of LLINs during travel. In section 7.6, implications of thesis findings are discussed including the linkage between low and high malaria transmission areas through travel. Section 7.7 presents the strengths of the thesis and highlights contributions by the thesis to current evidence on travel and the risk of malaria. Section 7.8 discusses the overall thesis limitations in addition to challenges discussed under chapters 4, 5 and 6. Finally, section 7.9 presents conclusions and general recommendations.

7.2 Summary of results

This thesis presents findings from a case-control study of 162 cases and 405 controls from Kampala city, a cohort of 906 participants across 3 sites in Uganda (PRISM 1), and a cohort of 531 study participants at a rural site in Uganda (PRISM 2). The case-control study evaluated participants from an urban setting, while the cohort studies evaluated participants from sites with varied malaria transmission intensity, as well as a rural area where intense malaria control with LLINs and IRS were implemented. It was possible to study travel and malaria in

Uganda because of the increased heterogeneity in malaria transmission following scaling up of widely used malaria control interventions and urbanisation that has led to environments where people who travel away from their homes may be at increased risk of malaria infection. Despite the relatively low burden of malaria in Kampala and in Tororo after the implementation of IRS, malaria cases continue to be diagnosed at health facilities in these areas. Analysis of data from this research project provided results that strongly suggest that travel was associated with an increased risk of malaria and identified personal protection measures that could be emphasised during overnight travel.

Using data from the Kampala case-control study, and the PRISM 1 cohort study at 3 sites in Uganda, the following research questions were addressed: 1) Is recent overnight travel associated with an increased risk of malaria in the urban capital of Kampala? 2) Is recent overnight travel associated with an increased risk of malaria among residents in rural areas of Uganda with varied malaria transmission intensity? and 3) Are there behavioural factors associated with travel in Uganda that might modify the risk of malaria infection?

Findings as presented in chapters 4 and 5 demonstrate that overnight travel, whether travelling from an urban setting or rural setting, was associated with an increased risk of malaria. Assessment of the association between recent overnight travel and malaria diagnosis in an urban setting revealed interesting findings. The odds of malaria in Kampala residents who travelled within the previous 60 days was much higher compared to those who did not travel. This suggests that travellers were likely to have acquired malaria infection while travelling away from Kampala and presented with malaria disease when they returned from their overnight trips. Further investigation of the associations between travel outside of the subcounty of residence and the incidence of malaria in a peri-urban and rural settings in Uganda, revealed that the incidence of malaria was much higher in the 1-60 days after

traveling compared to the period within 60 days before travel. The question as to why the risk of malaria was high when individuals travelled was addressed by the case-control study in Kampala through the identification of several factors that were associated with an increased odds of malaria. These factors include younger age below 16 years, travelling to districts not receiving IRS (indicating travel to a high malaria transmission destination), travelling for a longer duration of travel of 10 or more days, staying at a relative's place while travelling, and engaging in outdoor activities.

Furthermore, evaluation of the difference between behaviours at home and during travel of residents of rural Uganda (chapter 6), indicated that female participants were more like to use LLINs when at home than men while both genders were similarly unlikely to use LLINs when travelling. In addition, assessment of factors associated with increased odds of clinical malaria diagnosis among Kampala residents who travelled revealed failure to sleep under LLINs during travel as a significant risk factor. Further evaluation of factors associated with adherence to LLINs during travel indicated that study participants who travelled to non-IRS districts were more likely to sleep under LLINs during travel compared to those who travelled within Tororo district. Travelling for a longer duration of ≥ 7 days was significantly associated with sleeping under LLINs.

Overall, findings presented by this thesis provide evidence that 1) malaria cases diagnosed in Kampala (an urban setting) were strongly associated with recent overnight travel, 2) recent overnight travel was associated with increased incidence of malaria especially in Nagongera where IRS and universal LLIN distribution campaign have been implemented, 3) among travellers, failure to use LLINs during travel was associated with increased odds of malaria, 4) among travellers, those who were below age 16 years, travelling to a districts not receiving IRS, travelling for >= 10 days, sleeping at a relative's place, and engaging in outdoor activities at

increased odds of malaria and could potentially be targeted for personal protective measures such as use of LLINs, mosquito repellent sprays and chemoprophylaxis during travel.

During overnight travel, individuals are exposed to changes in their environment and there are several factors that might increase the risk of infection with malaria parasites (**Figure 7.1**).

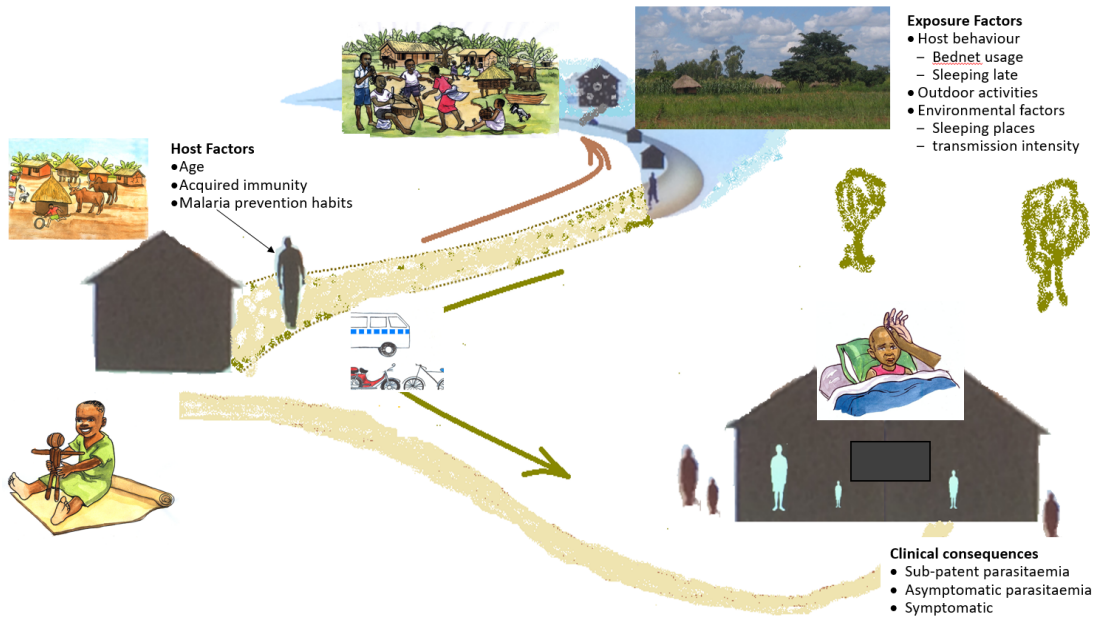

Figure 7.1. Events that occur when individuals travel and may partly explain the increased risk of malaria during overnight travel

In our study setting, people were likely to behave differently when they were at home compared to when they travelled. While at home, they were more likely to 1) use LLINs when they went to bed, 2) go to bed early, and 3) avoid outdoor activities that may expose them to mosquito bites. These behaviours were likely to be altered when people travelled, and may have led to increased exposure to mosquitoes and infection with malaria parasites while travelling. Behavioural factor that could increase exposure to malaria vector mosquitoes include engaging in late night outdoor activities like sitting in around a fire place during funeral rites, parties held in gardens, and sitting outdoors discussing with friends and family. The magnitude of exposure to mosquitoes and acquisition of malaria parasites infection was likely

to be influenced by malaria transmission intensity at the destination of travel. For example, travelling to lowland areas with high malaria transmission intensity relative to the level of transmission while at home, might increase exposure to mosquito vectors and increase the risk of infection with malaria parasites. These changes in environment and alterations in behaviour when individuals travel provide general understanding of events leading to the differences in malaria exposure during travel away from home.

7.3 Urbanisation and risk of malaria in Africa

Urbanisation has increased in Africa over the last three decades,[127-129,210] and in Uganda, people continue to migrate from rural to urban areas for many reasons such as better employment, good schools, and business.[211,212] There is increased evidence that urbanisation is associated with reduction in malaria transmission,[130,131] and yet malaria cases are diagnosed at health facilities within Kampala, an urban setting in Uganda. Kampala is the capital city and business hub of Uganda with people travelling daily between the city and other parts of the country. Kampala residents also maintain close ties with their relatives and friends living in various parts of the country and visit each other regularly especially during holidays. This thesis presents findings from a case-control study in Kampala that demonstrated that malaria diagnosed was strongly associated with recent travel, suggesting that malaria cases in Kampala are likely to have been acquired during travel as discussed in chapter 4. However, earlier studies had indicated that there was heterogeneity in malaria transmission in Kampala,[213] and the risk of malaria was related to where individuals lived. The incidence of malaria was higher in residents of slum areas near swamps compared to those living away from the swamp. This may suggest onward transmission of malaria due to availability of susceptible mosquito vectors in some areas of Kampala.

When individuals travel and get infected with malaria parasites, there is a risk of re-introducing parasites on returning home, and if the environment is conducive for onward transmission, may lead to persistence of malaria transmission. Findings discussed in chapter 4 suggest that many Kampala residents may be at risk of infection with malaria parasites while they are travelling away, and the cases diagnosed at health facilities are from a proportion that becomes symptomatic. It is possible that some individuals who become infected with malaria parasites while travelling away from home did not develop symptoms or seek treatment, and are harbouring parasites in Kampala. Such residents would contribute to the human infectious reservoir and onward malaria transmission.

Since immunity to malaria infection and illness is acquired gradually through repeated exposure to parasites over time,[97,115,214] residents of urban areas or places where malaria control has been scaled up and malaria transmission becoming very low, are likely to have waning immunity.[125,214] This happens in individuals who have been exposed to higher malaria transmission settings before and developed antimalarial immunity. In addition to existing residents, children born in these areas after malaria transmission has reduced significantly also lack immunity due to limited exposure to malaria parasites. This may be the reason why residents of Kampala and Tororo were prone to malaria infection and disease when exposed to malaria parasites during travel away from home.

7.4 Travellers may threaten malaria control

Increased risk of malaria associated with recent travel has been reported by many studies conducted across the world.[77,83,90,93,136,138-149,197] Human travel presents a major challenge to malaria control, elimination and maintaining a malaria free world. Currently available malaria control interventions have drastically reduced malaria transmission in some areas, but are likely to be inadequate in elimination of malaria due to many barriers including travel and

hence additional measures targeting travellers should be explored. Malaria prevention during travel is already emphasised mainly by residents of malaria-free countries when travelling to malaria endemic regions of the world.[183,215-217] This thesis provides evidence that local overnight travel plays an important role in malaria infection in Uganda. However, a comprehensive understanding of travel within Uganda and malaria burden would be helpful in sustaining reduction in malaria as Uganda strengthens efforts toward malaria control and elimination.

