

**Evaluating multi-level risk factors for malaria and arboviral infections in
regions of Tanzania**

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Abstract

Vector-borne diseases, such as those transmitted by mosquitoes, pose a significant public health concern in many countries worldwide. In this thesis, I explored the role of a number of risk factors defined at multiple scales on vector-borne disease prevalence, focusing on malaria and arboviral infections in several regions of North-Eastern Tanzania, with the principal aim of improving the overall diagnosis of febrile illness in this region.

First, I investigated the influence of household-wealth on prevalence of malaria and arboviral infections using principal component analysis (PCA), and then described the methodological challenges associated with this statistical technique when used to compute indices from smaller datasets. I then employed a multilevel modelling approach to simultaneously incorporate household-level anthropogenic factors and village-level environmental characteristics to investigate key determinants of *Anopheles* malaria vector density among rural households.

These analyses provided methodologically rigorous approaches to studying vector-borne diseases at a very fine-scale and also have significant public health relevance as the research findings can assist in guiding policy decisions regarding surveillance efforts as well as inform where and when to prioritize interventions.

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List of Abbreviations

CDC – United States Centers for Disease Control and Prevention

DHS – Demographic Health Survey

DHS – Demographic Health Survey

DTR – Diurnal Temperature Range

EIR – Entomological Inoculation Rate

ENM – Ecological Niche Model

EVI – Enhanced Vegetation Index

GIS – Geographic Information System

IRS – Indoor Residual Spraying

IVM – Integrated Vector Management

LLINs – Long-lasting Insecticidal Nets

LSMS – Living Standards Measurement Survey

OR – Odds Ratio

PCA – Principal Component Analysis

PCR – Polymerase Chain Reaction

RDT – Rapid Diagnostic Test

RR – Rate Ratio

SD = Standard Deviation

SDM – Species Distribution Model

SEP – Socioeconomic Position

SES – Socioeconomic Status

VBD – Vector-borne Disease

WHO – World Health Organization

General Introduction

The World Health Organization estimates that vector-borne diseases (VBDs) account for 17% of the global disease burden of all infectious diseases with approximately 50% of the world's population currently residing in regions where these diseases are highly endemic (Guzman & Istúriz, 2010; WHO, 2014a). VBDs pose major public health challenges in many parts of the world and are receiving renewed attention due to their expanding ranges and territories - fostered by changes in demographic patterns, socioeconomic infrastructure and the environment (Chipwaza et al., 2014; Gubler, 2002; Khan et al., 2014; Ogden, Lindsay, & Coulthart, 2015; Powers, 2015). Despite increasing global health and economic impacts resulting from VBD burden, these diseases remain extremely complex in their relationship with human populations, the changing environment, and the abundance and distribution of their vector species.

Continued investigation of human-vector-environment interactions is therefore critical for contemporary disease management efforts aimed towards refined predictive modeling tools and optimized, spatially-targeted control strategies. In particular, understanding these interactions is of critical importance because ecologically- and demographically-driven vector ecology may differ between local, regional, and global extents.

1.2 Mosquito-Borne Diseases & Public Health

Ninety percent (90%) of the global VBD burden is the result of mosquito-transmitted agents (McGraw & O'Neill, 2013). *Aedes aegypti*, *Aedes albopictus*, and *Anopheles* spp. mosquitoes are highly competent vectors that are responsible for the transmission of the most impactful VBDs such as malaria, and the arboviruses dengue and chikungunya. These infections not only affect

populations in tropical and sub-tropical regions, but their recent emergence in non-endemic parts of the world has created wider global public health and economic risks.

Malaria, caused by several species of the *Plasmodium* parasite, including *P. falciparum* and *P. vivax*, is considered to be deadliest VBD, causing tremendous morbidity and mortality to half of the world's population. Approximately 3.4 billion people in 106 countries and territories are considered to be at risk globally (WHO, 2014b), with the African continent bearing the brunt of the disease burden. In 2015, there were 214 million cases of malaria reported with 438,000 attributed deaths – 306,000 of these occurring among children under the age of 5 (WHO, 2015a). However, with global efforts to scale-up effective malaria control interventions in the last decade, reductions in malaria burden have been achieved. In Tanzania, East Africa, a highly malaria-endemic country, there is strong evidence that supports an overall decline in malaria prevalence. According to the 2011-12 HIV/AIDS and Malaria Indicator Survey, 9% of children under the age of 5 tested positive for malaria, with prevalence increasing with age (Tanzania Commission for AIDS et al., 2013). This reflects a 50% decrease in malaria burden since the previous survey in 2007-2008 (National Bureau of Statistics, 2008).

The observed decline is further supported by findings from local studies conducted throughout the country (Mboera, Mazigo, Rumisha, & Kramer, 2013). In the Korogwe district of north-eastern Tanzania, prevalence of malaria parasitaemia in the lowlands decreased from 78.4% in 2003 to 13.0% in 2008, and in the highlands from 24.7% to 3.1% (Mmbando et al., 2010). Similarly, in Muheza, a significant decline of *P. falciparum* transmission was documented during the period of 1992 – 2012 (Ishengoma et al., 2013). In another study in the Muleba district, an overall decline in malaria prevalence from 20.1% in 2007 to 6.6% in 2008 was reported (Mashauri et al., 2013). The number of deaths due to malaria has also significantly

decreased over the last decade as supported by findings from a recent systematic analysis of global malaria mortality (Murray et al., 2012).

Several arboviruses, however, have received increasing public health attention in recent years due to the global expansion of their *Aedes* mosquito vectors. Arboviruses are an important class of emerging infectious diseases that consist of viral infections commonly transmitted by arthropods, including: mosquitoes, ticks and sandflies. These infections are often asymptomatic, or can result in nonspecific flu-like symptoms, or range from a self-limiting febrile illness to more severe neurological sequelae including life-threatening encephalitis and hemorrhagic fevers (Gubler, 2002) - which can cause long-term physical and cognitive impairments or early death.

Dengue causes an estimated 50 -100 million cases annually in 128 countries (Brady et al., 2014; WHO, 2015b) and is often considered the most clinically important arboviral threat due to the reported 30-fold increase over the last decade (WHO, 2012). Previous studies have even suggested that the true burden of dengue is more than three times of that estimated by the WHO (Bhatt et al., 2013). Outbreaks of dengue have been reported in 2010 and 2012-2014 in Dar es Salaam, Tanzania, where prior to 2010, little was known about this virus in the country (Mboera et al., 2016). In one study, researchers reported the incidence of dengue in Tanzania to be much higher compared to Kenya and Angola (Vairo et al., 2016).

Re-emergence and introduction of the chikungunya virus into new regions have also led to devastating epidemics in several parts of the world including South Asia and the Indian Ocean (Gérardin et al., 2008; Thiberville et al., 2013), Italy (Rezza et al., 2007) and very recently to the Caribbean and the Americas where approximately 1.2 million suspected cases were reported to the Pan American Health Organization (CDC, 2014). Crump *et al.*, (2013) previously reported

that chikungunya was a highly prevalent yet frequently ignored cause of febrile illness in northern Tanzania compared to malaria - which was the clinical diagnosis of 61% of febrile cases, but the actual cause of only 1.6% of fevers (Crump et al., 2013). Very recently, Kajeguka *et al.*, (2016) reported a chikungunya sero-prevalence of 12.9% among febrile patients in north-eastern Tanzania– a statistic that is two times greater than previous reports (Kajeguka et al., 2016).

In Tanzania, arboviruses affect a significant proportion of the population, as such, further investigations into methods to improve surveillance and identify individuals most at risk is in the best interest of the public health. With the declining burden of malaria, the contribution of these arboviruses to febrile illness is a current concern with implications for proper diagnosis and case management. Fever misdiagnosis can have severe consequences for patients since the underlying cause of the fever will be left untreated and undiagnosed resulting in poor patient outcomes such as high case-fatality rates (Reyburn et al., 2004). Additionally, indiscriminate use of anti-malarial treatment due to inconsistencies in malarial diagnosis can facilitate resistance among parasites, thereby compromising the sustainability of current treatments (Reyburn et al., 2007), and adding pressure to already constrained resources in some regions (Mwanziva et al., 2011; Reyburn et al., 2004). Currently, artemisinin combination therapies (ACT) are highly effective in East Africa, but the utility of these drugs are of limited merit if misdiagnosis remains a prominent issue (Reyburn et al., 2007; Wiseman, Kim, Mutabingwa, & Whitty, 2006).

With respect to malaria, though significant reductions in disease burden has been observed throughout the country, complete malaria elimination may not be imminent due to the complex and multidimensional nature of this disease. Mboera *et al.*, (2013) outlines some of the major challenges impeding malaria elimination in this region alluding to how these issues directly and

indirectly affect humans, vectors, parasites and health systems (Mboera et al., 2013). Briefly, loss of protective immunity, patient-provider compliance with interventions and misuse of interventions, livelihood factors contributing to malaria transmission, mosquito resistance and behavioural changes, issues with health-care delivery, financial restraints, and presence of weak surveillance and evaluation systems were key issues outlined in their review (Mboera et al., 2013).

The need for more focused interventions to address the changing dynamics of malaria as well as improved surveillance efforts can result in further decline and potentially eliminate disease transmission. These interventions can only be effectively informed from sound studies that evaluate the key determinants of disease risk.

1.3 Critical Review of Risk Factors for Vector-borne Diseases in Tanzania

Identifying risk factors for malaria and arboviral illnesses is frequently prioritized among researchers in order to improve current control strategies and properly target the most disadvantaged populations. Owing to the dynamic nature of these VBDs, a comprehensive understanding of the risk factors that significantly influence disease rates and vector populations can be an instrumental step in devising more focused and sustainable control practices to tackle changing disease complexities.

A literature review of risk factors commonly explored among researchers for modelling malaria and arboviral vectors was performed and a summary of the key variables are described below. For the purpose of this review, risk factors were categorized into: (1) Individual-level risk factors and (2) Environmental risk factors.

1.3.1 Individual-level risk factors

There are a number of individual-and household-level risk factors associated with both malaria and arbovirus burden that are frequently investigated among epidemiologists with the overall aim of defining the most at-risk populations.

The majority of malaria associated mortality occurs among children under the age of 5, particularly in high transmission regions (World Health Organization, 2007). Recent studies, however, have highlighted an increase in the median age of children affected with malaria attributed to declines in transmission intensity (Haque et al., 2011; Kateera et al., 2015; Mosha, Sturrock, Greenwood, et al., 2014; Mtove et al., 2011; Rulisa et al., 2013; Winskill, Rowland, Mtove, Malima, & Kirby, 2011). Similar associations have also been reported between age and dengue risk. In one study investigating dengue sero-prevalence in Venezuela, researchers found that individuals 16 – 20 years old were more likely to be diagnosed with recent dengue infection compared to children under the age of 10 (Velasco-Salas et al., 2014). Consistent findings have also been reported among studies in Kenya (Mease et al., 2011), Tanzania (Vairo et al., 2016) and Zambia (Mazaba-Liwewe et al., 2014). Age has also been documented as an independent risk factor for chikungunya prevalence in some regions (Thiberville et al., 2013; Yusoff et al., 2013).

The gender and health information sheet published by the WHO suggests that both men and women are equally vulnerable to malaria infection, but pregnant women and individuals infected with HIV/AIDS are reported to be at an increased risk due to suppressed immune systems (World Health Organization, 2007). Local studies, however, have demonstrated differences in odds of infection between sexes. Among studies across Tanzania (Mosha, Sturrock, Brown, et al., 2014; Winskill et al., 2011), Senegal (Mosha, Sturrock, Brown, et al., 2014) and Bangladesh (Haque et

al., 2011), the odds of malaria infection was found to be lower among females compared to males. Similarly, for dengue and chikungunya, relationships between risk of infection and sex are inconsistent. Chipwaza *et al.*, (2014), Mazaba-Liwewe *et al.*, (2014) and Mease *et al.*, (2011) (Chipwaza *et al.*, 2014; Mazaba-Liwewe *et al.*, 2014; Mease *et al.*, 2011) among others, have reported insignificant statistical associations between sex and risk of either arboviral infection, while other researchers have reported otherwise (Thiberville *et al.*, 2013; Velasco-Salas *et al.*, 2014).

In Tanzania, a shift in the primary Anopheline vector species may potentially explain some of these inconsistent findings with regards to malaria. Current investigations suggest that *An. arabiensis* are now the dominant malaria vectors in regions where *An. gambiae sensu stricto* previously persisted (Derua *et al.*, 2012). Additionally, widespread use of insecticide treated bed nets and indoor residual spraying have been effective for deterring *An. gambiae* and *An. funestus* compared to *An. arabiensis* (Bayoh *et al.*, 2010; Russell *et al.*, 2011) – due to the exophilic nature of the latter species. This change in primary vector composition has significant implications for public health, as a different demographic are more likely to be affected than previously hypothesized – and current control methods may not be as successful in hindering disease occurrences.

Socioeconomic status (SES) is also frequently studied among researchers and accounted for in models due its potential role in modifying a number of disease relationships. The association between SES and malaria risk is well established, with individuals or households classified in lower socio-economic groups associated with a higher risk of infection (Marcia Caldas de Castro & Fisher, 2012; Gallup & Sachs, 2001; Ricci, 2012; Somi *et al.*, 2007; Teklehaimanot & Mejia, 2008; Yusuf, Adeoye, Oladepo, Peters, & Bishai, 2010). This is a result of the multiple pathways

that exist in which SES can influence disease risk such as: (1) the influence of SES on access to protective measures, with the argument that households with more resources are more likely to afford, comply and use the correct malaria prevention methods, such as bed nets and suitable malaria prophylaxis; (2) direct and indirect effects of SES on risk of infection such as ownership of a number of durable assets, household characteristics and crowding; and (3) the role of SES in access to accurate and prompt diagnosis, as well as appropriate malaria treatment (Dickinson, Randell, Kramer, & Shayo, 2012).

In arbovirus research, however, these relationships are less explicit. In a sero-prevalence study in Kenya, researchers were unable to find any significant associations between SES and risk of either dengue and chikungunya (Ochieng et al., 2015). Additionally, a recent systematic review by Mulligan *et al* (2014) also failed to conclude the presence of a significant association between poverty and dengue, mainly due to the inconsistent definition of poverty used among included studies (Mulligan, Dixon, Joanna Sinn, & Elliott, 2014).

SES also influences the structural quality of households, which is observed to be a key factor influencing both malaria and arbovirus risk. Lower SES households are often poorly constructed, for example, with perishable materials for roofs, walls and floors, or have holes and gaps in the walls, and have unscreened windows or doors that may facilitate an increased entry of vectors into homes to feed on humans. A plethora of studies have investigated the relationships between several of these structural features and risk of malaria (Atieli, Menya, Githeko, & Scott, 2009; Deressa, Ali, & Berhane, 2007; Haque et al., 2011; Keating, Macintyre, Mbogo, Githure, & Beier, 2009; Kirby et al., 2008; Lwetoijera et al., 2013; Sharma et al., 2015; Temu, Coleman, Abilio, & Kleinschmidt, 2012; Wanzirah et al., 2015; Yamamoto, Louis, Si, & Sauerborn, 2010;

Yé et al., 2006) and arboviruses (Mazaba-Liwewe et al., 2014; Mease et al., 2011; Ogoma et al., 2010; Velasco-Salas et al., 2014).

Local-scale environmental characteristics and features of the built-environment such as the water storage around the household (Mboera et al., 2016; Thomas et al., 2016; Tsuzuki, Duoc, Higa, Yen, & Takagi, 2009), factors related to household-sanitation (Castro et al., 2010; De Silva & Marshall, 2012; Nakkhara, Chongsuvivatwong, & Thammapalo, 2013), water supply and other infrastructural characteristics (Braga, Feitosa, Mariaturchi, & De, 2013; Velasco-Salas et al., 2014), livestock ownership (Deressa et al., 2007; Graves et al., 2009; Mayagaya et al., 2015) and presence of road ditches, tire tracks and animal foot-prints (Dutta et al., 2010; Imbahale et al., 2011; Nixon et al., 2014) can also influence risk of malaria and arbovirus infection through the creation of suitable habitats for their vectors to thrive.

Environmental risk factors

Climate variables such as: temperature and rainfall; environmental factors including land-cover, elevation and vegetation, and demographic factors such as urbanization, population density and land-use, are significant ecological variables that contribute to the spread of malaria and arboviral vectors. These factors all influence different aspects of a vector's ecology, and therefore have a critical impact on the parameters that define vector capacity including: vector density, survivorship, biting rate, extrinsic incubation period and vector competence (LaDeau, Allan, Leisnham, & Levy, 2015). Vector capacity is a measure of the efficiency of VBD transmission, and therefore important when considering human risk of disease. A summary of some of these critical environmental variables and their influence on disease transmission are discussed below.

