DISEASE ECOLOGY IN THE DEMOCRATIC REPUBLIC OF THE CONGO: INTEGRATION OF SPATIAL ANALYSIS WITH POPULATION SURVEILLANCE

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ABSTRACT

JANE P. MESSINA: Disease Ecology in the Democratic Republic of the Congo: Integration of Spatial Analysis with Population Surveillance
(Under the direction of Michael Emch)

In countries like the Democratic Republic of the Congo (DRC) that have limited public health infrastructures, only educated guesses have been made about the spatial distribution of important diseases. This research estimates the spatial distribution of HIV, malaria and anemia prevalence in the DRC, and determines the population, environmental and behavioral drivers underlying these distributions. Using molecular diagnostics from dried blood spots from a 2007 Demographic and Health Survey (DHS) and demographic data available from this survey, the primary research aims are addressed via spatial analysis and multilevel modeling. The creation of an extensive Geographic Information Systems (GIS) database and selection of individual questionnaire responses is informed by disease ecology theory. In addition to discerning patterns and drivers of disease prevalence in the DRC, this research demonstrates how well population-representative surveillance data can be used to improve understanding of disease transmission in other developing countries.

While older people were at greater risk for HIV and anemia, younger people were at greater risk for malaria. Individual wealth increased HIV risk, while it protected against malaria. Increased risk for anemia was found in certain cultural groups. Living near urban areas increased risk for HIV and decreased risk for malaria. Certain types of

agriculture were protective against anemia. Greater density of nearby conflict since 1994 decreased malaria risk and proximity to a refugee camp was protective against anemia in women. Certain population characteristics and behaviors were equally or more important at the community level as at the individual level. Greater individual wealth was protective against malaria along with the average wealth of the community in which one lived.

This research extends beyond the scope of what would have been possible with the DHS dataset alone. The molecular results for malaria parasitaemia as well as habitat data from a variety of sources contributed to the creation of a complex database which enabled all aspects of disease ecology to be explored.

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INTRODUCTION

Decades of chronic violence, population displacement, and destruction of the health care systems in the Democratic Republic of the Congo (DRC) have negatively affected the health of its population, with most deaths in the country occurring as a result of preventable causes such as infectious diseases and malnutrition [1, 2]. Unfortunately, in a setting such as the DRC, the collection of quality information regarding the health status of the population is difficult, and epidemiological studies have been hindered. As a result, only educated guesses have been made about the distributions and burdens of important diseases on the national and sub-national levels. Studies have most often used passively-reported data from sentinel clinics rather than active surveillance data, leading to a bias and lack of clarity in the spatial distribution of prevalence for many diseases in most sub-Saharan African countries. This lack of clarity impedes effective resource allocation, implementation of control measures and the monitoring and evaluation of interventions. Surveillance of the population for infectious and chronic diseases is important and necessary if public health and aid agencies are to efficiently plan programs to improve the health in the country.

The purpose of the research presented in the following chapters is to estimate the burden of important diseases in the DRC as well as to understand their etiologies in order to guide prevention strategies. This is done via population surveillance combined with spatial epidemiological methods and a disease ecology framework. Using molecular diagnostics from leftover dried blood spots from a 2007 Demographic Health Survey (DHS) for the DRC as well as population-based demographic data available from these surveys, the primary research aim is addressed via spatial analysis and multivariate statistical methods. The following chapters focus each on one sexually-transmitted disease (HIV), one vector-borne disease (malaria), and one chronic illness (anemia). This research not only provides important insight into the risk factors associated with these specific health problems in the DRC, but also explores the utility of implementing a spatial epidemiological and disease ecology approach to health data obtained via population surveillance, and how this approach may be extended to other diseases in the DRC and other countries where public health data infrastructure is poor. For each disease, the following questions are posed:

(1) What is the prevalence of the disease in the DRC, and how is it spatially distributed at the sub-national level?

(2) What individual and behavioral characteristics contribute to increased risk in individuals?

(3) What community-level factors contribute to increased risk in individuals, and how does their relative impact compare to that of individual-level factors?

These research questions were chosen to address gaps in the current literature related to HIV, malaria, and anemia in the Congo. For each disease, detailed studies of the spatial patterns of risk using population-based data are non-existent for the DRC. Estimates of disease burdens are essential in order to guide global health resource allocations. However, disease burden estimates depend on the availability of good incidence and prevalence data. For many infectious diseases in poor countries, data quality is poor, with incidence and prevalence data being extrapolated from convenience samplings or non-randomly selected sentinel populations. The WHO has relied on passive national reporting for many countries, causing global estimates of disease burden to be inaccurate. For example, the estimates for the global prevalence of malaria range from 50 million [3] to 515 million [4]. This is because extrapolations based on sentinel data are not representative of the entire population. This means investigators must make assumptions and adjustments to control for under-representation or over-representation of some groups, introducing large potential for bias. For example, with regard to HIV, it must be assumed that data derived from women attending antenatal care clinics may be a proxy for all adults. Population-based surveys represent a much wider proportion of the population than do antenatal clinics, since such surveys include men and non-pregnant women. Surveillance systems for infectious diseases in developing countries therefore need to be utilized or initiated in order to guide public health interventions on the global and sub-national levels. Demographic Health Surveys are well respected sources of population-based data on demography, reproductive health and HIV, and thus leveraging the DHS system conducted in the DRC in 2007 provides less biased prevalence data that enables better disease burden calculations and fairer, more well-informed allocation of resources.

Research Framework

The research presented in the following chapters relies upon the integration of a theoretical framework (disease ecology) with an analytical framework (spatial epidemiology). Disease ecology considers what population, behavioral, and habitat characteristics contribute to increased risk for diseases in populations, as well as how these factors interact with one another. Spatial epidemiology involves the description and analysis of geographically-referenced health or disease data. For each disease, disease ecology theory is used to guide decisions about what risk factors are to be considered, and models for understanding the spatial patterns and risk factors for these diseases are implemented using a spatial epidemiological approach.

Theoretical Framework: Disease Ecology

Disease ecology theory lies within the field of medical geography, and has several important contributors. Although some may go as far back as Hippocrates' awareness of the environment on human health, most accounts of early medical geography begin with Jacques May's *Ecology of Human Disease* [5]. Jacques May expressed in middle of the 20th century that diseases are the result of a collision between two or more life forms, including humans, vectors, reservoirs, intermediate hosts, and parasitic microorganisms (the causative agents of disease) [5, 6]. Although he did not refute the germ theory of disease, he called for a broader-scale understanding of disease causation. May explained that humans are tied "pathologically" to their culture, and thus the cultural ecology of disease is concerned with the way in which human behavior interacts with environmental conditions to affect disease outcomes in susceptible people. He published the first maps of pathogens and their vectors, and followed in the French tradition of geography by emphasizing the interaction between the human and physical domains. His definition of disease was the alteration of living cells or tissues such that their survival in their environment is jeopardized. He spoke of three types of stimuli: inorganic, organic, and social/cultural. Inorganic stimuli are physical environmental factors related to the weather, soil, water, etc., while organic stimuli are related to the interplay of organisms such as mosquitoes and parasites. Social/cultural stimuli are those adaptive and coping strategies that humans have developed to deal with the inorganic and organic stimuli.

In the 1960s, Rene Dubos expressed in his works, including a book entitled *Mirage of Health* [7], that health is related to one's ability to adapt to evolutionary stimuli coming from the environment, rather than an absolute lack of disease. Furthermore, he notes that in the modern world, these stimuli often come more from the built environment than the physical environment. Of particular interest in *Mirage of Health* is Dubos' discussion of the treatment of disease versus the promotion of health. He felt that antibacterial drugs were only one part in decreasing mortality from infections, and that it was more important to adapt human behavior against being infected in the first place. He recognized that human diseases and problems in general are of great ecological complexity, and any treatment cannot be effective in the long run unless the physical and social conditions that are responsible for them are treated, causing them to re-emerge. In addition, he expressed that infectious diseases replace one another: when a healthy human ecology does not exist, a new disease will emerge even if one is eliminated. Around the same time, Ralph Audy spoke of a “medical ecology” in which human populations were studied in relation to the environment and the populations of other organisms that affect human health [8-11]. In doing so, one may derive the distribution of diseases over the world and their behavior in any one community as it is influenced by historical and social human factors, as well as the environment and geography. Melinda Meade advanced the disease ecology framework for medical geography, holding to Ralph Audy’s definition of health as the individual’s ability to rally from a wide range of “insults,” being physical, psychological, social, chemical and infectious. Meade’s work synthesized that of May, Dubos, Audy and Pavlovsky, adding her own conceptualization that integrated May’s disease ecology framework more fully into geography. Meade established the “triangle of human ecology” which considers the population, behavioral, and environmental factors which interact and contribute to a specific disease outcome [12] and establish an overall state of health, and broadened the perspective by applying the disease ecology framework to chronic and degenerative diseases as well as infectious ones. Whether a disease is vectored or non-vectored, medical geography can help build hypotheses related to mechanisms and modes of transmission.

Population characteristics which may be considered are biological features which may increase or decrease a person’s susceptibility to a certain disease. This includes characteristics related to a person’s age, gender, or genetic makeup, for example. Behavioral factors to be considered relate to one’s social, cultural and even technological practices. Modes of transportation, time spent outdoors in the evenings, and use of bed nets are all behavioral characteristics which may affect malaria transmission, for example. In Meade's triangle, "environment" is considered in the broader sense, meaning an individual's habitat which may be social, built or natural (pertaining to the physical environment). Aspects of a person's environment may include, for example, the population density of their city or village, the socio-economic status of their neighborhood, or the amount and type of natural vegetation and fauna (which includes arthropods and microbes) by which they are surrounded.

An understanding of human behaviors is particularly important as humans not only dominate, but also consciously manipulate ecosystems, creating disturbances. Such disturbances result in the creation and/or modification of the environmental conditions and exposure patterns that result in spatial patterns of disease. They may result from behaviors such as deforestation, cultivation, irrigation, animal husbandry, and patterns of settlement, migration, transportation and trade. Audy specifically spoke of the consequences of such human behaviors on vector-borne diseases, as a domesticated landscape means the simplification of original ecosystems, leading to greater densities of fewer species and thus increased vector foci [9]. In general, any significant stress on the dynamic equilibrium between population, society and environment can produce domino-like effects on any part of the equilibrium. When combined with the academic realm of political economy, cultural ecology theory becomes known as "political ecology". This subfield is different from cultural ecology in that it explicitly focuses on the global economy as a major contributor to local cultures and ecologies. Thus, while political ecologists also study human-environment interactions, they specifically study the relationships between political, economic and social institutions with environmental issues and changes. While these topics highlight the importance of global and regional scale political and economic phenomena on local disease outcomes, they would also fall under the "behavior" rubric of Meade's triangle.

Whether examined politically or not, any human undertaking that changes the relationship between humans and their environment must be viewed in an ecological framework, as they lead to certain places being more vulnerable to disease than others. "Vulnerable places" are ones in which health disadvantages can arise from a combination of housing quality, employment opportunities, quality of health amenities and services, and the overall ecological state of a place, for example. As described above, disease ecologists do not view the environment in narrow physical terms, but rather consider elements such as lead and arsenic in pottery and piping as well as water, physical geography such as water bodies and climate zones, urbanized environments with both good and bad effects on health, and the policies, societies, and history which affect these environments. Being able to define these vulnerable places and delineate where they are on a map is of great importance to public health workers and epidemiologists.

Analytical Framework: Spatial Epidemiology

In contrast to medical geography which has maintained an ecological perspective in the study of disease patterns, the field of epidemiology has been primarily focused on study design and analytical methods, investigating disease etiology by seeking causal effects of specific agents or exposures. For medical geographers, then, epidemiology provides a valuable source of methods that can be incorporated to study health problems [13]. In his 2000 update on the spatial aspects of epidemiology and their interface with medical geography, Glass recommended a more active interaction between the two fields in light of recently created analytic methods in geography, such as those which identify disease clusters and those which enable estimation of outcomes in places where measurements have not been gathered [13].

In recent years, the interaction between the fields of epidemiology and medical geography has become known as spatial epidemiology, a framework in which geographically-referenced health or disease data is described and analyzed. In 2005, Ostfeld et al. described spatial epidemiology as the principal discipline for studying spatial heterogeneity of infectious diseases [14]. Study of the factors which affect the spatial distribution of disease agents, vectors, hosts, and sometimes reservoirs and the probability that these components will interact with one another are fundamental to spatial epidemiology. Major approaches to spatial epidemiology include disease mapping, surveillance and disease cluster analysis, and geographic correlation studies [15], all of which are incorporated into the research presented in the following chapters.

The spatial analysis of pathological factors, including vectors, reservoirs, and hosts, and their relationships to physical and cultural environments is fundamental to the study of the geography of disease. Cromley (2003) describes that while the mapping of the spatial distribution of disease and the geography of environmental risk has been common throughout history, the development of geographic information systems (GIS) has enabled the integration and analysis of spatially referenced data in ways not previously possible, and has therefore proven to be an exceptionally powerful tool in the field of medical geography, enabling the exploration of complex and locally variable ecological systems associated with vector-borne infectious diseases in particular [16].

The use of GIS in conjunction with spatial epidemiological methods is central to this study.

Background

Geographic, Political, and Socio-economic Context of the DRC

The DRC is located in central Africa, spanning between 5 degrees north and 13 degrees south of the equator. Its land area is 2,345,409 square kilometers, surrounded by the Republic of Congo, Angola, Central African Republic, Sudan, Uganda, Rwanda, Burundi, Tanzania, and Zambia (see Figure 1.1). In the center of the DRC lies a basin which covers 48 percent of the country's land area and whose average altitude is 350 meters. This basin is covered by a dense forest with several extensive marshes. A series of plateaus spreads out from the basin all the way to the bordering countries, with the exception of the eastern part of the country where mountains surpass 1,000 meters in altitude. A hot and humid climate dominates the majority of the DRC's land area, and in the basin, rainfall measurements may reach up to 2,000 millimeters annually [17]. As for its hydrographic characteristics, the DRC is essentially comprised of the Congo river basin, stretching 4,700 km and spanning from the east to the west of the country, emptying into the Atlantic ocean in the west. The Congo has a constant flow, as it is fed by rivers on both sides of the equator. With many navigable tributaries, it offers vast opportunity for transportation. Influenced by the topography, climate, and hydrography, the soil also offers varied mineral and agricultural potential.

The Congolese population has risen rapidly since the late 1950s, increasing from 13.5 million in 1958 to 64.4 million in 2007 according to the U.S. Census Bureau [18], of which nearly 7.9 million are in the city of Kinshasa, the country's capital. The population is very young overall, with nearly 50 percent of the population aged less than 15 years, and less than 5 percent aged 60 years or more. According to a 1984 census, around 30 percent of the population lived in urban areas. However, with population migration since the 1980s, the actual proportion of the population living in urban areas should be between 40-45 percent. This is particularly due to armed conflict in the eastern zone, but also a general global rural-to-urban migration phenomenon whereby people have sought greater economic opportunities. In terms of ethnic composition, the DRC has some 40 ethnicities which can be categorized into principal groups. French is the official language; however, there are four national languages including Kikongo (spoken in the west), Lingala (Kinshasa and the northeast), Tshiluba (south), and Swahili (east) [17].

Since the country gained independence in 1960, the DRC has experienced an extremely unstable political situation. The period between 1960 and 1965 is marked by a relentless battle between political parties of tribal or regional basis for power, and was ended by a military coup d'état. From 1965-1997, a totalitarian regime dominated the country. Towards the end of this period, the Conference on National Sovereignty initiated a democratic process, whose deadlock pushed the country into extreme political and social disorder. This period was ended when the Alliance of Democratic Forces for the Liberation of the Congo (AFDL) was put into power. From 1997-2006, a new political order was sought against the AFDL by rebels who were backed by the armies of several border countries [17]. This conflict provoked general insecurity, numerous population displacements, and vast loss of human life. Since 2007, efforts have been undertaken to unify the country and bring peace back to the entire DRC. However, at the time of the 2007 DRC Demographic and Health Survey, zones of insecurity still existed, especially in the provinces of Nord-Kivu and Sud-Kivu.

After a period of prosperity at the end of colonization which lasted until the early 1970s, the Congolese economy experienced a crisis characterized by negative growth and monetary instability, stemming mainly from a drastic drop in production, budgetary cutbacks, and excessive debt. This situation led to a large degradation of the population's buying power. Poverty rose and society remains plagued by large disparities, with difficult access to social services (water, electricity, basic health care, schooling of children) for most households. This situation is worsened by the destruction and lack of maintenance of infrastructure, and the elevated number of persons who have been severely victimized by the ongoing conflict. Nonetheless, the end of the war brings hope to many, with institutions put into place after the elections and a favorable reaction of the economy to political advancements.

HIV

According to the UNAIDS 2009 AIDS Epidemic Update, HIV prevalence in the DRC is lower overall than most African countries, with a rate of 1.3 percent in adults aged 15-49 compared to 5.2 percent in sub-Saharan Africa [19]. However, as many as 94,000 people were estimated to be living with HIV/AIDS in the country in 2007, with women accounting for more than half of the adults estimated to be living with the virus

[19]. While HIV surveillance data in the DRC are limited, prevalence estimates have suggested much higher HIV rates in the eastern urban regions of the country, as well as some higher rates in certain rural areas. HIV prevalence in the DRC and many other African countries has often been estimated using blood samples drawn from pregnant women in antenatal clinics. While these data have been shown to provide proximate estimates of prevalence in the overall population of women and men, there are obvious limitations in that these samples do not include women who are either not pregnant or do not attend antenatal care clinics, nor do they include men. Furthermore, pregnant women have been found to be at increased risk for new HIV infection [20], and knowledge of HIV status may also reduce a woman's fertility choices. Another important limitation to sentinel surveillance systems for HIV is that almost no information is collected about the demographic or behavioral characteristics of the individual women, nor is the geographic location of their place of residence. While broad regional estimates of prevalence may be possible from this type of data, its limitations present important obstacles when attempting to analyze the socio-demographic, behavioral and geographic determinants of HIV infection.

Population and behavioral factors and HIV

While poverty has been shown to be significantly related to risky sexual outcomes [21-23], the urban poor have been found to be more likely to be infected with HIV than rural poor in African countries such as Kenya, due to a greater incidence of multiple sexual partnerships in impoverished urban areas. High unemployment, financial insecurity, unstable wages, and a social context which promotes prostitution may explain such disparities across economic classes [21].

It cannot be ignored that the DRC has been in and out of war since the beginning of the HIV epidemic. Persistent conflict has led to the displacement of large numbers of refugees within central African countries, eliminating much of what little employment existed and placing an even greater demand on minimal health infrastructure. The combination of chaos, poverty, population displacement, and sexual violence would seem to provide a likely setting for high rates of HIV transmission; however as discussed earlier, the prevalence of HIV in the DRC is low in comparison to most sub-Saharan African countries. Surprisingly, most studies to date have found little or no relationship between HIV and conflict. A 2007 Lancet review article [24] found no relationship between seroprevalence and conflict. In this review, 65 studies in seven countries compared HIV seroprevalence before and after conflict, and in conflict areas or refugee camps compared to peaceful neighboring areas. Another study found no effect of widespread rape on HIV prevalence. This study suggested that even when 15% of women were raped by assailants with high HIV prevalence, overall HIV seroprevalence would only increase by 0.023% [25]. However, two studies from the DRC suggest that violence is indeed associated with an increased HIV seroprevalence, with increased HIV prevalence found among refugees compared to the general population [26, 27].

Also relevant to this study is recent research indicating that in many African countries, HIV prevalence is higher in women than in men [19, 28]; MacPhail et al. 2002; Glynn et al. 2001; Laga et al. 2001; Zierler and Krieger 1997; Berkley et al 1990). While it has been argued that gender norms and expectations have contributed to increased risk and societal vulnerability to HIV [23], Glynn et al. (2001) found that this gender discrepancy in HIV existed in Kenyan and Zambian adults despite age at sexual debut being similar in both genders and number of sexual partners being higher in men in some cases [29]. Furthermore, they found that prevalence was very high even among women reporting only one lifetime sexual partner and few instances of sexual intercourse. Therefore, it is possible that these seemingly important behavioral factors may not explain the differences between HIV prevalence in men and women.

The gender disparity in HIV prevalence in many African countries is likely due in greater part, then, to biological factors such as the greater ease with which HIV is spread from men to women than vice versa, especially during cases of forced sex or first female intercourse. Greater prevalence of other sexually transmitted diseases such as the herpes simplex in women may further increase their biological risk of HIV infection due to genital ulcers damaging the epithelial barrier [29, 30]. It has been argued, however, that this vulnerability to infection is worsened for women by social inequalities [31, 32].

Environmental factors and HIV

Past studies of HIV transmission in African countries have shown prevalence to be associated with such geographic factors as population mobility, migrant labor routes, and proximity to urban high-transmission areas [33-40]. Whereas population centers in east Africa are highly interconnected and the HIV epidemic has grown rapidly in this region of the continent, the low prevalence of the virus in the Democratic Republic of the Congo before and after the emergence of the pandemic may be due to the difficulty in travel between major population centers in central Africa [41]. Studies that map the geographic distribution of populations at greater risk of infection, such as non-circumcised males and commercial sex workers, have been important to our understanding spatial heterogeneity in rates of HIV prevalence [42, 43]. Significant correlations between HIV prevalence rates in South Africa and proximity of homesteads to primary or secondary roads have also been found using HIV data obtained from antenatal care clinics [44]. Unfortunately, the lack of precise spatially-referenced data in many African countries often hinders detailed geographic or ecological studies of HIV prevalence.