In chapter 5, findings from Tororo, a rural area in Uganda that had received intensified malaria control with LLINs and IRS, suggested that the risk of malaria was higher following travel away from home. Malaria exposure in these residents reduced drastically following scale up of control interventions and may have led to waning of acquired immunity against malaria parasites infection and disease like what has been reported elsewhere.[125,126] Residents of these areas are likely to be at an increased risk of malaria when they travel away from home to areas of higher malaria transmission intensity.

Malaria transmission intensity in Uganda has become increasingly diverse with some areas of near to zero transmission and others with highest transmission intensity reported globally.[28,43] Hence, people travelling within the country are at an increased risk of malaria infection when they travel to areas of higher transmission relative to their homes.[136,163] Similar finding have been reported from studies conducted on islands of Zanzibar in Tanzania and Bioko Island in Equatorial Guinea. [93,167,168,218] These areas showed clear distinction between low malaria burden on the islands and high malaria burden on the main land. Very few malaria cases were reported from the island following scale up of malaria control interventions and malaria elimination had become a possibility. However, malaria control efforts on Bioko Island in Equatorial Guinea were frustrated by imported malaria.[93,167,168,218] In 2015, Bradley *et al.*

evaluated the associations between malaria infection and travel to mainland Equatorial Guinea using Malaria Indicator Survey data collected in 2013 and 2014,[93] and demonstrated that malaria infection was associated with travel within the previous eight weeks to the mainland. Findings from a follow up study by *Guerra et al*. two years later conducted between 2015 and 2017 were consistent with a significantly higher odds of malaria among travellers than the rest of the population.[197] These findings suggest that travellers were responsible for imported malaria to the island, and if targeted for malaria prevention, cases would have decreased between 2014 and 2015.

In Zanzibar, several studies have shown that malaria elimination is possible,[219,220] if human movement could be considered to cater for imported malaria cases.[87,221] In 2011, Le Menach *et al.* demonstrated that residents returning to Zanzibar from malaria endemic regions contributed up to 15 times more imported cases than infected visitors.[76] The majority of malaria cases in Zanzibar were imported, advocating for implementation of control measures based on detecting imported malaria cases.

Collectively, these studies provide evidence that residents returning from trips away from the islands were likely to carry parasites back if infected. Onward transmission of parasites was possible as demonstrated by increased risk of malaria infection among non-travellers living in an area with a large number of travellers. The cross-sectional design of these studies could have led to selection bias by missing individuals who had travelled away from home, and further research with improved design was needed. This thesis has contributed to this evidence by presenting findings from 1) an urban setting where the odds of malaria was much higher in travellers compared to those who did not travel, and 2) a rural setting where malaria incidence was higher following travel compared to the period before travel. Factors that could have contributed to malaria risk among traveller included reduced adherence to LLINs as discussed under section 7.5.

7.5 Lack of LLIN use during overnight travel

Chapters 5 and 6, presents findings from analysis of behavioural factors that might influence the risk of malaria. Some factors like use of LLINs and engaging in outdoor activities were modifiable, and others like travelling to non-IRS districts were non-modifiable but had an effect on whether travellers used malaria prevention or not. Malaria prevention during travel was found to be lacking in both the urban setting of Kampala and rural Tororo. Although adherence to LLINs at home was low, travellers were additionally less likely to sleep under LLINs during travel compared to being at their homes as indicated under chapter 6. This might be attributed to limited access to LLINs following recent universal LLIN distribution campaign where one net was given per two household members and likely to leave no spare LLIN for the visitors. Even if travellers were willing to carry LLINs, there was no spare net to take while travelling. This may explain the increased odds and risk of malaria during travel as demonstrated by findings from the case-control study in an urban setting and a cohort study at 3 sites in rural Uganda.

The major prevention measure in Uganda available to people during travel is use of LLINs, and adherence could be hindered by several factors as discussed in chapter 6. Whether an individual adheres to LLINs or not during travel may be influenced by the reason for and destination of travel. For example, if people travel for a funeral rite or night parties where they stay outdoors for a whole night, they will most likely not use LLINs. However, if individuals travel for a conference and are booked in a hotel, they are likely to have a less risk of malaria exposure and infection. Outdoor activities are likely to increase exposure to mosquito vectors leading to a high chance of infection with malaria parasites.

During travel, there are circumstances that may negatively affect use of malaria preventive measures. Moreover, in areas where individuals are aware of benefits of malaria prevention during travel by using LLINs, they still have challenges of adherence to use of LLINs. Some studies have used focused group discussions and in-depth interviews to assess barriers and perceptions to malaria prevention when individuals travel away from their homes.[79,176,199] Two studies were carried out by *Monroe et al.*; the first study in Uganda,[79] the second in Zanzibar, Tanzania.[199] For both studies, data were collected using in-depth interviews and focused group discussions. In Uganda, the study was carried out in four districts between March 2012 and January 2013, and identified challenges to LLINs use during travel which included fear of appearing disrespectful, limited number of LLINs with no spare to carry, and not having a place to hang the net even if a traveller carried one.[79] In Zanzibar the study was carried out between December 2016 and May 2017, and findings were consistent with high levels of LLINs use while residents were at their homes but not as such when travelling away from home.[199]

Assessment of perceptions and attitudes of individuals towards malaria prevention during travel is vital if malaria preventive measures during travel are to be successful. A study conducted in south-eastern Tanzania by *Finda et al*. evaluated human behaviour and perceptions of malaria risk during outdoor and indoor activities using in-depth interviews, and findings were consistent with a good attitude towards adherence to LLINs use while at home.[176] However, adherence to LLINs use was not possible while people travelled away from their homes. Findings from this study provide more evidence that people are aware of the need to prevent malaria during travel.

Collectively, findings from these three studies agree with travellers having a good perception of malaria prevention during overnight travel, but there was reduced adherence to use of LLINs mainly as a result of limited availability and other logistical issues including having no place to hang the LLINs. This suggests that if LINs were adequately supplied, travellers would most likely use them to prevent malaria while away from home.

The destination of travel plays an important role in influencing use of malaria preventive measures during travel. In chapter 6, this thesis demonstrates that use of LLINs was higher if individuals travelled to districts not receiving IRS compared to when they travelled to districts receiving IRS. This suggests that travellers already know what areas in the country are likely to be high risk areas and those which are likely to be low risk. As malaria transmission in Uganda becomes increasingly heterogenous, there will be need to design risk maps and travel advice given to travellers on preventive measures to be taken depending on whether individuals are travelling to high or low malaria risk destinations. This is already practiced by travellers from malaria non-endemic countries to malaria endemic regions. A risk map is used to determine whether malaria prophylaxis is needed or not. In Africa, travel advice is given according to within country risk. In South Africa where malaria risk is minimal,[222,223] travel to higher risk countries, including Botswana[224] and Namibia,[225] as well as travel within South Africa, areas with high malaria risk are well-mapped and appropriate prevention measures suggested. Such data are lacking in Uganda, and findings presented by this thesis could initiate discussions towards compiling travel guidelines for travellers to different destinations within the country.

Most available and widely scaled up malaria control interventions including IRS and mass distribution of LLINs work at population level and have documented success towards malaria control.[37,38,40,46,49] However, use of these malaria control measures during travel is limited. IRS is implemented in a few selected districts, and as discussed under chapter 5 and 6, travellers report low adherence to use of LLINs. Besides, malaria control gains that have been achieved using IRS and LLINs should be maintained by additional malaria preventive measures especially when individuals travel away from home. Personal prevention measures like use of mosquito repellents and chemoprophylaxis are likely to play an important role in malaria prevention for targeted populations.[178,179]

Mosquito repellents and chemoprophylaxis are currently recommended by WHO for use by residents of non-malaria endemic countries travelling to malaria endemic areas,[178,226-228] and similar measures are yet to be emphasized for residents of malaria endemic countries. Although repellents have been widely used,[229] they have not been proven to be effective at community level.[182,230] These would provide additional malaria prevention not necessarily when individuals are at home, but while travelling away from home mainly before travellers are indoors or before going to bed where LLINs and IRS are utilised. Chemoprophylaxis involves regular administration of a drug to prevent malaria infection,[183,184] and standard recommendations include daily atovaquone-proguanil, daily doxycycline, or weekly mefloquine; primaquine and tafenoquine are also effective, but require confirmation of normal glucose-6-phosphate dehydrogenase levels before use.[231,232] Since currently available chemoprophylaxis regimen are supposed to be initiated shortly before the trip begins and continued for 1 – 4 weeks following completion of trip,[231] more friendly regimens will be required for residents of malaria endemic areas.

Much as suggestions to alternative approaches like use of repellents were made, research into acceptability and feasibility of these alternative malaria prevention measures in situations where LLINs are less likely to be used is needed. There are other innovative personal protective measures like portable/foldable LLINs that can be further studied. These do not need to be hung, but rather self-retaining/pop-up at sleeping places.

7.6 Implications of these findings

Findings presented in this thesis generally suggest that travellers within Uganda should be targeted for malaria prevention. For any additional malaria prevention measure to be sustainable, it should be implemented through the National Malaria Control Division (NMCD),

and our findings provide evidence that LLINs should be emphasised during travel. The NMCD should consider adding malaria prevention during travel to the malaria prevention messages already in existence, by encouraging travellers to carry an extra LLIN especially when travelling to high malaria transmission parts of the country. Whether travel increases the risk of malaria depends on the level of malaria transmission intensity at the location of origin and destination of travel. Travelling from a low malaria to a high malaria transmission area increases the risk of malaria infection since individuals are likely to have reduced immunity to malaria and are likely to get infected when exposed to malaria parasites. On the other hand, travelling from a high malaria to a low malaria transmission area intuitively increases the risk of introduction of parasites. Travellers from higher transmission areas area more likely to carry parasites to low-transmission areas. In both situations, malaria parasites are re-introduced in areas of low transmission and risk onward transmission. Hence, more emphasis on targeting travellers for malaria prevention whether from low or high malaria transmission areas.

To identify high-risk and low-risk areas of malaria importation, Wesolowski et al. analysed human travel patterns and movement of malaria parasites in Kenya, and identified two sources of parasite importation; individuals living in a low malaria transmission area (sink) visiting a high malaria transmission area and carrying parasites back home, and infected residents of a high malaria transmission area (source) visiting relatively low malaria transmission area and carrying parasites with them.[139] Although the risk of malaria is much lower in areas with low transmission intensity, such areas are at a risk of onward transmission when in presence of viable mosquito vector. It is worth considering maintenance of malaria control measures in areas with low transmission as well as targeting travellers.

Maintaining malaria control interventions at both sinks and sources has been supported by findings from work done by Prosper *et al.* Unlike the classic Ross MacDonald models that

described malaria transmission as determined by adult mosquito mortality rate,[15,233,234] a mathematical model was developed to analyse basic reproduction number (R_0) in two areas and compare low and high malaria transmission intensity.[235] The basic reproduction number was defined as an epidemiologically important threshold quantity that indicates whether malaria will persist or go extinct in a population, or a number of secondary cases resulting from one infectious case in an otherwise fully susceptible population.[235] If $R_0 > 1$, malaria will persist in the population, and if $R_0 < 1$ then malaria will become extinct. Findings from the model showed that malaria can persist in an area where $R_0 < 1$ if humans move between another area where $R_0 > 1$. Findings from this model suggest that for effective control, low transmission areas should be given attention as well if they are connected to high transmission areas through human movement.