I. Temperature

Temperature plays a significant role in the macro-spatial distribution of mosquito vectors and subsequent spatial and temporal heterogeneity of disease burden (Walker et al., 2013). Malaria prevalence depends significantly on the entomological inoculation rate (EIR) – defined as the average number of infectious mosquito bites per person per unit time, which is the product of the human biting rate (number of bites per person per day by vector mosquito), and the fraction of infectious mosquito vectors (sporozoite rate) (Kelly-Hope & McKenzie, 2009). These factors are inherently dependent on temperature, as low temperatures inhibit mosquito development at the larval stage – thereby influencing adult survivorship, while extremely high temperatures can influence the size and abundance of larval habitats (Walker et al., 2013), as well as contribute to significant mortality (Lyons, Coetzee, & Chown, 2013). Plasmodium development is also highly dependent on temperature both within the host and mosquito, with temperatures ranging from 16-32°C important for completing the extrinsic cycle (Eling, Hooghof, van de Vegte-Bollmer, Sauerwein, & van Gemert, 2001).

The thermal limits and effects of temperature on various life cycle stages for different *Anopheles* species have been described by previous authors (Christiansen-Jucht, Parham, Saddler, Koella, & Basáñez, 2014; Lyons et al., 2013; Lyons, Coetzee, Terblanche, & Chown, 2012). Briefly, environmental temperatures affect both the juvenile and adult stages of *An. gambiae* mosquitoes, resulting in major implications with respect to population dynamics and disease transmission (Christiansen-Jucht et al., 2014). Additionally, the larvae of both *An. arabiensis* and *An. funestus* are observed to survive higher temperatures compared to those of *An. gambiae*, owing to a higher upper thermal limit of the former vectors (Lyons et al.,

2012). This finding highlights significant physiological differences among *Anopheles* species – a factor that must be taken into account when assessing the responses to climate change.

Temperature also significantly influences arbovirus transmission and the distribution of *Aedes* mosquito vectors. The most widely studied and understood mechanisms of temperature on *Aedes* vectors are: the effects of female mosquito survivorship and the extrinsic incubation period (Brady et al., 2014) – defined as the incubation period between the time a mosquito ingests and infected blood meal and the time when that mosquito becomes infectious. Previous authors have demonstrated that increasing temperature can reduce EIP resulting in increased mosquito mortality (Rohani, Wong, Zamre, Lee, & Zurainee, 2009). More recent studies have demonstrated that diurnal temperature range (DTR) - defined as the difference between the daily maximum and minimum temperature - affects *Aedes* infection and transmission potential (Lambrechts et al., 2011), and the combined effect of temperature and DTR can influence vectorial capacity and dengue epidemic potential (Liu-Helmersson, Stenlund, Wilder-Smith, & Rocklöv, 2014). Key findings from a study by Liu – Helmersson *et al* (2014) indicate that in tropical regions with mean temperatures close to 29°C, a small DTR increases the dengue epidemic potential and a large DTR reduces it, while in cold to temperate or extremely hot climates, with mean temperatures far from 29°C, increasing DTR increases the dengue epidemic potential and the larger the DTR, the greater the dengue epidemic potential (Liu-Helmersson et al., 2014). Temperature also influences feeding behaviours of *Aedes* vectors, where temperatures < 15°C and > 36 °C have been documented to limit feeding activity among *Ae. aegypti* mosquitoes (Morin, Comrie, & Ernst, 2013) - a factor which directly affects the ability of these mosquitoes to reproduce resulting in significant implications with respect to population dynamics and distribution ranges.

II. Precipitation

Precipitation creates an essential habitat for the larval stages of mosquito populations and therefore is observed to be a significant predictor of vector distribution. Increased precipitation creates more breeding sites for vectors, thereby increasing their numbers and their probability of becoming infectious, but can also influence the stability and productivity of existing habitats (Afrane, Githeko, & Yan, 2012) since prolonged rains can wash away sites resulting in an overall decrease in larval abundance (Dlamini, Franke, & Vounatsou, 2015). *An. gambiae* is often found in environments with high humidity and rainfall, while *An. funestus* are more suited to regions with less rainfall (Imbahale et al., 2011). Distance to water/aquatic habitats are also documented to be significantly associated with malaria risk (Cottrell et al., 2012; Dlamini et al., 2015; Grillet, Barrera, Martínez, Berti, & Fortin, 2010).

For urban-dwelling mosquitoes like *Ae. aegypti* and to a lesser extent *Ae. albopictus*, water storing containers (such as buckets, used car tires, etc.) that hold and accumulate water following rains provides suitable breeding sites for their larvae to thrive. Kraemer *et al* (2015) previously alluded to the interspecies differences between *Aedes* vectors by observing that minimum precipitation had a stronger influence on *Ae. albopictus* due to this species' non-domesticated juvenile habitats compared to *Ae. aegypti* (Kraemer et al., 2015).

III. Urbanization

The degree of urbanization influences the availability of mosquito breeding sites, depending on mosquito ecology. With high rates of population growth and movement into urban cities, incorporating urbanization into models to predict vector distribution and density has become essential (Kigozi et al., 2015). The association between urbanization and malaria prevalence

have been previously studied across several spatial scales where the general consensus suggests that increased urbanization results in decreased malaria transmission due to the presence of less favourable habitats for their vectors (Alimi et al., 2015; Kigozi et al., 2015; Qi et al., 2012). In a systematic review on the factors contributing to urban malaria transmission in Sub-Saharan Africa, the researchers highlighted that the majority of urban breeding sites for malaria vectors are artificial, such as: urban agriculture, tire tracks, and ditches, and further concluding that low SES was a significant risk factor for malaria in peri-urban regions (De Silva & Marshall, 2012). At the local scale, however, the relationship between urbanicity and malaria risk is more complex due to differences in population characteristics and the environment between urban and rural communities (Kigozi et al., 2015)

Urbanization is also a key factor influencing *Aedes* vector density and distribution, as these species are highly adapted to urban environments due to the presence of suitable breeding sites for their larvae (i.e. water storing containers, car tires, flower pots etc.). In addition to urban settings being characterized by a higher presence of these suitable habitats, higher population densities create more opportunities to increase the feeding activity among vectors – with some researchers demonstrating that *Ae. albopictus* has a higher blood-feeding-rate in urban areas compared to rural regions (Valerio et al., 2010). With vectorial capacity and disease transmission significantly associated with urbanicity among *Aedes* vectors, this factor is key to understanding the spatial heterogeneity of arboviruses, especially with more regions shifting to urban areas (Li et al., 2014).

IV. Elevation

The landscape significantly influences the distribution of mosquito vectors and spatial patterns of disease incidence. Though often used as a proxy for small-scale climatic differences (temperature decreases and precipitation increases with increasing altitude, and *vice versa*), elevation limits the range of *Anopheles* and *Aedes* vectors and is considered a significant predictor in modelling vector distribution. Malaria is most common at lower elevations, due to the vectors' preference for warmer temperature (e.g., $> 16^{\circ}\text{C}$ for *Anopheles gambiae*), temperature limitations on parasite development (e.g., $> 21^{\circ}\text{C}$ for *Plasmodium falciparum*), high humidity ($> 80\%$ for adult mosquitoes) and presence of suitable aquatic habitats for their larvae (Grillet et al., 2010). In a study modelling the range of *Anopheles* vectors in South America, elevation was found to be the variable most responsible for predicting malaria, followed by temperature and precipitation (Alimi et al., 2015). Lower altitudes are often characterized by higher population densities thereby influencing both *Anopheles* and *Aedes* vector abundance. Similarly, the larvae of *Aedes* vectors also prefer lower elevations with a close proximity to a water source (Bian, Li, & Yan, 2006; Gama, Nakagoshi, & Islamiyah, 2013). At higher elevations fewer flat surfaces exist for water to accumulate, which further limits the number of breeding sites for mosquito vectors (Myers et al., 2009).

With a warming climate due to global warming, elevation thresholds are now being pushed allowing vectors to propagate to higher altitudes. In the Americas, the highest altitude reported for *Ae. aegypti* was 1,630 m in Mexico and 2,200 m in Colombia (Lozano-Fuentes et al., 2012), with recent studies reporting this species found at elevations of 1,700m – 2,130m in Mexico (Lozano-fuentes et al., 2014; Lozano-Fuentes et al., 2012). In one study investigating *Ae. aegypti* distributions at high elevations in Mexico, the researchers predicted that with current climatic

conditions, *Ae. aegypti* is expected to exist at a 300m higher altitude in the year 2100 compared to present day (Lozano-Fuentes et al., 2012).

V. Land-cover

Anopheles and *Aedes* mosquitoes have emerged to colonize a range of habitats due to their ability to adapt to new climatic conditions and thrive in a range of environmental landscapes. In a recent systematic review on land-cover and land-use factors and malaria in the Amazon, researchers concluded that incorporating landscape variables is important in order to effectively understand the ecology of malaria vectors (Stefani et al., 2013). Habitat preferences differ among *Anopheles* species with *An. arabiensis* often found in arid savannahs while *An. gambiae* sensu stricto (s.s.) prefers humid forest zones (Chirebvu & Chimbari, 2014). Additionally, as previously mentioned, density of *Anopheles* vectors is influenced by the presence of aquatic sites for ample breeding, making irrigated landscapes like swamplands and dams ideal for these mosquitoes to persist (Townes, Mwandama, Mathanga, & Wilson, 2013). Vegetation also influences vector abundance as canopy cover is observed in some studies to inhibit the occurrence of *Anopheles* larvae (Elleby & Feltelius, 2014) while short aquatic vegetation creates a suitable habitat for breeding (Chirebvu & Chimbari, 2014).

Density of *Aedes* vectors is also influenced by landscape characteristics and land-use changes. These urban and peri-urban mosquitoes can thrive in a range of habitats such as open land, mixed horticulture, to neglected agriculture and grass lands, in shaded regions and vegetated environments (Cheong, Leitao, & Lakes, 2014; Ling, Gruebner, Krämer, & Lakes, 2014). Additionally, with *Aedes* larvae dependent on the presence of aquatic habitats, marshes,

swamp lands and paddy fields are observed to be suitable habitats for these mosquitoes (Sarfranz et al., 2012).

1.3 Challenges to Current Modelling Approaches

Early epidemiological research on VBD primarily focused on identifying individual-level risk factors – assuming that disease causation could be determined at the level of the individual (Diez-Roux, 1998; Diez-Roux & Aiello, 2005). These individual-centred studies, however, ignored contextual factors that may have played an integral role in understanding the risk factors for a disease, resulting in an incomplete awareness of key disease determinants (Diez-Roux, 1998; Mauny, Viel, Handschumacher, & Sellin, 2004).

In the last decade, modeling the global extents and potential occurrence of VBDs has been the forefront of many research activities. Advances in statistical methods, availability of high resolution remotely-sensed environmental and climate data and access to comprehensive datasets and innovative software packages like Geographic Information Systems (GIS[®]), have allowed researchers to become fully equipped to explore a number of environmental predictors on risk of disease at a broader scale. Species distribution models (SDM) are examples of frequently used methods to characterize species-environment relationships and are now frequently employed as forecasting tools to provide detailed predictions of disease vectors (Elith et al., 2006; Howard, Stephens, Pearce-Higgins, Gregory, & Willis, 2014). SDMs - also referred to as bioclimatic models, climate envelope models, ecological niche models (ENMs), habitat models, or spatial models (Elith & Leathwick, 2009), function to model the geographic distribution of suitable habitat for a species with the aim of identifying relationships between known occurrence of a species (i.e. presence or absence) and environmental data (e.g. meteorological data, remote

sensing data) and using these relationships to make predictions for all un-sampled areas in the study region (Elith et al., 2006). These predictions are then used to improve detection of the spatial and temporal variation of ecological covariates that determine vector distribution or habitat suitability - usually at larger scales, such as at the country-wide or continental level (Acheson, Plowright, & Kerr, 2015; Drake & Beier, 2014; Kulkarni, Desrochers, & Kerr, 2010) in order to improve outbreak management and facilitate resource planning. The development of these global models have allowed for an improved understanding of the spatial epidemiology of both arboviral and malaria vectors, and have provided a medium to prioritize surveillance in regions where surveillance remains poor, or in areas where the disease and/or vectors are yet to be reported.

The majority of studies that model risk factors for VBDs frequently adopt a single analytical framework by focusing on one aspect of disease epidemiology, i.e. addressing risk factors at the individual- or at the environmental level. These investigations fail to explicitly model individual-level factors in combination with environmental predictors, and are becoming increasingly criticized due to their inability to explain observed variations in disease risk or vector abundance at a more local level. One major limitation to single-level analysis is that it is often impossible to determine whether observed variations in the outcome of interest are in fact due to individual differences, macro-level factors, or both (Dos Santos et al., 2014), with failure to discern these interactions resulting in inappropriate conclusions and poorly informed intervention strategies.

Due to the highly dynamic nature of VBDs, current control practices may also result in futile attempts of disease elimination. Several studies across countries in Africa report sustained malaria transmission even after improvements in vector control practices (De Beaudrap et al., 2011; Jagannathan et al., 2012; Mharakurwa et al., 2013; Mukonka et al., 2014). As such,

incorporating both individual and environmental predictors into disease models may provide researchers with a more holistic understanding of the key disease drivers to better inform public health policy and collectively reduce the burden of these diseases.

1.4 Research objective

The overall aim of this thesis is to investigate risk factors for malaria and arbovirus burden in Tanzania in order to inform selection of appropriate interventions and improve the overall diagnosis of febrile illness in this region. The onset of fever is highly characteristic of a number of VBDs, therefore, assessing factors that increase risk can have major implications with respect to patient care and disease management. This investigation can also allow researchers to discern differences in risk factors between malaria and arboviruses, and ultimately provide a medium to investigate the true underlying causes of febrile illnesses.

1.5 Overview of thesis organization

This thesis comprises of two manuscripts that contribute to the overall goal of our research, which is to explore risk factors for malaria and arbovirus burden in Tanzania. Each manuscript was prepared as a stand-alone research article for future publication and is presented under the following headings: Introduction, Methods, Results, Discussion and Conclusion. Complete reference lists and appendices for both manuscripts are presented at the end.

In the first manuscript, the association between socioeconomic factors (a factor measured at the household-level) and risk of malaria and arbovirus prevalence was investigated among two sites in north-eastern Tanzania: Hai and Lower Moshi. A composite measure to reflect SES was constructed using principal component analysis (PCA), and then assessed as an independent factor in final disease models. Specifically, this wealth proxy was used to determine whether the

socioeconomic position of a household significantly influenced the prevalence of malaria or arbovirus (dengue or chikungunya) in our study region.

This paper presents a more methodological take on the issues often faced by researchers who use smaller datasets to construct proxies for wealth. Our analysis explores how different combinations of socioeconomic indicators included in a wealth proxy can influence how households are classified based on wealth status and ultimately influence the strength of disease associations. Understanding which combination of socioeconomic indicators provide the most relevant information, and determining how sensitive wealth proxies are to the inclusion and exclusion of different indicators, can have pertinent implications for primary investigators particularly in regions where using PCA to construct a wealth status is frequently conducted to inform policy decisions.

For the purpose of this investigation, a dataset was obtained from a project funded by Grand Challenges Canada titled: Magnitude and risk factors associated with arbovirus infections in areas of different malaria endemicity in North Eastern and North-Western Tanzania. The rationale for using this dataset was that key information was available for both malaria and arbovirus burden allowing for a potential comparison of risk factors for these diseases. Ethics approval for the original study was obtained by the Ottawa Health Science Network Research Ethics Board (OHSN-REB) and the Tanzanian National Health Research Ethics Committee (NatHREC). A subset of this dataset was used in this manuscript to assess the association between household wealth and malaria and arbovirus prevalence in the Hai and Lower Moshi districts of north-eastern Tanzania.

Manuscript 2 builds on this risk factor analysis approach by incorporating predictors at both the household- and village-level. In this paper, the primary focus was modelling risk factors for malaria vector density among rural households in Muleba, Tanzania, where malaria presents a significant health problem. Modelling risk factors for vector density has important implications since malaria transmission depends on the presence and abundance of competent *Anopheles* vectors. Our rationale for conducting this investigation was to assess how risk factors defined at higher levels, i.e. village-level determinants, influence the variation in vector density observed at a more local-level, i.e. at the households, by simultaneously incorporating predictors defined at both of these levels.

A multi-level modelling method was used to concurrently investigate key predictors at both the household- and village-level on *An. gambiae s.s* density (defined as the mean number of host seeking-vectors per household) in our study region. This method provides a novel application to understanding how factors measured at different hierarchical scales can influence the observed rate of vectors at the level of the household. For this analysis, an entomological dataset was obtained from the study conducted by West *et al*, (2013) who previously investigated the protective effect of long-lasting insecticide treated nets (LLINS) and indoor residual spraying (IRS) in relation to risk factors for malaria infection in Muleba (West et al., 2013). Ethics approval was obtained for the original study from the ethics review committees of the Kilimanjaro Christian Medical College, the National Institute for Medical Research Tanzania and the London School of Hygiene and Tropical Medicine. This dataset is composed of a comprehensive list of household-level risk factors for modelling malaria risk in Muleba. Village-level risk factors including environmental and climate datasets were retrieved using remote sensing methods.