Malaria

The malaria disease agent is a protozoan (parasite) called *plasmodium*, of which there are four types: *plasmodium falciparum, plasmodium vivax, plasmodium malariae,* and *plasmodium ovale*. *Plasmodium falciparum* is the most dangerous form of malaria and the primary parasite in the DRC [44], with at least a ten percent mortality rate and the unique symptoms of disorientation, shock, coma, and kidney failure [12]. The disease agent is directly transmitted to humans via several species of *Anopheles* mosquitoes. Once a human is infected with the parasite, infection spreads to the liver and then into the bloodstream, where it can infect subsequent *Anopheles* vectors, thereby continuing the transmission cycle. *In utero* transmission from mother to child may also occur.

While 66 species have been documented in the DRC, the primary *Anopheles* vectors in Central Africa are *An. gambiae, An. funestus, An. moucheti,* and *An. nili,* with the first two being the most wide-spread and dominant [45]. *An. gambiae* accounts for most malaria transmission in the DRC, although *An. paludis* is also a locally-important vector in some parts. *An. gambiae* is known to bite outdoors in the early hours of the morning, and prefers sunlit water for breeding, while *An. Paludis* bites primarily in the middle of the night [46, 47]. These characteristics must be taken into consideration when assessing the human behaviors and physical ecology which may increase risk for malaria transmission.

Most symptomatic cases of malaria infection are manifest as uncomplicated fever, malaise, chills, anemia, and abdominal discomfort, and mortality is low if effective drugs are available. Drakeley et al. (2004) found in their Tanzania study that in the absence of effective antimalarial treatment, frequent or persistent subclinical malaria infection is in fact sufficient to maintain seropositivity (i.e. cause a person to have a positive reaction to a blood test). Once acquired, antibody responses to certain strains of malaria may persist for many years and even be lifelong [48]. However, when this is not the case and in the absence of effective drugs, vital organ dysfunction, unconsciousness, severe anemia, seizures, and hypoglycemia may result [49]. Children are most vulnerable to manifestation of symptoms and death [50, 51].

Malaria is the most serious vector-borne disease facing the world today, and is one of three principal causes of mortality in the Democratic Republic of the Congo (DRC) [52]. Accurate estimates of the epidemiology and burden of malaria are lacking, but the World Health Organization (WHO) estimates that in 2006, malaria caused 247 million clinical cases globally, killing nearly one million people, primarily children in sub-Saharan Africa [53]. Additionally, malaria morbidity contributes substantially to disease burden by chronically debilitating tens of millions with symptoms such as severe anemia [12]. In some endemic countries like the DRC, malaria accounts for up to 40 %

of public health expenditures and 30 to 50 % of hospital admissions [53]. The emergence of highly drug-resistant parasites [49, 54-57] underscores the need for prevention, and suites of preventive interventions have produced marked declines in malaria infections and mortality in several sub-Saharan African settings. In highly malarious countries like the DRC, efficient preventive efforts must be guided by understanding the geographic patterns of prevalence and the factors underlying these patterns.

Population and behavioral factors and malaria

Prevention of malaria transmission depends upon reducing numbers of and contact with malaria-bearing *Anopheles* mosquitoes as well as access to antimalarial drugs. Occupations which require working outside in areas which are highly suitable for malaria transmission may increase risk, especially if work is done in the early hours of the morning when *An. gambiae* feeds. The use of insecticide-treated nets for nighttime prevention of biting by mosquitoes is important, as well as indoor spraying to kill mosquitoes that may be on the walls ceilings of houses. Even in highland areas where it may be too cold for vectors to survive outside, inhabited houses may be warm enough to allow the vector to survive and the parasite to develop [58]. Unfortunately, poverty prevents not only governments from providing large-scale prevention programs, but also prevents families from being able to obtain the resources they need to protect their own households. Furthermore, there is an increased resistance of African species of *Anopheles* to the main insecticides such as DDT and pyrethroids and a lack of alternative insecticides [53]. The use of less expensive antimalarial drugs by impoverished families also increases parasite resistance. Therefore, wealth is an important factor that may contribute to differential prevalence of malaria infection across the country.

Human modification of the environment has created ever more favorable breeding conditions for *A. gambiae* in particular. Most importantly, forest clearing for agriculture has resulted in increased habitat in central African mountain valleys [59]. However, as emphasized by Packard (2008), armed conflicts have played a significant role in driving the resurgence and persistence of malaria in various parts of the world and may be more important in the DRC than forest clearing for agriculture. In the DRC, 45 percent of infant deaths are due to malaria as compared to 20 percent in the rest of Africa [60]. The mechanisms behind this relationship are manifold. Human populations have been dislocated from agricultural villages to more forested areas due to warfare. Health services and malaria control activities in conflict-ridden regions have also been weakened or destroyed. Poorer health care may lead to more deaths from malaria in these areas. Conflict and warfare have also destroyed the local ecology of many parts of the DRC, leaving agricultural fields untended and susceptible to collecting water in which mosquitoes may breed. Forest cover has also been destroyed in many areas by rebel groups who hide in parts of national forest reserves and cut down trees for firewood [61], thereby opening new areas up to sunlight which many species of *Anopheles* prefer for laying their eggs. Refugee camps may also be over-crowded areas with swampy and poor drainage as well as a lack of adequate medical resources. In camps located within mosquito flight range of forest, a high density of human hosts for the pathogen may make these risky areas for malaria transmission.

Environmental factors and malaria

The epidemiology of malaria is intimately tied to the ecology of its mosquito vectors. Thus, the ecological conditions that lead to higher malaria transmission in certain geographic areas than others are those conditions which support *Anopheles* breeding (such as wet and sunlit conditions) [62] and denser gathering of susceptible and infected people within flight range of these *Anopheles*. These may be both physical environmental factors and those which are related to the built environment.

Altitude has often been used as a proxy for malaria transmission, with highlands limited for transmission by their low temperatures [48, 62, 63]. However, even the highest elevations reached in the DRC would likely not inhibit the presence *Anopheles*. Vegetation, rainfall, and temperature data have also often been used to map malaria transmission and vector extent in African countries and regions [62, 64-70]. Deforestation is of particular concern in tropical areas, as deep tropical forests are a biological barrier for most of the primary malaria vectors in Central Africa (due to lack of sunlight), besides *A. nili* which can breed in shaded streams [45]. Therefore, deforestation may increase malaria transmission by creating suitable habitat for non-forest vector breeding, while a slight reduction may be seen in areas where *A. nili* is the main vector.

In terms of the built environment, urbanization has until recently been considered a limiting factor in malaria transmission, with reduced vector breeding habitat [59]. However, poor housing conditions, lack of plumbing, and poor drainage systems in many African countries such as the DRC provide surface water for vector breeding along with high potential for human contact [71-73]. As described above, the use of treated bed nets and larviciding has been recommended for the prevention of malaria transmission [72], but the cost of these measures is high. Furthermore, what has been proven to work in rural areas may not apply in the context of urban environments.

Anemia

Since 1985, global prevalence estimates for anemia have risen from 15 to 60-80% [74] and 40-80% of African women are estimated to be anemic (having a hemoglobin level of less than 11 g/dl in the blood) [75, 76]. In 1968, the World Health Organization defined nutritional anemia as "a condition in which the hemoglobin content of the blood is lower than normal as a result of a deficiency of one or more essential nutrients, regardless of the cause of such deficiency" [74]. Common deficiencies leading to anemia are iron and vitamin B-12 deficiencies, and major symptoms include fatigue, weakness, fainting, chest pain, and even heart attacks in severe cases. In women, anemia is associated with increased risk for maternal morbidity and mortality and lower productivity, and pregnant women are furthermore at higher risk for anemia. Maternal anemia may also lead to higher risks for premature births, perinatal and neonatal death, and low birth weight [75].

Population and behavioral factors and anemia

Hunger is common in Sub-Saharan Africa, with crop yields barely having increased since the 1970s. Anemia is but one of many manifestations of this chronic and ongoing problem [77]. Likewise, poor diet and nutrition are but one of many factors contributing to high prevalence of anemia in Sub-Saharan African countries like the

DRC. Other than nutritional deficiencies, the presence of malaria, HIV, intestinal parasites such as hookworm, schistosomes, other infectious diseases, and inherited anemia contribute to prevalence of anemia found in Sub-Saharan African populations [78-80]. Thus, although successful iron supplementation might eradicate anemia in countries without endemic malaria, a highly-malarious country like the DRC requires a more complex response to its anemia problem. Guyatt and Snow (2001) found that 26% of cases of anemia are due to malaria [78]. Ngnie-Teta et al. (2007) examined determinants of anemia at the individual, family, and community levels in Benin and Mali, finding diarrheal illness to be positively related with anemia [75, 76], while wealth and bed net usage were negatively associated with anemia (these authors did not have data on malaria infection). In a later paper, the same authors also found that women who spent their childhood in rural settings and those with low body mass indexes were more likely to be moderately-to-severely anemic [75].

Environmental factors and anemia

While much research has been conducted that relates to the epidemiology of anemia in Africa addressing relationships with malaria, HIV, and socio-demographic factors, little has been done regarding environmental, or habitat, risk factors. In his 1965 *Ecology of Malnutrition in West Africa*, Jacques May described the severe consequences of improper nutrition, including both hindered growth of individuals and that of national economies. While May’s work depicts the relationship between food supply, nutrition, geography, and tradition, his work focuses primarily on modes of combatting malnutrition and does not examine any potential causative relationships between habitat type and anemia specifically. Ngnie-Teta et al. (2007, 2009) conducted multilevel analyses that considered community-level effects on anemia, their study focused on population and behavioral factors available from a survey, and did not explicitly address the environment or spatial relationships. Consideration of these factors may be of particular importance in a country like the DRC where much of the agricultural land has been destroyed or abandoned in areas with high levels of conflict. Access to urban areas is also key under these circumstances, if families must obtain their food from towns or cities. This area represents a major gap in anemia-related literature which is addressed in the chapter on anemia.

Research Design

Demographic Health Surveys

MEASURE DHS (http://www.measuredhs.com) is run by Macro International, a research and evaluation company based in Maryland and collaborator on the larger project with which this research is associated. DHS's help to provide accurate information on population, health, and nutrition in developing countries via large representative population-based surveys, and also include the taking of blood samples for the surveillance of HIV since 2001.

The DRC DHS was based upon a stratified sampling design, with two stages in the major cities, and three stages in the villages and rural areas. With the exception of Kinshasa, each province was subdivided into three strata: major cities, towns, and rural areas. In total, 34 sampling strata were created within the eleven provinces. The basis of the sampling was a complete list of neighborhoods in major cities, and chiefdoms in the rural domain. This list of neighborhoods, towns, and chiefdoms was established for the 1984 DRC census, and has been updated in 2001 and 2005 for other surveys. In major cities, the first step involved selecting neighborhoods and the second step was to choose households from within these neighborhoods. In rural areas and cities, the first step was to select towns or chiefdoms, and the second step was to choose villages from within chiefdoms or neighborhoods from towns. Finally, households were chosen from within these villages and neighborhoods.

The geographic coordinates of the final 300 villages and neighborhoods (referred to throughout the following chapters as “communities” or “household clusters”) were retained, containing 9,000 households. No cluster contained more than 500 households, and 41 percent of households were in urban areas, corresponding with the percent of the population thought to live in urban areas overall. Of these 9,000 households, 99.3% were successfully identified and interviewed. This included 4,757 men aged 15-59 years, all of whom were tested for infection with HIV, as well as 9,995 women aged 15-49 years, half of whom were tested for HIV and anemia. The blood spots for these persons were made available and were tested for malaria by collaborators in the UNC-Chapel Hill Department of Epidemiology. By leveraging the DHS infrastructure, population-based data for malaria was obtained using leftover dried blood spots from the 2007 DRC DHS and molecular diagnostics.

Mapping of Prevalence in the DRC

National and sub-national HIV and malaria prevalence was computed using sample weights from the 2007 DRC DHS (prevalence of anemia was computed for the female population only). Using the geographic coordinates of the 300 household clusters, prevalence maps were produced for each disease. The purpose of these maps was for both visual and analytical purposes. Not only are they used to describe spatial patterns of prevalence and determine areas most in need of intervention, but prevalence values within certain distances from household clusters are determined from these maps and included in select risk models. This was done by creating smoothed surfaces of prevalence using inverse distance weighting (IDW) in the GIS software ArcGIS 9.3 (ESRI, Redlands CA). Creating a smoothed map is advantageous to simply mapping prevalence in each of the 300 clusters, as it allows for estimation of prevalence in areas between the clusters which were not sampled by the DHS. IDW is an appropriate interpolation technique for predicting prevalence in unmeasured locations because it places maximum influence on only the closest points to these locations. This is important when considering a study area as large as the entire DRC. Compared to other spatial interpolation techniques which eliminate high and low values, inverse distance weighting maintains the entire probability distribution of prevalence values, making it appropriate for active surveillance data.

Multivariate Statistical Methods

Initially, prevalence maps of each disease were overlaid with population and environmental ecosystem variables hypothesized to be related to them. These variables were then entered into hierarchical, or multi-level logistic regression models for each disease, in which individual-level factors are considered in conjunction with community-level, or habitat, factors. The dichotomous outcome variables were the presence or absence of each disease. For HIV, this was determined as part of the original DHS survey. For malaria, presence of any species of malaria parasite from real-time PCR testing has been determined by collaborators on this project. For anemia, hemoglobin levels which were recorded for women in the DHS survey were used to determine presence of anemia according to WHO standards. In addition, linear multilevel modeling was performed for anemia, with hemoglobin level as a continuous dependent variable. For HIV, separate models were also be carried out for men and women, as individual-level risk factors for a sexually-transmitted disease are expected to differ by gender. For anemia, only data for the female respondents was available; however, the greater prevalence of anemia in pregnant women merited additional models for pregnant women alone.

Multilevel analysis is the most appropriate method of analysis for these data because its nested structure requires simultaneous examination of group- and individual-level variables [81, 82]. Additionally, the multilevel approach produces correct standard errors and parameter estimates if outcomes for individuals within groups are correlated (and thus the standard regression assumption of independence of observations is violated). Conceptually, a multilevel model is a two-step set of equations, one explaining variation at the individual level, and the other explaining variation at the group level. In the first stage or level 1, a regression model is defined for individuals within each higher-level group (in this case, for each of the DHS clusters):

$$Y_{ij} = b_{0j} + b_{1j}I_{ij} + \varepsilon_{ij}$$

where Y_{ij} = the disease outcome variable for i^{th} individual in j^{th} cluster, I_{ij}= individual level variable for i^{th} individual in j^{th} cluster, b_{0j} = the cluster-specific intercept and b_{1j} = the cluster-specific parameter estimate of the individual level variable. In the second stage or level 2, each of the cluster-specific regression coefficients defined in the first equation are modeled as a function of cluster level variables:

$$b_{0j} = \gamma_{00} + \gamma_{01}G_j + U_{0j}$$

$$b_{1j} = \gamma_{10} + \gamma_{11}G_j + U_{1j}$$

where G_j = the cluster-level variable, γ_{00} = the common intercept across clusters, γ_{01} = the effect of the cluster-level predictor on the cluster-specific intercepts, γ_{10} = the common slope of the individual level variable across groups and γ_{11} = the effect of the cluster-level predictor on the cluster-specific slopes [83].

Bivariate correlations between all variables were tested prior to entering variables into the model in order to avoid multicollinearity. Models were be built in SAS v. 9.2 (SAS Institute, Cary, N.C.) using PROC GENMOD which uses the maximum likelihood method for model estimation and allows for the specification of multiple levels of analysis for logistic regression. All individual and community-level variables for which data is available and for which a relationship is hypothesized to exist with the disease outcomes of interest are diagrammed below. However, parsimonious models were be chosen using Akaike's Information Criterion (AIC) which makes a tradeoff between the precision and complexity of each model by taking into account both the log likelihood of each model and the number of parameters (lower AIC values are favored).

Conclusion

By examining the spatial patterns and population and ecological drivers of HIV, malaria, and anemia in the DRC, the research presented in the following chapters demonstrates the feasibility of using population-based behavioral and molecular surveillance data in conjunction with geographic methods to study sexually-transmitted, vector-borne, and chronic nutritional diseases in a developing country. For most diseases in developing countries, data quality is poor. Incidence and prevalence data are computed from non-randomly selected sentinel populations and are highly subject to investigator bias.

The use of active surveillance rather than passive reporting data is an important contribution of this research, as it enables the use of less biased prevalence data to better compute the disease burden of three important diseases in the DRC. It may also demonstrate the need for surveillance systems for infectious diseases to be implemented or improved in other developing countries. Current estimates for the prevalence of each disease vary widely, and more accurate estimates of disease burden are necessary for allocating health resources. Good spatial information and analyses are also necessary in order for the DRC government to focus its control efforts against these important health problems.

Figures

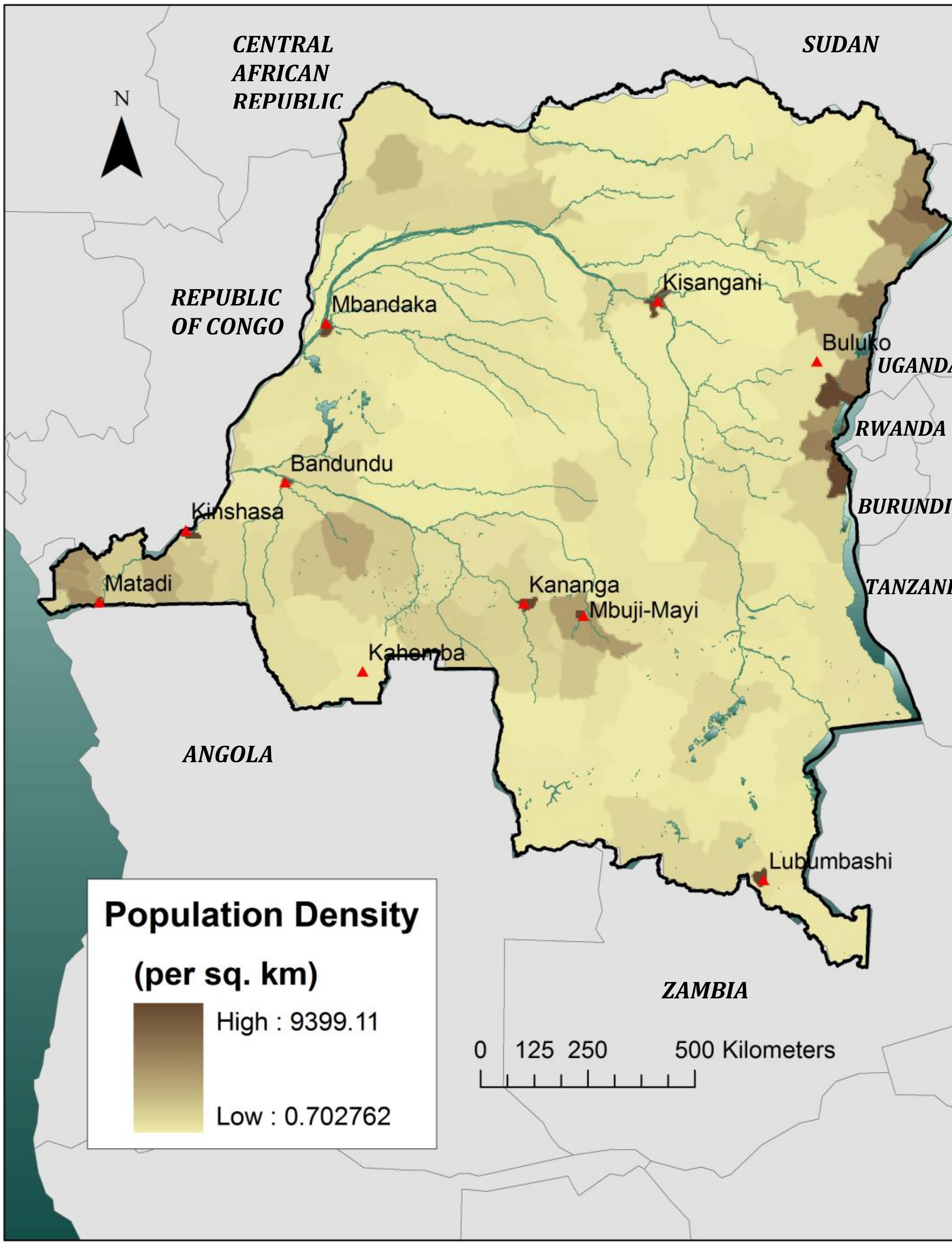

Figure 1.1. Map of study area.