Malaria control following travel can be hindered by the spread of other infectious diseases, with most recent evidence of the spread of coronavirus disease (COVID-19).[236-238] In attempt to control the spread of COVID-19, measures that were put in place have negatively affected malaria control in Uganda. These measures included quarantine of high-risk populations, contact tracing, case management of the confirmed cases, and limiting transmission through a country lockdown.

First, there was limited access to malaria case management during lockdown by restriction of movement to health facilities that lasted for three months, and health workers not able to get to work. Second, the most commonly presenting symptom for both COVI-19 and malaria being fever, the spread of COVID-19 will most likely overburden the health system. This may lead to increase in febrile patients requiring medical attention, increased number of malaria tests carried out, misdiagnosis of malaria, and stock-out of malaria drugs and other supplies. Areas

in Uganda that had received adequate control interventions are at a risk of increased malaria burden and individuals who travel between these areas may lead to further spread of malaria.

7.7 Strength of the thesis

As most malaria endemic countries work towards malaria control and elimination (and eradication), additional malaria control measures including targeting travellers will be required. However, there are limited data on travel patterns and the risk of malaria to inform policy on malaria prevention. This thesis responded to the evidence gap by presenting findings from 1) an urban setting in response to increased urbanisation and the risk of malaria, and 2) a longitudinal study of participants in which a gold standard measure (incidence of clinical malaria) was used to estimate the burden of malaria.

To our knowledge, this was the first study of its kind to evaluate travel between an urban setting (Kampala) and other parts of Uganda. As discussed in chapter 4, malaria exposure to Kampala residents has drastically reduced following increased urbanisation and scale-up of control interventions in Uganda,[158] and increased odds of malaria can be attributed to waning acquired immunity.

Again, to our knowledge, this thesis presents findings from the first prospective cohort study to evaluate overnight travel and the risk of malaria (chapter 5). All the three sites of varied malaria transmission intensity in Uganda were consistent with increased incidence of malaria within 60 days following travel compared to the period of 60 days before travel. This strongly suggests that malaria was acquired during travel and interventions aimed at malaria prevention in travellers would most likely play an important role in malaria control.

7.8 Limitations of the thesis

This thesis had several limitations which have been discussed under chapters 4, 5 and 6, and are discussed in details under this section. These limitations include difficulty in proper

measurement of travel, limited generalisability of results to other settings, inability to estimate the actual risk of malaria in Kampala and recall bias in a case control study, the study did not measure malaria transmission at destination of travel, malaria diagnosis was based on microscopy and not with a more sensitive molecular diagnostic test, and the study did not genotype malaria parasites to determine whether parasites were imported or locally transmitted.

First, the major limitation in studying travel is difficulty in finding a best way to measure human movement. People are always on the move for various reasons, and the ability to measure travel becomes very crucial for evaluating its associations with the risk of infectious diseases.

Travel can be estimated using several methods, including questionnaires, Global Positioning Systems (GPS), and mobile phone data.[87,139,155,239-242] Questionnaires involve data collection by interviewing participants but rely on the respondents' ability to recall places visited and details of travel. GPS enabled devices allow human movement to be tracked by measuring fine movements but can be limited by interference, short battery life, and errors during transformation of data collected.[241,243] Mobile phone data provide fine-scale and robust data but depend on mobile phone ownership suggesting that only data from adults may be obtained since mobile phone ownership in children living in malaria endemic areas is very low.[87] **Table 7.1** summarises advantages and disadvantages of using questionnaires, GPS trackers and mobile phone data.

	Advantages	Disadvantages
Questionnaire	• Well-structured. • Characterizes visits. • Most people are familiar with it.	• Relies on participants' ability to recall places they visited.
GPS data loggers	• Very accurate. • Fine-scale movement data.	• Poor signal issues and interference. • Participants forgetting to carry the device. • Device running out of battery. • Getting stolen. • Errors in transforming data into places visited.
Cell phone data	• Very accurate. • Fine-scale movement data.	• Children do not own phones. • Mothers may not travel with their phones. • Obtaining mobile phone data from the phone company might be problematic. • Poor phone signal issues in some areas. • People owning more than one phone from different mobile phone companies.

Table 7.1. Pros and cons of questionnaire, GPS data loggers and cell phone data in measuring human movement/mobility/overnight travel

Currently, there is no gold standard method of measuring travel. The choice of method used depends on the study environment and the study population. For example, in a country like Uganda where the population at risk (especially pregnant women and children) are less likely to either have mobile phones or carry GPS data loggers if provided, questionnaires would be the most appropriate data collection tools.

Second, findings presented came from studies that were carried out in areas with lower malaria burden compared to the rest of sub-Saharan Africa and may not be generalisable. Malaria parasitaemia in children under 5 years of age living is Kampala is one of the lowest compared to the rest of Uganda,[43] and Nagongera had received intensified malaria control using LLINs and IRS.[38] However, data from Kampala represent findings from an urban setting in Uganda, and cohorts were carried out at 3 sites of varied malaria transmission intensity, and results to a large extent represents findings from a wider range of transmission intensity.

Third, data collection for the case-control study had design related limitations which include inability to measure malaria risk but odds ratio instead, a possibility of recall bias, and the study was not able to adjust for seasonality in malaria transmission. The increased odds of malaria diagnosis observed referred to patients who attended Naguru hospital but not the general population in Kampala. Data on travel history were collected after participants had returned from their trips, and participants might have forgotten details about the trip. Furthermore, the study was carried out for two months and any seasonality in malaria burden from one year to another could have been missed. Nonetheless, the strong association between travel and malaria diagnosis suggests that malaria risk could have been significant as well.

Fourth, this study did not measure malaria transmission intensity at the origin and destination of travel. It is important to estimate EIR and parasite prevalence across Uganda so that assessment of the risk of malaria during travel adjusts for transmission intensity at the destination of travel. However, the areas which were not receiving IRS were considered to have high malaria transmission and travel to such destinations was associated with increased odds of malaria among those who travelled.

Finally, the study used RDT for the case-control study and microscopy for the cohort study, and did not have capacity to carry out malaria parasite genotyping to determine whether malaria parasites were locally acquired or travel related. However, the increased odds of malaria diagnosis in individuals with history of travel and the timing of the high incidence of malaria after travel compared to before travel strongly suggest that malaria parasites were acquired during travel.

These limitations demonstrate the difficulty in conclusively ascertaining that overnight travel was the only contributing factor of increased risk of malaria. Other factors such as malaria transmission at participants homes, parasite prevalence in individual before travel, and health care seeking behaviour might have led to malaria case diagnosis following travel away from home and diagnosis following travel. Findings presented in this thesis identified a target group for malaria prevention and suggests other factors that can be explored further if malaria control and eventually elimination in Uganda are to be realised.

7.9 Conclusions and recommendations

7.9.1 Conclusions

In conclusion, this thesis highlights evidence that overnight travel was strongly associated with increased risk of malaria. Particularly, in Kampala residents who were diagnosed with malaria

within 60 days following overnight travel. Similarly, residents of rural and peri-urban areas who presented with malaria disease were likely to have travelled within the previous 60 days. In both urban and rural settings, malaria infections were likely to have been acquired during travel suggesting that travellers could be targeted for malaria prevention. Factors that have been identified that influence the risk of malaria while travelling are categorised into modifiable and non-modifiable factors. Modifiable factors can be emphasized for malaria prevention during travel, and non-modifiable factors are equally important in identifying a population of travellers that can be targeted for additional personal malaria prevention measures during travel. As malaria burden reduces in areas that were previously high malaria transmission, overnight travel plays an increasingly important role in malaria risk especially in a setting where malaria control interventions have been implemented. Travellers could be targeted for malaria prevention. Overnight travel was associated with reduced adherence to LLINs which may partly explain the increased risk of malaria during travel.

7.9.2 Recommendations and future research

Previous studies evaluating mass distribution of mosquito repellents on top of IRS and LLINs did not show additional protection mainly due to issues with long time adherence.[230,244] However, there are limited data from studies evaluating use of mosquito repellents during overnight travel for people living in malaria endemic regions. This would include temporary use during overnight travel and stoppage on returning which will improve adherence. IRS protection starts when individuals are indoors and LLINs when they have gone to bed. In addition to mosquito repellents and chemoprophylaxis, more individual malaria protective measures should be assessed for use by residents of malaria endemic countries. In addition to malaria preventive measures that have been discussed, a travellers' kit that includes an LLIN, mosquito repellent cream or spray, an RDT test, and a course of ACT can be recommended as a measure to prevent malaria while travelling.

Malaria control interventions including IRS and use of LLINs are part of the main strategy for malaria control in Uganda and have proven to work at population level,[37,38,40,46,48,49] but not for malaria prevention at individual level especially during overnight travel. LLINs are a suitable malaria prevention measure that should encourage for use during overnight travel. In addition to emphasis on travellers carrying a spare LLIN with them, future work will be required to advocate for malaria prevention during travel.

Use of mosquito repellents and chemoprophylaxis are recommended by WHO for malaria prevention in residents of malaria non-endemic countries travelling to malaria endemic regions.[228,245] Further evaluations of these malaria prevention measures are needed for control of malaria in residents of malaria endemic countries during overnight travel away from their homes.