Ethics Approval

Ethical clearance for the secondary use of the datasets for manuscripts 1 and 2 was obtained following a delegated review by the Ottawa Health Science Network Research Ethics Board (OHSN-REB) (Protocol #: 20150788- 01H).

Preface – Chapter 1

The primary focus of this paper was to demonstrate the use of principal component analysis (PCA) for deriving a wealth index and assessing the association between this composite measure and the risk of malaria and arbovirus infection in Tanzania. This paper took on a more methodological scope to identify the key issues researchers face when employing PCA using smaller datasets. The primary objectives outlined in this paper are: 1) To identify which combination of variables collected during field research or community-based trials in rural or resource-poor settings provide the most relevant information for estimating household wealth; and (2) to determine how sensitive the derived wealth measures are to inclusion and exclusion of different socio-economic indicators.

For this investigation, Debora Kajeguka contributed the dataset and Dr. Manisha Kulkarni provided guidance and feedback in all aspects of the analysis. Ethics approval was obtained from the Kilimanjaro Christian Medical University College Research and Ethics Review Committee (Certificate No.658 KCMUC-CRERC), Ottawa Health Science Network Research Ethics Board (OHSN-REB) and the Tanzanian National Health Research Ethics Committee (NatHREC) for the initial study. Ethical approval for the secondary use of this dataset was obtained following a delegated review by Ottawa Health Science Network Research Ethics Board (OHSN-REB) (Protocol #: 20150788- 01H).

Principal Component Analysis of Socioeconomic Factors and their Association with Malaria and Arbovirus Risk in Tanzania: A Sensitivity Analysis

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Abstract

Background: Quantifying wealth-disease associations in low-and-middle-income (LMIC) countries poses many challenges due to the unavailability of reliable income and expenditure data. This limitation has prompted the use of an asset-based approach to create a wealth proxy using Principal Component Analysis (PCA); but the proper use of this method for smaller datasets remains unclear.

Objectives: (1) To identify which combination of variables collected during field research or community-based trials provide the most relevant information for estimating household wealth; and (2) to determine how sensitive the derived wealth measures are to inclusion and exclusion of different socio-economic indicators.

Methods: Participants with malaria-like symptoms were recruited from two rural health centres in Hai and Lower Moshi, Tanzania, and were administered a questionnaire to collect demographic and clinical information, as well as information on a number of household socioeconomic (SES) indicators. Blood samples were attained via finger prick to ascertain disease status using rapid diagnostic tests (RDTs) for malaria and IgM and IgG enzyme-linked immunosorbent assay (ELISA) tests for dengue and chikungunya.

PCA was used to construct 4 household-level wealth indices using different combinations of indicator variables and compared by: (1) percentage of variance explained; (2) presence of clumping and truncation; (3) possibility of stratifying households into 3 equal groups; (4) change in the categorization of households based on wealth status; and (5) strength of wealth-disease associations.

Results: Overall, Index 2 (durable assets and community infrastructural features) and Index 3 (durable assets and dwelling characteristics) provided less methodologically flawed representations of household-wealth compared to Index 1 (all SES indicators). Index 3 was associated with the lowest odds of malaria infection compared to Index 1 (OR = 0.15, CI: 0.07, 0.34) suggesting that the selection of wealth index may affect the interpretation of wealth-disease associations. None of the derived wealth-measures were significantly associated with arbovirus infection, but when assessed independently, roofing, specifically tiled or corrugated roofs, was associated with a 64% lower odds of arbovirus infection compared to households with thatched roofs (OR = 0.36, CI: 0.18, 0.74).

Conclusions: When using smaller datasets to characterize wealth-disease associations, the choice of the SES indicators included in a wealth-index influences the relative position of households in the overall wealth hierarchy, as well as the strength of disease associations. This can subsequently influence future resource planning activities and should be considered among investigators who use this approach to influence policy decisions in rural settings.

Introduction

Malaria, and arboviruses such as dengue and chikungunya, cause significant and increasing public health and economic challenges in many parts of the world due to the global expansion of

their mosquito vectors. Researchers are often interested in exploring risk factors for these infections in order to effectively inform control strategies and prioritize resources to reduce disease burden among the most affected populations. Socioeconomic factors are commonly explored in epidemiological research since they are likely to confound a number of disease associations (Howe, Hargreaves, Gabrysch, & Huttly, 2009; Howe, Hargreaves, & Huttly, 2008), as well as play a pivotal role in health resource planning and policy interventions (Howe, Hargreaves, Ploubidis, De Stavola, & Huttly, 2011). These factors are considered to be key determinants for a number of vector-borne diseases (VBDs) – diseases transmitted by mosquitoes and other arthropods – which currently plague the poorest and most vulnerable populations and collectively account for 17% of the global burden of all infectious diseases (WHO, 2014a).

In malaria research, poverty has been previously identified as a key risk factor influencing disease incidence (de Castro & Fisher, 2012; Gallup & Sachs, 2001; Ricci, 2012; Somi et al., 2007; Teklehaimanot & Mejia, 2008), with the mortality toll highest in the poorest 20% of the world (Gwatkin, Guillot, & Heuveline, 1999). Recent studies have even concluded that the causal link between poverty and malaria runs in both directions (Ricci, 2012; Sonko et al., 2014; Teklehaimanot & Mejia, 2008; Yusuf et al., 2010), creating a vicious cycle for the poorest segments of the population. In communities with a low socioeconomic position (SEP), opportune environments are created for vectors to thrive, which further hinders productivity and economic growth, thereby allowing these regions to persist in an impoverished state (Yusuf et al., 2010). Some researchers even argue that wealth may have a protective effect against malaria, with findings suggesting that in malaria endemic regions, children in the lowest socioeconomic

groups are twice as likely to be diagnosed with clinical malaria or test positive for malaria parasitemia compared to children in the highest socioeconomic group (Tusting et al., 2013).

Similar arguments can be made with respect to associations between wealth and arboviral infections, specifically dengue and chikungunya, two viruses that are transmitted by the same vector species. However, conflicting evidence exists suggesting that these relationships are less explicit. Chikungunya is often associated with poorer regions, with one study in India reporting that 80% of cases were among individuals below the poverty-line (according to the World Bank's definition of income less than \$1US per person per day), and with overall infection significantly more common in the lower income groups (Kumar et al., 2007). Additionally, following the epidemic in La Reunion in 2005 (Gérardin et al., 2008), investigators found that wealthier individuals were less affected by the outbreak compared to individuals with lower incomes (Setbon & Raude, 2008). In another study investigating dengue in the Americas, researchers found that the disparity in dengue prevalence between two countries with similar climate could be attributed to economic differences between these regions (Reiter et al., 2003). However, in a recent systematic review investigating the relationship between dengue and poverty, authors could not definitively conclude whether there was any significant relationship, mainly due to the inconsistent definitions of poverty among the included studies (Mulligan et al., 2014). Similarly, in a recent study exploring the sero-prevalence of dengue and chikungunya in Kenya, no significant association was found between socioeconomic status (SES) and either of these infections (Ochieng et al., 2015).

Despite the influence wealth may or may not have on an individual's risk of malaria or arboviral infection, estimating potential associations are in the best interest of public health. In low-and-middle-income (LMIC) countries, however, quantifying these associations poses many

challenges, especially since household income and expenditure levels – two frequently used indicators of wealth - are often unavailable, unreliable and difficult to measure (Houweling, Kunst, & Mackenbach, 2003). As a solution, investigators have opted to use an asset-based approach to estimate wealth using a multivariate statistical technique known as Principal Component Analysis (PCA).

Filmer and Pritchett first proposed the use of PCA to create a proxy for socioeconomic status (SES) in the absence of wealth indicators (Filmer & Pritchett, 2001; Mwageni et al., 2005). This method involves the use of asset-based indices and housing characteristics to create a wealth index that is indicative of long-run economic status (Bollen, Glanville, & Stecklov, 2002; Filmer & Pritchett, 2001; Howe et al., 2008), rather than using income or consumption-based indicators that are often subject to inherent biases and are seldom collected during field research (Howe et al., 2008; Vyas & Kumaranayake, 2006). The construction of a wealth index using PCA is commonly practiced among public health researchers for both primary and secondary data analysis as documented in a literature review in 2009, where approximately 294 papers cited the use this method (Howe et al., 2009). There are also well established guidelines on the appropriate methods to a construct a wealth index using PCA using a variety of statistical software packages (Filmer & Pritchett, 2001; Vyas & Kumaranayake, 2006).

The use of PCA is frequently adopted in larger datasets such as the Demographic and Health Survey (DHS) (Staveteig, Staveteig, & Staveteig, 2014), World Health Survey (WHS) of the World Health Organization and the World Bank's Living Standards Measurement Survey (LSMS) as a method to estimate wealth in LMIC (Filmer & Pritchett, 2001; Gunnsteinsson et al., 2010). However, using this method for smaller population-based studies, particularly in rural settings, may pose several methodological challenges. Our study therefore seeks to explore some

of these key issues by investigating the following questions: (1) which combination of variables collected using field research or community based trials provide the most relevant information for estimating wealth?; and (2) how sensitive are the derived wealth measures to inclusion and exclusion of different socio-economic indicators?

Methods

Study region

Information on the study region, participant recruitment and data collection are described in detail elsewhere (Kajeguka et al., 2016). Briefly, this study was conducted at three sites in north-eastern Tanzania: Bondo dispensary (Bondo, Tanga), Hai hospital (Hai, Kilimanjaro) and Tanganyika Plantation Corporation (TPC) hospital (Lower Moshi, Kilimanjaro) in May 2013, November 2013 and May 2014 respectively. For the current study, only data from Hai and TPC were used in analyses as these regions are geographically related (Figure 1.1), and likely so share similar demographic and environmental characteristics. These study sites were selected based on documented differences in precipitation and elevation levels resulting in differential malaria transmission, as well as previous evidence of malaria over-diagnosis and circulation of arboviruses (Crump et al., 2013).

Hai is characterized as a semi-urban region located at the south-western slope of Mount Kilimanjaro with varying malaria prevalence due to elevation differences. Historically, malaria transmission in this region has been described as moderately high or “meso-hyper-endemic” in lower altitude villages (750-1,000 m), moderate or “meso-endemic” in mid-altitude villages (1,001-1,250 m), low moderate or “hypo-endemic” in upper altitude villages (1,251-1,500 m), and non-endemic in highland villages (>1,500 m) (Chandramohan, Greenwood, Cox, Mswia, &

Setel, 2001). The Hai region, classified as a tropical savannah area, experiences two rainy seasons annually: long rains from March to June and short rains from November to December, with the uppermost zones receiving the highest amount rainfall on average.

Lower Moshi is located at the base of Mount Kilimanjaro with an elevation of 700-800m above sea level. This region experiences between 900-1200 mm of rainfall annually during the two rainy seasons from March to May and from November to December, with accumulating rainfall forming temporary pools for vector breeding. Malaria transmission occurs throughout the year in this region with relatively low parasitaemia (Shekalaghe et al., 2007; Shekalaghe et al., 2009) and low entomological inoculation rate (Kulkarni et al., 2006) mainly by the two primary *Anopheles* vectors: *An. arabiensis* and *An. funestus*.

Data Collection

In this study, our target population consisted of febrile individuals in the Kilimanjaro region. Prior to the administration of cross-sectional surveys, the community was made aware of the study objectives with the help of community leaders, and individuals between the ages of 2 – 70 years old with specific symptoms (including: fever, headache, rash, vomiting and joint pain) were encouraged to voluntarily attend the Hai or Lower Moshi health facility for testing. Written informed consent was obtained from all participants and from parents or guardians for children under the age of 18.

A questionnaire was administered to each participant in both rural health centres to collect demographic and clinical information including: age, sex and history of illness. Additionally, information indicative of household socioeconomic status such as: ownership of a number of durable assets (including: radio, TV, mobile phone, landline, bicycle, motorcycle, car, land,

another house), physical characteristics of the household (such as the materials the roof and walls were made from and presence of screened windows) and access to community infrastructure (including: electricity, water sources and toilet type) was also collected. Following this interview, each consenting participant provided a blood sample via finger prick to ascertain disease status. A subject number was assigned to each participant and used to label his or her blood sample. These samples were not linked with personal identifiers.

Data processing

Samples were tested for the presence of *P. falciparum* malaria antigens using rapid diagnostic tests (RDTs) (Wongsrichanalai, Barcus, Muth, Sutamihardja, & Wernsdorfer, 2007), and for IgM and IgG antibodies against dengue and chikungunya using direct enzyme-linked immunosorbent assay kits (ELISA) (Kuno, Gomez, & Gubler, 1991) (SD Inc, Gyeonggi-do, Korea) and Indirect ELISA kits (Prat et al., 2014) (SD, Gyeonggi-do, Korea and IBL international, Hamburg, Germany) respectively. Malaria positive participants were treated with anti-malarials according to the national guidelines (Kajeguka et al., 2016).

Presence of IgM antibodies suggests recent exposure to a disease, while IgG antibodies suggest past exposure. Individuals with a positive IgM and/or IgG result for dengue and/or chikungunya were pooled as arbovirus cases. Grouping of these cases was done to ensure there was a large enough sample to provide sufficient statistical power for analyses.

Construction of Wealth Indices

Principal Component Analysis (PCA) was used to construct the wealth indices in this study. Data management and all subsequent statistical analyses were done using SAS v 9.4 (SAS Institute, Inc., Cary, North Carolina). In total, four wealth indices were constructed using

different combinations of indicator variables, wherein the factor scores from the first principal component were used to compute the overall wealth index. The items included in each index are illustrated in Figure 1.2. The reference index, herein referred to as Index 1, was constructed by including the same items commonly used to generate a wealth index in DHS datasets, including: ownership of all durable assets (radio, TV, mobile phone, landline, car, bicycle motorcycle, land, other house), access to community infrastructure (private vs. public water supply, electricity and flush toilets vs. pit latrines); and physical characteristics of the household (wall material [cement vs. mud vs. thatched], roof material [tiled vs. thatched] and presence of screened windows). Subsequently, a series of 3 alternative indices were constructed by including the following items: (1) durable assets and community infrastructure– Index 2; (2) durable assets and dwelling characteristics – Index 3; and (3) dwelling characteristics – Index (4).

The alternative indices were constructed to determine which type of variables yielded the most relevant information to differentiate households based on their wealth status. Specifically, Index 2 excludes variables that are known to have a direct effect on the risk of VBD infection while Index 4 only included those variables. Generally, in exploratory analysis, variables that are considered to be direct determinants on the outcome of interest should not be included in an asset index, since this can lead to an inflation of measures of association (Houweling et al., 2003), and potentially hinder the distinction of households based on their socioeconomic position. The definition of “direct” and “indirect” depends on the outcome of interest. As suggested by previous research, household dwelling characteristics are hypothesized to have a direct association with prevalence of malaria and arboviruses due to the ecology of these VBDs (Deressa et al., 2007; Keating et al., 2009; Obaldia, 2015; Sharma et al., 2015; Wanzirah et al., 2015; Yadav, Dhiman, Rabha, Saikia, & Veer, 2014). With unscreened windows or poorly

constructed roofs for example, opportunities are created for mosquito vectors to enter homes and feed on humans, potentially transmitting diseases.

Index 3 excludes infrastructural characteristics since these indicators are often measured at the community level and may not necessarily reflect household wealth (Houweling et al., 2003).

Data Analysis

The four constructed wealth indices were explored both as continuous variables and by dividing them into tertiles (i.e. low, medium, high) to describe the SES of each household. Tertiles were used in this study rather than quintiles – which are commonly used in social epidemiology - due to the small sample size ($n = 551$). Index 1 was treated as the reference wealth index, and the indices were compared as follows:

1. Percentage of variance explained by the first principal component obtained from PCA.
2. Presence of clumping or truncation by visual inspection of histograms of the distribution of each wealth Index. [Note: Clumping and truncation are methodological limitations in PCA that may reduce the ability to distinguish households based on their wealth status. Clumping is described as households being grouped together in a small number of distinct clusters or socio-economic index score, while truncation implies an even distribution of socioeconomic position but over a narrow range (Vyas & Kumaranayake, 2006)].
3. Possibility of stratifying households into 3 equal wealth groups.
4. Change in categorization of households when using alternative indices compared to the reference index. This was done using cross tabulations to calculate the percentage of households remaining in the same tertile, and percentage that moved to adjacent tertiles.

5. Strength of the association between the medium and high wealth tertiles with prevalent malaria and arboviral cases compared to the low wealth tertile. Odds ratios (ORs) and 95% confidence intervals were calculated using logistic regression and were adjusted for age and sex.

Ethics approval

This study was part of a project partially funded by Grand Challenges Canada titled: Magnitude and risk factors associated with arbovirus infections in areas of different malaria endemicity in North Eastern and North-Western Tanzania. Ethical clearance was obtained by the Kilimanjaro Christian Medical University College Research and Ethics Review Committee (Certificate No.658 KCMUC-CRERC), Ottawa Health Science Network Research Ethics Board (OHSN-REB) and the Tanzanian National Health Research Ethics Committee (NatHREC).