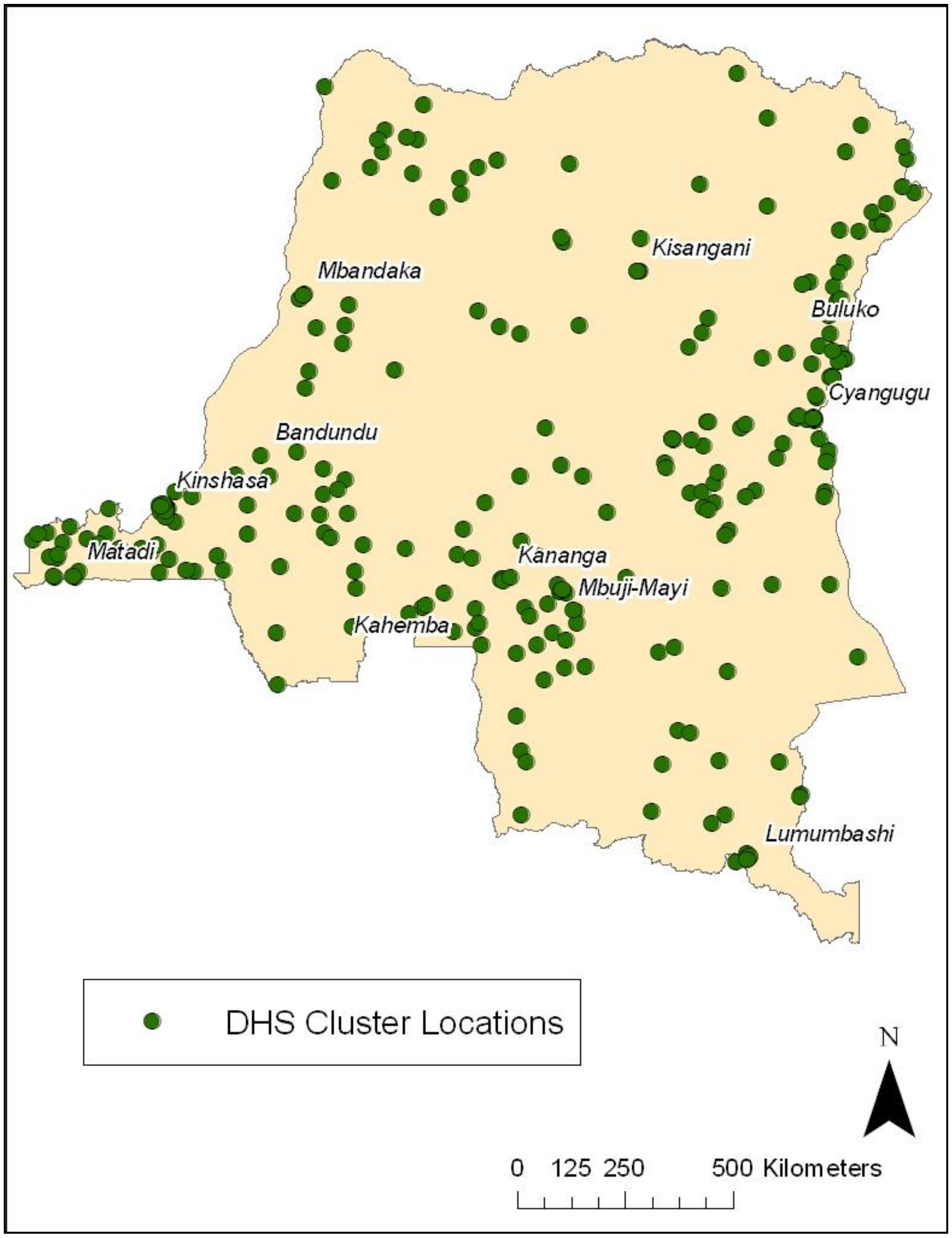

Figure 1.2. Cluster locations for the 2007 DRC DHS survey. N=300.

SPATIAL EPIDEMIOLOGY AND DISEASE ECOLOGY OF HIV IN THE DEMOCRATIC REPUBLIC OF THE CONGO

Background

According to the UNAIDS *2009 AIDS Epidemic Update*, HIV prevalence in the Democratic Republic of the Congo (DRC) is lower overall than most African countries, with a rate of 1.3 percent in adults aged 15-49 compared to 5.2 percent in sub-Saharan Africa[19]. However, as many as 94,000 people were estimated to be living with HIV/AIDS in the country in 2007, with women accounting for more than half of the adults estimated to be living with the virus [19]. While HIV surveillance data in the DRC are limited, prevalence estimates have suggested much higher HIV rates in the eastern urban regions of the country [84], as well as some higher rates in certain rural areas.

Spatial Patterns and Geographic Drivers of HIV Risk

Poverty and economic deprivation have been shown to be complex yet important factors related to HIV transmission, making the study of HIV and AIDS in Africa an area in need of collaboration between natural and social scientists, including geographers [31]. Geography plays an important role in HIV transmission in the Democratic Republic of the Congo and other central African countries. While population centers in east Africa are highly interconnected and the HIV epidemic has grown rapidly in this region of the continent, the low prevalence of the virus in the Democratic Republic of the Congo before and after the emergence of the pandemic may be a reflection of the difficulty in travel between major population centers in central Africa [41]. Although geographic factors are important indicators of HIV prevalence, there is little current research that uses spatial analytical techniques to study local heterogeneity of HIV prevalence rates in Africa. A 2002 review by Tanser and Leseuer of the application of Geographic Information Systems (GIS) to public health problems in Africa found only one study which incorporated GIS in analyzing factors related to HIV prevalence. This study [85] found a significant correlation between HIV prevalence rates in South Africa and proximity of homesteads to primary or secondary roads using HIV data obtained from antenatal care clinics. Since 2002, the number of national-level studies using spatial analytical methodologies to study HIV prevalence has increased [32, 86-89]; however, to date no detailed spatial studies have been published on HIV prevalence in the DRC.

Past studies of HIV transmission in African countries have shown prevalence to be associated with geographic factors including population mobility, migrant labor routes, and proximity to urban high-transmission areas [33, 35-39, 90]. Studies that map the geographic distribution of populations at greater risk of infection, such as non-circumcised males and commercial sex workers, have been important to our understanding spatial heterogeneity in rates of HIV prevalence [42, 43]. Unfortunately, the lack of precise spatially-referenced data in many African countries often hinders detailed geographic studies of HIV prevalence.

Socio-behavioral Factors and HIV Risk

While poverty has been shown to be significantly related to risky sexual outcomes [21-23], the urban poor have been found to be more likely to be infected with HIV than rural poor in African countries such as Kenya, due to a greater incidence of multiple sexual partnerships in impoverished urban areas. High unemployment, financial insecurity, unstable wages, and a social context which promotes prostitution may explain such disparities across economic classes [21].

It cannot be ignored that the DRC has been in and out of war since the beginning of the HIV epidemic. Persistent conflict has led to the displacement of large numbers of refugees within central African countries, eliminating much of what little employment existed and placing an even greater demand on minimal health infrastructure. The combination of chaos, poverty, population displacement, and sexual violence would seem to provide a likely setting for high rates of HIV transmission; however, we have discussed that the prevalence of HIV in the DRC is low in comparison to most sub-Saharan African countries. Surprisingly, most studies to date have found little or no relationship between HIV and conflict. A 2007 Lancet review article [24] found no relationship between seroprevalence and conflict. In this review, 65 studies in seven countries compared HIV seroprevalence before and after conflict, and in conflict areas or refugee camps compared to peaceful neighboring areas. Another study found no effect of widespread rape on HIV prevalence. This study suggested that even when 15% of women were raped by assailants with high HIV prevalence, overall HIV seroprevalence would only increase by 0.023% [25]. However, two studies from the DRC suggest that violence is indeed associated with an increased HIV seroprevalence, with increased HIV prevalence found among refugees compared to the general population [26, 27].

Also relevant to this study is recent research indicating that in many African countries, HIV prevalence is higher in women than in men [19, 28-30, 91-93]. While it has been argued that gender norms and expectations have contributed to increased risk and societal vulnerability to HIV [23], Glynn et al. (2001) found that the gender discrepancy in HIV existed in Kenyan and Zambian adults despite age at sexual debut being similar in both genders and number of sexual partners being higher in men in some cases [29]. Furthermore, they found that prevalence was very high even among women reporting only one lifetime sexual partner and few instances of sexual intercourse. Therefore, it is possible that these seemingly important behavioral factors may not explain the differences between HIV prevalence in men and women.

The gender disparity in HIV prevalence in many African countries is likely due in greater part, then, to biological factors such as the greater ease with which HIV is spread from men to women than vice versa, especially during cases of forced sex or first female intercourse. Greater prevalence of other sexually transmitted diseases such as the herpes simplex in women may further increase their biological risk of HIV infection [29, 30]. It has been argued, however, that this vulnerability to infection is worsened for women by social inequalities [31, 32].

Gaps in Recent Literature

HIV prevalence in the DRC and many other African countries has often been estimated using blood samples drawn from pregnant women in antenatal clinics. While these data have been shown to provide proximate estimates of prevalence in the overall population of women and men [94], there are obvious limitations in that these samples exclude women who are either not pregnant or do not attend antenatal care clinics, and also exclude men. Furthermore, pregnant women have been found to be at increased risk for new HIV infection [20], and knowledge of HIV status may also reduce a woman's fertility choices. Another important limitation to sentinel surveillance systems for HIV is that almost no information is collected about the demographic or behavioral characteristics of the individual women, nor is the geographic location of their place of residence. While broad regional estimates of prevalence may be possible from this type of data, its limitations present important obstacles when attempting to analyze the socio-demographic, behavioral and geographic determinants of HIV infection.

The current study used a 2007 population-based household survey of the DRC to examine the individual and community-level factors that increase an individual's risk for HIV infection. Geographic coordinates of the survey communities were used to map prevalence of HIV infection in the country and compute rates for areas surrounding an individual's community, as well as to explore a number of additional geographic variables thought to be related to HIV risk. These were explored in conjunction with several demographic and behavioral characteristics extracted from the survey. Improved surveillance systems in the DRC and other African countries have the potential to greatly enhance understanding of the determinants of HIV infection as well as the spatial patterns of prevalence, therefore contributing to improved allocation of public health resources in the future.

Methods

The 2007 DRC Demographic Health Surveillance (DHS) survey was a population-based, nationally representative survey linking individual HIV test results to that individual's responses to an array of socio-demographic and behavioral characteristics. With the exception of Kinshasa which is the country's densely-populated capital, each of the country's eleven provinces was divided into three strata: major cities, towns, and rural areas, with a total of 34 enumeration areas created. The basis for these areas was the 1984 DRC census which contained a complete list of neighborhoods in the major cities and towns, along with territories and chiefdoms in rural areas. Neighborhoods were selected from cities and towns, and villages were selected from territories or chiefdoms. Within these neighborhoods or villages, households were then selected. The population sampling scheme did not exclude those areas heavily plagued by conflict; however, it must be considered that conflict does affect short-term migration patterns and the sampling scheme could not account for changes in the underlying population distribution since the 1984 census.

In total, nine thousand households in 300 communities (neighborhoods or villages) were targeted for the 2007 DRC. DHS (3,690 in urban areas and 5,310 in rural areas). Of these 9,000 households, 99.3% were successfully identified and interviewed. This included 4,757 men aged 15-49 years, all of whom were tested for infection with HIV, as well as 9,995 women aged 15-49 years, half of whom were tested for HIV. Of the nearly 9,000 individuals tested for HIV, 1.3 % were found to be HIV-positive, with 0.9 % of men testing positive and nearly double the proportion in women at 1.6 [17].

Geographic coordinates of the 300 community centroids (mean centers) were also collected. For privacy purposes, these geographic coordinates were randomly displaced by 5 km in rural areas and 2 km in urban areas. The number of respondents in each community ranged from 14 to 53 individuals, with an average of 30 individuals per community. The proportion of HIV-positive individuals was computed for men, women, and both genders combined for each community taking the sampling weights of the survey into account. A visualization of the spatial patterns of HIV prevalence in the DRC was created by computing smoothed surfaces of prevalence for men, women, and both genders combined using inverse distance weighting (IDW) in the GIS software ArcGIS 9.3 (ESRI, Redlands CA). IDW uses nearby values to predict those in unmeasured locations. In this procedure, known values closest to the unmeasured location have greater influence on the interpolated values than those farther away. The estimated prevalence in a minimum of 2 and a maximum of 15 of the nearest communities were used to interpolate prevalence for each unmeasured 1 km-by-1 km cell in the resulting surfaces. As subsequently discussed in further detail, these smoothed surfaces were also used to create regional-level HIV prevalence predictor variables to be included in individual-level multivariate statistical models of HIV risk.

GIS layers for water bodies and roads were obtained from the Digital Chart of the World (DCW) (ESRI 1991) and used to compute the distance from the community centroid to the nearest primary or secondary road, nearest river or major water body, and nearest city in kilometers. These geographic indicators serve as measures of proximity to trade and migratory routes. It must be noted that while the DCW dataset is the most recent data available for determining the locations of roads, it is likely that in the face of economic collapse many of the roads have changed or disappeared since 1991.

A geographic database of armed conflict and refugee camp locational data was also compiled. The Armed Conflict Locational Event Dataset (ACLED) includes locations, dates, and additional characteristics of individual battle events in states affected with civil war [95]. The current dataset covers eight conflict areas in West and Central Africa including the DRC and surrounding countries from 1960 through 2006. We computed the number of conflict events within several distance buffers of the community centroids (10, 25, and 100 km), aggregating events temporally into two categories: (1) all events between 1960 and 2006, and (2) only those events occurring within ten years of the survey year (since 1997). The locations of current refugee camps in the DRC and all surrounding countries were also obtained from the United Nations Human Rights Council. The distance of a community centroid to a refugee camp was computed, as well as the density of camps within the three different distance buffers.

Additional geographic indicator variables computed for this study included the average HIV prevalence and population per square kilometer within 10-, 25- and 100-kilometer buffers around each survey community. These were computed using the interpolated surfaces described above and a DRC population density grid [96].

Both the female and male questionnaires asked detailed questions regarding socio-demographic characteristics, sexual activity and risk behaviors associated with HIV/AIDS. Items related to the age, gender, education, wealth, sexual behaviors and rural or urban residence of the respondents were extracted from the survey and examined in conjunction with the computed geographic indicator variables for their relationship to HIV risk in DRC individuals.

The socio-demographic, behavioral and geographic indicators described above were entered into three multivariate logistic regression models using Stata v. 10 (StataCorp LP, College Station TX), including separate models for men and women as well as a model for both genders combined. Correction for unobserved random errors at the community level was included as part of all three models. The dependent variable in all models was the individual's HIV status, and independent variables initially entered into the models included the respondent's age, gender (combined model only), education in single years, total number of lifetime sex partners, the distance of the respondent's community to the nearest road, water body, and city, the population density and HIV prevalence within the 10-, 25-, and 100-km buffers, the described conflict-related variables, and whether the respondent resides in a rural or urban community. Although the DHS survey provided many behavioral variables of interest, due to the low prevalence of HIV in both men and women in the DRC, only very simple statistical models with a small number of parameters could be used to predict risk for infection and the variables described above were considered to be the most important to enter into our models. The best-fitting model for the entire survey population as well as each gendered subset was selected using Akaike's Information Criterion (AIC), a goodness-of-fit statistic which favors parsimony by making a tradeoff between the precision and complexity of each model [97].

Results

Spatial Patterns in DRC HIV Prevalence

Figure 2.1 highlights the differences in the spatial patterns between male and female HIV prevalence in the DRC. While overall, the southwest and northeast regions of the country exhibit low HIV prevalence, areas of high prevalence are not identical for men and women. While there tends to be higher infection amongst women in the upper northeast corner of the country, this is an overall low-prevalence region for men. On the contrary, the southernmost tip of the country near Lubumbashi is an area of high prevalence for men, while similar high rates are not seen for women. Overall, heterogeneity in prevalence for the entire population, as well as for men and women separately, can be seen upon examination of these interpolated surfaces, with estimated rates as high as 30.5 percent in some areas as compared to the country's overall HIV prevalence of less than 2 percent. Such patterns indicate that some factor or set of factors is contributing to increased risk in certain parts of the country.

Multivariate Analyses

Five variables were retained in both the overall and female-only models, while only three variables were retained in the male-only model after selection using the AIC criterion (Table 2.1). In all models, individual HIV outcome is the dependent variable. No relationship was found in any of our models between HIV status and education, population density, urban versus rural place of residence, or conflict or refugee camp density. When men and women were considered together, age, the total number of lifetime sexual partners, and HIV prevalence within 25 kilometers of one's community were all positive predictors of HIV infection, while distance to a city was a negative predictor of infection. Men were also 71 percent less likely than women to be infected with HIV in the DRC.

Considered separately, the model results for both men and women indicate that an individual's total number of lifetime sexual partners as well as the HIV prevalence within 25 kilometers of his or her community were both positively associated with the probability of being infected with HIV, with the total number of lifetime sexual partners further showing a correlation of more than double the magnitude with women's HIV prevalence than men's. In women, age and distance to a river or water body were also positively associated with HIV infection, while distance to a city was negatively associated. In men, wealth was positively associated with HIV infection, while it was not an important factor in a woman's likelihood of being infected with HIV.

Discussion

This study has shown the importance of considering socio-demographic, behavioral and geographic factors in conjunction when determining individuals' risk for HIV infection in the DRC. Individual characteristics and sexual behaviors including age, gender, wealth and number of sexual partners are shown to be associated with increased risk for HIV infection. Most notably, our findings in the DRC are concurrent with recent literature highlighted in the background section of this paper citing that women are significantly more likely to become infected with HIV in many sub-Saharan African countries. We also noted in our results that the total number of lifetime sexual partners showed a correlation of more than double the magnitude with women's HIV prevalence than men's, supporting the literature discussed in the background section of this paper which suggests women may be biologically at greater risk for HIV infection than men. The significant positive relationship between age and HIV status in the women-only and overall models indicates that exposure time is important in terms of the likelihood of becoming infected with the virus. The greater significance and magnitude of the relationship with this variable in the women-only model may be related to more lifetime exposure to partner violence with increasing age [98]. Our results also indicate that wealth shows no relationship in our overall or women-only models; however, a significant positive relationship was found between wealth and men's HIV status, likely due to increased travel and extramarital sex for wealthier men [99, 100].

It is also notable that the prevalence of HIV within 25 kilometers of an individual's community is highly significant in all three models. The greater significance of the 25-kilometer buffer of HIV prevalence in comparison to the 100-km buffers is unsurprising in that people in the DRC are likely partaking in sexual intercourse with people who live closer to them rather than farther away. While small sample sizes within community household clusters may have led to exaggerated rates in certain areas, a particularly high HIV prevalence in one 25 km area is not likely to have a significant impact in a model in which persons are grouped into 300 clusters.

While living in an urban community itself is not a significant contributor to any of the three models, the negative association found with the geographic variable (distance to a city) in the overall and women-only models indicates that even individuals who do not live in urban areas may be exposed to higher risk for HIV infection if they live close to these urban areas and their associated high-risk sexual networks. It is not clear why this variable is not significant when men's HIV status is modeled alone, but its significance in the women-only and overall model indicates that access to or living in cities places DRC individuals at a greater risk for HIV transmission.

When controlling for other factors, the average population density near an individual's community and distance to roads are not shown to be significant, and the distance to the nearest river is shown to be positively associated with female HIV prevalence, which is contrary to the expected relationship. A large portion of the DRC is without primary or secondary roads, particularly in the central part of the country where the Congo River Basin lies. The terrain and climate of the basin present severe barriers to road construction, and persistent economic mismanagement and domestic conflict has led to serious underinvestment in transportation for many years, with only 2,250 km of paved roads, few of which remain in good condition [101]. In contrast, the DRC has thousands of kilometers of navigable waterways, and thus water transport has traditionally been the primary means of moving about the country. Therefore, it is surprising that the distance to a river is not negatively associated with HIV infection overall or in men, and furthermore that it is positively associated with HIV infection in women, as access to transportation routes would likely increase one's exposure to trade and migratory routes as well as facilitate access to high-risk urban areas. Further information about the navigability of the rivers in the DCW dataset would be necessary in order to fully assess this relationship. Understanding of women's role in trade, care-seeking behaviors, and use of rivers in escaping conflict areas may be important as well.

A finding meriting further study is the relationship between conflict and HIV prevalence in the DRC. While the current study found no relationship between proximity to or density of conflict events within several distances, either since 1960 or since 1997, or the proximity to or density of current refugee camps, patterns of migration between neighboring countries and high-conflict regions would be necessary in order to confirm our findings that conflict does not seem to affect HIV prevalence in the DRC. A limitation of the current study also lies in the fact that no information about personal experience of violence, especially rape, was available and should be addressed in order to confirm our findings. It is also possible that population movement from conflict plays an important role in the spatial distribution of HIV clades, or subtypes, found across the country [102], even if it does not affect overall HIV prevalence. This is a question that also merits further research.

The geographic pattern of prevalence estimated using the DHS survey results confirms the 2005 WHO estimates from antenatal clinic data that higher HIV prevalence exists in the eastern parts of the DRC. However, much more locally heterogeneous patterns are found in this study by using a population-based survey and spatial statistical techniques, and most importantly the differences in the locations of high-prevalence areas for men and women are highlighted. Having access to counseling, testing services and treatment facilities is essential for controlling HIV incidence. Understanding the geographic distribution of prevalence may be of great importance when determining where to locate these services. In particular, our study findings show that targeting interventions and sentinel surveillance sites may be most effective if they were to be implemented in the north eastern border of the Orientale province, the eastern border of

Kivu, and the southeast corner of Equateur. High rates in the southern portions of Kasai-Occidental and Kasai-Oriental as well as in the southern tip of the Katanga province near Lubumbashi should also be considered for sentinel surveillance.

Conclusion

To date, this is the largest study examining factors associated with increased risk for HIV infection in the DRC. A better understanding of the geographic distribution of HIV-infected populations is essential in assessing the magnitude of the epidemic in certain parts of the DRC and for allocating better treatment and support services to those who are infected. This study has exhibited the potential for using spatial methods in conjunction with population-based surveys in order to help predict HIV prevalence based on known socio-demographic, behavioral and geographic factors associated with risk. While the region of residence was included in the DHS survey, using region as a predictor in the analysis would not have allowed such a fine scale computation of geographic variables which were built into the GIS according to communities rather than large-scale regions. Modeling the spatial distribution of HIV prevalence at the local scale allows for the exploration of sub-regional heterogeneity in the DRC, an important step towards efficient intervention planning.