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Appendices

Appendix 1. Screening questionnaire for the case-control study in Kampala

	Question	**Response**
1	Study ID	Participant unique ID
2	Patient's initials	Participant initials
3	screening date	Date
4	Gender	Male/Female
5	Date of birth	Date
6	Please re-enter Date of Birth?	Date
7	Diagnostic test for malaria	Yes/No
8	Age 6 months or more	Yes/No
9	Body weight of 4kg or more	Yes/No
10	Resident of Kampala Capital City for at least the last 3 months	Yes/No
11	History of fever within the last 24 hours/ axillary temperature of 37.50 C or more	Yes/No
13	Able to speak English or Luganda (a local language spoken in Kampala)	Yes/No
14	Prior participation in the study	Yes/No
15	Evidence of chronic illnesses that can explain the history of fever/documented temperature such as AIDS and sickle cell disease	Yes/No
16	Willingness to provide informed consent for self or child participant or assent for minors?	Yes/No

	Was consent signed after the date screening began? If yes, record date consent signed below	
18	All criteria for study inclusion met? If yes, complete screening log and proceed to the Travel form	Yes/No
19	Date that consent was signed	Date

Appendix 2. Travel questionnaire for the case-control in Kampala

	Question	**Variable Codes**
1	Study ID	Participant ID
2	Initials	Participant initials
3	Date	Date
4	Date of birth	Date
Overall assessment		
5	Malaria test done	BS/RDT
6	Malaria test results	Positive/Negative
7	Status	Case/Control
Demographics		
8	Subcounty/Division	Name of the subcounty of residence
9	Parish	Name of the parish of residence
10	Village	Name of the village of residence
11	Village code	Village code
Travel		
12	Did the study participant travel and spend at least a night outside of Kampala city within the last 2 months? (if yes, complete the number of trips and the travel form provided)	Yes/No
13	Number of Trips made	Number
14	Who is the respondent	1-Participant, 2-Parent, 3-Older sibling, 4-Guardian

15	Notes	Other relevant comments
STUDY PARTICIPANT TRAVEL HISTORY - this section repeated for each trip		
16	Trip Number (beginning at 1)	Number
17	Date of first night away	Date
18	Date of last night away	Date
19	Number of days (length of trip)	Number
20	District	Name of the district travelled to
21	Subcounty/Division	Name of the sub-county travelled to
22	Parish	Name of the parish travelled to
23	Village	Name of the village travelled to
24	Village code	Code of the village travelled to
25	Main reason for travel (circle one answer)	1-work/trading, 2-visiting relatives/friends 3-funeral rites, 4-school, 5-holiday 6-Conference/worship/church 7-partying/wedding/cultural gathering 8-accompanying parents/guardians 9-Other
26	Specify other reason for travel	Reason
27	Participant's perception of the risk of getting malaria at the place they have travelled to	1-No risk, 2-Low risk, 3-Medium risk 4-High risk, 5-Unknown
Information for each day		
Day number _____		

28	Where did the participant stay overnight?	1-Hotel, 2-Friend/relative's home, 3-school, 4-Hospital, 5-Church, 6-Camp, 7-Other
29	Specify other place the participant stayed overnight	Name of the place
30	Time participant had diner	Time
31	Time study participant went to bed	Time
32	Measures taken by the study participant to prevent malaria during travel (Indicate all that apply)	1-None, 2-Slept Under a bednet 3-Used mosquito repellents 4-Used mosquito coils 5-take antimalarial drugs, 6-other
33	Any other measures take to prevent malaria	Measures
34	What did the study participant do between dinner and going to bed? (Indicate all that apply)	1-Helped with work in the household 2-Sat in the gardens discussing 3-have a drink with friends, 4-stay at work, 5-listen to news/watch TV 6-Go to bed right away

Appendix 3. Clinic visit case record form for the cohort study

UMSP: PRISM-COHORT STUDY STUDY ID [PC]-[___]-[___]-[___] Patient Initials [___][___] Date of Visit [___]/[___]/[___]
Site ID Last First day month year

CLINIC VISIT FORM

If adult participant, remember to ask if there are any new members of the household 6 months – < 10 years of age study who should be screened for enrollment – if yes, add information to Household Census and Locator Log and either screen today or make appointment

Date of last encounter* [___][___]/[___][___]/[___][___]
day month year

Last routine assessment □ finger prick □ venipuncture
* Perform finger prick if more than 28 days since last done and venipuncture if more than 80 days since last done

Medical care outside the study clinic since last seen? □ Yes □ No (If no skip below)

Where care given	Date	Diagnosis	BS done
	/ /		Y / N / ?
	/ /		Y / N / ?

Antimalarial therapy given outside study since last seen? □ Yes □ No (If no skip below)

Antimalarial	Dose	Date last given
		/ /
		/ /

Did the study participant sleep under an ITN last night? □ Yes □ No

VITAL SIGNS (temperature mandatory; height and weight should be done at time of routine assessment; others are optional)

Temperature (°C) [___].[___] Height (cm) [___][___][___] Weight (kg) [___][___].[___] HR [___][___][___]/minute BP (mm Hg) [___][___]/[___][___] RR [___][___]/minute

CLINICAL ASSESSMENT

Parameter	Circle one	Duration (days)	Parameter	Circle one	Duration (days)	NOTES
Fever	Y / N / NA		Cough	Y / N / NA		
Fatigue/ malaise	Y / N / NA		Headache	Y / N / NA		
Abdominal pain	Y / N / NA		Joint pains	Y / N / NA		
Anorexia	Y / N / NA		Muscle aches	Y / N / NA		
Vomiting	Y / N / NA		Seizure	Y / N / NA		
Diarrhea	Y / N / NA		Jaundice	Y / N / NA		*Initials:*

Did the study participant travel and spend at least a night outside the sub-county since last encounter? □ Yes □ No – if yes and complete a travel form provided →

Number of trips made: [___]
Who is the respondent? □Participant □Caregiver □Older sibling □Other
If respondent is a caregiver, what is the relationship with the participant?

NEW DIAGNOSIS AND MEDICATION RECORD (add additional pages if needed)

New Diagnosis	Code	Medication	Code	Dose	Frequency	Duration

Malaria visit type (tick one)

□ No malaria diagnosed today
□ New episode of uncomplicated malaria > 14 days since last episode = AL
□ New episode of complicated malaria > 14 days since last episode = Quinine
□ Uncomplicated malaria 14 or fewer days since last episode = Quinine
□ Complicated malaria 14 or fewer days since last episode = Quinine
□ Uncomplicated malaria during the 1st trimester of pregnancy = Quinine

Routine visit type (tick one)

□ No routine assessment done
□ Sent to lab for finger prick
□ Sent to lab for venipuncture (BS, FP, Hb, immunology)
□ Sent to lab for T Cell Study (BS, FP, ACD Tube(s))

Hospitalizations (tick one)

□ Patient not referred to hospital
□ Patient referred for hospitalization (complete hospitalization form)

LABORATORY TESTS

Blood smear reading □ urgent □ routine

Test	Result	Initials
Parasite density (/ul)		
Gametocytes (Y/N)		
Parasite Species		
Hemoglobin (g/dL)		

Date of next scheduled visit:

[___][___]/[___][___]/[___][___]
day month year
Initials:

Version 4.0 dated 01 July 2016 *Entered* ____ *Date* __/__/__ *Verified* ____ *Date* __/__/__

STUDY ID |___|--|___|___|___| Patient Initials |___|___| Date of Visit |___|___|/|___|___|/|___|___|
Site ID — Last First — day month year

TRAVEL FORM	To be completed if there is a history of travel. Please complete one form per trip* in chronological order. Trip number |___| (1 = 1st trip, 2 = 2nd trip, etc.)

* trip defined as one or more consecutive nights spent in a single sub-county outside of a participant's primary residence

Study participant travel history	
Date of first night away	Date: |___|___|/|___|___|/|___|___| *(DD/MM/YY) (Day 1 below)*
Date of last night away	Date: |___|___|/|___|___|/|___|___| *(DD/MM/YY) (Last day below)*
Name of the district and sub-county travelled to	District: _____Code: |___|___|___|___| Sub-county: _____Code: |___|___|___|___|
Main reason for travel (circle one answer)	1 = work/trading; 2 = visiting relatives/friends; 3 = funeral rites; 4 = school; 5 = holiday; 6 = conference/workshop/church; 7 = partying/wedding/cultural gathering; 8 = accompanying parents/guardians; 9 = Other_____
Participant's perception of the risk of getting malaria at the place they have travelled to (circle one answer)	1 = No risk; 2 = Low risk; 3 = Medium risk; 4 = High risk; 5 = Unknown

		Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Date (add additional pages if trip more than 7 days)		/ /	/ /	/ /	/ /	/ /	/ /	/ /
Where did the participant stay overnight	1 = Hotel; 2 = Friend/relative's home; 3 = School; 4 = Hospital; 5 = Church; 5 = Camp; 6 = Other_____	|___|	|___|	|___|	|___|	|___|	|___|	|___|
Time participant had diner	1 = before 5pm; 2 = 5 pm to <9 pm; 3 = 9 pm to 12 mid-night; 4 = After mid-night; 5 = did not have diner	|___|	|___|	|___|	|___|	|___|	|___|	|___|
Time study participant went to bed	1 = before 5pm; 2 = 5 pm to <9 pm; 3 = 9 pm to 12 mid-night; 4 = After mid-night; 5 = did not go to bed	|___|	|___|	|___|	|___|	|___|	|___|	|___|
Measures taken by the study participant to prevent malaria during travel *(Indicate all that apply)*	1 = None 2 = Slept under a bednet 3 = Used mosquito repellents 4 = Used mosquito coils 5 = Take antimalarial drugs 6 = Other_____		___
___
___			___
___
___			___
___
___			___
___
___			___
___
___			___
___
___			___
___
___	
What did the study participant do between dinner and going to bed? *(Indicate all that apply)*	1 = Helped with work in the house/hotel 2 = Sat in the gardens discussing 3 = Have a drink with friends 4 = Stay at work 5 = Listen to news/watch TV 6 = Go to bed right away		___
___
___			___
___
___			___
___
___			___
___
___			___
___
___			___
___
___			___
___
___	
Notes:								

Initials: _____

Appendix 5. LSHTM ethics approval for the case-control study in Kampala

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
United Kingdom
Switchboard: +44 (0)20 7636 8636

www.lshtm.ac.uk

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

Observational / Interventions Research Ethics Committee

Dr Emmanuel Arinaitwe
LSHTM

28 May 2019

Dear Emmanuel

Study Title: Factors associated with the risk of malaria during recent overnight travel out of Kampala city: a case control study in Kampala, Uganda

LSHTM Ethics Ref: 16625

Thank you for responding to the Observational Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document Type	File Name	Date	Version
Protocol / Proposal	Factors associated with the risk of malaria during recent overnight travel out of Kampala city - Study protocol	05/12/2018	1.0
Protocol / Proposal	Screening form	05/12/2018	1.0
Protocol / Proposal	Travel form	05/12/2018	1.0
Information Sheet	5. Participant informed consent form IDI	12/12/2018	1.0
Information Sheet	1. Participant informed consent form (Adult)	14/12/2018	1.0
Information Sheet	2. Participant informed consent form (Child)	14/12/2018	1.0
Information Sheet	3. Participant assent form	14/12/2018	1.0
Protocol / Proposal	7. IDI topic guides	21/12/2018	1.0
Investigator CV	Emmanuel Arinaitwe _CVFeb2019	14/02/2019	1
Information Sheet	Appendix C_Participant assent form	15/04/2019	2.0
Information Sheet	Appendix E_Participant informed consent form IDI	15/04/2019	2.0
Protocol /	Factors associated with the risk of malaria during recent overnight travel out of	29/04/2019	2.0

Proposal	Kampala city - Study protocol		
Protocol / Proposal	Factors associated with the risk of malaria during recent overnight travel out of Kampala city - Study protocol_EDITS HIGHLIGHTED	29/04/2019	2.0
Information Sheet	Appendix A_Participant informed consent form (Adult)	29/04/2019	2.0
Information Sheet	Appendix B_Participant informed consent form for children below 8 years (Child)	29/04/2019	2.0
Covering Letter	Response to LSHTM Ethics committee and MHREC comments	01/05/2019	1.0

Suggestion: Given that you are no longer conducting FGD we would encourage you to actively plan for more than 10 IDIs to ensure that you obtain sufficient data to answer the 2 qualitative objectives.