Results

Characteristics of the study population and frequency of durable assets, dwelling characteristics and community infrastructural features among households are presented in **Table 1.1**. In total, 551 individuals were surveyed in Hai (n = 240) and Lower Moshi (n = 331) where the overall prevalence of malaria and sero-prevalence of arboviruses was 9.1% (n=50) and 25.2% (n=139), respectively. All of the confirmed malaria cases in this study were from participants in Lower Moshi, while 43.2% and 56.8% of arboviral cases were from Hai and Lower Moshi, respectively.

In this study, 60 % (n = 330) of the participants were female and 80.4% of the population was over the age of 15 (n=443). A majority of the households in both communities owned most of the durable assets with the exception of a landline (n = 4) and car (n = 38). These variables

were therefore not included in the final PCA models. The percentage of households owning a bicycle and owing another house was substantially different between communities with 38.3% and less than 1.0% of households owning a bicycle and another house respectively in Hai compared to 75.2% and 29.6% respectively in Lower Moshi.

More than 50% of the households in Hai and Lower Moshi were constructed with cement walls (74.0% and 50.7%, respectively) and tiled or corrugated roofs (95.4% and 76.5%, respectively). However, only 35.8% of households in Hai had screened windows compared to 60.8% in Lower Moshi. With respect to community infrastructure, most of the households in both communities had to access to electricity (63.3% in Hai and 57.6% in Lower Moshi). In Hai, however, most households relied on private water sources (68.3%) but used pit latrines (53.1%), whereas in Lower Moshi, public water sources (65.9%) and pour toilets (56.0%) were more common.

Table 1.2 shows the weights assigned to the indicators in each wealth index and the total variance explained by the first principal component. Index 2 and Index 3 did not differ considerably from each other or from Index 1, where the total variance explained was 25.7%, 21.4% and 24.2%, respectively. Inclusion of only dwelling characteristics, i.e. variables that are documented to have a direct effect on malaria and arboviral risk (Index 4) was associated with the highest overall variance explained (53.7%). When examined graphically, however, Index 1 displayed significant clumping, with households clustered at the highest and lowest ends of the distribution (**Figure 1.3**). Index 3 was less truncated than Index 2 suggesting that inclusion of dwelling characteristics and exclusion of community features allowed for a better discrimination of households based on their socioeconomic position. Similarly, Index 4 demonstrated clumping, although less than Index 1, and was more truncated than Index 3.

It was possible to distinguish three, equally sized wealth groups using Index 1, 2 and 3 (**Table 1.3**), suggesting that these combinations of indicator variables were appropriate for wealth construction. Index 4, however, showed a less equal distribution compared to the other indices, with more households grouped in the middle tertile. The categorization of households into wealth tertiles was sensitive to the choice of Index used (**Table 1.4**). Between 24% and 37% of households were re-categorized into a different wealth group when compared with Index 1. Most households moved to an adjoining tertile, with the largest changes were observed for Index 4 where 25.4% of households shifted one tertile and 12% shifted two tertiles.

When examining the associations between the derived wealth indices and prevalent malaria cases, Indices 2, 3 and 4 were observed to show significant inverse relationships (**Table 1.5**). These associations remained consistent even after adjusting for sex and age. Overall, Index 2 showed a stronger gradient in the distribution of prevalent malaria cases across all socioeconomic groups (i.e. medium, high), while for Index 1, all malaria cases (n=50) were observed to be among individuals classified in the lowest wealth tertile. Compared to households in the lowest wealth tertile, households categorized in the medium wealth tertile had a 65% (OR = 0.35; 95% CI: 0.18, 0.69), 85% (OR = 0.15, CI: 0.07, 0.34) and 94% (OR = 0.06; 95% CI: 0.02, 0.17) decreased odds of malaria for Index 2, 3, and 4 respectively. None of the derived wealth indices were observed to be significantly associated with risk of arboviral infection (**Table 1.5**).

Discussion

By comparing different sets of variables for the construction of a wealth index measure, our analysis demonstrated that the wealth indices constructed using smaller datasets from

community-based studies may be markedly influenced by the choice of the socioeconomic indicators included in each measure. In this study, combining variables similar to the DHS wealth measures (Index 1) (Staveteig et al., 2014) resulted in a poor discrimination of households based on their wealth status. Index 2, 3 and 4, which were constructed using subsets of these variables, provided a better overall estimate of wealth in the sampled population compared to Index 1. Contrary to previous research that has demonstrated the robustness of these methods (Filmer & Pritchett, 2001; Vyas & Kumaranayake, 2006), several studies support our findings that differentiating households based on their wealth status, and subsequently differentiating the poor from the very poor, depends on the socioeconomic indicators included in each index (Houweling et al., 2003; Howe et al., 2008; Howe et al., 2011). Houweling *et al.*, (2003) previously demonstrated through the construction of three wealth indices that the choice of assets included in each index altered household rankings into distinct wealth groups and often changed the magnitude and direction of poor-rich inequalities (Houweling et al., 2003). Boccia *et al.*, (2013) also reported similar findings when comparing two alternative wealth indices for investigating wealth associations with tuberculosis prevalence (Boccia et al., 2013).

In our study, Index 2, which consisted of durable assets and community infrastructural features, and Index 3, which consisted of durable assets and dwelling characteristics, consistently provided less methodologically-flawed representations of household wealth compared to Index 1. Inclusion of assets was common between these two indices, suggesting that in a wealth index for smaller datasets, combining socioeconomic indicators that have no direct relationship with the outcome of interest is appropriate. However, determining which other variables can be added to these composite measures warrants closer investigation. One can argue that the solution to this goes back to the primary research interests. Like ours, if the goal is largely exploratory, i.e.

comparing how different combinations of socioeconomic indicators influence risk of disease, then the methods here are likely reasonable. By independently analyzing different sets of socioeconomic indicators, we were able to elucidate the relative importance of different components of wealth and their potential associations with disease risk. Since measuring household-wealth in LMIC was our primary investigation, Index 3, which only includes household-level socioeconomic indicators, provided the most relevant estimation of wealth in our study regions.

In our study, we observed that wealth measures that included household-level socioeconomic indicators, with or without household assets (Index 3 and 4), were associated with a lower odds of malaria in wealthier households compared to the wealth index that included community-level indicators (Index 2). Due to sample size limitations, however, we were unable to identify the independent effect of these risk factors and their interactions at different levels. We were also unable to find any significant associations between any of the composite wealth measures and risk of arboviral infection among our study population. This finding is supported in the literature in the context of other endemic countries (Ochieng et al., 2015). However, the capacity of these viruses to spread to new regions, including poorer regions of several high-income countries, suggests that although socioeconomic factors may not be the principal drivers for infection they may influence whether these diseases persist in the adopted regions.

Another question often faced by researchers is whether or not indicators included in a composite wealth index can also be treated as independent variables in a disease model. From a statistical standpoint this poses many challenges. Since the variability is already captured, these ‘double-counted’ variables can make disease associations difficult to interpret (Howe et al., 2008). However, if investigators are interested in exploratory risk factor analysis, excluding

variables of interest from the composite wealth measure and assessing them independently may provide clues to causal mechanisms responsible for disease risk. To illustrate this point, we constructed separate multivariate models for arbovirus prevalence using Index 2 and 3, where the variables excluded from the wealth measures were assessed as independent risk factors in the final models (See Table A.1). Consistent with other studies (Mazaba-Liwewe et al., 2014; Mease et al., 2011), the results from these analysis showed that the type of roofing, specifically tiled or corrugated roofs, was associated with a 64% decreased odds of arbovirus prevalence compared to households with thatched roofs.

Our study suffers from a number of limitations, the first owing to the relatively small sample size - studies that have adopted similar methods to explore wealth associations have often used larger datasets (Gunnsteinsson et al., 2010; Houweling et al., 2003) allowing their findings to be much more rigorous. However, independent researchers that conduct smaller-scaled studies such as community-wide interventions or use hospital record datasets are likely to face some of the methodological issues highlighted in this paper; therefore, our study provides a theoretical framework of some of the potential issues these researchers might face particularly in a more rural setting. In addition to the sample size, there is also a selection bias with respect to the study sample. A possibility exists that the individuals recruited in this study present a biased estimate of the underlying wealth distribution in these communities. These recruited participants may come from households that are less rural and therefore have better access to roads or transportation to get to these health facilities. Additionally, these individuals may reside closer to these health centres and are therefore more likely to go see a health professional when symptoms appear (Lowassa et al., 2012).

Another major limitation is attributed to the cross-sectional nature of the study design. Cross-sectional studies are limited in their ability to ascertain a causal association between exposures and outcome since information on these variables are collected at the same time; therefore, issues of reverse causality are not without mention. Finally, some authors have argued that the use of PCA may not be the most appropriate data reduction method particularly for discrete data (Laura D Howe et al., 2008), but this hypothesis is beyond the scope of this paper.

Conclusion

Investigating wealth-disease associations is an important activity for public health policy in order to optimize resource allocation and ultimately reduce the burden of disease among vulnerable populations. When using smaller datasets to characterize these associations, the choice of the socioeconomic indicators included in each wealth index influences the relative position of households in the overall wealth hierarchy, as well as the strength of disease associations. For the purpose of this study, Index 3 which included durable assets and dwelling characteristics provided the best representation of household-wealth in our study regions.

Ultimately determining which combination of variables provides the most information depends on the research interests of investigators. Though it may seem appealing to combine a number of different wealth indicators, researchers should be wary of the methodological challenges this may present.

Tables

Table 1.1 Characteristics of sampled households in the Kilimanjaro region

	Total (n=551)	Hai (n = 240) n (%)	Lower Moshi (n=311) n (%)
Sex			
Male	221	79 (32.9)	142 (45.7)
Female	330	161 (67.1)	169 (54.3)
Age			
2 – 5	43	30 (12.5)	13 (4.2)
6 – 15	65	24 (10.0)	41 (13.2)
> 15	443	186 (77.5)	257 (82.6)
Durable Assets			
Radio	454	207 (86.3)	247 (79.4)
TV	260	119 (49.6)	141 (45.3)
Mobile Phone	474	204 (85.0)	270 (86.8)
Landline	4	3 (1.3)	1 (0.3)
Bicycle	326	92 (38.3)	234 (75.2)
Motorcycle	115	38 (15.8)	77 (24.8)
Car	38	23 (9.6)	15 (4.8)
Land	326	158 (65.8)	166 (53.6)
Other House	94	2 (0.8)	92 (29.6)
Dwelling characteristics:			
Cement Walls	331	174 (74.0)	157 (50.7)
Mud walls	137	50 (21.3)	87 (28.1)
Stick/ Thatched Walls	77	11 (4.7)	66 (21.3)
Tiled / Corrugated Roof	467	229 (95.4)	238 (76.5)
Thatched Roof	84	11 (4.6)	73 (23.5)
Screened Windows	275	86 (35.8)	189 (60.8)
Community Infrastructure			
Electricity	331	152 (63.3)	179 (57.6)
Private Water source	270	164 (68.3)	106 (34.1)
Public Water source	281	76 (31.7)	205 (65.9)
Pour Toilet	286	112 (46.9)	174 (56.0)
Pit latrine/ No facility	264	127 (53.1)	137 (44.1)

Table 1.2 Comparison of factor scores and variances of wealth indices constructed using principal component analysis (PCA)

	INDEX 1	INDEX 2	INDEX 3	INDEX 4
Assets				
Radio	0.44459	0.49943	0.54983	
TV	0.73186	0.82526	0.67974	
Mobile Phone	0.30351	0.33968	0.43954	
Bicycle	0.03982	0.15024	0.22334	
Motorcycle	0.23124	0.38916	0.35759	
Land	-0.01374	0.07257	0.07295	
Other House	0.26242	0.37495	0.40303	
Community Infrastructure				
Electricity	0.74361	0.77395		
Water source (Private/Public)	0.57866	0.55405		
Toilet type (Pour / Pit latrine)	0.64347	0.54485		
Dwelling characteristics				
Wall type (Cement/Mud/Thatched)	0.65799		0.58862	0.853
Roof type (Tiled/Corrugated/Thatched)	0.61995		0.57711	0.41413
Screened Windows	0.28353		0.39991	0.84244
Indicators, n	13	10	10	3
Variance of 1st PC, %	24.22%	25.69%	21.43%	53.68%

Table 1.3 Distribution of sampled households in each wealth group

	Low	Medium	High
	n (%)	n (%)	n (%)
Index 1	181 (33.3)	183 (33.7)	179 (33.0)
Index 2	181 (33.3)	181 (33.0)	185 (33.7)
Index 3	181 (33.3)	181 (33.3)	182 (33.5)
Index 4	163 (29.9)	205 (37.6)	177 (32.5)

Table 1.4 Percentage change of households into different wealth tertiles, by wealth index

Wealth Index	% in same tertile	% moving 1 tertile	% moving 2 tertiles
Index 1	REF	REF	REF
Index 2	76.4%	17.3%	6.3%
Index 3	74.6%	18.8%	6.6%
Index 4	62.6%	25.4%	12.0%

Table 1.5 Association between household wealth indices and prevalent malaria and arboviral cases by wealth index

	Malaria				Arbovirus			
	Unadjusted		Adjusted		Unadjusted		Adjusted	
Wealth Index^a	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Index 1 (REF)								
Medium	N/A	N/A	N/A	N/A	0.75	0.47, 1.18	0.74	0.47, 1.18
High	N/A	N/A	N/A	N/A	0.64	0.40, 1.03	0.62	0.39, 1.01
Index 2								
Medium	0.38	0.20, 0.74*	0.35	0.18, 0.69*	1.02	0.64, 1.61	1.00	0.63, 1.59
High	0.08	0.02, 0.25*	0.06	0.02, 0.22*	0.71	0.44, 1.15	0.70	0.43, 1.14
Index 3								
Medium	0.15	0.07, 0.34*	0.15	0.07, 0.34*	0.69	0.43, 1.11	0.69	0.43, 1.10
High	N/A	N/A	N/A	N/A	0.69	0.43, 1.10	0.68	0.43, 1.10
Index 4								
Medium	0.07	0.03, 0.17*	0.06	0.02, 0.17*	0.96	0.60, 1.51	0.95	0.60, 1.51
High	N/A	N/A	N/A	N/A	0.67	0.41, 1.11	0.66	0.40, 1.09

^a = wealth index derived using Principal Component Analysis (PCA); OR = Odds Ratio; REF = Reference; CI = confidence interval; N/A = not applicable; * = p-value < 0.05

Figures

Figure 1.1 Location of study sites in Tanzania. The inset map details Hai and Moshi district locations in North-Eastern Tanzania