Tables

Table 2.1. Parameter estimates for the variables retained in the logistic regression models explaining individual HIV in men and women.

Parameter	Men	Women	Men and Women
Individual-Level Variables			
Age		0.042***	0.020*
Wealth index (1-5)	0.370**		
Total lifetime no. of sexual partners	0.016*	0.034*	0.015*
Male	N/A	N/A	-0.710**
Community-Level Variables			
Distance to a city (km)		-0.005***	-0.004***
Distance to a water body (km)		0.117*	
HIV prevalence within 25 km of community (%)	0.491***	0.370***	0.430***
Number of Observations	3325	3849	7174
Log Likelihood	-172.5	-309.3	-490.7
AIC	351.0	628.6	991.4

****=p<.001 **=p<.01 *=p<.05*

Figures

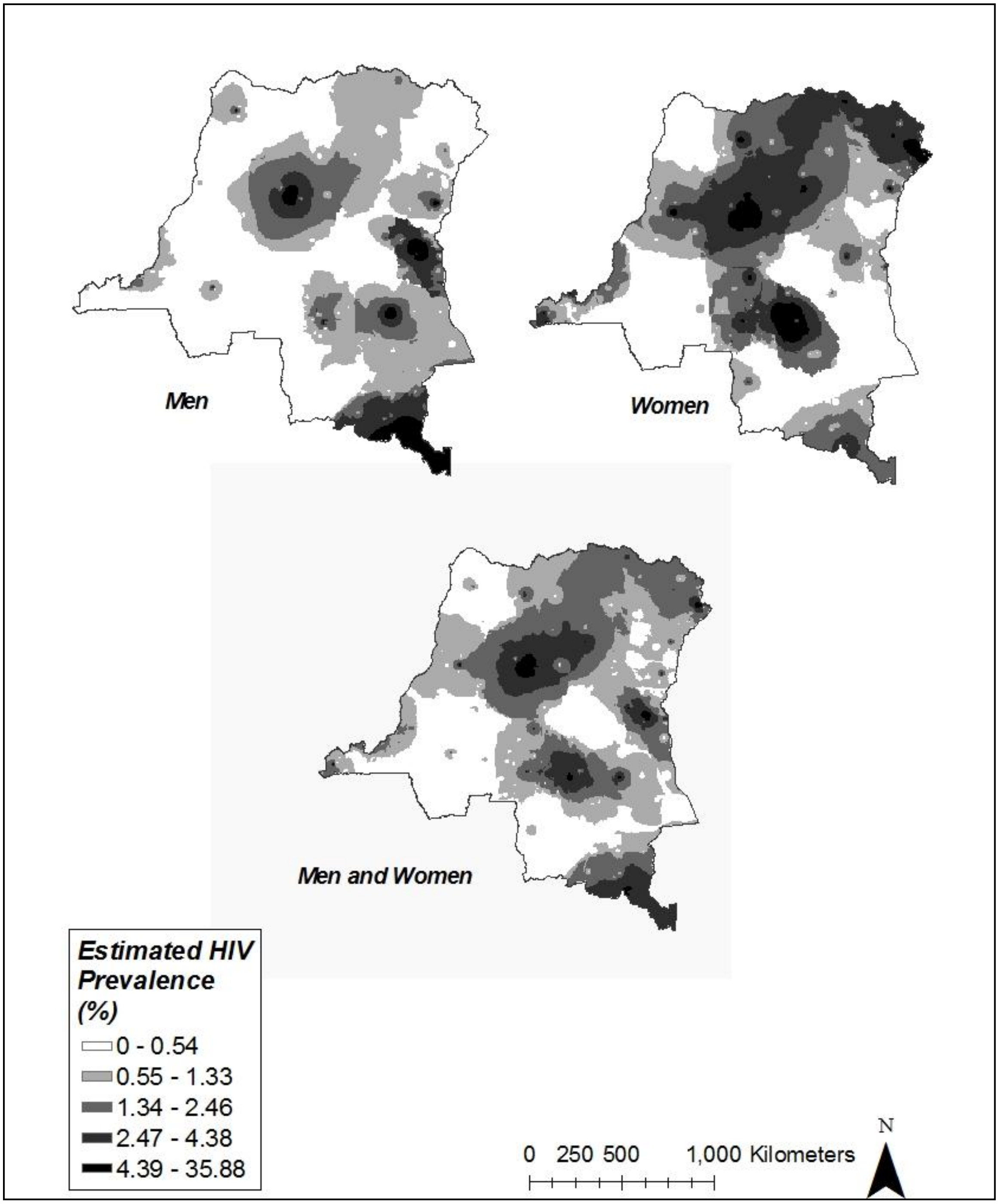

Figure 2.1. Interpolated surfaces of HIV prevalence in the DRC.

SEXUALLY-TRANSMITTED VERSUS VECTOR-BORNE DISEASE ECOLOGY: INTRODUCING THE PHYSICAL ENVIRONMENT

Health and the environment are inextricably linked. However, the term "environment" is broad, and may refer to either the physical or built environment, or both. In general, the physical environment refers to the living and non-living things which occur *naturally* on the earth's surface or in its atmosphere. It is a system in which living species interact with one another, and usually is thought of as a realm in which human intervention has not occurred in large amounts, although there are almost no "natural" systems on earth which humans have not influenced. The built environment, on the other hand, is generally thought of as the realm in which humans have had a strong influence. This would include, for example, permanent village settlements, urbanized areas, or cultivated areas. The social environment is part of the built environment as well, including support systems, health services, crowding, or isolation, for example.

As HIV is a virus which is transmitted directly from human to human via sexual activity, the physical environment has less to do with spatial patterns of prevalence than the built and social environments. The only way HIV can be transmitted from person A to person B is either directly, or via intermediate human hosts (See Figure 3.1). As was seen in the previous chapter, it was still important to study the relationship between one's habitat and risk for HIV; however, the environment being examined was primarily the built environment. The DRC provides somewhat of an exception with regard to the study of sexually-transmitted diseases in that water bodies such as rivers and tributaries are the main form of transportation in the country. Thus, proximity to a naturally-occurring water body was important to transmission of HIV. However, it was for humans' use of these water bodies and not the living matter which occurs in them that a significant relationship with disease transmission was found.

Unlike HIV, malaria is a disease which is strongly influenced by the natural environment, specifically for the living and non-living matter which exists within it and solely for humans' use of it. This is due to the fact that malaria is a vector-borne parasite which is transmitted not from humans to humans, but rather from humans to mosquitoes and then to humans again (See Figure 3.2). Thus, the promotion or inhibition of mosquito breeding and habitat establishment are extremely important when considering where malaria will occur in the physical environment. Factors which promote or inhibit mosquito breeding and habitat are discussed in the following chapter. Specific habitat requirements for each species of mosquito are different, including the amount of sun, organic matter, salt tolerance, depth and amount of flow of rivers and water bodies, presence of fish and other arthropods, vegetation to lay eggs on. Many specific needs determine what species can breed abundantly where, and changing a specific characteristic, not just exposure to sun but turbidity, or intersection of vegetation, can alter species location.

A study of the cultural ecology of malaria also differs from that of HIV in that it requires characterization of another species -- a vector -- in the disease system. Therefore, not only human population density matters, but the density of certain species of mosquito also matters. Each mosquito population has its own age structure and life table as well as feeding and egg-laying behaviors. The baseline population dynamics of the vectors must be established, as any change in infections may be due to alteration of the vector ecology, rather than human immunity.

Based upon understanding of the transmission cycles of these two very different infectious diseases, one can begin to formulate potential disease ecologies. While many of the built environment factors considered for HIV continue to be important in the consideration of malaria, a new challenge is to incorporate the complex and ever-changing physical environment. This is done in the following chapter.

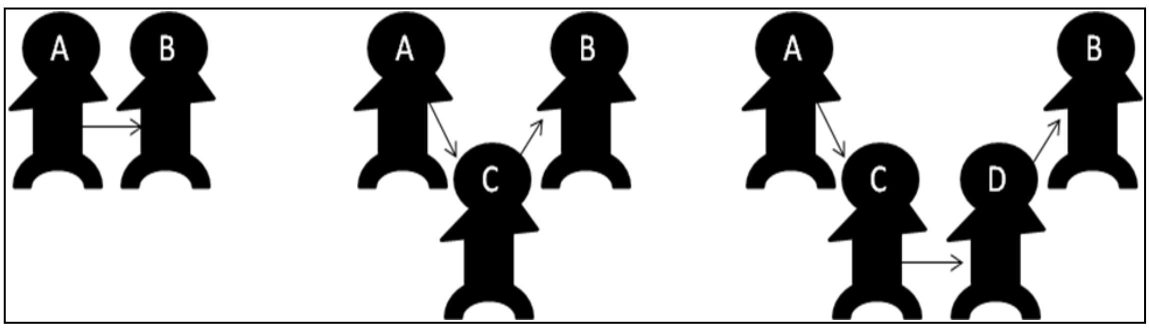

Figure 3.1. Transmission of HIV between two persons, A and B.

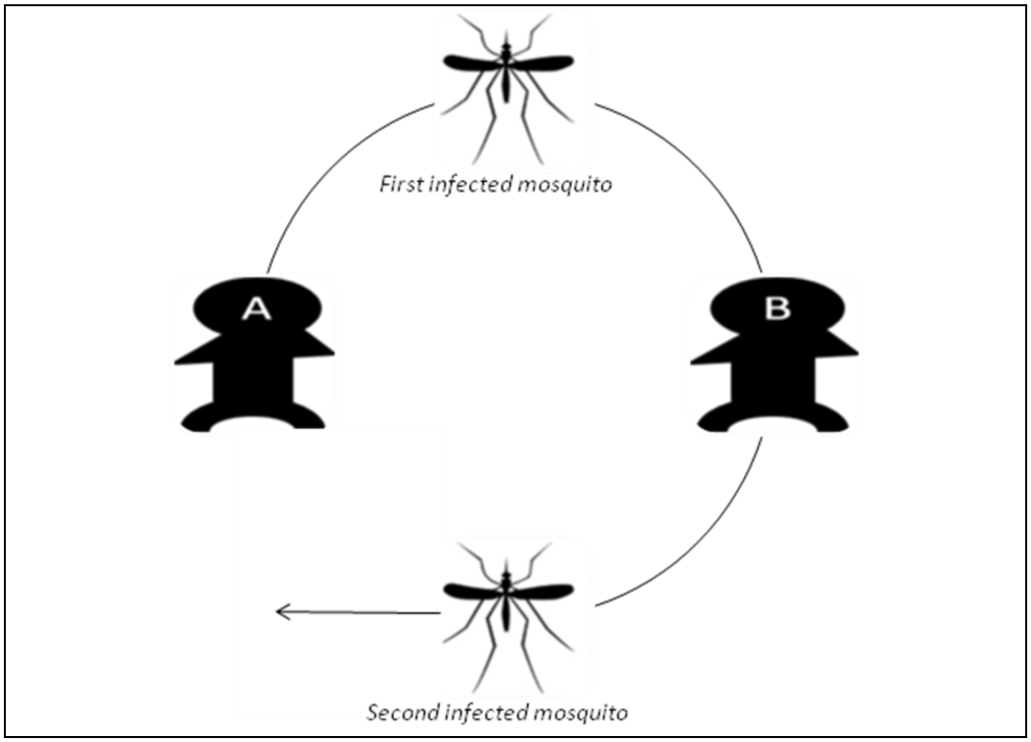

Figure 3.2. Transmission of malaria between two persons, A and B.

SPATIAL EPIDEMIOLOGY AND DISEASE ECOLOGY OF MALARIA IN THE DEMOCRATIC REPUBLIC OF THE CONGO

Background

Malaria is the vector-borne disease causing the most deaths in the world today, and is one of three principal causes of mortality in the Democratic Republic of the Congo (DRC)[52, 103]. The World Health Organization (WHO) estimates that in 2006, malaria caused 247 million clinical cases and killing nearly one million people globally -- primarily children in sub-Saharan Africa [51, 53]. The Roll Back Malaria program estimates even more global cases for 2006 at 300-500 million.[104]. Additionally, malaria morbidity contributes substantially to disease burden by chronically debilitating tens of millions with symptoms such as severe anemia [12]. In some endemic countries like the DRC, malaria accounts for up to 40 % of public health expenditures and 30 to 50 % of hospital admissions [53]. The emergence of highly drug-resistant parasites [49, 54-57] underscores the need for prevention, and suites of preventive interventions have produced marked declines in malaria infections and mortality in several sub-Saharan African settings[105-115]. In highly malarious countries like the DRC, efficient intervention and preventive efforts must be guided by understanding the geographic patterns of prevalence and the factors underlying these patterns. Even in highly malarious countries like the DRC, malaria prevalence varies across space. Thus prevalence maps are needed in order to focus interventions in regions where they are most needed.

Infection with malaria parasites is dependent on mosquito and human factors. Environmental factors such as land cover, rainfall, altitude and temperature affect Anopheles breeding and have been used to predict malaria transmission risk. Areas with greater amounts of precipitation and higher temperatures are expected to have greater malaria prevalence, as these conditions favor breeding of many Anopheline species as well as parasite reproduction within the mosquitoes [48, 58, 62-70]. Agriculture and urbanization may affect malaria transmission as well; highly cultivated areas have increased suitable habitat for most of the primary vectors, which are non-forest and prefer sunlight, while urbanized areas tend to have reduced vector breeding habitat, although decreased sanitary conditions in urban areas may promote vector breeding in some instances by promoting the presence of stagnant water. In general, however, *Anopheles* species are rural breeders, as their eggs must be deposited on vegetation and their larvae require some nutrition. Malaria in cities in Africa is usually acquired in rural areas, or mining areas, and brought in [59, 71, 116-121]. Conflict and warfare have also altered the local ecology of many parts of the DRC, leaving agricultural fields untended and susceptible to collecting water in which mosquitoes may breed [61]; however, a major focus of humanitarian efforts in war-affected regions is in preventing and treating malaria [122]. Human behaviors such as the use of bed nets (now proven to be even more effective if impregnated with insecticides) and access to anti-malarial drugs are also vital for reducing risk.

Though the DRC is one of the most highly malaria-endemic countries in Africa [123] the limits and intensity of transmission within the country are unknown. For many infectious diseases in developing countries, data quality is poor because it is extrapolated from convenience samplings or non-random sentinel populations. Estimates of disease burden depend upon reliable prevalence data. While several studies have delineated endemic malaria zones in Africa, none have used population-based molecular diagnostics to produce detailed estimates of the spatial patterns and drivers of malaria prevalence. To this end, surveillance systems for infectious diseases in developing countries like the DRC can guide public health interventions at the sub-national scale. Demographic and Health Surveys (DHSs) are well established sources of population-based data on demography, reproductive health and HIV, and thus can provide unbiased prevalence data to enable better disease burden calculations and more well-informed allocation of resources [17]. This study uses specimens collected from the 2007 DRC DHS to provide estimates of malaria prevalence across the country.

Methods

Demographic and health surveys

The DHS provides accurate demographic data in developing countries via large representative population-based surveys and in some countries also includes blood sampling for HIV surveillance. By leveraging the DHS infrastructure, molecular diagnostics for malaria were employed using leftover dried blood spots from the 2007 DRC DHS. Nine thousand households were surveyed. Of these, 9,000 households, 99.3% were successfully identified and interviewed. This included 4,757 men aged 15-

59 years, all of whom were tested for HIV infection, as well as 9,995 women aged 15-59 years, half of whom were tested for HIV. The age distribution of the sampled respondents is presented in Figure 4.1. A chi-square test of age distribution by geographic cluster revealed no significant differences in the distribution between clusters ($p=0.343$). Genomic DNA was extracted from the dried blood spots for testing in real-time PCR assays for *Plasmodium falciparum*, *Plasmodium malariae,* and *Plasmodium ovale* [124, 125]. *Plasmodium vivax* is rarely detected in Western and Central Africa [126]. Malaria parasitemia was thus determined for each individual who had been tested for HIV. The DHS was taken in urban Kinshasa during the rainy season (January 31-March 8, 2007). The remainder of the country was surveyed May-August, 2007 which is mostly dry season. Data on clinical symptoms are unavailable in the DHS database.

<u>Mapping of malaria prevalence in the DRC</u>

Geographic coordinates of clusters of households were collected with global positioning system receivers. To ensure privacy, the coordinates of these 300 communities were randomly displaced by 5 km in rural areas and 2 km in urban areas. The number of respondents per community ranged from 14 to 53, with an average of 30. Malaria prevalence was computed for each community using the survey's sampling weights. A smoothed map of the spatial pattern of malaria prevalence in the DRC was then created in a geographic information system (GIS) using inverse distance weighting (IDW) spatial interpolation in ArcGIS 9.3 (ESRI, Redlands CA). IDW uses nearby values to predict prevalence in unmeasured locations. In our analysis, the prevalence values of the 12 closest communities to an unmeasured location were used to interpolate its prevalence value, with closer communities having a greater influence than those farther away. While other interpolation methods, such as kriging may be appropriate in certain instances, this method produces smoothed maps, eliminating areas of extremely high and low prevalence values from the interpolated surfaces. Inverse distance weighting maintains the entire probability distribution of prevalence values, for which we have authentic data to support.

<u>Assessing drivers of malaria prevalence in the DRC</u>

A multivariate analysis was conducted to estimate factors driving malaria prevalence in the DRC. A diagram of factors considered is provided in Figure 4.2 according to population, behavior, and habitat/environmental categories.

Ecological database creation

All population and behavioral variables were obtained from the DHS survey, as well as the time to a water source, time to a health facility, average wealth index and bed net use by community, altitude, and urban versus rural community type. The wealth index was computed by scoring households according to goods owned (television, radio, car, furniture, etc.) and lodging characteristics (electricity, drinking water, toilet type, roof material, and cooking fuel type) using principal components analysis and then classifying the scores into quintiles, resulting in an index of 1-5 ranging from the poorest to the richest. The remaining habitat/environment variables were computed in the GIS. GIS layers for water bodies, roads, and cities were obtained from the Vector Map Level 0 (VMAP0) of the National Imagery and Mapping Agency (NIMA, 1997) and used to compute the distance from the community centroid to the nearest primary or secondary road, nearest river or major water body, and nearest city in kilometers. The same was done for towns (NIMA, 2003). Notably, while the VMAP0 dataset is the most recent available for determining the locations of roads, it is likely that in the face of economic collapse many of the roads have changed or disappeared since 1997.

A GIS database of armed conflict and refugee camp locations was also compiled in order to examine the effects of ongoing warfare in the DRC on malaria transmission. The Armed Conflict Locational Event Dataset (ACLED) includes locations and dates of individual battle events and rebel activity in states affected with civil war [127]. For the DRC and its surrounding countries, information dating from 1960 onward is available. Fighting in the eastern DRC increased in 1994, and conflict variables were computed between 1994 and 2006 (the year before the DHS survey was conducted). The variables used in this study included battle events within 100 km, rebel activities within 100 km, and all conflict events combined within 100 km. This distance was chosen as population migration from conflict is expected to occur across larger distances. The locations of current and recently closed (post-2004) refugee camps and settlements in the DRC and its surrounding countries were obtained from the United Nations Human Rights Council. Recently closed camps were included as they were likely still inhabited. The distance of a community centroid to a refugee camp was computed, as well as the density of camps within 100 km of the communities. Figure 4.3 shows the locations of battle events, rebel activity, and refugee camps as described here. A community's location within rebel territory as defined by Coghlan *et al* was also computed in the GIS [1, 2]. Rebel territory was defined by these authors as of 2001.

Additional geographic variables included the accumulated rainfall (mm) at the community centroid for the month prior to interview and average air temperature (degrees Celsius) at the month of interview, both computed using Tropical Rainfall Measuring Mission data (TRMM) (NASA, 2009). The percent of agricultural land cover within 10 km of one's community (representative of the average Anopheles flight distance range) was computed using LANDSAT TM images from the years 2000-2001 which were classified using FAO/UNEP international standards by FAO Africover [128]. The population per square kilometer within 25 km of each survey community was also computed using a population density grid [96].

Statistical methods

The population, habitat, and behavioral indicators diagrammed in Figure 4.2 were entered into a multilevel logistic regression model. Individual response variables related to age, gender, HIV status and behaviors were entered into the model along with the array of community-level variables. The dichotomous outcome variable was the presence or absence of any species of malaria parasite from real-time PCR testing. Multilevel analysis was chosen because the nested structure of the data required simultaneous examination of group- and individual-level variables [81, 82]. Additionally, the multilevel approach produces correct standard errors and parameter estimates if outcomes for individuals within groups are correlated (and thus the standard regression assumption of independence of observations is violated). Conceptually, a multilevel model is a two-step set of equations, one explaining variation at the individual level, and the other explaining variation at the group level. Bivariate correlations between all variables were tested prior to entering variables into the model in order to avoid multicollinearity. The model was built in SAS v. 9.2 (SAS Institute, Cary, N.C.) using PROC GENMOD. The best-fitting model was chosen using Akaike's Information Criterion (AIC) which favors parsimony by making a trade-off between the precision and complexity of each model (lower AIC values are favored).

The relationship between conflict and malaria prevalence was then tested with a geographically-weighted regression (GWR), which estimates local models for each community and its neighbors as defined by a spatial weighting matrix [129]. In this analysis, the dependent variable was community malaria prevalence and the independent variables were the number of battle events within 100 km since 1994 along with community averages for other variables found to be significant in the multilevel model. A regression estimate was calculated at each observation and the local parameter estimates are displayed. The results allowed us to determine if the direction of the relationship found between conflict and malaria prevalence was stationary across space.