After ethical review

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the Committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

An annual report should be submitted to the committee using an Annual Report form on the anniversary of the approval of the study during the lifetime of the study.

At the end of the study, the CI or delegate must notify the committee using an End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: http://leo.lshtm.ac.uk

Additional information is available at: www.lshtm.ac.uk/ethics

Yours sincerely,

Professor John DH Porter
Chair

ethics@lshtm.ac.uk
http://www.lshtm.ac.uk/ethics/

Improving health worldwide

Appendix 6. Mulago Hospital Research and Ethics committee approval for the case-control study in Kampala

TELEPHONE: +256-41554008/1
FAX: +256-414-5325591
E-mail: admin@mulago.or.ug
Website: www.mulago.or.ug

IN ANY CORRESPONDENCE ON THIS SUBJECT PLEASE QUOTES NO…

MULAGO NATIONAL REFERRAL HOSPITAL
P.O. Box 7051
KAMPALA, UGANDA

THE REPUBLIC OF UGANDA

5th June, 2019

Dr. Emmanuel Arinaitwe
Principal Investigator
Infectious Diseases Research Collaboration.

Dear Arinaitwe,

Re: Approval of Protocol MHREC 1592: "Factors Associated with Risk of Malaria during Recent Overnight Travel out of Kampala City: A Case Control Study in Kampala, Uganda".

The Mulago Hospital Research and Ethics Committee reviewed your proposal referenced above and granted approval of this study in its 89th sitting held on 3rd April, 2019. The conduct of this study will therefore run for a period of one (1) year from 5th June, 2019 to 4th June, 2020.

This approval covers the protocol and the accompanying documents listed below;

- Informed consent for adult study participants
- Informed consent for parent/guardian of child below 8 years
- Assent form for minors
- Informed consent for participation in IDIs
- Topic guide for IDI
- Qualitative data collection matrix

This approval is subjected to the following conditions:

1. That the study site may be monitored by the Mulago Hospital Research and Ethics Committee at any time.
2. That you will be abide by the regulations governing research in the country as set by the Ugandan National Council for Science and Technology including abiding to all reporting requirements for serious adverse events, unanticipated events and protocol violations.
3. That no changes to the protocol and study documents will be implemented until they are reviewed and approved by the Mulago Hospital Research and Ethics Committee.
4. That you will submit this approved protocol and all accompanying documents for approval to UNCST before starting the study. In case of studies involving drug and medical devices, approval must be obtained from the National Drug Authority before starting the study.
5. That you provide quarterly progressive reports and request for renewal of approval at least 60 days before expiry of the current approval.

Vision: "To be the leading centre of Health Care Services"

6. That you provide an end of study report upon completion of the study including a summary of the results and any publications.
7. That you will include Mulago Hospital in your acknowledgements in all your publications.

I wish you the best in this Endeavour.

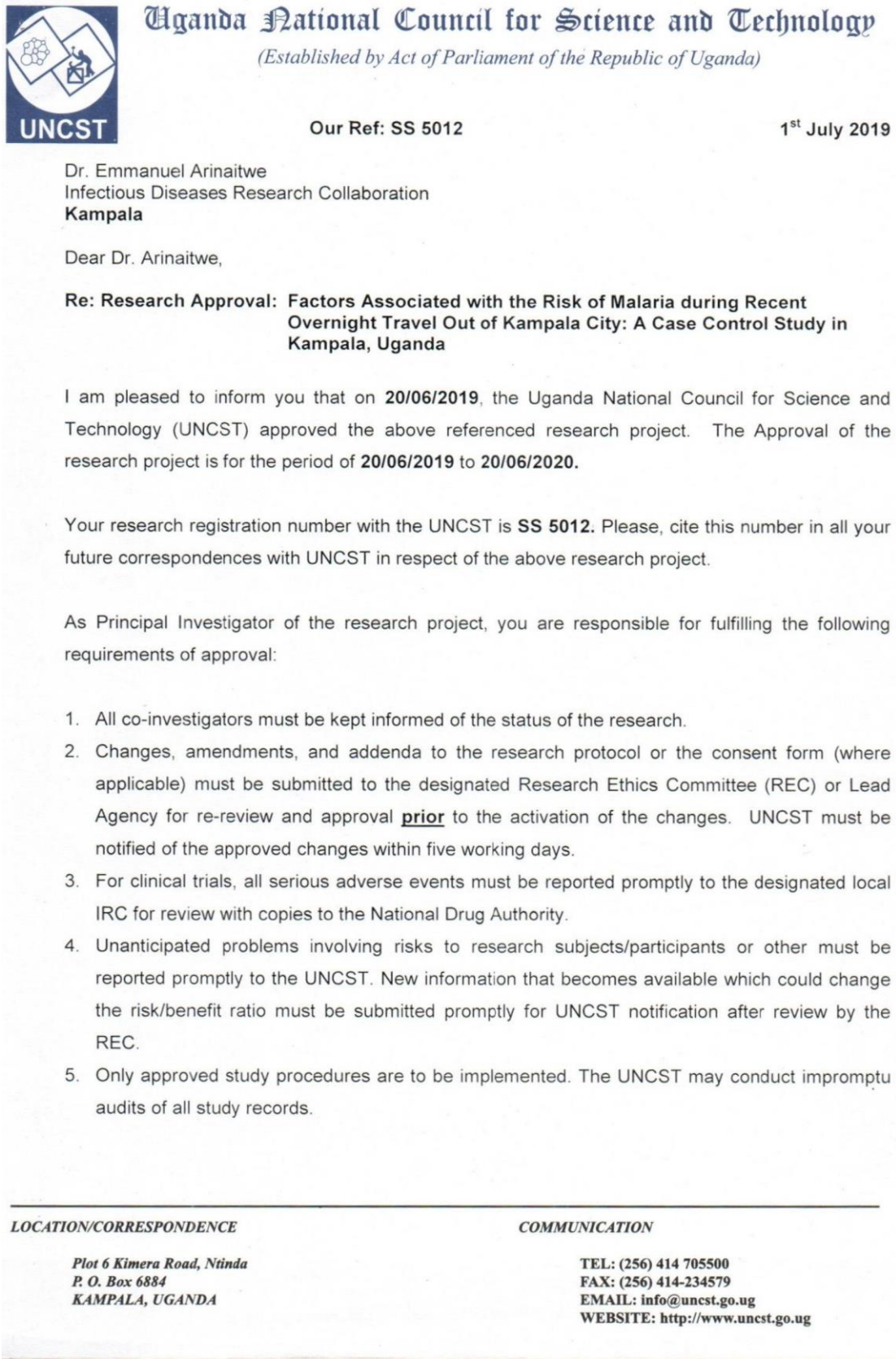
[redacted]

APPROVED
APPROVAL DATE EXPIRY DATE
05 JUN 2019 04 JUN 2020
MULAGO RESEARCH ETHICS COMMITTEE
IRC

DR. NAKWAGALA FREDERICK NELSON
CHAIRMAN- MULAGO HOSPITAL RESEARCH & ETHICS COMMITTEE.

Vision: "To be the leading centre of Health Care Services"

Appendix 7. Uganda National Council for Science and Technology approval for the case-control study in Kampala



Uganda National Council for Science and Technology

(Established by Act of Parliament of the Republic of Uganda)

UNCST

Our Ref: SS 5012 **1st July 2019**

Dr. Emmanuel Arinaitwe
Infectious Diseases Research Collaboration
Kampala

Dear Dr. Arinaitwe,

Re: Research Approval: Factors Associated with the Risk of Malaria during Recent Overnight Travel Out of Kampala City: A Case Control Study in Kampala, Uganda

I am pleased to inform you that on **20/06/2019**, the Uganda National Council for Science and Technology (UNCST) approved the above referenced research project. The Approval of the research project is for the period of **20/06/2019** to **20/06/2020.**

Your research registration number with the UNCST is **SS 5012.** Please, cite this number in all your future correspondences with UNCST in respect of the above research project.

As Principal Investigator of the research project, you are responsible for fulfilling the following requirements of approval:

1. All co-investigators must be kept informed of the status of the research.
2. Changes, amendments, and addenda to the research protocol or the consent form (where applicable) must be submitted to the designated Research Ethics Committee (REC) or Lead Agency for re-review and approval **prior** to the activation of the changes. UNCST must be notified of the approved changes within five working days.
3. For clinical trials, all serious adverse events must be reported promptly to the designated local IRC for review with copies to the National Drug Authority.
4. Unanticipated problems involving risks to research subjects/participants or other must be reported promptly to the UNCST. New information that becomes available which could change the risk/benefit ratio must be submitted promptly for UNCST notification after review by the REC.
5. Only approved study procedures are to be implemented. The UNCST may conduct impromptu audits of all study records.

LOCATION/CORRESPONDENCE

Plot 6 Kimera Road, Ntinda
P. O. Box 6884
KAMPALA, UGANDA

COMMUNICATION

TEL: (256) 414 705500
FAX: (256) 414-234579
EMAIL: info@uncst.go.ug
WEBSITE: http://www.uncst.go.ug

Uganda National Council for Science and Technology
(Established by Act of Parliament of the Republic of Uganda)

6. An annual progress report and approval letter of continuation from the REC must be submitted electronically to UNCST. Failure to do so may result in termination of the research project.