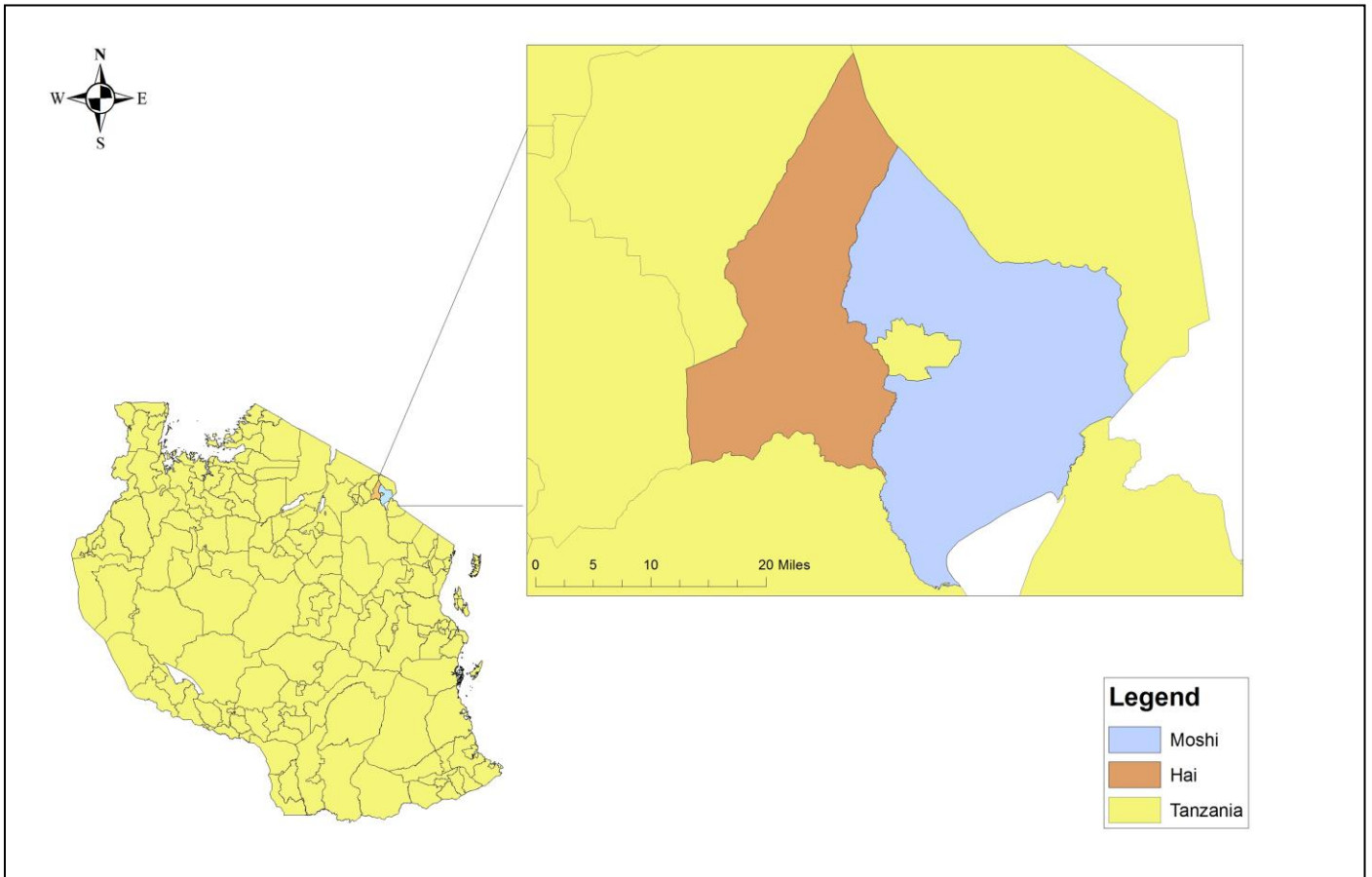


Figure 1.2 Items included in each wealth-index

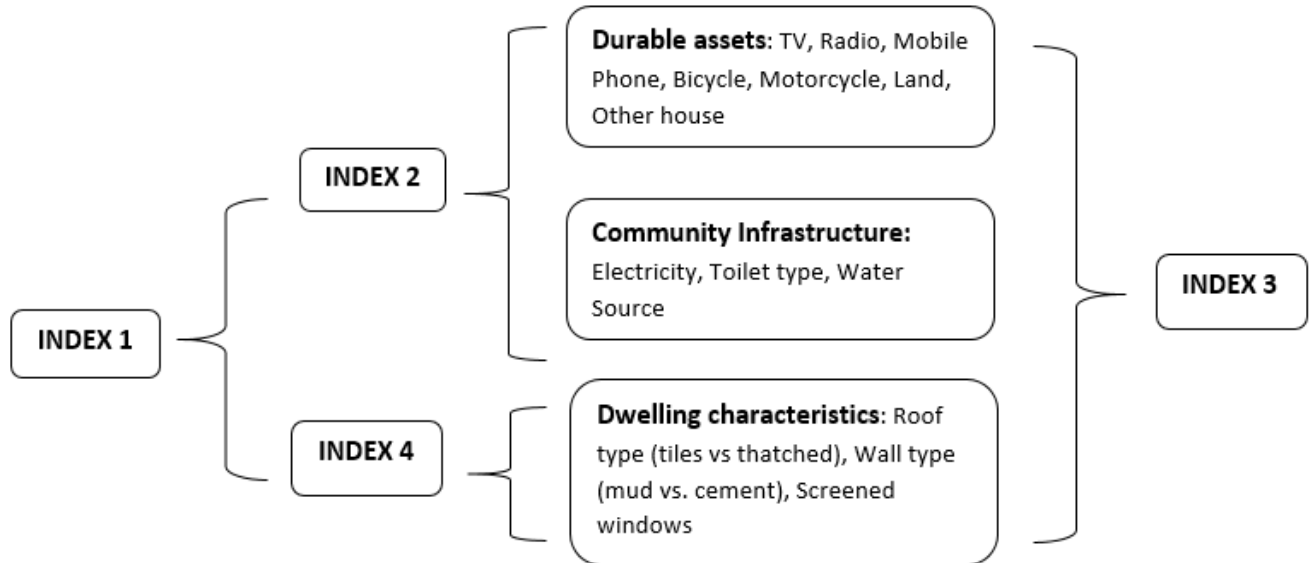
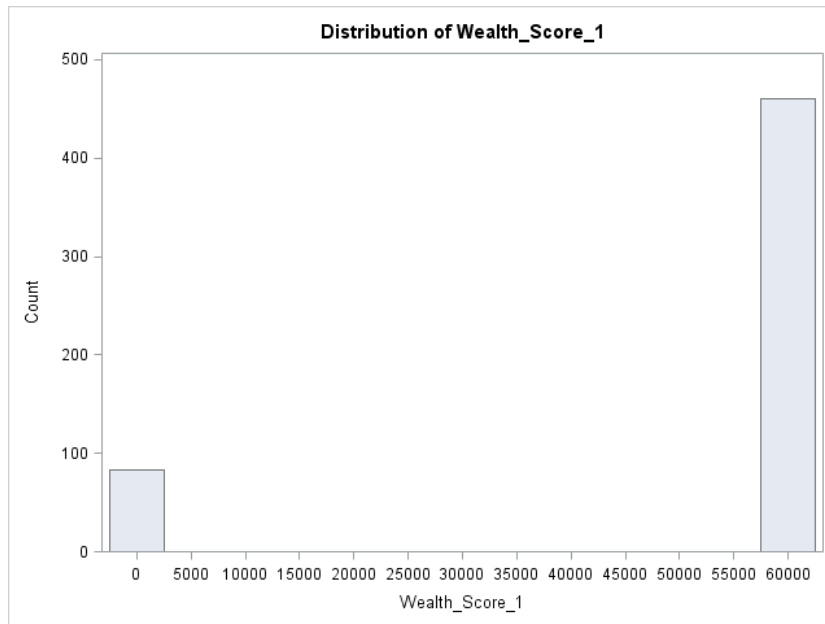
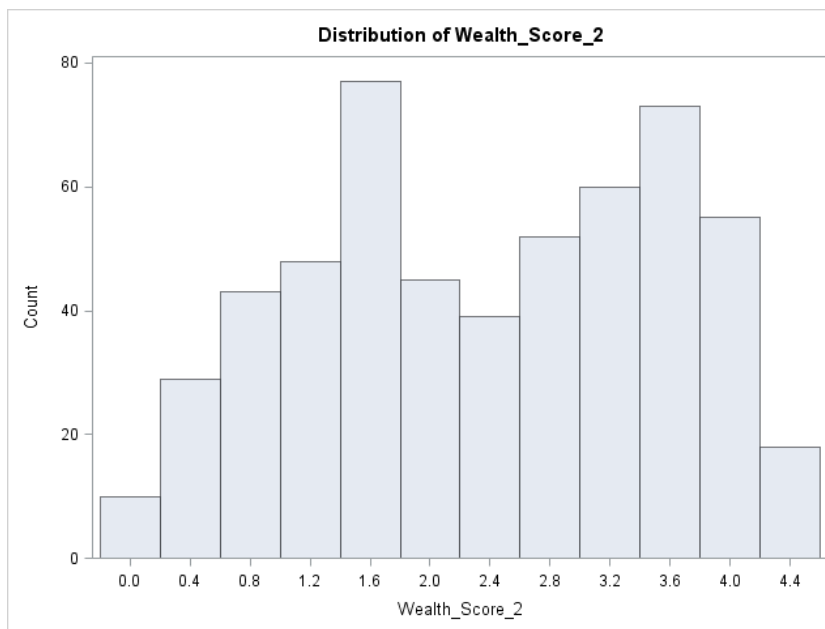


Figure 1.3. Household wealth distribution for all indices. **(1)** Index 1 = reference index (includes durable assets, community infrastructure and dwelling characteristics); **(2)** Index 2 = includes only asset variables; **(3)** Index 3 = includes only household socioeconomic indicators; and **(4)** Index 4 = includes indicators with direct effects of risk of malaria and arbovirus infection

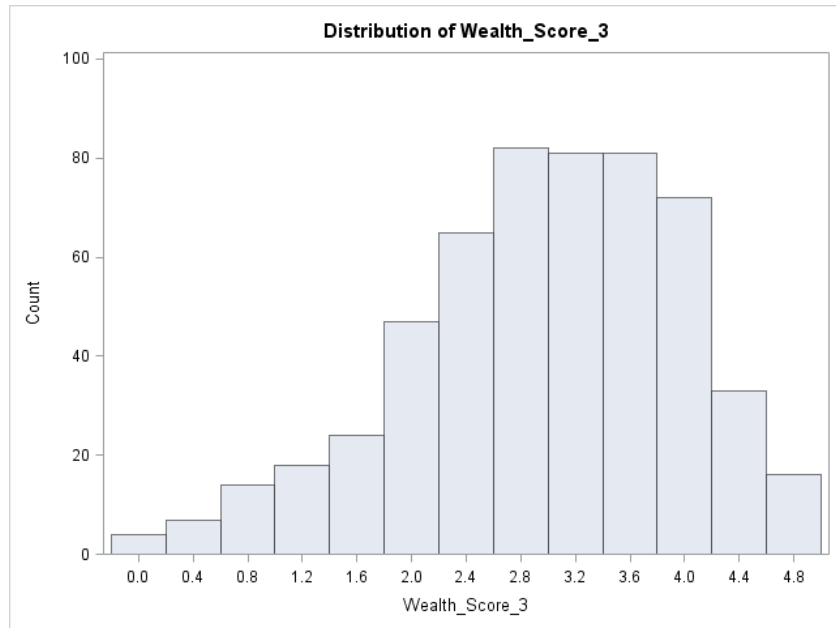
(1)



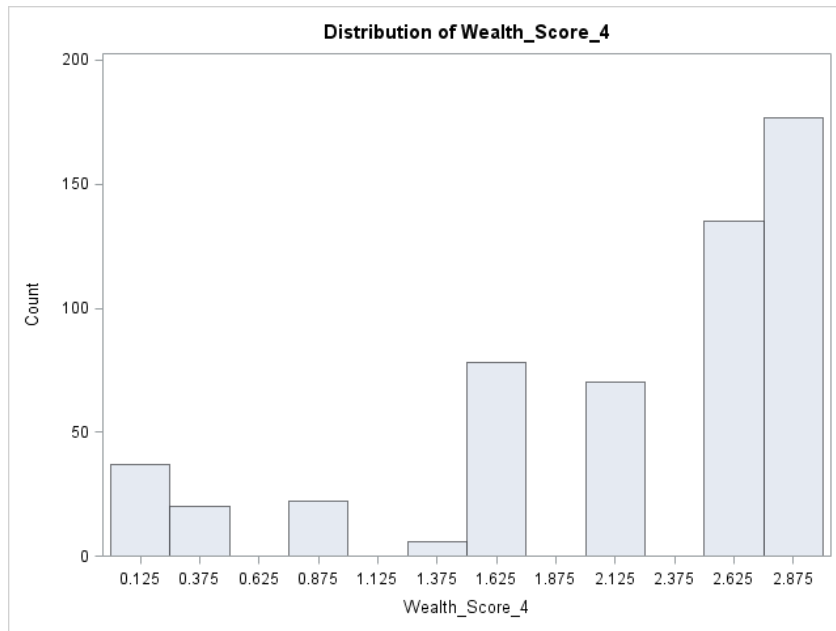
(2)



(3)



(4)



Bridge to Chapter 2

In the previous article, principal component analysis (PCA) was used to derive a household wealth proxy, which was subsequently assessed as an independent risk factor in final disease models. Though the primary objectives of this paper were to discuss the methodological challenges associated with the use of PCA, the association between socioeconomic status (a household-level risk factor) and risk of malaria and arbovirus infection was also demonstrated

To build on this analysis, the subsequent paper adds a second level to the disease model. Using another dataset obtained from researchers in Muleba, household- and village- level risk factors were incorporated into a multilevel regression model to examine their effect on *Anopheles* mosquito density in a region where malaria continues to persist. Modelling risk factors associated with the density of malaria vectors at the household level is a critical investigation as human risk of infection with malaria is highly dependent on the presence and abundance of these vectors.

Preface – Chapter 2

This paper highlights the use of multilevel modelling for understanding key risk factors that influence density of *Anopheles* mosquito vectors in a region of Tanzania with malaria continues to pose a significant health concern. Modelling vector density is a warranted investigation as malaria transmission intensity and hence human risk of infection is highly dependent on the presence and abundance of these vectors. The primary objective of this paper was: to examine the contribution of household- and village-level factors on the density of malaria vector mosquitoes (mean number per household) in the Muleba district of Tanzania using a multilevel regression approach.

Dr. Natacha Protopopoff provided the entomological dataset that was used for the development of multilevel models. Dr. Alexander Watts and Dr. Rahim Moineddin provided GIS and statistical guidance for data collection and model building, and Dr. Manisha Kulkarni provided guidance and feedback in all aspects of the analysis. Ethics approval for the original study that produced the dataset was obtained from the ethics review committees of the Kilimanjaro Christian Medical College, the National Institute for Medical Research Tanzania and the London School of Hygiene and Tropical Medicine. Ethical approval for the secondary use of this dataset was obtained following a delegated review by Ottawa Health Science Network Research Ethics Board (OHSN-REB) (Protocol #: 20150788- 01H).

Exploring the determinants of malaria vector density among rural households in Muleba, Tanzania – a multilevel analysis

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Abstract

Background: The abundance of *Anopheles* mosquito species that transmit malaria is a key determinant of disease transmission and hence risk of infection among populations living in malaria-endemic regions. Investigating the factors associated with malaria vector abundance within households requires a holistic understanding of social and ecological determinants at both the local and global scales. Multilevel modelling provides a novel platform to perform such investigations and has become a powerful statistical tool among public health researchers in the last decade.

Objective: To examine the contribution of household- and village-level factors on the density of malaria vector mosquitoes (mean number per household) in the Muleba district of Tanzania using multilevel modelling.

Methods: In a sample of 1,775 households from 39 villages in a rural district of Tanzania, a two-level modelling process is presented based on baseline data from a previously conducted cluster randomized trial assessing malaria vector control interventions. The trial collected entomological data from each household and conducted a cross-sectional survey to collect information on a variety of infrastructure and socio-economic factors from household members. Data on

household-construction variables were also collected and used to create an index for housing-quality using principal component analysis (PCA). The distance of each household to the nearest water source and road was calculated using ArcGIS v 10.2. At the village scale, climate and environmental data including: temperature, precipitation, land-cover, elevation, vegetation and population density were retrieved from remotely sensed datasets and processed using zonal statistics in ArcGIS. Multilevel models were constructed using negative binomial regression and rate ratios were calculated from fixed effects estimates. As a sensitivity analysis, models were stratified by season to investigate key risk factors in the dry season versus during the rains. All statistical analyses were conducted using SAS v 9.4.

Results: Cattle ownership and rainy season were significantly associated with density of malaria vectors among sampled households, with the rate of vectors among households with cows 1.93 times the rate of vectors in cattle-free homes (RR = 1.93, 95% CI = 1.16, 3.21), and the rate of vectors during the long and short rains 10.6 (RR = 10.6, 95% CI = 7.09, 15.61) and 1.4 (RR = 1.4, 95% CI = 1.00, 1.93) times the rate of vectors in the dry season, respectively. When stratified by season, both household- and village-level factors were found to be responsible for driving vector density during the dry season, particularly cattle ownership (RR= 3.53; 95% CI = 1.63, 7.63) and temperature (RR = 1.25; 95% CI = 1.03, 1.52). During the rainy season, however, only household-level risk factors were significantly associated with rate of malaria vectors: housing-quality (RR= 0.54, 95% CI = 0.29, 0.89), ownership of goats (RR = 1.80; 95% CI = 1.27, 2.54) and distance to water sources (RR=2.22; 95% CI = 1.14, 3.98).

Conclusions: Household malaria vector density in a rural area of Tanzania is significantly influenced by factors operating at both the local household-level and regional village-level, and the influence of these factors varies depending on the season. Therefore, simultaneous

examination of risk factors defined at multiple levels is imperative to allow for a complete understanding of key drivers of malaria vector density in order to effectively design suitable interventions to reduce or eliminate disease transmission.

Introduction

Malaria in Africa is principally caused by the protozoan parasite, *Plasmodium falciparum*, and transmitted to humans by the bite of a female mosquito of the *Anopheles gambiae* complex. Malaria transmission relies on human-vector contact, and can be measured by the entomological inoculation rate (EIR), which is a product of the human-biting rate (number of bites per person per night) and the sporozoite rate (proportion of infective mosquitoes). The human-biting rate can be estimated by measuring the density of malaria vectors within households, and thus malaria vector density is a critical factor in determining human risk of infection (Kilama et al., 2014).

Malaria is considered to be the deadliest vector-borne disease causing significant morbidity and mortality to half of the global population. Approximately 3.4 billion people in 106 countries and territories (WHO, 2014b) are considered to be at risk globally, with the African continent bearing the brunt of the disease burden. In 2015, there were 214 million cases of malaria reported with 438,000 attributed deaths – 306,000 of these occurring among children under the age of 5 (WHO, 2015a).