The study was approved by the Institutional Review Board of the University of North Carolina.

Results

GIS mapping of malaria prevalence

The IDW interpolation mapping results are shown in Figure 4, with a range of 0-82% prevalence estimated across the DRC. Of those people who had malaria, 98% had either mono- or mixed infections with *Plasmodium falciparum* [124]. The center and east-central regions of the country are areas of low prevalence, as well as the urban areas near Kinshasa and Lubumbashi. The northern part of the country has particularly high prevalence, as do the more rural regions near Kinshasa and Lubumbashi.

Multivariate analysis

Table 4.1 shows the descriptive statistics and p-values for two-sample t-tests for unequal variances for the 29 variables entered into the initial multilevel model, as well as the hypothesized direction of association between exposure and outcome. The three conflict variables computed were highly correlated, and thus the battle events variable was chosen over rebel activity and combined conflict types since it most improved model fit. In the final model, 7746 respondents were included due to missing values for certain variables. Of these respondents, 2268 or 29.3% were parasitemic. The final model contained 25 variables, of which 8 were statistically significant at $p < 0.05$. The results of this model are shown in Table 4.2, with statistically significant parameters highlighted in bold.

Individual-level variables that significantly reduced the odds of having malaria include lower age and lower household wealth. Males were nearly 24 % more likely to be infected than women. Four community-level covariates reduced the odds of individual parasitemia: altitude, the number of battles since 1994 within 100 km of a community, average wealth index of the community, and the percent of respondents in one's community having slept under an untreated bed net the previous night. While untreated bed nets were highly protective on a community level, they were not at an individual level, and insecticide-treated nets were never protective. Living further from a town slightly increased one's odds of parasitaemia.

To further examine the association between conflict and parasitaemia, a sensitivity analysis was conducted in which the communities falling within the regions of Nord-Kivu and Sud-Kivu were removed from the analysis, as these regions have experienced heavy amounts of fighting but are also characterized by higher elevations and thus less risk of malaria. Despite this, the relationship between proximity to a battle and malaria risk remained significant at $p < .05$.

The GWR analysis demonstrated the spatial distribution of the association between malaria prevalence and conflict (Figure 5). The negative association was maintained throughout the country after controlling for age, wealth, bed net ownership, gender, distance to a town, and altitude at the community level (variables found to be significant in the multilevel model).

Discussion

The maps and model terms presented in this paper provide important insight into the factors that contribute to increased risk for malaria in certain populations and regions of the DRC. Malaria risk was found to vary geographically and to be dependent on a variety of individual-level and community-level variables. As expected, living further from a town was associated with higher rates of malaria prevalence, actually indicating *Anopheles* are rural habitat breeders. Malaria risk also decreased with increasing altitude [48, 58, 63]. Younger males were found to have the highest risk of malaria, possibly due to occupational exposures or decreased use of health care services[130]. While negative associations with malaria parasitaemia were found between rainfall and temperature, these variables were not significant after controlling for other factors.

Several factors were protective at the community level but not the individual level. Notably, living in a wealthier community more greatly decreases one's odds of having malaria than individual wealth, suggesting that better-off people living in impoverished neighborhoods are still at increased risk. In other words, the general prevalence of infection in the surrounding population is very important for the probabilities of getting malaria. While individual bed net usage was significant when entered alone into the model, it was no longer significant when community bed net ownership was entered, indicating multicollinearity. Inclusion of the community bed net ownership variable provided better model fit and was thus retained. Therefore, while individual bed net ownership is important, community ownership was a stronger predictor of parasitaemia, indicating that herd immunity may be occurring within communities. Community effects on disease transmission have been reported for a variety of diseases and settings, especially for infectious diseases [131-134].

Surprisingly, the use of untreated nets was negatively associated with parasitaemia while the use of treated nets was not. Since there is evidence that insecticide-treated nets are more protective against malaria [134], this finding may be attributable to the fact that roughly twice the number of respondents slept under untreated nets as compared to treated nets (see Table 4.1). It is also possible that the treated nets were introduced to villages or neighborhoods that were identified as producing a lot of sick children, so hyperendemic neighborhoods were most likely to get the treated nets. However, individuals remained at greater risk as the community around them had higher prevalence.

Most notably, the level of conflict since 1994 occurring within 100 km of one's community was negatively associated with an individual's malaria risk in the majority of the DRC. This relationship persisted even when areas having the greatest potential to contribute to confounding (the Kivu provinces) were excluded. The GWR analysis indicated that the negative direction of the relationship was in fact stationary across the entire country.

While the relationship between conflict and infectious disease has been explored in past research [135, 136], to date no studies have compared localized density of conflict with malaria parasitaemia. The inclusion of conflict variables in the models was intended to determine the outcome of parasitemia in places that have long been characterized by conflict. The nature of these places indeed differ from those areas little-affected by conflict, and this study has shown such differences to be relevant to the understanding of the drivers of parasitemia in the DRC, even while controlling for other factors. The inclusion of conflict density in these models is not, however, intended to indicate a direct causal relationship between specific past battle events and individual malaria parasitemia. Possible explanations for this observed association include population migration away from and increased humanitarian efforts in places having experienced large amounts of fighting. Displacement from rural areas due to conflict may lead to less dense human host populations for malaria transmission in zones of insecurity [137]. The focus of humanitarian efforts in war-affected regions on preventing and treating malaria [122] may also underlie the findings, although further knowledge of the geography and practices of humanitarian agencies in the DRC would be necessary to support this premise.

This study has several limitations. Because the presence of clinical symptoms is unknown in this study, our maps highlight where prevention may be most effective but not necessarily where treatment is most needed. Lack of blood sampling from children is also an important limitation to consider, as they suffer the greatest risk for illness and death from malaria. If children had been included in this study, malaria prevalence values might be expected to change and the significance and magnitude of parameter estimates in our models may have been different. There has only been one published report looking at age stratification of PCR-positive malaria, and the difference between adults and children was minor [138]. Data were also limited in that individuals could only be located to the centre of their community, and not to their actual place of residence. Furthermore, mobility and migration characteristics are unknown for the individuals in our dataset. Consequently, several of the geographic variables we computed (distance to a road, water body, city or town, agricultural land cover, and altitude) may lack precision and could affect the terms of these variables in the multivariate models. Finally, with the exception of Kinshasa, the study was conducted during the dry season, so the annual peak prevalence may be higher than reported here.

Conclusion

There is much uncertainty regarding the spatial distribution of important endemic tropical diseases like malaria on the global, national, and sub-national levels. Studies have most often used passively-reported data from sentinel clinics rather than active surveillance data, leading to a bias and lack of clarity in the spatial distribution of malaria prevalence in sub-Saharan African countries. Effective resource allocation and implementation of control measures are thus hindered. While projects such as Mapping Malaria Risk in Africa (MARA) [139] and the Malaria Atlas Project (MAP) [140] have delineated endemic zones for presence of the Plasmodium parasite in sub-Saharan Africa, these projects have not employed molecular diagnostics in the DRC to determine infection in the human population. Furthermore, the Malaria Atlas Project found high levels of uncertainty in the DRC, with the fewest data points per land area of any country. Despite the limitations discussed above, this study provides the most accurate population-based estimates to date of where illness from malaria occurs in the DRC and what factors contribute to the estimated spatial patterns. In addition to increasing understanding of patterns and drivers of malaria in the DRC, this study provides an example of how population-representative surveillance can improve understanding of infectious disease prevalence and transmission.

This research demonstrates the feasibility of using population-based behavioral and molecular surveillance in conjunction with geographic methods to study endemic infectious diseases. Estimates for the prevalence of malaria are vague or unavailable in countries such as the DRC, and thus more accurate estimates of disease burden are necessary for allocating health resources. It is also important to study sub-national patterns in disease prevalence, as malaria is an infectious disease spread by the bite of an infected vector and tends to be highly geographically localized. This study suggests that spatial information and analyses can enable the DRC government to focus its control efforts against malaria.

Tables

Table 4.1. Descriptive statistics for variables entered into the initial multilevel model.

	Malaria-Positive	Malaria-Negative	p-value	Expected Relationship
Individual-*/Level Variables				
Age in single years - mean	28.6	30.2	<.0001	-
Male - %	52	47	<.0001	+
HIV-positive - %	1	2	0.0053	-
Education in single years -mean	5.7	6.7	<.0001	-
Wealth index (1-5) - mean	2.7	3.3	<.0001	-
Number of household members - mean	6.3	6.8	<.0001	+
Household has bed net - %	28	37	<.0001	-
Number of household bed nets - mean	.4	.6	<.0001	-
Number of kids under bed net previous night - mean	.21	.29	<.0001	-
Respondent slept under treated bed net last night - %	5.1	7.0	0.0010	-
Respondent slept under untreated bed net last night - %	10.2	15.2	<.0001	-
Child with fever past 2 weeks didn't use antimalarial - %	48	44	0.0023	+
Community-Level Variables				
Time to water source (minutes) – mean	31.1	26.9	<.0001	+
Time to get to health facility (minutes) - mean	72.3	53.1	<.0001	+
Distance to a town (km) -mean	45.4	30.4	<.0001	+
Distance to a city (km) - mean	139.5	106.9	<.0001	+
Distance to a river or water body (km) - mean	2.7	2.5	0.0136	+
Distance to a road (km) - mean	1.9	1.8	0.0923	+
Population density (pop/sq km) within 25 km - mean	257.9	525.1	<.0001	-
Refugee camps within 100 km of community - mean	2.5	3.3	<.0001	+ / -
Conflict events since 1994 within 100 km of community - mean	26.9	62.9	<.0001	+ / -
Within rebel territory (as defined in 2001) - %	45	45	0.7101	+ / -
Average wealth index of community (1-5) - mean	2.7	3.3	<.0001	-
Percent agricultural land cover within 10 km - mean	11.9	13.0	<.0001	+
% in community under treated net last night – mean	4.6	5.5	<.0001	-
% in community under untreated net last night - mean	9.0	12.1	<.0001	-
Altitude (km) - mean	66.8	78.2	<.0001	-
Urban community - %	34	50	<.0001	-
Accumulated rainfall, month prior to interview (mm) - mean	87.5	86.8	0.0092	+
Average air temperature, month of interview (C) - mean	24.1	23.4	<.0001	+

Table 4.2. Results of the final multilevel logistic regression model. AIC for the original model was 8608.32.

Parameter	Beta Estimate	Odds Ratio	95% Lower	95% Upper	p-value
Individual-Level Variables					
Age in single years	**-0.0228**	**0.9775**	**0.9725**	**0.9826**	**<.0001**
Wealth Index (1-5)	**-0.1140**	**0.8923**	**0.8313**	**0.9577**	**0.0016**
Male	**0.2129**	**1.2373**	**1.1103**	**1.3787**	**0.0001**
Education in single years	-0.0140	0.9861	0.9711	1.0013	0.0724
HIV-Positive	-0.4008	0.6698	0.4040	1.1104	0.1202
Time to water source (minutes)	-0.0017	0.9983	0.9962	1.0005	0.1238
Number of household members	-0.0067	0.9933	0.9760	1.0109	0.4554
Household has bed net	0.0729	1.0756	0.8706	1.3289	0.4993
Number of household bed nets	-0.0356	0.9651	0.8663	1.0751	0.5185
Slept under treated net last night	-0.1558	0.8557	0.6602	1.1092	0.2391
Slept under untreated net last night	-0.1188	0.8880	0.7170	1.0998	0.2765
Child with fever past 2 weeks did not use anti-malarial	-0.0535	0.9479	0.8482	1.0593	0.3451
Community-Level Variables					
Distance to a town (km)	**0.0031**	**1.0032**	**1.0002**	**1.0061**	**0.0374**
Number of battles since 1994 within 100 km	**-0.0068**	**0.9932**	**0.9907**	**0.9957**	**<.0001**
Average community wealth index (1-5)	**-0.1714**	**0.8425**	**0.7422**	**0.9563**	**0.0080**
Percent in community sleeping under untreated net last night	**-0.0154**	**0.9847**	**0.9758**	**0.9936**	**0.0008**
Altitude (m)	**-0.0004**	**0.9996**	**0.9994**	**0.9998**	**0.0008**
Refugee camps within 100 km	-0.0068	0.9932	0.9827	1.0039	0.2114
Percent agricultural land cover within 10 km	0.0008	1.0008	0.9997	1.0018	0.1429
Percent in community sleeping under treated net last night	-0.0054	0.9946	0.9822	1.0072	0.3988
Percent agricultural land cover within 10 km	0.0007	1.0007	0.9997	1.0018	0.1592
Accumulated rainfall, month prior to interview (mm)	-0.0005	0.9995	0.9986	1.0003	0.2375
Avg. air temp, month of interview (C)	0.0295	1.0299	0.9814	1.0808	0.2207

Deviance	8579.83
Pearson Chi-Square	7756.28
Log Likelihood	-4289.91
AIC	604.83

Figures

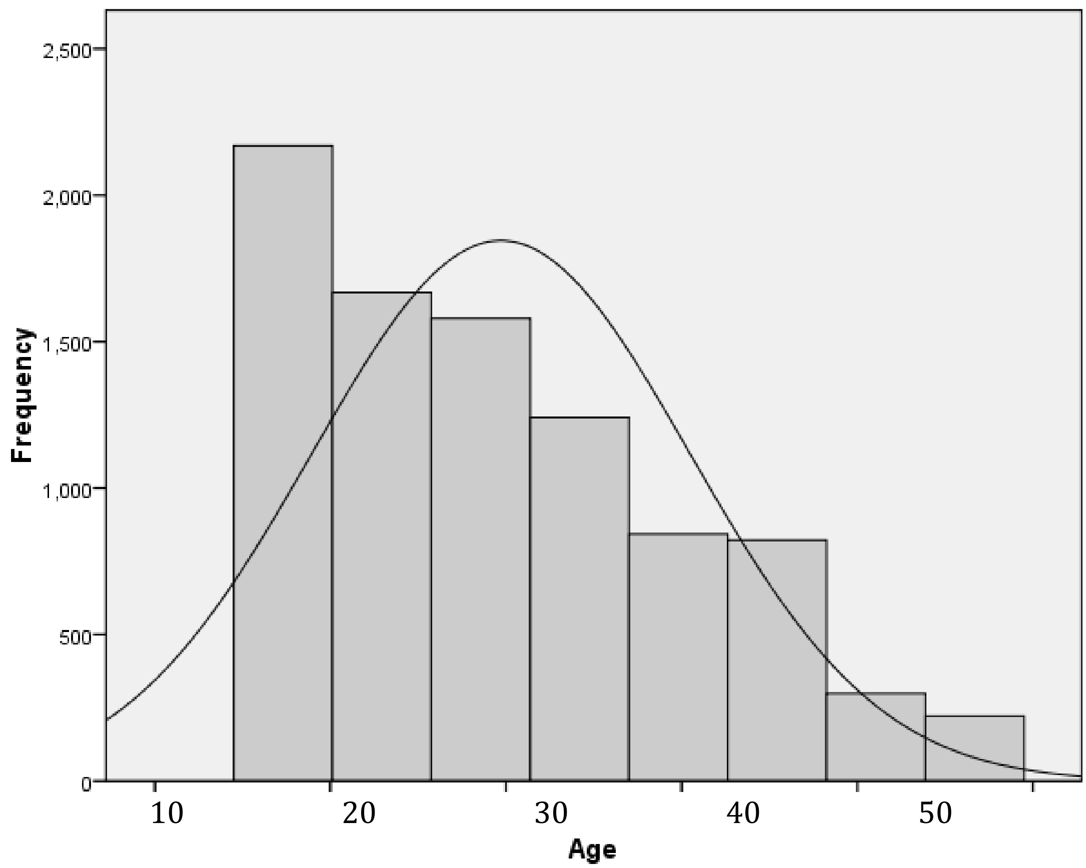

Figure 4.1. Age distribution of the sampled population in the DRC DHS dataset displayed against the normal curve. Mean age is 29.71 years (Standard Deviation = 10.755).

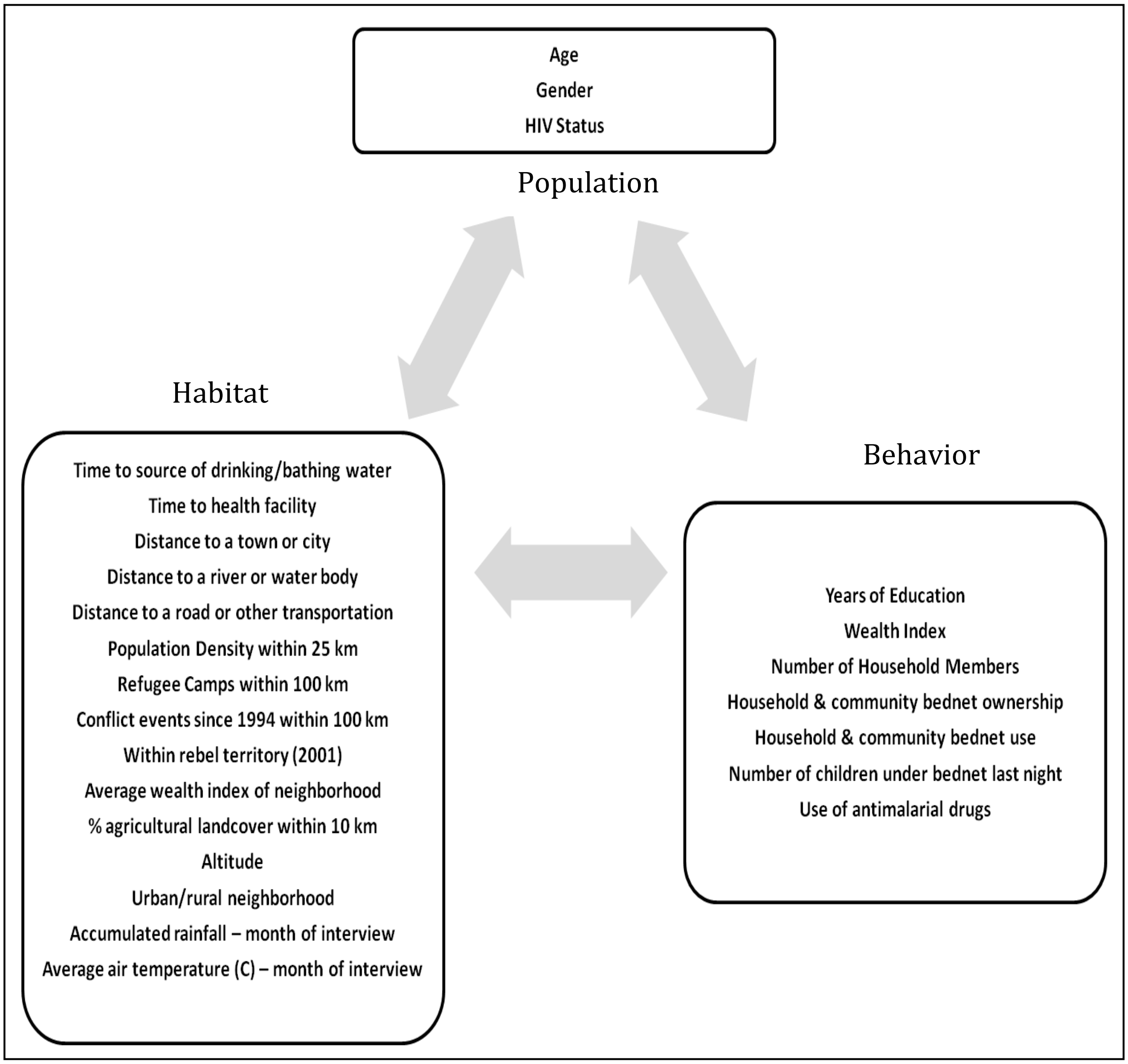

Figure 4.2. Variables entered into multivariate analyses according to population, habitat, and behavioral characteristics.

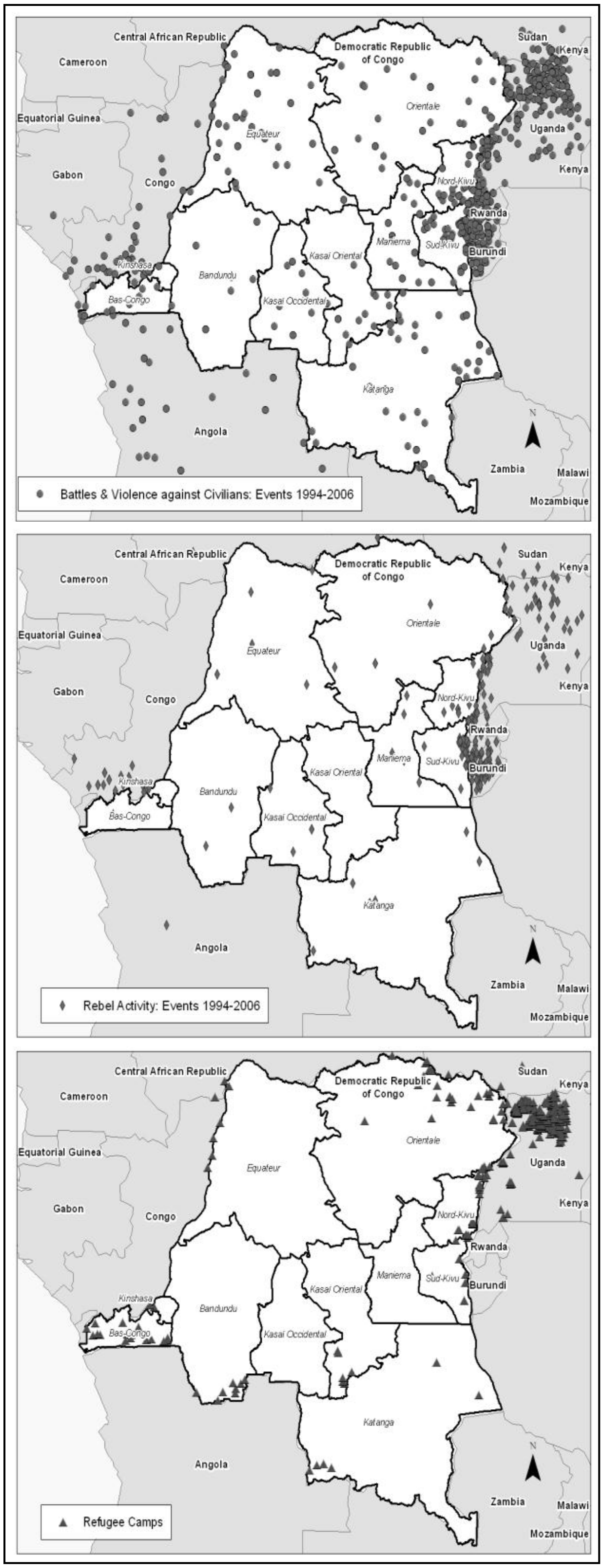

Figure 4.3. Conflict and Refugee Camps in the DRC.