Below is a list of documents approved with this application:

	Document Title	Language	Version	Version Date
1.	Research proposal	English	2.0	April 2019
2.	Research participant Informed Consent Form (ICF) (adult)	English	1.0	December 2018
3.	Research participation ICF (child)	English	1.0	December 2018
4.	Assent form for children aged 8 years and more	English	1.0	December 2018
5.	ICF for in – depth interview	English	2.0	April 2019

Yours sincerely,

[redacted]

Isaac Makhuwa
For: Executive Secretary
UGANDA NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

Copied to: Chair, Mulago Hospital, Research Ethics Committee

LOCATION/CORRESPONDENCE

Plot 6 Kimera Road, Ntinda
P. O. Box 6884
KAMPALA, UGANDA

COMMUNICATION

TEL: (256) 414 705500
FAX: (256) 414-234579
EMAIL: info@uncst.go.ug
WEBSITE: http://www.uncst.go.ug

Appendix 8. London School of Hygiene and Tropical Medicine approval for the cohort study

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
United Kingdom
Switchboard: +44 (0)20 7636 8636

www.lshtm.ac.uk

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

Observational / Interventions Research Ethics Committee

Dr Sarah Staedke
Clinical Senior Lecturer
Department of Clinical Research (CRD)
LSHTM

15 June 2016

Dear Sarah

Study Title: Cohort studies to estimate malaria incidence and morbidity in three different epidemiological settings in Uganda

LSHTM Ethics Ref: '10305 (A429-5943) - 6'

Thank you for your application for the above amendment to the existing ethically approved study and submitting revised documentation. The amendment application has been considered by the Observational Committee.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above amendment to research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval for the amendment having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document Type	File Name	Date	Version
Other	DMID 10-0063 Cohort Study PROTOCOL vers 7.0 5_May_2016_CLEAN	05/05/2016	7.0
Other	DMID 10-0063 Cohort Study PROTOCOL vers 7.0 5_May_2016_trackchanges	05/05/2016	7.0
Other	ICF PRISM Cohort Study PARENTAL Monthly Enhanced Surveillance Sampling Consent Additional up to June 2017_CLEAN	05/05/2016	3.0
Other	ICF PRISM Cohort Study PARENTAL Monthly Enhanced Surveillance Sampling Consent Additional up to June 2017_Trkchg	05/05/2016	3.0
Other	PRISM Cohort Study ADULT Monthly Enhanced Suveillance Sampling Consent additional up to June 2017_CLEAN	05/05/2016	3.0
Other	PRISM Cohort Study ADULT Monthly Enhanced Suveillance Sampling Consent additional up to June 2017_Trkchg	05/05/2016	3.0
Other	Travel form_OVERNIGHT TRAVEL_FINAL	05/05/2016	1.0
Other	PRISM Photography Audio taping and Filming ICF version 5_May_2016	05/05/2016	1.0

After ethical review

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the Committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

At the end of the study, the CI or delegate must notify the committee using an End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: http://leo.lshtm.ac.uk

Additional information is available at: www.lshtm.ac.uk/ethics

Yours sincerely,

[redacted]

Page 1 of 2

[redacted]
Professor John DH Porter
Chair

ethics@lshtm.ac.uk
http://www.lshtm.ac.uk/ethics/

Improving health worldwide

Appendix 9. Makerere University School of Medicine Research and Ethics Committee approval for the cohort study

MAKERERE UNIVERSITY
P.O. Box 7072 Kampala, Uganda
E-mail: rresearch9@gmail.
Phone: 256 414 533541
Fax: 256 414 541036/0414 532204

COLLEGE OF HEALTH SCIENCES
SCHOOL OF MEDICINE

RESEARCH ETHICS COMMITTEE

June 2, 2016

Prof. Moses Kamya
Department of Internal Medicine

Category of review
[ ] Initial review
[ ] Continuing review
[X] Amendment
[ ] Termination of study
[ ] SAEs

Dear Prof. Kamya,

Re: REC REF No. 2011-167

Title: Cohort studies to estimate malaria incidence and morbidity in three different epidemiological settings in Uganda"

Your proposal entitled "**Cohort studies to estimate malaria incidence and morbidity in three different epidemiological settings in Uganda" was** initially reviewed and approved by the School of Medicine Research and Ethics Committee on June 23, 2011.

On April 28th, 2016, you requested for permission to make some changes in the study and informed consent documents: to update the protocol version date and number to Version 6.1 dated March 15, 2016, to add an objective aimed at assessing for the associations between overnight travel in Uganda and the risk of malaria infection, to extend the study participant follow up period for Nagongera cohort to June 30, 2017- this will provide an opportunity for the study team to document the transitioning epidemiology of malaria in this setting and measure the trends over time following intervals of indoor residual spraying, page 16: to include a background section on overnight travel and malaria infection, page 17: to add the rationale for assessing overnight travel and malaria infection, page 19: to add objectives for overnight travel and infection with malaria parasites, page 20, 23 and 30: to clarify that the follow up period for Walukuba and Kihihi cohorts will be up to July 01. 2016, page 26: to update routine blood draw schedule for Nagongera site to reflect finger pricks for the 2 monthly visits done in between visits where phlebotomy is done every 3 months, page 28: to change the 3 monthly clinic visits to monthly routine visits, page 36 and 37: to add a methods section for the overnight travel and malaria infection study and to add the photographic informed consent document.

The committee considered these changes on 2nd June, 2016. On behalf of the committee, I am glad to inform you that these changes have been approved. You may now proceed with the study. But forward regular reports on your study to the committee.

MAKERERE UNIVERSITY
SCHOOL OF MEDICINE
APPROVED
02 JUN 2016
RESEARCH & ETHICS COMMITTEE
P.O.BOX 7072, KAMPALA

Yours sincerely,

[redacted]

Assoc. Prof. Ponsiano Ocama
Chairperson School of Medicine Research & Ethics Committee

Appendix 10. Uganda National Council for Science and Technology approval for the cohort study

Uganda National Council for Science and Technology

(Established by Act of Parliament of the Republic of Uganda)

Our Ref: HS 1019 **29th August 2016**

Dr. Moses Kamya
Principal Investigator
MU-UCSF Malaria Research Collaboration
Mulago Complex
Kampala

Dear Dr. Kamya,

RE: COHORT STUDIES TO ESTIMATE MALARIA INCIDENCE AND MORBIDITY IN THREE DIFFERENT EPIDEMIOLOGICAL SETTINGS IN UGANDA

This is to acknowledge receipt of the notification of amendments in your letter dated **20th June 2016** made to the above protocol. The Uganda National Council for Science and Technology (UNCST) has no objection to the amendments made to the study.

Yours sincerely,

[redacted]

Hellen .N. Opolot
for: Executive Secretary
UGANDA NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

LOCATION/CORRESPONDENCE

Plot 6 Kimera Road, Ntinda
P. O. Box 6884
KAMPALA, UGANDA

COMMUNICATION

TEL: (256) 414 705500
FAX: (256) 414-234579
EMAIL: info@uncst.go.ug
WEBSITE: http://www.uncst.go.ug

Appendix 11. Research participation consent form (adult) for the case-control study in Kampala

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Project Title:	Factors associated with the risk of malaria during recent overnight travel out of Kampala city: a case control study in Kampala, Uganda
Funding Source:	Fogarty International Centers training grant
Site of Research:	Naguru general hospital, Kampala
Principal Investigator:	Emmanuel Arinaitwe, MBChB, MPH
Version number:	2.0
Version date:	29 April 2019

Why is this study being done?

You are being asked to participate in this study because you came for a malaria test at Naguru general hospital. The main purpose for this research is to understand better how people get infected with malaria when they travel out of Kampala. This study is being done by researchers from Infectious Diseases Research Collaboration and London School of Hygiene and Tropical Medicine. The study is sponsored by the Fogarty International Center training grant.

Before you decide if you want to participate, we would like to explain the purpose of this study, how the study will be done, any risks and benefits to you. This is a consent form and

gives information about this study. You are free to ask questions at any time. After this consent form is read to you, and your questions have been answered, we will ask if you want you to be in the study. Medical research includes only people who choose to take part. Take your time to make your decision about participating. You may discuss your decision with your family and friends before making the decision. If you have any questions, you may ask the researcher team. You may ask us to return at any time to consent and enrol for the study. If you agree to participate, we will ask you to sign this consent form. You will get a copy of this form to keep.

How many people will take part in this study?

We plan to select 162 people with a positive test for malaria and 405 people with a negative test for malaria. Both children and adults will be given a chance to participate in this study.

What will happen if I take part in this study?

If you agree to participate in this study, a member of our study team will ask you questions about whether you travelled out of Kampala city and spent at least one night away within the last 2 months or not. If you did travel, we shall be interested in knowing more about the places you travelled to, the dates of your travel, time you went to bed and time you woke up, whether you slept under a bednet or not, and the activities that were involved in before going to bed.

All of the data collected will be kept confidential and will not be shared with anyone other than members of our study team. Participation in this study is voluntary. Whether you decide to participate or not will not affect your medical care received at Naguru general hospital. You can end your participation at any time and we will not ask you additional questions about your travel. However, we hope you will answer all the questions about your recent trip since your participation is very important.

How long will I be in the study?

It will take about 30 minutes to discuss the study and answer questions from the questionnaire. There will be no follow up required unless you have specific questions that you would want to be addressed later.

What side effects or risks can I expect to have from being in the study?

- ***Questionnaires*:** Answering some questions may make you feel uncomfortable. You may refuse to answer any question or stop at any time. This will not result in any penalty or other disadvantage for you or your child at this hospital or any other hospital.
- ***Confidentiality*:** Other people may learn that you are part of this study in case they see us talking to you. However, we will not allow people who are not working for the study to see any information collected from you.

Are there benefits to taking part in the study?

There is no direct benefit to you for participating in the study but the information which we get from this research will help Uganda and other countries to understand the risk of malaria during travel and discuss best way to prevent malaria during travel.

What other choices do I have if I do not take part in this study?

If you decide you don't want to be part of the study or decide to withdraw your participation at any time and for any reason, this will not affect your access to medical care at Naguru general hospital.

Will information about me be kept private?

Information collected from the households will only be accessed by the people working on the study. Only study staff will be able to link the participant data by use of assigned study numbers. The universities and research organizations running this study are not allowed to let others to know the identity of the people in the study. The information from the study linking the data to you will be kept in a limited access, locked office and will only be able to be seen by study team members.

The findings from this study will be shared with Uganda Ministry of Health officials and may be published in a medical journal. We will not identify you by name. After the study is completed, study findings can be accessed by calling Dr Arinaitwe at Infectious Diseases Research Collaboration (IDRC) on 0752-900078.

What are the costs of taking part in this study?

You will not be charged for taking part in the study.

Will I be paid for taking part in this study?

You will not be paid for participation in the study. However, at the end of answering the questionnaire, study participants will be given 10,000 Uganda Shillings as compensation for their time and for transport costs.

What are my rights if I take part in this study?

We will let you know quickly about new information that might change your wish for continued participation in the study.

Who can answer my questions about the study?

Dr. Arinaitwe and his staff can answer questions you have about the study. You may call Dr. Arinaitwe at Infectious Diseases Research Collaboration (IDRC) on 0752-900078. We will give you free access to a telephone line. For questions about your rights as a research participant, you may also contact Dr. Fred Nakwagala on 0772-325869. Dr. Nakwagala is the Chairman of the Mulago Hospital Research and Ethics Committee which approved this study.

CONSENT: WHAT YOUR SIGNATURE OR THUMBPRINT MEANS

A copy of this consent form will be given to you. Your signature or thumbprint below means that you have had this study explained to you. Your signature or thumbprint below means you have had the opportunity to ask questions. If you wish to participate in this study, you should sign or place your thumbprint below.

Name of the study participant (printed)

Signature or Fingerprint of the study participant Date

Name of Study Staff Administering Consent (printed) Position/Title

Signature of Study Staff Administering Consent Date

*If the participant is unable to read and/or write, an impartial witness should be present during the informed consent discussion. After the written informed consent form is read and explained to the participant, and after they have orally consented to their participation in the study and have either signed the consent form or provided their fingerprint, the witness should sign and personally date the consent form. By signing the consent form, the witness

attests that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the participant, and that informed consent was freely given by the patient and parent or guardian.