Researchers are often interested in modelling risk factors for malaria in order to optimize the utility of health interventions, particularly among vulnerable populations. These risk factors range from local-household determinants such as: population demographics, socioeconomic status (SES), structural and built-environmental characteristics, to regional or global-scale

environmental factors including: temperature, precipitation, humidity, land-cover/land-use and elevation. The presence and abundance of *Anopheles* mosquito populations are also important determinants of malaria transmission in a given area, and can be used to identify areas and populations at risk.

In order to predict malaria occurrence over a global scale, several investigators and research groups have made significant strides in modelling the potential occurrence of malaria vectors using niche modelling approaches, remotely sensed datasets, and compendiums of observed malaria occurrences (Hay et al., 2010; Sinka, 2013; Sinka et al., 2010, 2011, 2012). However, due to the highly dynamic, multi-dimensional etiology of the disease, these multi-regional scale investigations may not completely explain the observed spatial variations in malaria vector populations, and hence malaria risk, at a more local scale. Generally, at a local scale, it is expected that malaria transmission should be spatially heterogeneous given environmental variability and biological factors influencing the presence and abundance of competent mosquito vectors (DaSilva et al., 2004; De Beaudrap et al., 2011; Jagannathan et al., 2012; Mharakurwa et al., 2013; Wendy Prudhomme O'Meara, Mangeni, Steketee, & Greenwood, 2010; Ojuka et al., 2015).

Multilevel modelling provides a novel platform to perform such investigations as it allows researchers to simultaneously examine risk factors defined at multiple levels in order to isolate key predictors that influence disease risk (Mauny et al., 2004) across sampling or spatial scales. Recognizing the inter-related regional- and local-scale factors on which *Anopheles* mosquito vectors depend, a more nuanced method for modelling disease vectors could provide researchers with a holistic understanding of the key ecological and spatial drivers of malaria risk. This type of analysis is most appropriate for data with a hierarchical structure, such as in settings where

lower-level units (e.g. households) are nested within higher-level units (e.g. villages). This modelling method, though commonly practiced in the social sciences, has become a powerful statistical tool in public health research in the last decade (Mauny et al., 2004), with several studies adopting these approaches to understand the risk factors for malaria in order to properly design effective interventions and reduce the burden of this disease (Da Silva-Nunes et al., 2008; Dos Santos et al., 2014; Haque et al., 2011; Lawpoolsri et al., 2010; Nixon et al., 2014; Peterson, Borrell, El-Sadr, & Teklehaimanot, 2009; Pullan, Bukirwa, Staedke, Snow, & Brooker, 2010; Woyessa, Deressa, Ali, & Lindtjorn, 2013).

These models can also assist in informing the scale at which particular malaria control interventions are targeted, i.e. at a local/household- or individual-level vs. a regional/village-level. Current interventions that have been successful in significantly reducing the burden of malaria worldwide (Chanda, Mukonka, Kamuliwo, Macdonald, & Haque, 2013; D'Acremont, Lengeler, & Genton, 2010; Masaninga et al., 2013; Wendy P O'Meara et al., 2008; WHO, 2014b) include: increased coverage and deployment of long lasting insecticide treated bed nets (LLINs), indoor residual spraying (IRS), environmental management of mosquito breeding sites and use anti-malarial combination therapy (ACT). Rigorous research is still needed to determine the most efficient and cost-effective level of implementation to reduce the burden of this disease particularly in regions where malaria still persists despite increased coverage of current control methods.

We applied a multilevel modelling approach to examine the contribution of household- and village-level factors on *Anopheles* mosquito density in a rural area of Tanzania, where malaria is a significant health concern. Our approach differs from many models used in malaria research that focus on predicting the presence/absence of malaria vectors with the two-fold aim of

developing risk maps and characterizing species-environment relationships, in order to forecast potential disease outbreaks. Seldom do these studies look at the relative abundance or density of vectors in a given location, which may be a more relevant proxy for disease transmission at smaller spatial scales, and better predictor of human malaria incidence or prevalence.

Methods

Study Area

The study region has been described in detail by previous authors (Protopopoff et al., 2015; P. A. West et al., 2012; P. a West et al., 2013). Briefly, the Muleba District (1° 45' S 31° 40' E) of the Kagera Region is located on the western shore of Lake Victoria in north-eastern Tanzania. In the Kagera Region, about 91% of the population resides in rural communities with an average of 4.7 persons per household. Kagera is home to a relatively young population with 48% of the residents aged 15 years or younger and about 4% over the age of 65 (Tanzania National Bureau of Statistics 2012 Census).

Muleba District is situated at an altitude of 1,100 – 1,600 m above sea level and covers a total area of 3,550km² with a population of 540,310 people (Tanzania National Bureau of Statistics 2012 Census) (Figure 2.1). Malaria transmission is reported to occur throughout the year in this region, often peaking after the short and long rains in October-December and March-May, respectively. The prevalence of malaria in Muleba was found to be 9.3% in February 2011 and 22.8% in June 2011 (West et al., 2013).

Data sources

Entomological data

To explore the factors that influence malaria vector density among households in Muleba, entomological data collected during the pre-intervention arm of a cluster randomized controlled trial were used. The trial was conducted in 2011-2012 and compared the combined use of long-lasting insecticide treated nets (LLINs) and indoor residual spraying (IRS) versus LLINs alone on malaria infection prevalence in children. The results of this investigation are discussed elsewhere (West et al., 2013), and the entomological outcomes have been recently published (Protopopoff et al., 2015).

Vector control in Muleba has been effectively facilitated through ongoing IRS since 2007, potentially altering the composition and abundance of the primary malaria vectors in this region. Therefore, the rationale for using baseline entomological data was to ensure that there were enough mosquito catches to provide a large enough sample size and allow sufficient statistical power, while avoiding any intervention effect on vector populations (Protopopoff et al., 2015).

In the trial, each sampled household was enumerated using hand-held Global Positioning System devices (Garmin *etrex legend H*®, Garmin International Inc. USA) and mapped to form 50 clusters with buffer zones at 1-km wide surrounding core sampling areas of 2-km diameter. Monthly entomological surveys were conducted for seven months at baseline in 20 randomly selected clusters, in which 8 houses selected at random were monitored for mosquito density using Centers for Disease Control (CDC) light traps. Once informed consent was obtained from the household residents, a light trap was installed at the foot of a bed occupied by a family member sleeping under a treated or untreated net, and operated from dusk to dawn to collect host-seeking mosquitoes.

Identification of mosquito collections was done using a simplified morphological key adapted from Gillies and Coetzee (Gillies & Coetzee, 1987), followed by ELISA tests for detection of *P. falciparum* circumsporozoite protein (Pf-CSP) (Wirtz et al., 1987). A real-time PCR Taq Man assay was also used to distinguish between the two sibling species, *An. gambiae* s.s. and *An. arabiensis* (Bass, Williamson, & Field, 2008).

A total of 12,926 mosquitoes were collected, of which 45.3% were identified as *Anopheline* and 54.5% as *Culicine*. *An. gambiae* s.s represented 98.3% (n=5,763) of the total *Anopheline* catches and *An. funestus* accounted for only 0.2%. Due to interspecies differences in host and habitat preferences, multilevel models were only constructed using female *An. gambiae* s.s. (n=5,709) counts as the outcome of interest.

Household data

Data from the baseline cross-sectional survey were used to evaluate the association between household-level factors and vector density. In each selected household, a questionnaire was administered to collect information on a variety of infrastructure and socio-economic factors, the number of inhabitants per house, presence of livestock inside or outside the home, malaria prevention measures used by household members (including: indoor residual spraying [IRS] in 2010 or 2011, ownership and usage of bed nets), and house construction materials.

Village-level data

At the village level, remotely-sensed measures of elevation and land cover, and global spatial layers of human population density, climate (mean annual temperature and mean annual precipitation) and vegetation were extracted for the study region (**Table 2.1**). These variables have been previously identified as key risk factors influencing *Anopheles* spp. density at regional

and local scales due to their involvement in different aspects of mosquito vector ecology (LaDeau et al., 2015).

Data analysis

Household- level predictors of malaria vector density

The physical features of each household were used to create a housing-quality index by performing a principal component analysis (PCA). PCA is a multivariate statistical technique that reduces the number of variables in a dataset by extracting a weighted linear combination from those variables that provide the most information (Filmer & Pritchett, 2001; Vyas & Kumaranayake, 2006). The variables combined to create the index included: wall type (brick/cement, grass/banana leaves or mud); roof material (metal sheets or grass, papyrus, banana leaves); wall integrity (plastered or damaged); type of eaves (open/closed); ceilings (intact / damaged or absent); and screened windows (presence or absence). The first principal component was used to generate the housing-quality index for each household, which was further divided into quintiles and assessed as an independent risk factor for vector density. The purpose of creating this index was to capture micro-level risk factors that would potentially explain differences in vector density at the household scale. PCA analyses were performed using SAS v 9.4.1.

The distances of each household to the nearest road and watershed were calculated to evaluate impact of road ditches and aquatic habitat on vector density, respectively. This distance was calculated in ArcGIS v 10.2.1 (ESRI, Redlands, USA), using the near function in the spatial analytics tool box. The data sources of these distance measures are presented in **Table 2.1**.

Village-level predictors of vector density

Village boundaries were artificially created around aggregated household with similar GPS coordinates using the point to polygon function in the spatial analytics toolbox of ArcGIS v 10.2.1. 100-m buffers were then processed around each village polygon and zonal statistics were used to extract the values of each environmental variable per buffer.

Statistical Analysis

Statistical analyses were conducted using SAS v 9.4.1. Only households with non-missing data ($N=1,775$) were retained in the analysis. Descriptive statistics were used to describe the characteristics of the sample including the frequency of the household- and village-level predictors. Vector density in this study was defined as the number of female *An. gambiae* s.s mosquitoes collected per household per night (also referred to as ‘rate of vectors’) and associations were measured using rate ratios. Table 2.2 shows the frequency and bivariate analysis between all household- and village level predictors and vector density.

Data were aggregated at the household-level due to some households being sampled more than once. The number of times a household was sampled was added as an offset term in the model in order to estimate the number of mosquito vectors per household. Due to the over-dispersion of the data, a negative binomial model was used in the analysis. Univariate analyses were initially performed for each explanatory variable, and significant variables with a p -value < 0.20 were considered for inclusion into final multivariate models. A two-level modelling process was performed by following the steps outlined in **Figure 2.2** using the proc glimmix function in SAS v 9.4.1.

First, Model 1 was constructed by including all the household-level predictors that were found to be significant at the univariate level as well as variables identified in the literature as having a plausible biological relationship with vector density. Model 2 was then fit by including all the variables in Model 1 plus the village-level predictors identified as significant in the bivariate analysis. Mean precipitation was not included in the final model since the variable ‘season’ was used as a proxy for rainfall.

The fixed effects estimates were converted into rate ratios by taking the exponentials of the estimates obtained from the *proc glimmix* procedure and examined to identify significant variables. The random effects were then explored by comparing the variance explained by each model. Model fit was then assessed by comparing the ratio of the generalized chi-square statistic and its degree of freedom, with a value close to 1 indicating that the data has been modelled appropriately. In this study, the random slope was not significant in any model; therefore the final model was reduced to a random intercept model.

As a sensitivity analysis, the modelling methods described previously were repeated and stratified by season. Households sampled during the long- and short-rains were combined as the ‘rainy’ season. This analysis was done to explore potential differences in risk factors for malaria vectors at the level of the household depending on the season.

Model equations

Final models were constructed using a multilevel mixed effects negative binomial regression in order to simultaneously analyze the association between household-level predictors and village-level predictors on vector density, while accounting for variation between and within these levels.

The final multilevel models can be conceptualized in the following two-step process:

1. Separate level-1 unit (household) regressions models are developed for each level-2 unit (village):

$$Y_{ij} = b_{0j} + b_{1j}X_{ij} + \epsilon_{ij}, \epsilon_{ij} \sim N(0, \sigma^2) \quad (a)$$

Where:

Y_{ij} = outcome for the i th household in the j th village;

X_{ij} = value of i th household-level predictor in the j th village;

b_{0j} = intercept of the j th village following normal distribution with mean b_0 and variance σ^2 ;

b_{1j} = regression coefficient for X_{ij} , for the j th village;

ϵ_{ij} = household-level errors (sometimes referred to as micro-errors), are assumed to be independent and normally distributed with mean of 0 and variance of σ^2 .

2. The household-level regression coefficients (b_{0j} and b_{1j}) are then modelled as functions of village level variables:

$$b_{0j} = Y_{00} + Y_{01}G_j + U_{0j}, U_{0j} \sim N(0, \tau_{00}) \quad (b)$$

$$b_{1j} = Y_{10} + Y_{11}G_j + U_{1j}, U_{1j} \sim N(0, \tau_{11}) \quad (c)$$

Where:

b_{0j} = intercept of the j th village unit;

b_{1j} = slope of the j th village unit;

G_j = value of the village-level predictor;

Y_{00} = overall mean intercept adjusted for G ;

Y_{10} = overall mean intercept adjusted for G;

U_{0j} , U_{1j} = village-level errors (sometimes called macro-errors), are assumed to be independent and normally distributed with mean of 0 and variance of τ_{00} and τ_{11} respectively. These macro errors are assumed to be independent across levels and independent of micro errors (Mason & Entwisle, 1984).

By substituting equations (b) and (c) into equation (a), an alternative presentation of the multi-level model is provided as follows:

$$Y_{ij} = Y_{00} + Y_{01}G_j + Y_{10}X_{ij} + Y_{11}G_jX_{ij} + U_{0j} + U_{1j}X_{ij} + \epsilon_{ij} \quad (d)$$

The final model includes the effects of village-level variables (Y_{01}), household-level variables (Y_{10}), and their interaction (Y_{11}) on the household-level outcome (Y_{ij}). This model is composed of fixed and random components (hence often termed “mixed effects” or “mixed models”), where the coefficients Y_{01} , Y_{10} , Y_{11} are common to all households irrespective of their village (fixed effects), and the random intercept component U_{0j} , and random-slope component U_{1j} , varies across groups (random-effects). This modelling framework provides a unique approach to understanding village-level effects on the outcome of interest after adjusting for household-level variables, as well as understanding inter-household and inter-village variability (Diez-Roux & Aiello, 2005).

Ethics Approval

Ethical approval was obtained for the original study from the ethics review committees of the Kilimanjaro Christian Medical College, the National Institute for Medical Research Tanzania and the London School of Hygiene and Tropical Medicine. This study constituted a secondary

analysis of data with ethical approval granted by the Ottawa Health Sciences Network Research Ethics Board.

Results

Characteristics of study sample

1,775 households in 39 villages were included in the analysis; 447 of these households were sampled more than once, with one household sampled 5 times. Each household had approximately six individuals, owned 2 LLINs and kept livestock, particularly goats (51.6%) and poultry (57.1%) (**Table 2.2**). On average, each house was 1,992 m away from the nearest road and 3,539 m away from the nearest water source. Coverage of IRS was high among all households both in 2010 and 2011 (92.1% and 95.3%, respectively). With respect to village-level characteristics, 44.2% of households were sampled in the dry season, 16.1% during March-May (long rains) and 49.8% during October-December (short rains). The mean annual temperature and precipitation during this study period were 20.4°C and 1,404 mm respectively. Additionally, the mean elevation and mean population density of each village were found to be 1,341.8 m and 132.4 people per square km, respectively. All of the villages were classified as being a savannah or cropland, with a mean enhanced vegetation index (EVI) of 0.42%. Mean vector density was 1.67 (SD=14.7) in the dry season and increased to 4.42 (SD =24.3) in the rainy season (long and short rains).

Results of the bivariate analysis (Table 2.2) suggested that a number of household- and village-level predictors were significantly associated with vector density and were subsequently considered for inclusion into final multilevel models. These variables included: use of LLINs,

housing quality, distance to roads and water, season, elevation, land cover, temperature, precipitation and EVI ($p < 0.20$).

Multivariate, multi-level models

Based on the model results, malaria vector density was influenced by factors at both the household and village levels (**Table 2.3**). The variables in Model 1 included: mean use of LLINs, the housing quality index (both significant at the univariate level), mean number of individuals per household, ownership of cows, and distance to roads and waterways. Of these predictors, the housing-quality index and distance to roads were the only variables significantly associated with vector density. Households classified in the highest housing-quality quintile were observed to have 0.62 the rate of *Anopheles* vectors compared to households in the lowest housing-quality quintile (RR = 0.62; 95% CI = 0.37, 0.88), while households with the furthest distance from roads were also observed to have 2.65 the rate of vectors compared to households closest to roads (RR=2.65, 95% CI=1.09, 6.48).

In Model 2, cow ownership was observed to be significantly associated with vector density, where households with cows were found to have 1.93 times the rate of vectors among households without cows (RR = 1.93, 95% CI = 1.16, 3.21). With respect to village-level predictors, the rainy season was the only variable found to be significantly associated with the rate of vectors ($p < 0.0001$), with long rains and short rains associated with 10.6 (RR = 10.6, 95% CI = 7.09, 15.61) and 1.4 (RR = 1.4, 95% CI = 1.00, 1.93) times the rate of vectors, respectively, compared to the dry season.