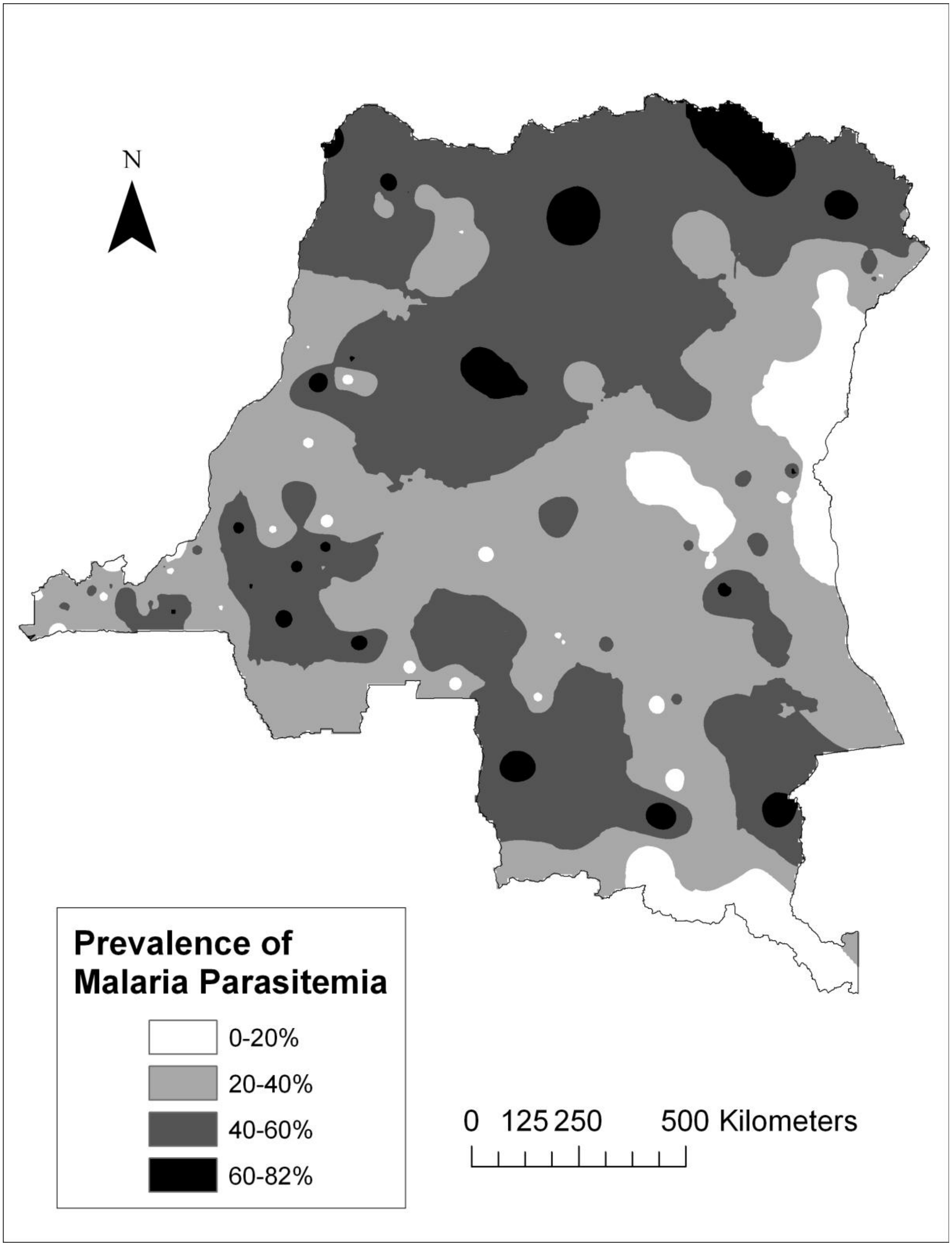

Figure 4.4. Inverse distance-weighted surface of malaria prevalence, DRC 2007.

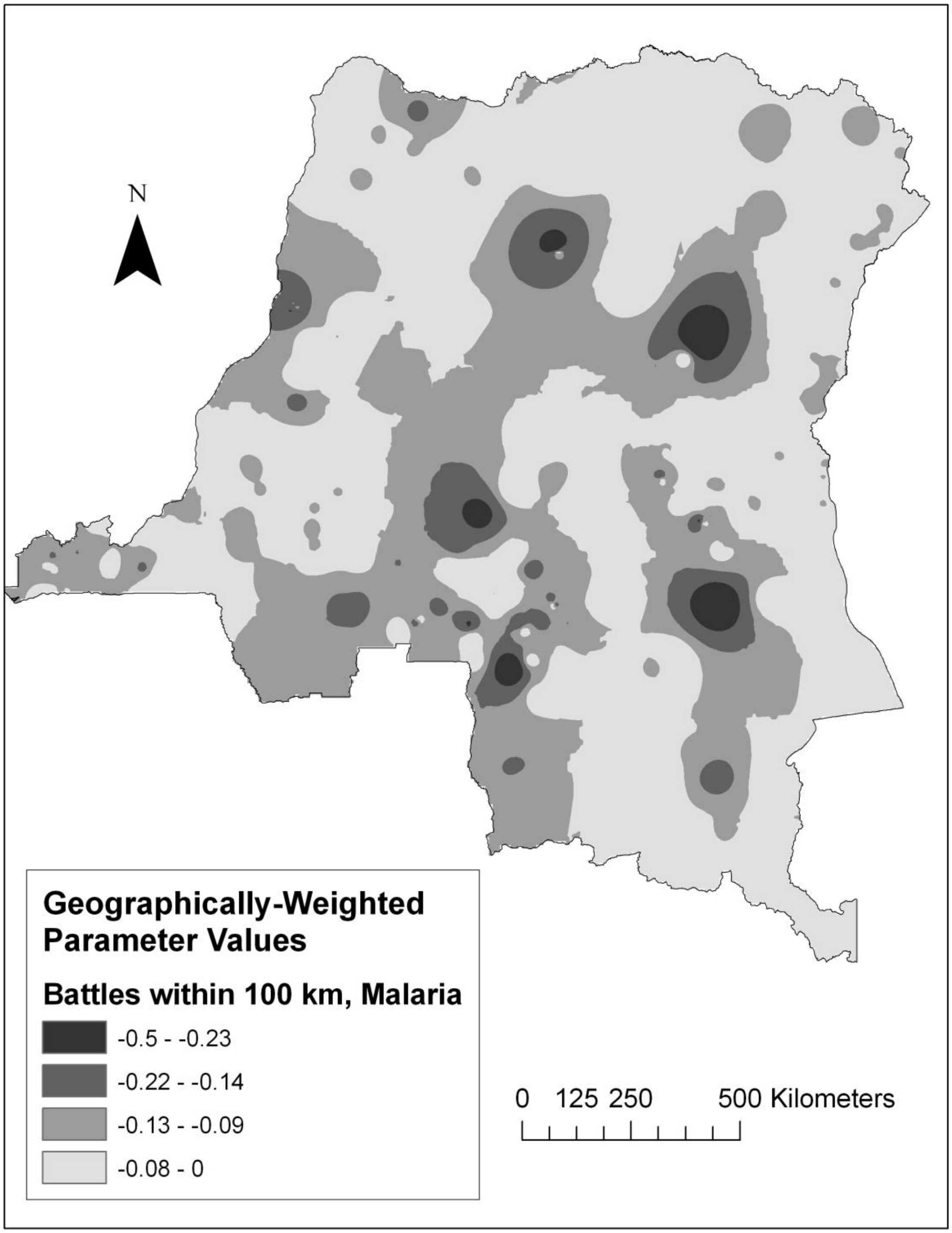

Figure 4.5. Local parameter estimates for the relationship between 2007 malaria prevalence and the number of battles occurring within 100 km since 1994. Areas where a positive relationship was found are highlighted in white.

INFECTIOUS VERSUS CHRONIC DISEASE ECOLOGY AND EPIDEMIOLOGY

When one considers disease ecology, it is often primarily applied to infectious diseases or those related specifically to environmental hazards such as pollution-related illnesses. As can be seen in the previous chapters, disease ecology theory was indeed a valuable framework for studying a sexually-transmitted and a vector-borne disease, as the populations, their behaviors, and their habitat were all relevant to understanding risk for infection and the spatial patterns of prevalence. However, this theory is less often used in the consideration of chronic or nutritional-related illnesses.

Behaviors are the aspect most commonly referred to when studying the epidemiology of chronic or nutritional diseases. For example, habits such as smoking, lack of physical activity, unhealthy diet, and alcohol use are repeatedly mentioned with regard to heart disease. Education is also considered, as it can be used to inform people about those behaviors which put them at greater risk for certain health problems or deficiencies.

Less consideration is generally given in chronic disease epidemiology to factors that extend beyond the individual. With increasing regard for the field of environmental health, this is beginning to change in recent years. However, the disease ecology framework presents an improvement over either environmental health or traditional chronic disease epidemiology alone, as it considers not only individual behaviors and not only the environment, but the importance of both and the interaction between the two.

In the case of a disease such as anemia in women, there are of course many behavioral factors to be considered such as dietary variety and wealth, and individual factors such as pregnancy or infection with other diseases which may lower the hemoglobin content of the blood. However, as is be discussed in the following chapter, habitat-related factors are rarely considered in this type of chronic or nutritional disease. Often researchers do not consider the fact that where one lives, especially in a country like the DRC where transportation is difficult and access to a varied diet may be impossible, may make all the difference when understanding women's risk for anemia. This is explored in detail in the following chapter.

SPATIAL EPIDEMIOLOGY AND DISEASE ECOLOGY OF ANEMIA IN THE DEMOCRATIC REPUBLIC OF THE CONGO

Background

Anemia is a global public health problem. Since 1985, global prevalence estimates for anemia have risen drastically [74]. Estimates are particularly high in sub-Saharan Africa, where 40-80% of women are estimated to be anemic [75, 76]. In the Democratic Republic of the Congo (DRC), 52.8% of non-pregnant women and 67.3% of pregnant women were estimated by the WHO to be anemic (less than 11 g/dl hemoglobin in the blood), making anemia a severe public health problem in the country [141]. In women, anemia is associated with increased risk for maternal morbidity and mortality and lower productivity, and pregnant women are furthermore at higher risk for anemia. Maternal anemia may also lead to higher risks for premature births, perinatal and neonatal death, and low birth weight [75]. Common symptoms include fatigue, weakness, fainting, chest pain, and even heart attacks in severe cases.

There are a variety of causes of anemia. The principal cause, malnutrition, is common in sub-Saharan Africa [77]. Anemia in adults is also the result of deficiencies in specific nutrients such as iron and vitamin B-12. Infectious diseases such malaria, HIV, schistosomiasis, and hookworm have also been implicated as major causes of anemia [78-80]. However, the relative contributions of these different potential causes to the overall anemia burden are not known.

Public health interventions which may control anemia include iron supplementation, mass de-worming, and malaria control programs. Iron supplementation programs are common and thought to mitigate anemia, although the evidence for their success is mixed [74, 142, 143]. More information about the causes of anemia could lead to more appropriate applications of these interventions.

Guyatt and Snow (2001) estimated that 26% of cases of anemia in pregnant women in sub-Saharan Africa are due to malaria [78]. However, there has never been a large epidemiological study there to correlate the risks of malaria and anemia. Here, using data from the 2007 DRC DHS, we map anemia and assess the relative importance of malaria, other population and behavioral factors, and habitat, as cofactors for anemia.

The examination of population, behavioral, and habitat factors simultaneously falls under the medical geographic theory of the cultural ecology of disease. Although several twentieth century scientists including Jacques May, René Dubos, and Ralph Audy contributed to the formulation of this theory, it was synthesized by Melinda Meade in the 1970s [144] and continues to be an important focus of medical geographic research [12, 145-152]. Meade established the "triangle" of human ecology in which habitat, population, and cultural behavior are considered as three nexuses. Habitat is meant as the social, natural, and built environments in which people live, population considers humans as biological organisms with age, gender, and genetic characteristics which make them more or less likely to be hosts of specific diseases, and behavior encompasses the beliefs, social organization, and technologies specific to a culture in which a disease may occur.

While much past work has focused on the population and behavioral factors associated with anemia risk and prevalence [78, 153-159], little work has explicitly explored habitat factors in relation to anemia. While Jacques May described the ecology of malnutrition in west Africa up until 1965 [160], no studies have explicitly explored the prevalence of anemia in African women from a disease ecology perspective. This represents an important gap in anemia literature. Other important gaps include a lack of estimation of highly localized anemia rates in any sub-Saharan African country, as well as an absence of studies which unambiguously examine the relationship between malaria parasitemia and blood hemoglobin levels. Although malaria is commonly thought to contribute to higher anemia prevalence, previous work has been anecdotal and not based upon molecular surveillance.

Here, in addition to estimating the burden of anemia in the DRC at the country level as well as the local scale, population factors such as pregnancy malaria parasitemia, as well as behavioral factors such as cultural affiliation and wealth are explored. We also explore habitat factors such as proximity to urban areas and refugee camps, agricultural land cover, and population density in relation to anemia prevalence. As a result, this study offers a broader, more comprehensive understanding of the determinants of anemia prevalence in the DRC using a framework which has not been used to study anemia in past literature and including factors which have not yet been examined in relation to the disease.

Methods

Demographic and health surveys

The DHS provides accurate demographic data in developing countries via large representative population-based surveys and in some countries also includes blood sampling for HIV surveillance. Nine thousand households were surveyed and 99.3% were successfully identified and interviewed. This included 9,995 women aged 15-59 years, 4,638 of whom were tested for HIV and had the hemoglobin content of their blood recorded using a portable device (HemoCue). Hemoglobin level was communicated immediately to all participants, and those with severe anemia (<7g/dL for non-pregnant women, and < 9g/dL for pregnant women) were referred to local medical care facilities. Of these 4,638 women, 526 reported being pregnant at the time of the interview. Genomic DNA was extracted from the dried blood spots for testing in real-time PCR assays for Plasmodium falciparum, malariae, and ovale [124, 125]. Malaria parasitaemia was thus determined for each individual who had been tested for HIV as well. Data on clinical symptoms are unavailable in the DHS database.

Mapping of anemia prevalence in the DRC

Geographic coordinates of clusters of households were collected with global positioning system receivers. To ensure privacy, the coordinates of these 300 communities were randomly displaced by 5 km in rural areas and 2 km in urban areas. The number of female respondents per community ranged from 6 to 30, with an average of 15. Anemia prevalence was computed for each community using the survey's sampling weights and altitude-adjusted hemoglobin levels. The percent anemic in each cluster was computed with a cutoff of 11 g/dl hemoglobin in the blood. A smoothed map of the spatial pattern of anemia prevalence in the DRC was then created in a geographic information system (GIS) using inverse distance weighting (IDW) spatial interpolation in ArcGIS 9.3 (ESRI, Redlands CA). IDW uses nearby values to predict prevalence in unmeasured locations. The prevalence values of the 12 closest communities to an unmeasured location were used to interpolate its prevalence value, with closer communities having a greater influence than those farther away. Compared to other spatial interpolation techniques which eliminate high and low values, inverse distance weighting maintains the entire probability distribution of prevalence values, making it appropriate for active surveillance data.

Assessing drivers of anemia prevalence in the DRC

All population and behavioral variables were obtained from the DHS survey, as well as the time to a health facility and urban versus rural community type. The wealth index was computed by scoring households according to assets (television, radio, car, furniture, etc.) and household characteristics (electricity, drinking water, toilet type, roof material, cooking fuel type) using principal components analysis and then classifying the scores into quintiles, resulting in an index of 1-5 ranging from the poorest to the richest. The culture of origin was reported by individuals in the DHS questionnaire according to eight options: Bakongo, Basele, Bas-Kasai and Kwilu-Kwongo, Cuvette Centrale, Ubangi, Uele (Lac Albert), or Lunda.

The remaining habitat/environment variables were computed in the GIS. A GIS database of armed conflict and refugee camp locations was compiled in order to examine the effects of ongoing warfare in the DRC on anemia outcome. The Armed Conflict Locational Event Dataset (ACLED) includes locations and dates of individual battle events and rebel activity in states affected with civil war [95]. For the DRC and its surrounding countries, information dating from 1960 onward is available. Fighting in the eastern DRC increased in 1994, and conflict variables were computed between 1994 and 2006 (the year before the DHS survey was conducted). The variables used in this study included battle events within 100 km, rebel activities within 100 km, and all conflict events combined within 100 km. This distance was chosen as population migration from conflict is expected to occur across larger distances. The locations of current and recently closed (post-2004) refugee camps and settlements in the DRC and its surrounding countries were obtained from the United Nations Human Rights Council. Recently closed camps were included as they were likely still inhabited. The distance of a community centroid to a refugee camp was computed, as well as the density of camps within 100 km of the communities.

The percent of agricultural land cover within 25 km of one's community was computed using 2005 GlobCover data which was classified using the UN Land Cover Classification System by the Global Land Cover Network [161]. The population per square kilometer within 25 km of each survey community was also computed using a population density grid [96]. Areas with international aid were delineated by health district by the DRC Minister of Health as of 2003. Aid agencies included Cooperation Belge, African Development Bank (BAD), 9th European Union Development Fund

(FED9), USAID, Minimum Partnership Program for Transition and Recovery (PMPTR), Emergency Multisectorial Rehabilitation and Reconstruction Project (PMURR), Corporation for Technical Cooperation (GZT), and Health Sector Rehabilitation Support Project (PARSS). Dominant agricultural type was derived by Consultative Group on International Agricultural Research (CGIAR) from the U.S. Geological Survey (USGS) Earth Resources Observation System (EROS) Data Centre (EDC) 1998, 1 km resolution, global land cover characteristics database. Agricultural types included forest, highland perennial, cereal root crop mixed, maize mixed, root crop, and tree crop. The majority of the DRC's land area is dominated by forest agriculture.

Statistical methods

The population, habitat, and behavioral indicators listed in Table 6.1 were entered into four multilevel regression models: (1) logistic regression with dichotomous dependent variable for anemia (<11 g/dl Hb) or no anemia in all women (2) linear regression with a continuous hemoglobin level dependent variable in all women (3) logistic regression with dichotomous dependent variable for anemia or no anemia in pregnant women, and (4) linear regression with a continuous hemoglobin level dependent variable in pregnant women. Individual response variables related to age, gender, pregnancy, HIV and malaria status and behaviors were entered into the model along with the array of community-level variables. Multilevel analysis was chosen because the nested structure of the data required simultaneous examination of group- and individual-level variables [81, 82]. Additionally, the multilevel approach produces correct standard errors and parameter estimates if outcomes for individuals within groups are correlated (and thus the standard regression assumption of independence of observations is violated). Multilevel models consist of two sets of equations, one explaining variation at the individual level, and the other explaining variation at the group level. Bivariate correlations between all variables were tested prior to entering variables into the models in order to avoid multicollinearity. The models were built in SAS v. 9.2 (SAS Institute, Cary, N.C.) using PROC GENMOD and using the sampling weights of the survey. The best-fitting models were chosen using Akaike's Information Criterion (AIC).

Results

Anemia prevalence

Table 6.1 provides descriptive statistics for all variables entered into the analysis according to population, behavioral, and environment (habitat) categories. Table 6.2 of weighted frequency computations showed that in all women, 29.1% were anemic at less than 11 g/dl Hb, while 0.7% were severely anemic at less than 8 g/dl Hb. In pregnant women, 56.8% were anemic while 0.5% were severely anemic. The IDW interpolation mapping results for anemia at less than 11 g/dl Hb in all women are shown in Figure 5.1, with a range of 0-92% prevalence estimated across the DRC. In general, the southeast portion of the country has lower anemia rates, as well as areas surrounding major cities. The central part of the country contains higher anemia rates.

Table 6.3 provides a summary of the percent who were found to be anemic (< 11g/dl Hg) or severely anemic (< 8 g/dL Hg) according to malaria parasitemia. Slightly more women with malaria parasitemia were found to be anemic than those who were not parasitemic (34.5% as compared to 31.2%). In pregnant women, 60 percent of those with malaria parasitemia were found to be anemic as compared to 54.7 percent of non-parasitemic pregnant women. All differences between groups were significant at $p < 0.01$.