Name of Person Witnessing Consent (printed)

Signature of Witness Date

Appendix 12. Research participation consent form (child) for the case-control study in Kampala

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Project Title:	Factors associated with the risk of malaria during recent overnight travel out of Kampala city: a case control study in Kampala, Uganda
Funding Source:	Fogarty International Centers training grant
Site of Research:	Naguru general hospital, Kampala
Principal Investigator:	Emmanuel Arinaitwe, MBChB, MPH
Version number:	2.0
Version date:	29 April 2019

To be read aloud to the parent (any age) or guardian age 18 years or older:

Why is this study being done?

Your child is being asked to participate in this study because you brought your child for a malaria test at Naguru general hospital. The main purpose for this study is to help us understand better how people get infected with malaria when they travel out of Kampala and how malaria can be prevented during travel. This study is being done by researchers from Infectious Diseases Research Collaboration and London School of Hygiene and Tropical Medicine. The study is sponsored by the Fogarty International Center training grant.

Before you decide if you want your child to participate, we would like to explain the purpose of this study, how the study will be done, any risks and benefits to you. This is a consent form and gives information about this study. You are free to ask questions at any time. After this consent form is read to you, and your questions have been answered, we will ask if you want you to be in the study. Medical research includes only people who choose to take part. Take your time to make your decision about participating. You may discuss your decision with your family and friends before making the decision. If you have any questions, you may ask the researcher team. You may ask us to return at any time to consent and enrol for the study. If you agree to have your child participate, we will ask you to sign this consent form. You will get a copy of this form to keep.

How many people will take part in this study?

We plan to select 162 people with a positive test for malaria and 405 people with a negative test for malaria. Both children and adults will be given a chance to participate in this study.

What will happen if my child takes part in this study?

If you agree to have your child participate in this study, a member of our study team will ask you questions about whether your child travelled with someone out of Kampala city and spent at least one night away within the last 2 months or not. If your child did travel, we shall be interested in knowing more about the places your child travelled to, the dates of travel, time of going to bed and time of waking up, whether your child slept under a bednet or not during travel, and the activities that your child was involved in before going to bed.

All of the data collected will be kept confidential and will not be shared with anyone other than members of our study team. Participation in this study is voluntary. Whether you decide to have your child participate or not will not affect the medical care your child will be receiving

at Naguru general hospital. You can end your child's participation at any time and we will not ask you additional questions about your child's travel. However, we hope you will answer all the questions about your child's recent trip since your child's participation is very important.

How long will my child be in the study?

It will take about 30 minutes to discuss the study and answer questions from the questionnaire. There will be no follow up required unless you have specific questions that you would want to be addressed later.

What side effects or risks can I expect my child to have from being in the study?

- ***Questionnaires*:** Answering some questions may make you feel uncomfortable. You may refuse to answer any question or stop at any time. This will not result in any penalty or other disadvantage for you or your child at this hospital or any other hospital.
- ***Confidentiality*:** Other people may learn that your child is part of this study in case they see us talking to you. However, we will not allow people who are not working for the study to see any information collected from you.

Are there benefits to taking part in the study?

There is no direct benefit to your child for participating in the study but the information which we get from this research will help Uganda and other countries to understand the risk of malaria during travel and discuss best way to prevent malaria during travel.

What other choices does my child have if I do not allow my child to take part in this study?

If you decide you don't want your child to be part of the study or decide to withdraw your child's participation at any time and for any reason, this will not affect your child's access to medical care at Naguru general hospital.

Will information about my child be kept private?

Information collected from the households will only be accessed by the people working on the study. Only study staff will be able to link the participant data by use of assigned study numbers. The universities and research organizations running this study are not allowed to let others to know the identity of the people in the study. The information from the study linking the data to your child will be kept in a limited access, locked office and will only be able to be seen by study team members.

The findings from this study will be shared with Uganda Ministry of Health officials and may be published in a medical journal. We will not identify your child by name. After the study is completed, study findings can be accessed by calling Dr Arinaitwe at Infectious Diseases Research Collaboration (IDRC) on 0752-900078.

What are the costs of taking part in this study?

You will not be charged for your child's taking part in the study.

Will I be paid for my child's taking part in this study?

You will not be paid for your child's participation in the study. However, at the end of answering of the study questionnaire, you will be given 10,000 Uganda Shillings as compensation for their time and for transport costs.

What are my child's rights if I decide that my child should take part in this study?

We will let you know quickly about new information that might change your wish for your child's continued participation in the study.

Who can answer my questions about the study?

Dr. Arinaitwe and his staff can answer questions you have about the study. You may call Dr. Arinaitwe at Infectious Diseases Research Collaboration (IDRC) on 0752-900078. We will give you free access to a telephone line. For questions about your rights as a research participant, you may also contact Dr. Fred Nakwagala on 0772-325869. Dr. Nakwagala is the Chairman of the Mulago Hospital Research and Ethics Committee which approved this study.

CONSENT: WHAT YOUR SIGNATURE OR THUMBPRINT MEANS

A copy of this consent form will be given to you. Your signature or thumbprint below means that you have had this study explained to you. Your signature or thumbprint below means you have had the opportunity to ask questions. If you wish your child to participate in this study, you should sign or place your thumbprint below. All children 8 years or older will be asked to give separate assent to be in the study.

Name of Participant (printed)

Name of Parent/Guardian (if study participant is a child)

Signature or Fingerprint of Parent/Guardian Date

Name of Study Staff Administering Consent (printed) Position/Title

Signature of Study Staff Administering Consent Date

*If the parent/guardian is unable to read and/or write, an impartial witness should be present during the informed consent discussion. After the written informed consent form is read and explained to the parent/guardian, and after they have orally consented to their child's participation in the study and have either signed the consent form or provided their fingerprint, the witness should sign and personally date the consent form. By signing the consent form, the witness attests that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the parent/guardian, and that informed consent was freely given by the patient and parent/guardian.

Name of Person Witnessing Consent (printed)

Signature of Witness Date

Appendix 13. Assent form for minors aged 8 years to 17 years for the case-control study

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Project Title:	Factors associated with the risk of malaria during recent overnight travel out of Kampala city: a case control study in Kampala, Uganda
Funding Source:	Fogarty International Centers training grant
Site of Research:	Naguru general hospital, Kampala
Principal Investigator:	Emmanuel Arinaitwe, MBChB, MPH
Version number:	2.0
Version date:	15 April 2019

A. Why have we met you?

- We want to tell you about something we are doing called a research study. A research study is when doctors collect a lot of information to learn more about something. After we tell you about it, we will ask if you want to be in this research study or not.
- Your parent/guardian has said yes to your being in this study. That is why we want to talk with you more.

B. Why are the doctors doing this study?

- Dr Emmanuel Arinaitwe and others want to know if you get malaria when you travel and spend a night out of Kampala. We also want to know if you are

likely to get malaria when you travel to some areas compared to other areas in Uganda.

- We are gathering this information from children and old people. After we have asked you some questions, we shall let you know whether you can be part of this study or not.

C. What is expected of you if you are involved in this study?

We shall ask you a few questions if you say yes to being in this study. It will help us to know if you visited any place outside Kampala. If you did, we shall write down the place you visited and the dates of your visit. We shall also ask you about the time you went to bed during your visit and the time you woke up. She shall discuss anything else that can make you get malaria during your visit.

D. What will you gain from this research?

There will be no direct gains. Answers to the questions from this study will help us know better if people can get malaria while visiting places out of Kampala or not. This will help us in finding ways to stop malaria during travel.

E. Can this research cause harm?

Sometimes some questions we ask you may make you uncomfortable, but we shall make sure this does not happen many times.

F. How long will you be in this study?

The doctors will take about 30 minutes talking with you about this study and asking you about the places you visited outside Kampala city.

G. Do you have any questions?

You may ask questions any time either now or later. You can talk to me or somebody else.

H. Must you be in this research?

- No. No one will force you into this research study. Do not feel ashamed to tell us. Remember you may accept now and later refuse; however, it is your own choice if you are not interested in this study or if you change your mind later. Let us know whether you have accepted or not.
- The study staff will give you a copy of this agreement

I. Will I be paid to be in this study?

You will not be paid. We know that you have spent time answering some questions and we shall give you 10,000 shilling to cover your transport back home.

Consent to Join the Research

I have explained the study to _____ (*print name of child here*) in language he/she can understand, and the child has agreed to be in the study.

_____

Signature or Fingerprint of Participant Date

_____

Signature of Person Conducting Assent Discussion Date

_____

Name of Person Conducting Assent Discussion (*print*)

Appendix 14. Research participation consent form (Adult) for the cohort study

Project Title:	Cohort studies to estimate malaria incidence and morbidity in three different epidemiological settings in Uganda
Funding Source:	National Institute of Allergy and Infectious Diseases (NIAID)
Site of Research:	Nagongera sub-county, Tororo
Principal Investigator:	Moses Kamya, MBChB, MMed, PhD; Grant Dorsey, MD, PhD
Version Date:	3.0, 22 March 2016

This is a request to allow your child to participate in research to exam how your child's body fights malaria illness. The researchers from Makerere University (MU), the University of California, San Francisco (UCSF), the London School of Hygiene and Tropical Medicine (LSHTM), and Uganda Malaria Surveillance Project (UMSP) will explain this research to you. Medical research includes only people who choose to take part. Take your time to make your decision about participating. You may discuss your decision with your family and friends and with your health care team. If you have any questions, you may ask the researchers.

What will be done if I agree for my child to participate?

If you agree and your child is found eligible to participate, you will be asked to bring your child to the clinic one time every month for up to June 30, 2017. When you bring your child for this visit, study staff will collect about 5-10 mls (1-2 teaspoons, for children less than 5 years of age) or 10-15 mls (2-3 teaspoons, for children 5-10 years of age) of blood from your child's arm every 3 months, and a small amount of blood from your child's finger every 2 months in between 3 monthly blood collections. This blood draw should take no more than 30 minutes

at each visit. You will not be told the results from the laboratory research. After all tests are done, instead of discarding your leftover specimens we will save them in what is called a "specimen bank" for possible future research if you have previously agreed on a separate consent for your child's specimens to be stored. If you agree, we shall also ask your child to carry a small gadget (smaller than a mobile phone) to help us know where your child goes, and how much risk this mobility adds to your child's infection with malaria.

Are there risks?

The needle stick may hurt. There is a small risk of bruising, and a rare risk of infection.

Are there benefits?

There is no benefit to your child. The blood will be used only for research. However, we hope we will learn something that will contribute to the advancement of science and understanding of health and disease.

Will my child be paid for taking part in this study?

Study participants will not be paid for participation in the study. However, study participants will be given 10,000 Uganda Shillings as compensation for your time and transport costs for each visit.

What financial issues should I consider before donating my child's specimens?

You will not be charged for donating your child's specimens. You will not be paid for donating your child's specimens. If the data or any new products, tests or discoveries that result from this research have potential commercial value, you will not share in any financial benefits.