Analysis of the random effects showed that the variation in vector density at the household level was associated with other predictors at the village level. The variance of Model 1 was 4.57

(SE=1.26) compared to the smaller but significant variance for Model 2: 2.05 (SE = 0.74). The addition of these village-level predictors was therefore responsible for explaining 55% of the variation between the models. Model fit statistics suggested that both models were explaining the data properly (Generalized chi-square = 0.81).

Table 2.4 shows the results of the full multilevel models stratified by season. In the dry season, ownership of livestock (cows and goats) and temperature were found to be significantly associated with the density of malaria vectors. Households owning cows had 3.53 (RR = 3.53, 95% CI=1.63, 7.63) the rate of malaria vectors compared to households with no cows, and household with goats had 0.51 the rate of malaria vectors compared to household with no goats (RR = 0.51, 95% CI = 0.31, 0.82). Additionally, every 10°C increase in temperature was associated with a 1.25 (RR = 1.25, 95% CI = 1.03, 1.52) times increase in the rate of malaria vectors.

In the rainy season, however, houses of the highest quality had 0.54 (RR = 0.54, 95% CI = 0.29, 0.89) the rate of malaria vectors compared to households of the lowest quality, and households with goats had 1.80 (RR = 1.80, 95% CI = 1.27, 2.54) the rate of malaria vectors compared to households with no goats. Households between 1,566 – 3,079 m away from waterways also had 2.22 (RR = 2.22, 95% CI = 1.14, 3.98) the rate of malaria vectors compared to houses closest to waterways.

Discussion

Malaria transmission is spatially, epidemiologically, and ecologically complex. In Tanzania, a growing body of evidence supports an overall decline in malaria prevalence following improvements in vector-control. According to the 2011-12 HIV/AIDS and Malaria Indicator

Survey, 9% of children under the age of 5 tested positive for malaria, with prevalence increasing with age (Tanzania Commission for AIDS et al., 2013). This reflects a 50% decrease in malaria burden since the previous survey in 2007-2008 (NBS, 2008). Despite this overall reduction, some geographic areas still experience a substantial number of cases. In Muleba, previous malaria epidemics were reported in 1997 and 1998 (Garay, 1998), and more recently in 2006 and 2010 (West et al., 2013), which were prompted by a number of factors including: immigration from malaria endemic regions, ecological changes, poor surveillance efforts and degraded health systems and healthcare infrastructure (Kinung'hi et al., 2010; Mboera & Kitua, 2001). With this disease still persisting in Muleba, the current study aimed to identify key determinants that influence the density of malaria vectors at a given household – as this factor is critical in influencing malaria transmission intensity and significantly impacts the incidence of malaria infection and burden of disease in a given population.

The findings of our study demonstrate that the density of malaria vectors at a given household was associated with multiple social and ecological factors operating at different hierarchical levels. At the household-level, the housing-quality index - a composite measure describing the structural integrity of each home – was significantly associated with rate of malaria vectors, with the highest quality homes associated with a decreased rate of vectors. These findings complement multiple studies which describe similar results (Deressa et al., 2007; Keating et al., 2009; Obaldia, 2015; Sharma et al., 2015; Wanzirah et al., 2015; Yadav et al., 2014). For example, mud, thatched and grassed roofs (Temu et al., 2012; Yé et al., 2006), unplastered and cracked walls (Yamamoto, Louis, Sie, & Sauerborn, 2010; Yusuf et al., 2010), damaged or absent ceilings (Ernst et al., 2009), open eaves (Chirebvu & Chimbari, 2014) and unscreened windows and doors (Atieli et al., 2009; Kirby et al., 2009) have been shown to allow

increased entry of *Anopheles* mosquitoes into houses to potentially feed on humans. These building materials are often associated with socio-economic status (SES) and are frequently included in composite measures to reflect household wealth (Krefis et al., 2010; Sonko et al., 2014; West et al., 2013), with poorer households most likely constructed with materials that increase the risk of invasion by vectors.

In model 2, cattle ownership, a factor measured at the household-level, was identified as a key predictor influencing the rate of malaria vectors. The presence of anthropogenic sources of water to accommodate livestock can potentially explain these findings. Feeding or drinking troughs, water tanks or other reserves (Oladepo, Tona, Oshiname, & Titiloye, 2010), as well as divots created by animal hoof-prints (Dlamini et al., 2015; Dutta et al., 2010; Vanwambeke et al., 2007), can serve as suitable aquatic habitats for mosquito larvae to thrive, thereby increasing the likelihood of these vectors to persist in environments where livestock are reared. Previous studies that have investigated associations between cattle ownership and risk of malaria infection in Africa have yielded mixed results, with some findings suggesting that owning cattle creates a substitute host for mosquitoes to feed on, ultimately reducing malaria burden for humans (Bulterys, Mharakurwa, & Thuma, 2009; Iwashita et al., 2014; Kaburi et al., 2009), while other investigators argue that with cattle increasing vector abundance, more opportunities are created for these mosquitoes to feed on human hosts – a phenomenon known as “zoopotentiality” (Iwashita et al., 2014). However, our study focused on household density of malaria vectors rather than measurement of malaria transmission, therefore, the likelihood of zoopotentiality is outside the scope of our key objectives

Season, specifically the long rains, was also significantly associated with a higher rate of malaria vectors compared to the dry season. This finding is ecologically plausible since rainfall

is essential for creating suitable breeding sites for the larval stages of mosquito populations. Stratifying our analyses by the season provided a clearer picture of the key differences in risk factors for vector density during the dry vs. the rainy season. Intervention efforts are frequently scaled up during the rainy season due to increases in vector abundance facilitated by favourable environmental conditions. However, sustaining malaria control interventions during the dry season is crucial and properly targeting households at highest risk in the dry seasons can potentially reduce the burden caused by these vectors before the onset of the rains. In our study, during the dry season – when there is less naturally occurring water - we found livestock ownership and temperature to be significantly associated with vector density.

With the assumption that with livestock comes anthropogenic aquatic habitats, our findings are consistent with previous reports that highlight water and temperature as the primary ecological drivers of *Anopheles* density (Stresman, 2010). In the rainy season, however, only household-level risk factors were found to be significantly associated with vector density, specifically housing-quality, livestock ownership (goats) and distance to aquatic habitats.

The presence of aquatic habitats or temporary breeding sites are primary drivers of vector density during both seasons, which is ecologically justifiable as supported by a plethora of studies that have investigated similar associations (Cottrell et al., 2012; Geissbuhler et al., 2009; Grillet et al., 2010; Gu et al., 2008). Additionally, temperature was found to be significant in the dry season, suggesting that when water and habitat are not plentiful, temperature is the main driver that significantly influences malaria vector density. This finding implicitly alludes to the critical role temperature plays in vector ecology, specifically its involvement in every stage of the mosquito life cycle. Temperature affects *Anopheles* survivorship directly during the juvenile and adult stages and indirectly during the larval stages (Christiansen-Jucht et al., 2014), as well

as the proliferation of malaria *Plasmodium* parasites in the vector (Stresman, 2010) - thereby influencing malaria transmission intensity. The entomological inoculation rate (EIR) – defined as the average number of mosquito bites per person per unit time, also highly dependent on environmental temperatures. Temperature also influences the macro-spatial distribution of mosquito vectors (regional, district, continental) and subsequent spatial and temporal heterogeneity of disease burden (Walker et al., 2013).

Our research findings can contribute to a better overall understanding of how the utility of interventions can be optimized if targeted at multiple levels. Currently, IRS, LLINs and environmental management of mosquitoes or larval source management (LSM) have been successful in providing protection against malaria vectors; however, deployment of these interventions solely at one level, i.e. at the household or at the community, may result in futile attempts towards complete malaria elimination. A combination of these interventions, or concurrent use of some of these methods, can potentially aid in reducing the burden of disease caused by these vectors. This strategy is known as integrated vector management (IVM), and is endorsed by the WHO as a means of optimizing the use of resources for vector control and improving the capacity of current malaria control methods (World Health Organization, 2012). The primary findings of this study support this recommendation by the WHO to integrate vector management strategies in Muleba, where malaria continues to persist, with attention to seasonal differences in the drivers of vector density and the importance of livestock ownership.

Our study is not without limitations. From the dataset used in our analysis, we were unable to identify risk factors for other *Anopheles* species present in this area due to the relatively small sample of mosquito catches. Though our study region may have been primarily affected by *An. gambiae*, assessing risk factors for competing strains, particularly *An. arabiensis*, may have

provided some insights into key biological distinctions between these species. Potential interactions between risk factors at the same level were also not examined and should be considered in future studies. The study design and data sources also limit the findings of our study, since the data provided by primary researchers were not collected for the purpose of multilevel modelling (Protopopoff et al., 2015; West et al., 2013). For our analysis, defined geographical village boundaries were not used, but polygons were artificially created around aggregated households to represent villages. This method may have resulted in a misspecification of village-level attributes potentially affecting our results.

Conclusions

In summary, the findings of our study have pertinent implications for vector-borne disease research. Our use of a multi-level modeling approach provided a novel application of exploring key built-environment, climatic and anthropogenic risk factors responsible for malaria vector density in rural Tanzania. We were able to demonstrate that the rate of malaria vectors at a given household (analogous to vector density) was a complex, multi-factorial process. By adopting multi-level regression analyses, we simultaneously investigated household- and village-level risk factors that influence density of malaria vectors as well as elucidated differences in risk factors between the dry and rainy seasons.

To the best of our knowledge, this paper is the first to demonstrate the use of multilevel regression methods to predict household density of malaria vectors in a rural setting, as previous work in this area has often focused on disease or fever prevalence as the primary outcome of interest (Da Silva-Nunes et al., 2008; Dos Santos et al., 2014; Haque et al., 2011; Lawpoolsri et al., 2010; Nixon et al., 2014; Peterson et al., 2009; Pullan et al., 2010; Woyessa et al., 2013).

Further research is warranted to optimize this approach to inform the development of integrated malaria vector control strategies.

Tables

Table 2.1 Description of data sources for the climatic and environmental variables

Variable	Time Period	Spatial Resolution	Source
Temperature	2011	1 km	WorldClim http://www.worldclim.org/bioclimate
Precipitation	2011	1 km	WorldClim http://www.worldclim.org/bioclimate
Elevation	2011	90 m	Shuttle Radar Topography Mission (SRTM) http://www.cgiar-csi.org/data/srtm-90m-digital-elevation-database-v4-1#download .
Population Density	2011	1 km	LandScan http://web.ornl.gov/sci/landscan/http://web.ornl.gov/sci/landscan/
Land cover	2001 - 2012	500 m	MODIS https://lpdaac.usgs.gov/dataset_discovery/modis/modis_products_table/mcd12q1
Vegetation	2011	250 m	USGS Earth Explorer http://earthexplorer.usgs.gov/
Distance to roads	-	-	DIVA-GIS http://www.diva-gis.org/datadown
Distance to waterways	-	-	DIVA-GIS http://www.diva-gis.org/datadown

Table 2.2 Frequency and bivariate analysis of household and village level predictors of malaria vector density in Muleba, Tanzania

VARIABLES	N (%)	BIVARIATE ANALYSIS		
		OR	95% CI	p-value
Household-level characteristics				
Number of people per HH				
Mean [SD]	5.61 [2.09]	0.9	0.79, 1.02	0.27
Use of LLINs				
Mean [SD]	1.59 [0.94]	0.74	0.63, 0.86	0.13*
Indoor Residual Spraying				
2010	1506 (92.1)	1.18	0.17, 8.19	0.76
2011	1572 (95.3)	1.19	0.46, 3.10	0.55
Household-Index**				
0	327 (20.6)	REF		0.17*
1	544 (34.3)	1.11	0.37, 3.43	
2	361 (22.8)	0.97	0.23, 3.94	
3	353 (22.3)	0.33	0.10, 0.90	
Livestock				
Cows	176 (10.7)	1.23	0.84, 1.83	0.41
Goats	852 (51.6)	0.88	0.78, 1.00	0.26
Chicken	942 (57.1)	1.09	0.60, 1.96	0.79
Pigs	235 (14.2)	1.51	0.81, 2.83	0.52
Other animals	213 (13.2)	1.3	0.53, 3.19	0.57
Distance to Roads(m)				
Mean [SD]	1992.4 [1418.24]			
≤ 709 m	443 (25.0)	REF		0.13*
710 - 1823 m	445 (25.0)	1.1	0.68, 1.76	
1824 - 3049 m	443 (25.0)	1.44	0.86, 2.45	
> 3049 m	444 (25.0)	2.76	1.22, 6.27	
Distance to Waterways(m)				
Mean [SD]	3539.04 [2338.42]			0.19*
≤ 1565 m	443 (25.0)	REF		
1566 - 3079 m	444 (25.0)	2.18	1.28, 3.73	
3080 - 5252 m	444 (25.0)	1.13	0.59, 2.16	
> 5252 m	444 (25.0)	2.73	0.88, 8.47	
Village-level characteristics				
Rain Season				0.11*
Dry	784 (44.2)	REF		
Long rains	285 (16.1)	9.46	8.04, 11.1	
Short rains	706 (39.8)	1.42	0.85, 2.35	

Elevation (m)				
Mean [SD]	1341.83 [122.73]			
≤ 1251 m	465 (26.2)	REF		0.17*
1252 - 1292 m	415 (23.4)	1.14	0.15, 8.6	
1293 - 1469 m	467 (26.3)	0.14	0.02, 0.83	
> 1469 m	428 (24.1)	0.06	0.01, 0.36	
Population Density				
Mean [SD]	132.42 [104.04]			
≤ 51	456 (25.7)	REF		0.22
52 - 113	421 (23.7)	0.12	0.03, 0.42	
114 - 176	548 (25.8)	0.75	0.13, 4.51	
> 176	440 (24.8)	0.05	0.01, 0.16	
Land-cover classification				
Savannah				
Mean [SD]	0.5 [0.39]	5.09	2.79, 9.29	0.16*
Cropland				
Mean [SD]	0.44 [0.39]	0.28	0.15, 0.51	0.23
Annual Temperature (C*10)				
Mean [SD]	203.75 [7.20]	1.21	1.07, 1.27	0.04*
Annual Precipitation (mm)				
Mean [SD]	1404.16 [143.01]	0.99	0.99, 1.00	0.12*
Enhanced Vegetation Index (EVI) (%)				
Mean [SD]	0.42 [0.04]			
≤ 0.40%	593 (33.4)	REF		0.12*
0.41-0.44	593 (33.4)	0.15	0.04, 0.63	
> 0.44	589 (33.2)	1.01	0.18, 5.60	

HH = Household; LLIN = Long-lasting insecticide treated nets; SD = Standard Deviation; ** = Housing-index derived using Principal Component Analysis (PCA); OR = Odds Ratio; 95% CI = 95% Confidence Interval; p-value – obtained from Type 3 Analysis of Effects; * p-value < 0.20

Table 2.3 Predictors of malaria vector density obtained from mixed effects negative binomial regression modelling

Fixed Effects	MODEL 1			MODEL 2		
	RR ^a	95% CI	p-value ^b	RR ^a	95% CI	p-value ^b
<i>Household-level characteristics</i>						
Number of people per HH [per increase in 1 person]	1.00	0.93, 1.08	0.93	1.03	0.96, 1.11	0.43
Use of LLINs [per increase in 1 LLIN]	1.02	0.85, 1.22	0.83	1.08	0.90, 1.29	0.39
Household-Index**						
0	REF		0.04*	REF		0.40
1	1.07	0.72, 1.58		1.01	0.69, 1.49	
2	1.26	0.83, 1.92		0.98	0.64, 1.50	
3	0.62	0.37, 1.03		0.68	0.40, 1.14	
Livestock						
Cows	1.28	0.76, 2.14	0.35	1.93	1.16, 3.21	0.01*
Distance to Roads (m)						
≤ 709 m	REF		0.06	REF		0.18
710 - 1823 m	0.90	0.54, 1.50		0.80	0.48, 1.34	
1824 - 3049 m	1.79	0.86, 3.72		1.49	0.69, 3.23	
> 3049 m	2.65	1.09, 6.48		2.04	0.80, 5.20	
Distance to Waterways (m)						
≤ 1565 m	REF		0.09	REF		0.10
1566 - 3079 m	1.71	0.98, 2.97		1.86	1.06, 3.28	
1380 - 5252 m	1.00	0.39, 2.55		2.66	0.93, 7.59	
> 5252 m	1.08	0.93, 1.27		2.71	0.92, 7.95	
<i>Village-level characteristics</i>						
Rain Season						
Dry				REF		<0.0001*
Long rains				10.52	7.09, 15.61	
Short rains				1.39	1.00, 1.93	
Elevation (m)						
≤ 1251 m				REF		0.62
1252 - 1292 m				2.04	0.37, 11.28	
1293 - 1469 m				2.73	0.19, 40.07	
> 1469 m				1.03	0.02, 57.04	
Population Density						
≤ 51				REF		0.48
52 - 113				0.46	0.08, 2.81	
114 - 176				1.84	0.29, 11.57	
> 176				1.49	0.21, 10.47	0.69