<u>Multivariate analysis</u>

Figure 5.2 provides a summary of variables which significantly increased risk for anemia in any of the four models, those which significantly decreased risk, and those for which no significant relationship was found in any model. Tables 6.4 through 6.7 show the results of the multilevel regression models. Of the 4,368 female respondents, 4,356 were included in the first two models due to missing values. Of the 526 pregnant respondents, 497 were included in the second two models.

In all women, being pregnant significantly increased the likelihood of being anemic; specifically, pregnant women were at 3.74 times the risk of being anemic at less than 11 g/dl Hb than non-pregnant women and also had significantly lower hemoglobin levels overall. This merited examination of separate models for pregnant women alone whose results are discussed later. Older women had an increased prevalence of anemia, along with Lunda women who exhibited lowered hemoglobin levels. At the community level, living in a community dominated by maize mixed agriculture significantly decreased women's odds of being anemic at less than 11 g/dl Hb (74% less likely) as well as increased their hemoglobin levels. Conversely, living in a community dominated by tree crop agriculture significantly increased women's odds of being anemic at less than

11 g/dl Hb (68% more likely) as well as decreased their hemoglobin levels. Highland perennial and root crop agriculture were associated with decreased odds of being anemic as well. While urban residence was not associated with increased or decreased risk for anemia in all women, living in a more densely populated area increased the odds of being anemic. Living farther from a refugee camp was associated with lowered hemoglobin levels.

In pregnant women, more significant associations were found with the continuous hemoglobin dependent variable than the dichotomous anemia variable. This is probably due to the fact that the number of pregnant women is relatively small (526). Having a refrigerator was significantly associated with both decreased odds for anemia (29.5% less likely) as well as increased hemoglobin levels. Women who identified themselves as Bakongo, Cuvette Centrale, or Ubangi exhibited significantly decreased hemoglobin levels, as well as more educated women. At the community level, both living in an urban area and one with increased agriculture put a women at decreased odds of being anemic as well as increased her hemoglobin levels overall. More densely populated areas exhibited increased odds for anemia as well, and living further from a town significantly increased women's hemoglobin levels. Tree crop agriculture was associated with decreased hemoglobin levels as well. People in these areas need money to buy food and such expenditures, especially by men for women and children, may not be a priority.

Strikingly, malaria parasitemia was not associated with anemia in any of the models. Even when high parasite density alone was examined (defined as a cycle threshold (Ct) value lower than 30), it remained a non-significant predictor of anemia in all women and pregnant women alone (data not shown). High parasite density was rare in the sample population, with 135 women, including 19 pregnant women, found to have such high levels of parasites in their blood. Other factors which were not associated with anemia include HIV and wealth. Among habitat variables, the time to a health facility, nearby conflict density, and presence of international aid also had no significant effect on anemia or hemoglobin level outcome.

Discussion

Anemia prevalence was found to vary geographically and to be dependent on a variety of individual-level and community-level variables. At the individual level, several population and behavioral factors were found to be significant. While pregnancy was unsurprisingly the single largest risk factor for being anemic, it is remarkable that malaria parasitemia was not a significant predictor of anemia outcome even at high parasite levels which may be more indicative of clinically-significant infections. Unfortunately, the presence of malaria symptoms was not reported as part of the DHS survey.

Our study also highlighted that several cultural affiliations were found to be associated with increased anemia risk. The Bakongo people, centered mainly in the southwest region of the country near Kinshasa, are known to have dietary habits different than those of the eastern regions, with diets consisting primarily of dry fish and fewer vegetables. Lunda cultural affiliation is highly correlated with tree crop agriculture in the south of the DRC, which may explain increased anemia risk in Lunda people as well. Owning a refrigerator was important for pregnant women, indicating that their ability to keep a wider variety of foods in their household may have prevented pregnancy-related anemia. Household wealth and number of household members did not affect anemia prevalence, while education unexpectedly decreased hemoglobin levels in pregnant women although had no effect in the majority of the models. The importance of cultural affiliation with respect to anemia outcome was the most exceptional finding of this study at the individual-level.

At the community level, several habitat factors were found to be associated with anemia outcome. Most interestingly, agricultural land cover and agricultural type were important predictors of anemia risk, highlighting the importance of a community's local supply of nutritious foods. In table 6.8 the average iron content per metric ton of output for each agricultural type is estimated using the USDA's Nutrient Data Laboratory [162], according to the types of crops included under each agricultural category in the CGIAR database. While the actual output per CGIAR agricultural type is not known, it can be seen that overall, tree crop agriculture produces the least amount of iron-rich nutrients. While forest agriculture is associated with a relatively high iron content, it is likely not associated with reduction in anemia risk due to the foraging nature of the system, whereby individuals are not obtaining high amounts of each product. Although highland perennial crops contain overall low average iron content per metric ton of output, Jacques May's 1965 work emphasized that Belgian policy pre-independence created an elite class of Congolese in which the prosperity generated from cash crops was concentrated [160]. This legacy may persist in highland perennial areas in the eastern part of the Congo, where coffee is a dominant cash crop.

Both living in an urban area and one with more agriculture are associated with decreased risk for anemia. This indicates that while living in an urban area associated with increased access to a variety of foods (both financially and in terms of proximity) is protective, if one does not live in a city, having greater access to agricultural outputs is important, especially if they are not forest or certain tree crops. This would mean that living in a smaller town would be disadvantageous to one's nutritional health, as these people would not have access to large amounts of imported food as in the highly urban areas, nor large amounts of agricultural outputs as in the more rural areas. This is supported by our results which show that after controlling for urban residence, living closer to a town is actually detrimental for pregnant women's health, and less densely populated areas have decreased risk for anemia. Although international aid was not an important predictor of anemia risk, living closer to a refugee camp improved hemoglobin levels of the women in our study, meaning that access to the food and disease-prevention resources provided in these camps is important to consider for a nutritional disease in the DRC. This may also indicate that international aid agencies are not being successful at providing nutrition for individuals in the communities where they are located. Overall, our study highlights that community access to a nutritional diet is extremely important in preventing anemia in women.

While the amount and types of agriculture in a community were important along with cultural affiliation (which clusters geographically), individual wealth was not significantly associated with anemia. This indicates that dietary habits and customs which dominate one's community, regardless of individual wealth, are extremely important in determining anemia outcome in the DRC. In other words, some communities are likely to be more fit against nutritional deficiencies than others.

An important limitation of this study was the inability to more comprehensively measure other correlates of anemia (i.e. B12, folate, hookworm infection, etc.). Data were also limited in that individuals could only be located to the center of their community, and not to their actual place of residence. This may affect several of the community-level variables which were computed in the GIS, such as agricultural land cover and type, distance to a town or refugee camp, and population density. However, compared to current global estimates which are not based upon population-representative data and do not consider local-scale geographic heterogeneity, this study provides the best estimates to date of the populations and locations at highest risk for anemia in the DRC.

Conclusion

Effective resource allocation and implementation of control measures is important for a disease that affects 29% of all women in the DRC and 57% of pregnant women. Prevalence in pregnant women is similar to the WHO estimates for Africa as a whole from 1993-2005 at 56%, although striking when compared to a global rate of 42% and especially rates in North America and Europe (6% and 19%, respectively) [141]. Despite the limitations discussed above, this study provides the most accurate population-based estimates to date of where anemia occurs in the DRC and what factors contribute to the estimated spatial patterns. The map and model terms presented in this paper provide important insight into the factors that contribute to increased risk for anemia in certain populations of the DRC. In addition to increasing understanding of patterns and drivers of anemia in the DRC, this study provides an example of how population-representative surveillance can be combined with spatial analyses under a disease ecology framework to improve understanding of the burden of chronic nutritional diseases.

This research demonstrates the feasibility of using population-based health and behavioral surveillance in conjunction with geographic methods to study an important and ongoing problem in a developing country. Estimates for the prevalence of anemia are vague or unavailable in countries such as the DRC, and thus more accurate estimates of disease burden are necessary for allocating health resources. This study also demonstrates the importance of studying sub-national patterns in disease prevalence, as several geographically localized variables were found to be important predictors of anemia risk. Spatial information and analyses can aid the DRC government in focusing its control efforts against anemia.

Tables

Table 6.1. Descriptive statistics for variables entered into multilevel regression models.

	Anemic (<11 g/dl Hb)	Not Anemic (>11 g/dl Hb)	Model Level
Population			
Average Age (years)	29.1	28.3	1
Average Body Mass Index	21.3	22.1	1
% HIV-Positive	29.9	33.1	1
% Malaria-parasitemic	33.5	30.2	1
% Pregnant	20.0	7.2	1
Behavior			
Avg. Wealth Index (1-5)	3.0	3.1	1
Avg. Years of Education	4.8	5.1	1
Avg. Births in Past Year	0.2	0.2	1
Avg. Births in Past 5 Years	1.0	0.9	1
Avg. # of Household Members	6.9	6.9	1
% Owning Refrigerator	23.1	19.2	1
% Bakongo (N& S of River)	10.5	8.9	1
% Cuvette Centrale	14.7	9.0	1
% Ubangi	9.4	6.7	1
% Uele; Lac Albert	2.9	6.9	1
% Kasai	26.3	33.5	1
% Lunda	0.9	1.1	1
% Basele	8.0	8.0	1
% Bas-Kasai & Kwilu-Kwongo	25.4	18.5	1
Habitat			
Avg. Distance to a City (km)	118.5	111.8	2
Avg. Distance to a Town (km)	40.5	37.3	2
Avg. Time to Health Facility (min)	63.9	56.7	2
Avg. # Conflict Events since 1994 w/in 100 km	28.4	36.9	2
Avg. Pop. Density within 25 km (pop/sq. km)	475.8	421.3	2
Avg. Agricultural Landcover w/in 25 km (km2)	3.0	562.7	2
% Urban	40.8	46.1	2
% with International Aid	66.5	66.0	2
% Forest Agriculture	83.3	69.0	2
% Highland Perennial Agriculture	2.6	6.7	2
% Maize Mixed Agriculture	1.9	6.5	2
% Cereal Root Crop Mixed Agriculture	2.6	3.2	2
% Root Crop Agriculture	7.5	12.3	2
% Tree Crop Agriculture	1.7	0.8	2

Table 6.2. Weighted frequency of anemia and mean hemoglobin level by pregnancy status.

	Anemic at <11 g/dl Hb - % (N)	Anemic at <8 g/dl Hb - % (N)	Average Hb level (g/dl)
Pregnant	56.8 (298.24)	0.5 (11.3)	10.6
Not Pregnant	29.1 (1193.7)	0.7 (88.7)	11.8

Table 6.3. Anemia and severe anemia prevalence according to malaria parasitemia. P-values for Pearson's chi-square test are given.

		Malaria Parasitemia in all Women	
		No (weighted N=3114)	**Yes** (weighted N=1435)
Anemic	% (N)	31.6% (984)	34.5% (495)
	95% CI	29.6%-32.8%	32.1%-37.0%
	p-value		<.0001
Severely anemic	% (N)	0.7% (23)	0.6% (8)
	95% CI	0.4%-1.0%	0.2%-1.0%
	p-value		<.0001
		Malaria in Pregnant Women	
		No (weighted N=325)	**Yes** (weighted N=192)
Anemic	% (N)	54.7% (178)	60.0% (115)
	95% CI	49.3%-60.0%	52.8%-66.6%
	p-value		0.007
Severely anemic	% (N)	0.4% (1)	0.5% (1)
	95% CI	0.0%-1.1%	0.0%-1.4%
	p-value		<.0001

Table 6.4. Multilevel logistic regression results with anemia defined as less than 11 g/dl Hb; all women. Parameters significant at p=.05 or better are highlighted in bold.

Parameter	**Odds Ratio**	**95% Lower**	**95% Upper**	**p-value**
Intercept	0.2367	0.1032	0.5428	0.0007
Individual-Level Variables				
Age	**1.0127**	**1.0022**	**1.0233**	**0.0178**
Currently Pregnant	**3.7430**	**2.7701**	**5.0581**	**<.0001**
Has Refrigerator	1.0720	0.9832	1.1687	0.1151
Births in Past 5 Years	1.0375	0.9385	1.1469	0.4717
Body Mass Index	1.0000	0.9997	1.0003	0.8903
Years of Education	0.9767	0.9496	1.0044	0.0983
Culture of Origin: Bakongo	1.3596	0.9338	1.9794	0.1090
Culture of Origin: Bas-Kasai & Kwilu-Kwongo	1.2609	0.9207	1.7267	0.1485
Culture of Origin: Cuvette Centrale	1.3531	0.9639	1.8993	0.0806
Culture of Origin: Ubangi	1.2933	0.8032	2.0826	0.2900
Culture of Origin: Uele; Lac Albert	0.7661	0.5324	1.1022	0.1510
Community-Level Variables				
Population Density within 25 km	**1.0003**	**1.0001**	**1.0005**	**0.0030**
Highland Perennial Agriculture	**0.4247**	**0.2639**	**0.6834**	**0.0004**
Maize Mixed Agriculture	**0.2607**	**0.1311**	**0.5185**	**0.0001**
Root Crop Agriculture	**0.6208**	**0.3922**	**0.9828**	**0.0419**
Tree Crop Agriculture	**1.6839**	**1.2173**	**2.3291**	**0.0016**
Cereal Root Crop Mixed Agriculture	0.7722	0.4669	1.2772	0.3140
Urban	0.7984	0.6027	1.0575	0.1164
Distance to a Refugee Camp	1.0012	1.0000	1.0024	0.0501
Agricultural Land Cover within 25 km	1.0000	0.9997	1.0003	0.9854
Has International Aid	1.1663	0.8591	1.5831	0.3241

N=4356 (out of 4638)
Initial Model: 33 parameters, AIC = 5127.7974
Final Model: 21 parameters, AIC= 5121.4494

Table 6.5. Multilevel linear regression model with continuous hemoglobin level outcome variable; all women.

Parameter	**Beta Estimate**	**95% Lower**	**95% Upper**	**p-value**
Intercept	113.9761	106.4643	121.4878	<.0001
Individual-Level Variables				
Currently Pregnant	**-13.0274**	**-15.3883**	**-10.6666**	**<.0001**
Culture of Origin: Lunda	**-7.2706**	**-13.7798**	**-0.7614**	**0.0286**
Culture of Origin: Bakongo	-3.6384	-7.7915	0.5147	0.0860
Culture of Origin: Cuvette Centrale	-2.3271	-7.8774	3.2232	0.4112
Culture of Origin: Ubangi	-3.5231	-8.8272	1.7810	0.1930
Culture of Origin: Uele; Lac Albert	2.0519	-2.1626	6.2665	0.3400
Culture of Origin: Kasai	1.2866	-2.2284	4.8016	0.4731
Births in Past Year	-0.8847	-3.0373	1.2680	0.4205
Body Mass Index	0.0019	-0.0017	0.0056	0.2992
Years of Education	0.0672	-0.2551	0.3896	0.6828
Community-Level Variables				
Distance to a Refugee Camp (km)	**-0.0127**	**-0.0247**	**-0.0007**	**0.0373**
Highland Perennial Agriculture	**7.2711**	**2.2012**	**12.3411**	**0.0049**
Maize Mixed Agriculture	**11.4979**	**6.4935**	**16.5023**	**<.0001**
Tree Crop Agriculture	**-5.6149**	**-8.4299**	**-2.7999**	**<.0001**
Urban	2.2115	-0.5637	4.9866	0.1183
Distance to a City (km)	0.0088	-0.0082	0.0258	0.3091
Population Density within 25 km	-0.0014	-0.0037	0.0009	0.2254
Agricultural Land Cover within 25 km	0.0000	-0.0024	0.0023	0.9881

N=4356 (out of 4638)
Initial Model: 33 parameters, AIC = 40780.3114
Final Model: 18 parameters, AIC = 40775.3978

Table 6.6. Multilevel logistic regression results with anemia defined as less than 11 g/dl Hb; pregnant women only.

Parameter	Odds Ratio	95% Lower	95% Upper	p-value
Intercept	2.4663	0.3473	17.5157	0.3668
Indivdual-Level Variables				
Has Refrigerator	**0.7154**	**0.5463**	**0.9369**	**0.0149**
Age	0.9809	0.9449	1.0184	0.3139
HIV-Positive	0.6283	0.3435	1.1492	0.1315
Births in Past Year	2.2710	0.8325	6.1941	0.1092
Malaria Parasitemia	1.2960	0.7537	2.2282	0.3484
Body Mass Index	0.9997	0.9988	1.0007	0.5736
Years of Education	1.0509	0.9823	1.1241	0.1498
Number of Household Members	1.0665	0.9612	1.1834	0.2248
Culture of Origin: Bakongo	3.4823	0.7344	16.5122	0.1161
Culture of Origin: Cuvette Centrale	1.5275	0.7116	3.2789	0.2770
Culture of Origin: Ubangi	1.8329	0.8693	3.8644	0.1114
Culture of Origin: Uele; Lac Albert	1.9531	0.6800	5.6086	0.2136
Community-Level Variables				
Urban	**0.4933**	**0.2863**	**0.8498**	**0.0109**
Population Density within 25 km	**1.0007**	**1.0002**	**1.0012**	**0.0096**
Agricultural Land Cover within 25 km	**0.9993**	**0.9988**	**0.9999**	**0.0162**
Cereal Root Crop Mixed Agriculture	**0.1326**	**0.0185**	**0.9485**	**0.0441**
Maize Mixed Agriculture	0.6211	0.1577	2.4464	0.4960
Distance to a Refugee Camp (km)	1.0019	0.9990	1.0049	0.2029
Conflict Events since 1994 within 100 km	0.9979	0.9941	1.0017	0.2728

N=497 (out of 526)
Initial Model: 30 parameters, AIC =644.044
Final Model: 19 parameters, AIC= 636.5256

Table 6.7. Multilevel linear regression model with continuous hemoglobin level outcome variable; pregnant women only.

Parameter	Beta Estimate	95% Lower	95% Upper	p-value
Intercept	106.3526	93.1556	119.5496	<.0001
Individual-Level Variables				
Has Refrigerator	**1.7749**	**0.3091**	**3.2407**	**0.0176**
Years of Education	**-0.7671**	**-1.3075**	**-0.2268**	**0.0054**
Culture of Origin: Bakongo	**-18.1920**	**-32.1398**	**-4.2441**	**0.0106**
Culture of Origin: Cuvette Centrale	**-5.8628**	**-10.4353**	**-1.2903**	**0.0120**
Culture of Origin: Ubangi	**-9.3103**	**-16.6813**	**-1.9394**	**0.0133**
Births in Past 5 Years	-1.0755	-2.8709	0.7199	0.2404
Malaria Parasitemia	-2.5313	-6.4037	1.3411	0.2001
Body Mass Index	0.0000	-0.0063	0.0062	0.9902
Community-Level Variables				
Urban	**6.0013**	**1.5086**	**10.4940**	**0.0088**
Distance to a Town (km)	**0.0475**	**0.0097**	**0.0852**	**0.0138**
Agricultural Land Cover within 25 km	**0.0051**	**0.0018**	**0.0084**	**0.0026**
Cereal Root Crop Mixed Agriculture	**19.9249**	**3.9417**	**35.9082**	**0.0146**
Tree Crop Agriculture	**-14.0964**	**-17.9475**	**-10.2454**	**<.0001**
Highland Perennial Agriculture	9.7585	-0.1519	19.6690	0.0536
Maize Mixed Agriculture	3.9994	-4.6567	12.6554	0.3652

N=497 (out of 526)
Initial Model: 30 parameters, AIC=4344.9692
Final Model: 19 parameters, AIC=4339.1694

Table 6.8. Average iron content by agricultural type.

Agriculture Type	Average grams of Iron per metric ton of output
Cereal-Root Crop	16,442
Maize Mixed	14,318
Root Crop	12,354
Forest	8,352
Highland Perennial	7,636
Tree Crop	1,090

Figures

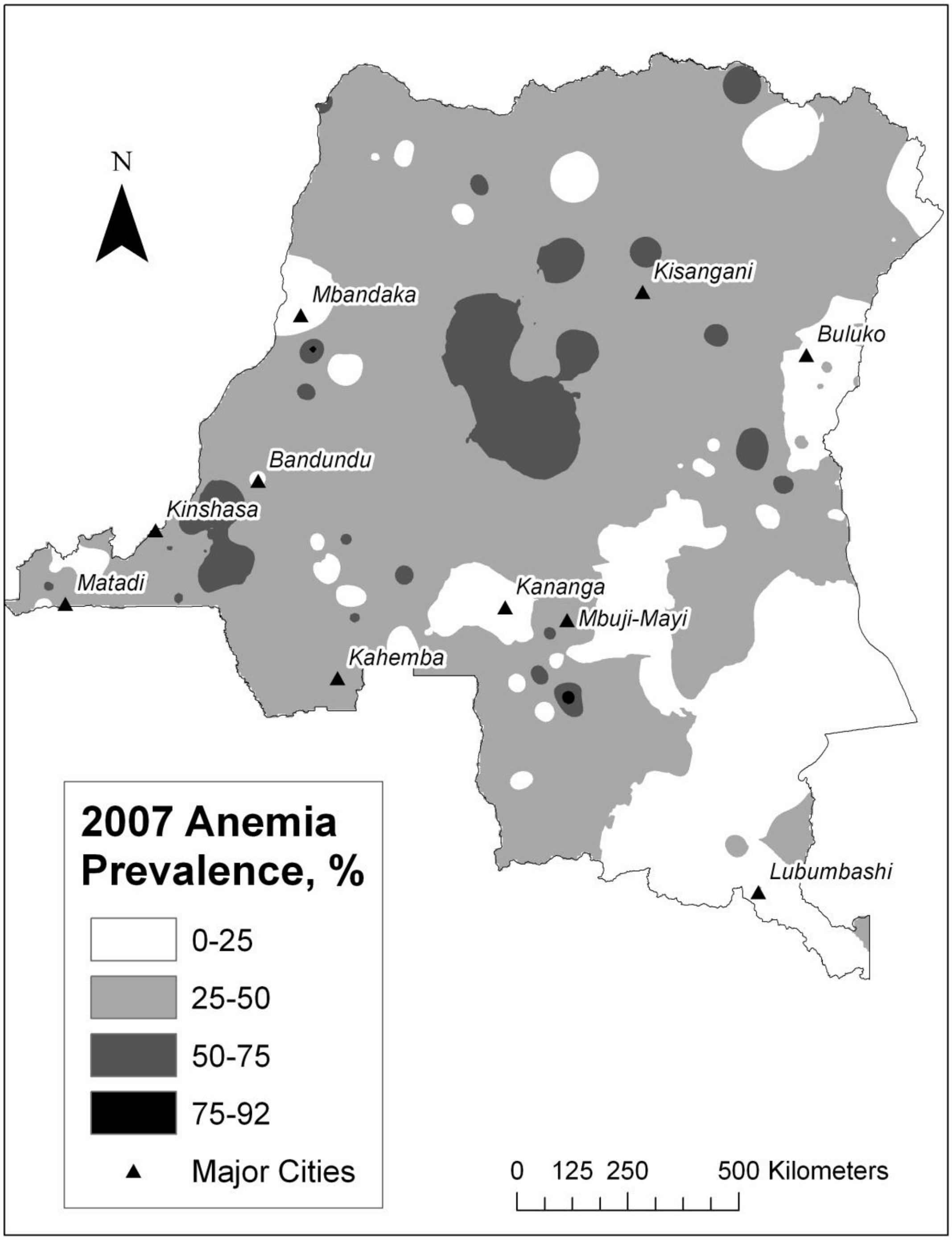

Figure 5.1. Inverse distance-weighted map of anemia prevalence in the DRC, 2007.