What are my child's rights if he/she take part in this study?

Taking part in this study is your choice. You may choose either to have your child take part or not to take part in the study. No matter what decision you make, there will be no penalty to

you or your child and you or your child will not lose any of your regular benefits. Your child can still get medical care from our institution. In the case of injury resulting from this study, your child does not lose any legal rights to seek payment by signing this form.

Who can answer my questions about the study?

Dr. Kamya and staff are available to explain this study to you and answer your questions. If you have any other questions about the information here, you may call Dr. Kamya (telephone 0414-541188 or 0414-533200) at Mulago Hospital. You may also contact Prof. James Tumwine (0414-533541) at the Makerere University Research and Ethics Committee which approved this study for questions about participants' rights and research-related harm.

FREEDOM TO REFUSE

You are free to change your mind about allowing use of your samples in future research at any time. If you change your mind, contact Dr. Moses Kamya (telephone 0414-541188 or 0414-533200).

WHAT YOUR SIGNATURE OR THUMBPRINT MEANS

A copy of this consent form will be given to you. Your signature or thumbprint below means that use of your child's specimens for future research has been explained to you. It also means that you have had the opportunity to ask questions. You have also had time to think about if you want your child to be in the study or not. If you wish to allow your child's blood samples to be used for future research, you should sign or thumbprint below.

Name of child (printed)

Name of Parent/guardian (printed)

Signature or Fingerprint * of Parent/guardian Date

Name of Study Staff Administering Consent (printed) Position/Title

Signature of Study Staff Administering Consent Date

Name of Translator

Signature of Translator Date

*If the parent or guardian is unable to read and/or write, an impartial witness should be present during the informed consent discussion. After the written informed consent form is read and explained to the participant and parent or guardian, and after they have orally consented to allow their child's specimens to be used for future laboratory testing and have either signed the consent form or provided their fingerprint, the witness should sign and personally date the consent form. By signing the consent form, the witness attests that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the patient, parent or guardian, and that informed consent was freely given by the patient, parent or guardian.

Name of Person Witnessing Consent (printed)

Signature of Person Witnessing Consent Date

Appendix 15. Research participation consent form (parent/guardian) for the cohort study

Project Title:	Cohort studies to estimate malaria incidence and morbidity in three different epidemiological settings in Uganda
Funding Source:	National Institute of Allergy and Infectious Diseases (NIAID)
Site of Research:	Nagongera sub-county, Tororo
Principal Investigator:	Moses Kamya, MBChB, MMed, PhD; Grant Dorsey, MD, PhD
Version Date:	3.0, 22 March 2016

This is a request to allow your child to participate in research to exam how your child's body fights malaria illness. The researchers from Makerere University (MU), the University of California, San Francisco (UCSF), the London School of Hygiene and Tropical Medicine (LSHTM), and Uganda Malaria Surveillance Project (UMSP) will explain this research to you. Medical research includes only people who choose to take part. Take your time to make your decision about participating. You may discuss your decision with your family and friends and with your health care team. If you have any questions, you may ask the researchers.

What will be done if I agree for my child to participate?

If you agree and your child is found eligible to participate, you will be asked to bring your child to the clinic one time every month for up to June 30, 2017. When you bring your child for this visit, study staff will collect about 5-10 mls (1-2 teaspoons, for children less than 5 years of age) or 10-15 mls (2-3 teaspoons, for children 5-10 years of age) of blood from your child's arm every 3 months, and a small amount of blood from your child's finger every 2 months in between 3 monthly blood collections. This blood draw should take no more than 30 minutes at each visit. You will not be told the results from the laboratory research. After all tests are

done, instead of discarding your leftover specimens we will save them in what is called a "specimen bank" for possible future research if you have previously agreed on a separate consent for your child's specimens to be stored. If you agree, we shall also ask your child to carry a small gadget (smaller than a mobile phone) to help us know where your child goes, and how much risk this mobility adds to your child's infection with malaria.

Are there risks?

The needle stick may hurt. There is a small risk of bruising, and a rare risk of infection.

Are there benefits?

There is no benefit to your child. The blood will be used only for research. However, we hope we will learn something that will contribute to the advancement of science and understanding of health and disease.

Will my child be paid for taking part in this study?

Study participants will not be paid for participation in the study. However, study participants will be given 10,000 Uganda Shillings as compensation for your time and transport costs for each visit.

What financial issues should I consider before donating my child's specimens?

You will not be charged for donating your child's specimens. You will not be paid for donating your child's specimens. If the data or any new products, tests or discoveries that result from this research have potential commercial value, you will not share in any financial benefits.

What are my child's rights if he/she take part in this study?

Taking part in this study is your choice. You may choose either to have your child take part or not to take part in the study. No matter what decision you make, there will be no penalty to you or your child and you or your child will not lose any of your regular benefits. Your child

can still get medical care from our institution. In the case of injury resulting from this study, your child does not lose any legal rights to seek payment by signing this form.

Who can answer my questions about the study?

Dr. Kamya and staff are available to explain this study to you and answer your questions. If you have any other questions about the information here, you may call Dr. Kamya (telephone 0414-541188 or 0414-533200) at Mulago Hospital. You may also contact Prof. James Tumwine (0414-533541) at the Makerere University Research and Ethics Committee which approved this study for questions about participants' rights and research-related harm.

FREEDOM TO REFUSE

You are free to change your mind about allowing use of your samples in future research at any time. If you change your mind, contact Dr. Moses Kamya (telephone 0414-541188 or 0414-533200).

WHAT YOUR SIGNATURE OR THUMBPRINT MEANS

A copy of this consent form will be given to you. Your signature or thumbprint below means that use of your child's specimens for future research has been explained to you. It also means that you have had the opportunity to ask questions. You have also had time to think about if you want your child to be in the study or not. If you wish to allow your child's blood samples to be used for future research, you should sign or thumbprint below.

Name of child (printed)

Name of Parent/guardian (printed)

Signature or Fingerprint * of Parent/guardian Date

Name of Study Staff Administering Consent (printed) Position/Title

Signature of Study Staff Administering Consent Date

Name of Translator

Signature of Translator Date

*If the parent or guardian is unable to read and/or write, an impartial witness should be present during the informed consent discussion. After the written informed consent form is read and explained to the participant and parent or guardian, and after they have orally consented to allow their child's specimens to be used for future laboratory testing and have either signed the consent form or provided their fingerprint, the witness should sign and personally date the consent form. By signing the consent form, the witness attests that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the patient, parent or guardian, and that informed consent was freely given by the patient, parent or guardian.

Name of Person Witnessing Consent (printed)

Signature of Person Witnessing Consent Date

Appendix 16. Research participation assent form for the cohort study

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Project Title:	Cohort studies to estimate malaria incidence and morbidity in three different epidemiological settings in Uganda
Funding Source:	National Institute of Allergy and Infectious Diseases (NIAID)
Site of Research:	Nagongera sub-county, Tororo Walukuba sub-county, Jinja Kihihi sub-county, Kanungu
Principal Investigator:	Moses Kamya, MBChB, MMed, MPH; Grant Dorsey, MD, PhD
Version Date:	2.0, May 10, 2013

J. Why have we met you?

- We want to tell you about something we are doing called a research study. A research study is when doctors collect a lot of information to learn more about something. After we tell you about it, we will ask if you'd like to be in this research study or not.

K. Why are the doctors doing this research?

- Dr. Moses Kamya and others want to know how many times children get sick with malaria. We also want to know if malaria causes bad results like low blood levels among children like you.

- We are following and gathering this information from boys and girls of your age for two years. After we have asked you some questions, we shall let you know whether you can be a part of this research or not.

L. What is expected of you if you are involved in this research?

Only if you have accepted to be included in this research study will the following happen to you:

1. At the beginning of the research, we shall ask you a few questions on how you are feeling.
2. We shall also take off some blood from your arm to be placed in a small container. This blood will be used to estimate your blood counts and also carry out some other tests which may be useful to you or help us understand how malaria affects our blood count levels.
3. After you have been included in the study, we shall request you to come to our clinic every 3 months even if you are not sick. This is because we want to know how you are and also ask you a few questions about your health. At this visit, we will take a small drop of blood from your finger and your arm to monitor the progress of your blood count level and also carry out some other tests
4. You will be asked to come to the clinic whenever you are not feeling well. We shall take off some blood from your finger to look for malaria parasites and also check your blood counts if the clinician thinks you could be having malaria.
5. If you have malaria, you will be asked to swallow some pills. If you are very sick, you will be admitted to hospital and you may be given medication through a drip attached to your arm.

M. What will you gain from this research?

- You will receive free medical attention from the clinic for all you illnesses. The outcome of this research is that children will get assistance from other countries, including Uganda, in knowing better how much malaria is in your area.

N. Can this research cause harm?

- You will feel pain when the sample of blood is being removed from your finger or arm, but it will subside quickly. Sometimes some questions we ask you may make you uncomfortable but we shall make sure this does not happen many times.

O. How long will you be in this study?

- For two to four years or when you reach 11 years old or when the doctors or your parents or guardians decide you should leave the study, whatever occurs first. The doctors may write a referral letter for you to continue with the same treatment if it is needed.

P. Do you have any questions?

- You may ask questions any time either now or later. You can talk to me or somebody else.

Q. Must you be in this research?

- No. No one will force you into this research study. Do not feel ashamed to tell us. Remember you may accept now and later on refuse, however it is your own choice if you are not interested in this study or if you change your mind later. Let us know whether you have accepted or not.
- The research staff will give you a copy of this agreement

<u>Consent to Join the Research</u>

I have explained the study to _____ (*print name of child here*) in language he/she can understand, and the child has agreed to be in the study.

Signature or Fingerprint of Participant Date

_____

____

Signature of Person Conducting Assent Discussion Date

_____

Name of Person Conducting Assent Discussion (*print*)

Appendix 17. WHO guidelines for malaria treatment[164]

Treating uncomplicated *P. falciparum* malaria

Treatment of uncomplicated P. falciparum malaria

Treat children and adults with uncomplicated *P. falciparum* malaria (except pregnant women in their first trimester) with one of the following recommended ACTs:

- artemether + lumefantrine
- artesunate + amodiaquine
- artesunate + mefloquine
- dihydroartemisinin + piperaquine
- artesunate + sulfadoxine–pyrimethamine (SP).

Strong recommendation, high-quality evidence

Duration of ACT treatment

ACT regimens should provide 3 days' treatment with an artemisinin derivative.
Strong recommendation, high-quality evidence

Revised dose recommendation for dihydroartemisinin + piperaquine in young children

Children weighing <25kg treated with dihydroartemisinin + piperaquine should receive a minimum of 2.5 mg/kg bw per day of dihydroartemisinin and 20 mg/kg bw per day of piperaquine daily for 3 days.
Strong recommendation based on pharmacokinetic modelling