Land-cover classification				
Savannah [per 1% increase]		2.05	0.42, 10.09	0.38
Annual Temperature (°C*10)		1.21	0.97, 1.50	0.88
[per increase in 10°C]				
EVI (%)				
≤ 0.40%		REF		0.70
0.41-0.44		0.88	0.16, 4.75	0.88
> 0.44		0.50	0.08, 3.14	0.46
Random Effects				
Variance (SE)		4.77 (1.27)		2.75 (0.9)
Model-fit statistics, X ²		0.81		0.82

HH = Household; LLINs = Long-lasting insecticide treated nets; ** = Housing-index derived using Principal Component Analysis (PCA); REF = Reference; ^a= Rate Ratio; CI = confidence interval; ^b =p-value obtained from fixed effects estimates; * = p-value < 0.05; SE = Standard Error; X² = Chi-squared test

Table 2.4. Predictors of malaria vector density stratified by rain season in Muleba, Tanzania

	DRY SEASON			RAINY SEASON		
	RR ^a	95% CI	p-value ^b	RR ^a	95% CI	p-value ^b
Household characteristics						
Number of people per HH [per increase in 1 person]	1.06	0.95, 1.19	0.32	1.00	0.91, 1.09	0.95
Use of LLINs [per increase in 1 LLIN]	0.93	0.70, 1.25	0.65	1.00	0.81, 1.24	0.97
Indoor Residual Spraying 2011	1.51	0.51, 4.43	0.46	-	-	-
Household-Index**						
0	REF		0.10	REF		0.25
1	1.57	0.84, 2.96		0.88	0.57, 1.38	
2	0.84	0.40, 1.75		0.94	0.59, 1.50	
3	0.66	0.29, 1.52		0.54	0.29, 0.89*	
Livestock						
Cows	3.53	1.63, 7.63	< 0.01*	1.11	0.61, 2.04	0.73
Goats	0.51	0.31, 0.82	0.01*	1.80	1.27, 2.54	<0.001*
Distance to Roads(m)						
≤ 709 m	-	-	-	REF		0.23
710 - 1823 m	-	-		1.12	0.61, 2.06	
1824 - 3049 m	-	-		1.80	0.77, 4.21	
> 3049 m	-	-		2.72	1.00, 7.26	
Distance to Waterways(m)						
≤ 1565 m	-	-	-	REF		0.10
1566 - 3079 m	-	-		2.22	1.14, 3.98*	
3080 - 5252 m	-	-		2.13	0.77, 6.54	
> 5252 m	-	-		1.68	0.54, 5.19	
Village-level characteristics						
Elevation (m)						
< 1251 m	REF		0.61	REF		0.77
1256 - 1292 m	1.19	0.26, 5.41		2.54	0.42, 15.25	
1293 - 1469 m	2.32	0.26, 21.02		1.53	0.11, 20.29	
> 1469 m	0.77	0.02, 27.90		1.69	0.03, 97.89	
Land-cover classification						
Savannah [per 1% increase]	4.43	0.95, 20.52	0.06	0.77	0.19, 3.15	0.71
Annual Temperature [per increase in 10°C]	1.25	1.03, 1.52	0.03*	1.19	0.96, 1.49	0.12
EVI (%) < 0.40%	REF		0.78	REF		0.50

0.41-0.45	0.59	0.13, 2.61	0.82	0.14, 4.72
> 0.45	0.77	0.17, 3.48	2.56	0.45, 14.62
Random Effects				
Variance		4.47 (1.15)		3.25 (1.01)
Model-fit statistics, X^2		0.85		0.83

HH = Household; LLINs = Long-lasting insecticide treated nets; ** = Housing-index derived using Principal Component Analysis (PCA); REF = Reference; ^a= Rate Ratio; CI = confidence interval; ^b =p-value obtained from fixed effects estimates; * = p-value < 0.05; SE = Standard Error; X^2 = Chi-squared test

Figures

Figure 2.1. Location of study area in Muleba District, Tanzania. The inset map details Muleba's location in North-Eastern Tanzania

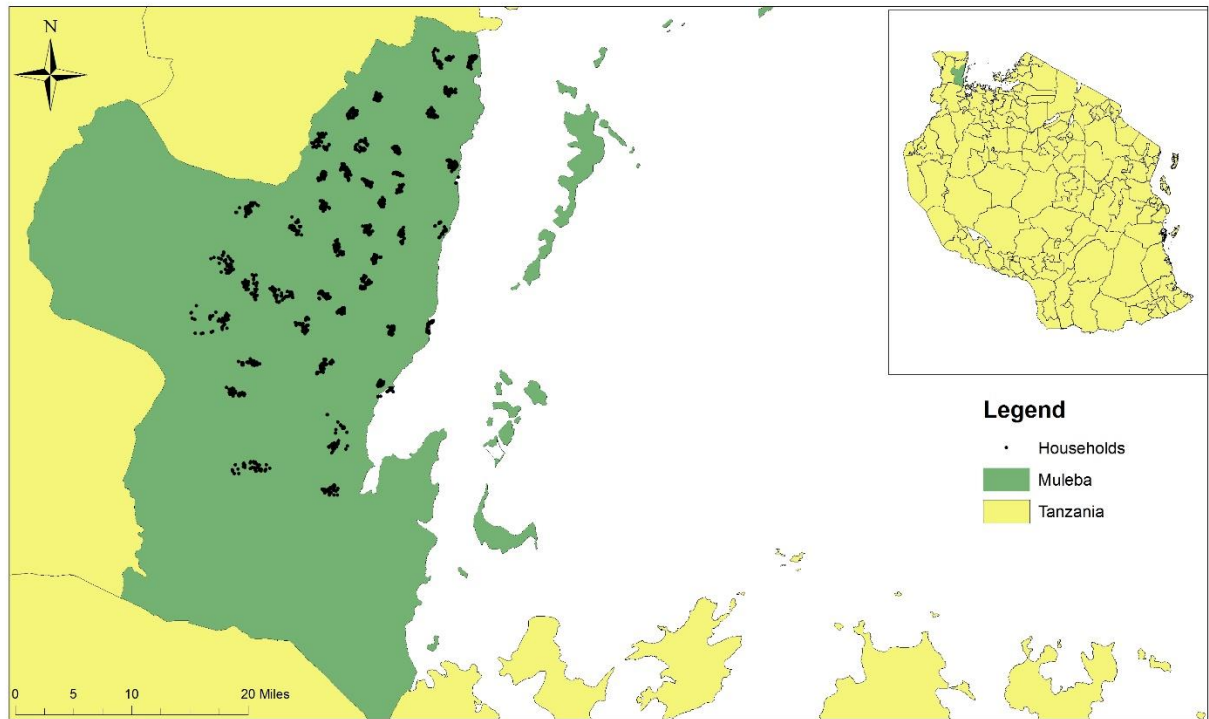
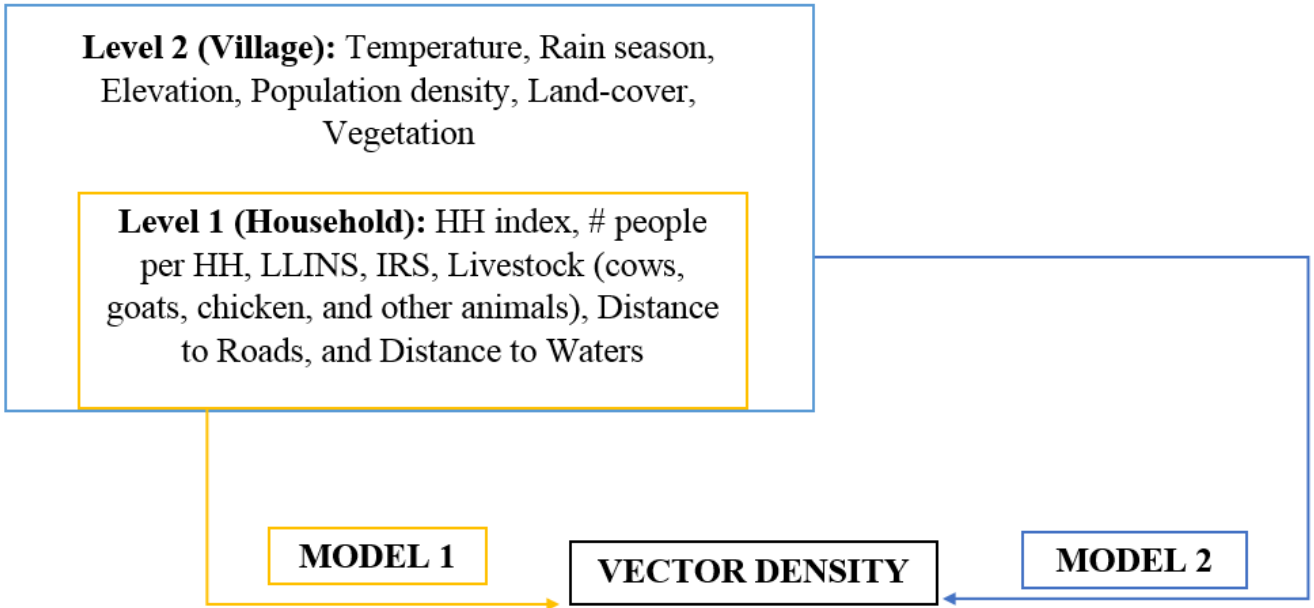


Figure 2.2. Analytical Framework: Hierarchical model of proposed risk factors for malaria vector density in Muleba, Tanzania



General Discussion

Investigating risk factors for malaria and arboviral illnesses in Tanzania is of critical public health importance as these diseases continue to persist despite increased coverage and investment in vector-control initiatives. In order to prioritize resources, target the most vulnerable populations and improve the utility and effectiveness of current interventions, it is fundamental to understand disease determinants defined at multiple levels (i.e. individual/local/global scales). The manuscripts that comprise this thesis tackle this issue by identifying key risk factors for malaria and arboviral disease burden and transmission among rural communities in northern Tanzania, and also address several gaps in the way risk factors are often investigated in current studies.

In Chapter 1, the associations between socioeconomic risk factors and malaria and arboviral infections were investigated in the Kilimanjaro region. Principal Component Analysis (PCA) was used to construct household wealth proxies using different combinations of socioeconomic indicators with the goal of: (1) identifying which combination of variables collected during field research or community based trials provided the most relevant information for estimating wealth; and (2) determining how sensitive the derived wealth measures were to inclusion and exclusion of different socio-economic indicators. The methods described herein for the construction of a wealth index in rural settings from smaller datasets brings attention to the challenges researchers face when studying or modelling risk factors for VBDs. Future studies should carefully consider the type of socioeconomic indicators that should be included in a wealth index as this can significantly impact the strength of disease associations, and subsequently influence policy decisions to address inequities.

Moreover, the findings of this study highlighted the importance of considering socioeconomic status in interventions for malaria, as all the derived wealth measures were found to be significantly associated with malaria risk (i.e. lower odds of malaria infection amongst people living in wealthier households). Specifically, our results suggested that in a region where resources are limited, focus should be placed on household infrastructure in order to reduce the burden of malaria. Tusting *et al* (2015) recently conducted a systematic review and meta-analysis comparing modern vs. traditional houses and their influence on malaria burden. These authors concluded that compared to traditional houses, residents in modern homes had a 42% lower odds of malaria infection and 54-65% lower incidence of clinical malaria (Tusting et al., 2015). Improvements in the quality of housing to reduce malaria burden is also supported in the multi-sectoral action framework for malaria proposed by Roll Back Malaria Foundation and the United Nations Development Program(RBM/UNDP, 2013). Housing quality was also observed to be a key risk factor influencing the density of malaria vectors in Muleba (Chapter 2) further supporting our recommendation for improvements in household infrastructure.

Additional research is still needed, however, to assess the cost-effectiveness of improved housing in this region; this is an important investigation that should be considered in future studies. The Roo *Pfs* trial in West Africa is one example of a recent ongoing investigation (Pinder et al., 2016): this study aims to assess whether improved housing coupled with LLIN use provides additional protection against malaria in The Gambia, compared to LLIN use alone; with the secondary objective of assessing the cost effectiveness of improved housing and mechanisms for scaling up housing interventions (Pinder et al., 2016).

The association between wealth and arbovirus burden, however, was less explicit in our study. None of the derived wealth indices were found to be significantly associated with

arbovirus infection, but household socioeconomic indicators, specifically the type of roof that homes were constructed with was found to have a significant association. Consistent with our models for malaria, investments in household-level infrastructure may potentially reduce the burden of arboviral illnesses in this region. Improvements in the structural integrity and quality of households can be pivotal towards reducing the prevalence of dengue and chikungunya, though further research may be needed to quantify whether improvements in housing as a protective measure is the most beneficial intervention for our study population.

Chapter 2 aimed to build on this risk factor analysis by adding another level into the disease model. Rather than investigating risk factors solely at one level, as in Manuscript 1 (i.e. at the household level), this study incorporated both household-level and village-level or environmental risk factors into a multilevel model in order to create a more complete understanding of significant determinants of vector density at a given household in Muleba. The findings of this study suggested that the density of malaria vectors was significantly influenced by factors operating at both the household and village levels; therefore, only investigating one type of factor (i.e. anthropogenic vs. environmental) may not necessarily reflect the key drivers of vector density and hence malaria transmission.

In our study, when both household- and village-level factors were included in the multi-level model, livestock ownership (a household-level factor) and rain season (a village-level factor) were the variables responsible for influencing the rate of vectors within households. Stratifying the analysis by season allowed us to draw significant conclusions regarding the type of interventions that should be scaled-up during the dry vs. during the rains, and the level at which these strategies should be targeted. Livestock and temperature (household- and village-level factors, respectively) were found to be significantly associated with the rate of malaria vectors in

the dry season; while housing-quality, livestock and distance to water sources (all household-level factors) were significant factors during the rainy season. These findings allude to the importance of scaling-up surveillance among livestock farmers as this risk factor was observed to increase the density of vectors among households irrespective of the season. Additionally, these results support the proposed use of IVM as a method to reduce the burden of malaria vectors in Tanzania, as a simultaneous or combined use of existing interventions is the hallmark of this strategy.

One of the main strengths of this thesis is that it provides a methodologically rigorous approach to studying vector-borne diseases at a very fine-scale. The use of PCA and multilevel modelling constitutes a novel application for investigating key risk factors for malaria and arboviral diseases, as well density of disease vectors in rural villages in Tanzania. This thesis also has significant public health relevance as the research findings can assist in guiding policy decisions regarding surveillance efforts as well as informing where and when to prioritize interventions. Currently, high-level policy decisions are often fuelled by research that adopts a global analytical framework, but with the persistence of diseases despite improvements in vector control, these fine-scale studies can be instrumental in understanding the spatial variation of observed disease burden at the district-level, the level at which most public-health decision making takes place. In developing countries, the methods used to characterize disease associations are complicated by the lack of complete and reliable datasets, as well as the availability of appropriate statistical techniques. This thesis addresses some of these issues and provides strategies and recommendations for future researchers.

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Appendix 1

Table A.1 Association between Index 2 and prevalent arboviral cases

	Arbovirus			
	Unadjusted		Adjusted	
	OR	95% CI	OR	95% CI
Index 2				
Low	REF	REF	REF	REF
Medium	1.24	0.75, 2.04	1.23	0.74, 2.03
High	0.84	0.49, 1.47	0.85	0.49, 1.46
Wall Type				
Cement Walls	1.85	0.87, 3.93	1.87	0.87, 3.97
Mud walls	1.22	0.54, 2.76	1.22	0.53, 2.77
Stick/ Thatched Wall	REF	REF	REF	REF
Roof Type				
Tiled / Corrugated Roof	0.38	0.19, 0.76*	0.36	0.18, 0.74*
Thatched Roof	REF	REF	REF	REF
Screened Windows	0.77	0.52, 1.15	0.77	0.52, 1.15

OR = Odds Ratio; REF = Reference; CI = confidence interval; N/A = not applicable; * = p-value < 0.05

Table A.2 Association between Index 3 and prevalent arboviral cases

	Arbovirus			
	OR	Unadjusted 95% CI	Adjusted OR	95% CI
Index 3				
Low	REF	REF	REF	REF
Medium	0.71	0.42, 1.18	0.71	0.42, 1.19
High	0.69	0.37, 1.27	0.69	0.38, 1.28
Electricity	1.42	0.87, 2.32	1.4	0.86, 2.30
Water Source				
Private Water source	0.81	0.53 1.24	0.81	0.86, 2.30
Public Water source	REF	REF	REF	REF
Toilet type				
Pour Toilet	0.77	0.49, 1.21	0.77	0.49, 1.2
Pit latrine/ No facility	REF	REF	REF	REF

OR = Odds Ratio; REF = Reference; CI = confidence interval; N/A = not applicable