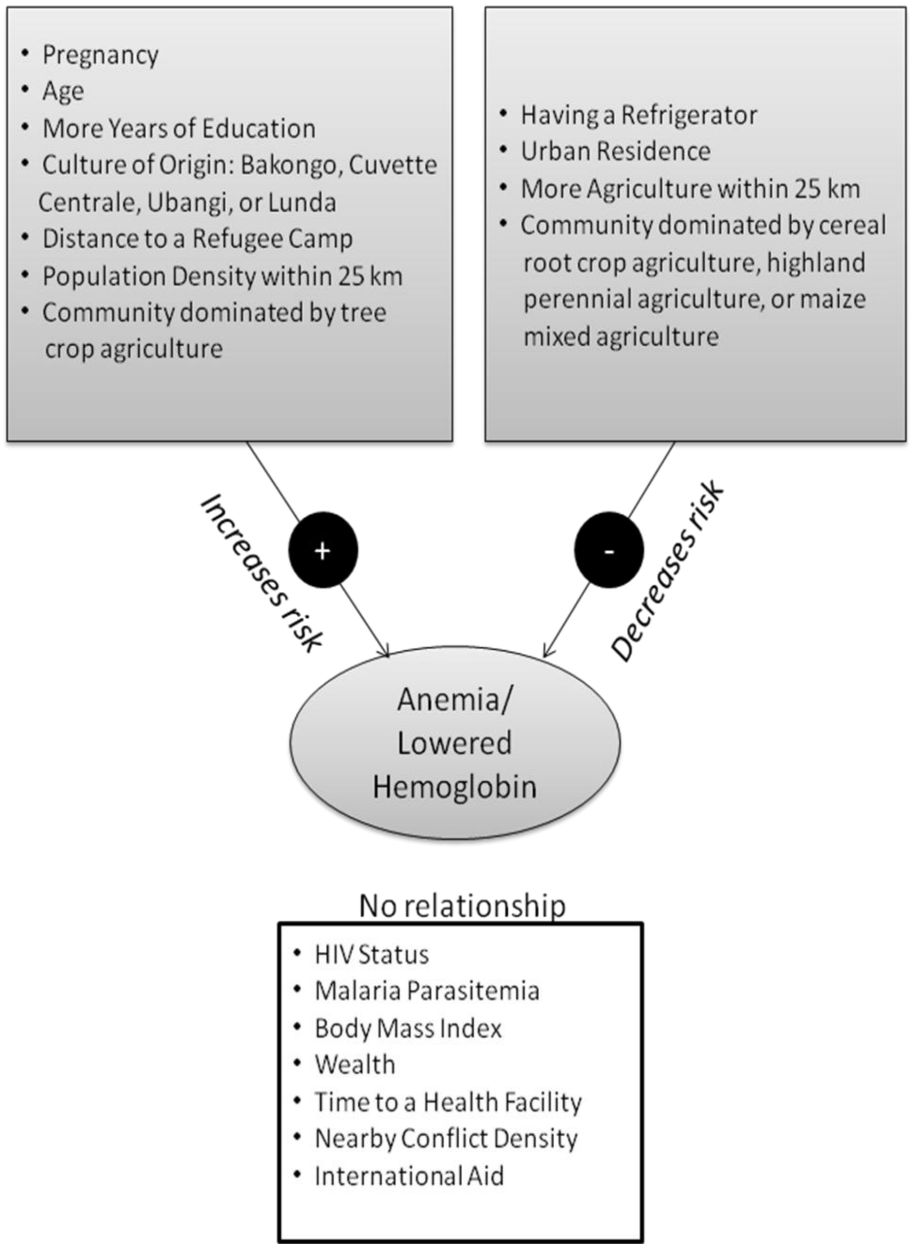

Figure 5.2. Variables entered into multivariate analyses according to their found relationship with anemia and/or lowered hemoglobin risk.

CONCLUSION

It is clear from this research that the spatial patterns of several important diseases in the Democratic Republic of the Congo (DRC) involves a complex set of interactions between humans, their behaviors, and their habitat. Drivers and indicators of risk were identified in this study, and evidence was provided that individual characteristics and behaviors, as well as aspects of the built and natural environment, are all important when considering risk for human affliction with HIV, malaria, and anemia. By illustrating the spatial patterns of risk for these three diseases in 2007 and revealing factors which may increase risk, this research has increased understanding of human illness in the culturally- and ecologically-complex DRC.

In the first part of each substantive chapter of this study, spatial analyses were performed to determine the distribution of human illness from HIV, malaria, and anemia across the DRC. Using inverse-distance weighted interpolation of prevalence within DHS sample clusters, it was confirmed that the spatial distribution these diseases in 2007 exhibited extremely non-uniform patterns. Overall, HIV prevalence ranged from 0-36% across the DRC, with lower rates found in the southwest portion of the country. Higher rates of HIV were found in the north and west of the country. By examining male and female infection separately, it was determined that high HIV rates found in Kivu are likely accounted for by high prevalence of male infection, while higher rates of female infection are found further to the north as well as to the south of this male HIV hotspot. As for malaria, a range of 0-82% prevalence was estimated across the DRC. The centre and east-central regions of the country are areas of low prevalence, as well as the urban areas near Kinshasa and Lubumbashi. The northern part of the country has particularly high prevalence, as do the more rural regions near Kinshasa and Lubumbashi. Finally, anemia prevalence was found to range from 0-92% across the DRC, with the highest prevalence generally being found in the center of the country. The southeast portion of the country has lower anemia rates, as well as areas surrounding major cities. Heterogeneous patterns of all three diseases indicated that the characteristics of certain places and the populations within those places were causing them to be at greater risk, and potential risk factors were explored in greater detail.

Geographic Information Systems (GIS) and multivariate statistical analysis were essential in exploring the complex and locally variable ecological systems associated with HIV, malaria, and anemia. Several multilevel models were derived in order to examine the determinants of risk for each disease while accounting for the clustering in the sample design. Three categories of potential explanatory factors (population, behavioral, and habitat) were included in all models according to Melinda Meade's formulation of disease ecology theory [12, 145]. While there was some overlap in the variables explored for each disease, the choice of variables was tailored to the human ecology of each disease as determined based upon the published literature. The varied model inputs and outputs highlight the fact that the DRC hosts a complex set of processes which affect the health of its human populations, and generalization of the factors increasing risk for all three diseases may not be appropriate. However, it is possible to compare and contrast the findings of the models for each different disease.

At the individual level, the population and behavioral nodes of the disease ecology triangle were explored in detail for each disease. While older people were at greater risk for HIV and anemia, younger people were at greater risk for malaria. With a much greater proportion of the population being in the younger age groups, the risk for malaria in large numbers of people is significant. Individual wealth was a risk factor for HIV, while it was protective against malaria. This underscores the importance of understanding behavioral aspects of the disease ecology for different disease types. While wealth is a privilege that enables some to obtain resources to protect themselves from a vector-borne disease like malaria, this privilege puts some men at disproportionate risk for a sexually-transmitted disease if it is abused for the purposes of paying for sex from sex workers. Another interesting finding at the individual level was the increased risk for anemia in certain cultural groups. Little is published regarding the customs of individual Congolese cultural groups, and this area presents a significant opportunity for future research and field work, and would perhaps be best situated within the field of medical anthropology.

At the community level, the habitat node of the disease ecology triangle was explored. While living in a community located near a city increased risk for HIV, living near a town decreased risk for malaria. Both of these findings were in line with recent literature described in the introduction. HIV is associated with high-risk sexual networks typically found in large urban areas, while the conditions of rural areas (vegetation, standing water) are more conducive to malaria transmission. The relationship between anemia and urban vs. rural residence was found to be more complex, however. While living in an urban area associated with increased access to a variety of foods is protective, if one does not live in a city, having greater access to agricultural outputs is important, especially if they are not forest or tree crops. Living in a smaller town would thus be disadvantageous to one's nutritional health, as these people likely do not have access to large amounts of imported food as in the highly urban areas, nor large amounts of agricultural outputs as in the more rural areas. Certain types of agriculture are particularly protective against anemia, being cereal, highland perennial (e.g., coffee beans), or maize agriculture.

The findings that greater density of nearby conflict since 1994 decreased malaria risk and that proximity to a refugee camp was protective against anemia in women were important but should be explored in greater detail. These findings support the possibility that in conflict and refugee zones, anti-malarial drugs and nutrition are being provided in greater amounts to nearby residents. Unexpectedly, conflict density and proximity to refugee camps had no effect on HIV risk; it is likely with this disease that analysis of individual cases of violence (both domestic and from conflict) against respondents would be associated with increased risk for HIV. However, this type of reporting was not done for those tested for HIV in the 2007 DRC DHS. It must be reiterated that no connection was hypothesized or found to exist between individual battle events and disease outcome. Rather, the effects of long-lasting and ongoing conflict in certain areas were hypothesized to have some effect on the nature of certain places such that human health would be affected. This hypothesis was ultimately supported by findings of this dissertation, particularly with regard to malaria.

Also of interest are findings that certain population characteristics and behaviors were equally or more important at the community level as at the individual level. For example, greater individual wealth was protective against malaria along with the average wealth of the community in which one lived. Along with the finding that community bed net use was protective against malaria as well, this indicates the existence of a "herd immunity" effect in which those who do not have the resources to protect themselves against mosquitoes may be protected by others' efforts at decreasing exposure to the malaria-bearing vectors. HIV prevalence within one's community was also an important risk factor for HIV, even after controlling for individual risky behaviors such as the total lifetime number of sexual partners. Thus, those who live in neighborhoods with HIV prevalence must limit their risky sexual behaviors to a greater extent.

Overall, this research has shown that the DRC hosts a multitude of landscapes which may be considered "vulnerable" for HIV, malaria, and anemia. While the creation of a GIS database enabled characterization of the landscape, the way in which people may have adapted or altered their behavior in order to cope with vulnerability was explored in detail thanks to the extensive DHS dataset. In the case of HIV and malaria, this involves avoiding contact with certain pathogens. To decrease risk of infection with HIV, people in the DRC must adapt their sexual behaviors, particularly in high-prevalence areas. The fact that the prevalence of HIV within 25 kilometers of an individual's community is a highly significant predictor of individual risk is a unique finding which underscores the need for greater adaptive behaviors in localized high-risk areas. Public health efforts aimed at increasing awareness of safer sexual behaviors specifically in these areas may help reduce transmission. While a relatively low prevalence of HIV exists in the country as a whole, the pattern of prevalence is spatially heterogeneous, and demographic, behavioral, and habitat factors play an important role in determining the geographic patterns of high and low prevalence. The demographic characteristics of individuals within certain communities proved to be an important component in predicting prevalence. Those who were able to adapt their behaviors to avoid high-risk areas and keep a relatively low number of sexual partners were more likely to be protected from HIV infection. Population growth and urbanization are important habitat factors to consider as well; however, the poor economic state of the DRC has generally prevented these phenomena from occurring. With few roads, urban centers remain disconnected and hotspots of high HIV prevalence remain relatively isolated. The physical ecology of the DRC is also important to understanding the transmission of HIV, as rivers and waterways remain the primary method of transportation in the country. Ease of access to urban areas is an important consideration as well as residence within these areas. While individuals may reduce the risk of transmission by limiting their number of sexual partners, from a municipal or public health standpoint, prevention becomes more complicated. Increasing connectedness of urban centers is desirable for economic purposes, and thus should not be discouraged. However, campaigns specifically aimed at educating the residents of high-risk areas about the importance of limiting sexual partnerships may help decrease transmission within these areas. These programs should be adapted to the culture of the area, with a regional if not highly localized focus.

In terms of malaria, parasites are brought into contact with the humans in the landscape via mosquitoes, which are more prevalent in certain areas than others due to appropriate ecological conditions. These conditions may include deforestation, greater amounts of water for depositing larvae, and lower altitude. However, appropriate ecological conditions are not the only consideration when determining if humans come into contact with mosquitoes. The way in which people adapt their behaviors in vulnerable areas is of great importance as well. Specifically, community use of bed nets protects not only those under the bed nets, but the population of the community as a whole. The fewer humans who serve as reservoirs for the parasite, the fewer mosquitoes will subsequently transmit the virus to new hosts. Thus, while individual access to a bed net is desirable, it is more important from a public health standpoint that a minimum number of residents of a community be provided with these nets and are educated as to the importance of proper usage. Demographically, younger individuals and males are at greater risk, meaning that bed net campaigns may be focused not only in the highest-risk areas shown on the map, but also toward these high-risk populations. Poorer families and communities within high-risk zones should also be preferentially targeted for prevention purposes, as these populations generally have decreased access to anti-malarial drugs, bed nets, and screens for their homes. It should also be the goal of public health officials and international aid agencies to increase efforts in conflict-free zones, as this study indicates that areas that have been little-affected by conflict over the past two decades may be neglected with regard to malaria prevention campaigns.

As regards anemia in DRC women, prevalence involves a complex interaction between one's demographic characteristics, behaviors, and place of residence. Demographically, pregnancy is unsurprisingly the single greatest risk factor for anemia. Behaviorally, anemia differs by cultural affiliation. This is likely due to dietary habits, and highlights the importance of adapting any anemia prevention efforts to the local culture of a given community. In terms of place of residence, living in an urban area with large varieties of food is protective against nutritional deficiencies which lead to anemia. Failing that, local access to nutritionally rich agricultural types is important in the DRC. This includes most types of agriculture other than tree crops and forest scavenging. Unfortunately, the majority of the DRC is dominated by forest agriculture, and deeply forested areas tend to be particularly isolated from urban areas. It is difficult for individuals to adapt their behaviors in these areas when transportation to urban zones is extremely limited and poverty is abundant. Therefore, public health efforts at decreasing anemia in pregnant women may be most needed in rural areas without nutritionally rich varieties of agriculture.

To conclude, the DRC presents researchers, public health officials, and international aid agencies with a cultural ecologic conundrum in terms of disease prevalence. While the people in the DRC may do what they can in order to adapt to vulnerability for several diseases, only some of which are HIV, malaria, and anemia, poor economic conditions may inhibit the sustainability of any top-down approach. Changes must be appropriate in terms of local culture, behavior, and societal norms in order to be maintained through time. From a research standpoint, the choice of appropriate spatial methods is integral to the creation of distribution maps for any disease, as is the implementation of disease ecology theory in the formulation of models and interpretation of results. Understanding the three nodes of the disease ecology triangle – population, behavior, and habitat – is essential in terms of researching potential risk factors. While data for all possible risk factors is not always obtainable, the creation of an extensive GIS database and selection of individual questionnaire responses may be extremely well-informed by disease ecology theory. This research presents an example which extends beyond the scope of what would have been possible with the DHS database alone. The molecular results for malaria parasitaemia as well as habitat data obtained from a variety of other sources contribute to the creation of a unique and complex database.. Such complexity sets this research apart from other work done using DHS surveys.

Study Limitations

Several limitations were discussed in previous chapters, particularly the absence of relevant data which was a problem for each of the three diseases. For HIV, lack of information about personal violence, namely rape, presents a “behavioral” gap in the data. It is likely that experiences of forced sex would increase one’s likelihood of being infected with HIV; unfortunately, however, those DHS respondents chosen to participate in the domestic violence module were not those for whom blood spots were collected to test for HIV. This is an important limitation of the DHS dataset. Information about migration, particularly due to mass population movements resulting from conflict, would also have been extremely valuable in understanding HIV exposure, as one’s place of residence in 2007 may not necessarily be indicative of the location of infection with the virus.

As regards malaria, while the DHS survey provided rich information about bed net and anti-malarial drug usage, most questions were concerned with children. This is unsurprising, as children are at the greatest risk for malaria in sub-Saharan Africa.

However, as our blood spot samples were limited to adults who were tested for HIV, inference had to be made in several cases between children's bed net and drug usage and that of adults. Either blood sampling from children or direct questions about adults' protective behaviors against malaria would have the potential to improve the strength of the findings presented regarding malaria. A further limitation concerning malaria findings was that clinical symptoms were also unknown in this study. The DHS survey was not intended for the type of analysis presented here, with blood spots being reused for PCR testing of malaria parasitaemia. Therefore, while the risk factors determined to be significant for asymptomatic malaria parasitaemia may not be the same if the outcome variable had been actual illness from malaria. Nonetheless, the combination of DHS and molecular data enabled the most spatially-detailed understanding of malaria prevalence and risk for the DRC to date.

Similar to the malaria study, the anemia study was limited in its lack of inclusion of information about children's anemia outcome, with children being at great risk for the disease along with women. Men were also not tested for anemia; while men are at decreased risk for anemia overall (much of it resulting from pregnancy as was seen in this study), information about their hemoglobin levels would have had the potential to alter or support findings for women. While the dominant agricultural type of one's community was determined in this study, more direct data about individual and household dietary habits would also have the potential to significantly enhance the findings of the anemia research.

Some general limitations apply to all aspects of this research as well. Firstly, data were limited in that individuals could only be located to the center of their community, and not to their actual place of residence. This may have affected several of the community-level variables which were computed in the GIS, such as population density, climate factors and distances to roads, towns, or refugee camps. While the complexity of the databases created for each disease is indeed what sets this study apart, it may also present a limitation in that many datasets came from different sources and were collected in different years. However, the most recently available datasets were used for all variables and justification for the choice of years for certain variables was presented when necessary.

Directions for Future Research

One area for future research was shared for all diseases, and that is the potential for a much more detailed analysis of conflict and disease in the DRC. HIV did not exhibit any association with density of past conflicts nearby or location of refugee camps, while malaria was associated with decreased risk in zones of high conflict density and anemia was associated with decreased risk in locations close to refugee camps. These findings are limited by the type of data which was available, and also by a lack of information about how individuals may have been directly affected (or not) by conflict or refugee camps. However, the fact that associations were found undoubtedly merits further research. Detailed field research involving interviews and characterization of levels of insecurity or effects of such insecurity in one's place of residence could tease out the reasons behind the found associations (or lack thereof). Unfortunately, field research in zones of insecurity may present obvious obstacles and would need to be carried out with caution.

As with any study involving cross-sectional data, the most important direction for future research is the study of future years of data. The next planned DHS for the DRC will take place in 2012, and the findings of this study may be contrasted with or supported by an additional year of data. Although the survey will not be aimed at the same set of individuals or even communities, it will nonetheless be country-wide and will be appropriate for a similar type of study as was carried out here, making comparisons possible. If resources available, any number of other diseases may be studied in the same fashion as well, either with 2007 or 2012 data. This research has shown the strengths of combining survey and molecular data with geographic and multivariate statistical methods to study sexually-transmitted, vector-borne, and chronic or nutritional diseases. Using the frameworks of spatial and epidemiology and given the appropriate data, many other types of infectious or chronic diseases may be studied in relation to their spatial distribution and potential risk factors. Therefore, this study has not only contributed new and important information regarding HIV, malaria, and anemia in the DRC, but has also provided a framework for increasing knowledge and understanding of any number of diseases, in the DRC and beyond.

REFERENCES

1. Coghlan, B., et al., *Mortality in the Democratic Republic of Congo: a nationwide survey.* Lancet, 2006. **367**(9504): p. 44-51.

2. Coghlan, B., et al., *Update on mortality in the Democratic Republic of Congo: results from a third nationwide survey.* Disaster medicine and public health preparedness, 2009. **3**(2): p. 88-96.

3. *Malaria, reported cases.* Earth Trends, 2001. **2008**(Journal Article).

4. Snow, R.W., et al. *The public health burden of plasmodium falciparum malaria in Africa: deriving the numbers*. in *Disease Control Priorities Project*.

5. May, J.M., *The ecology of human disease: studies in medical geography*. 1958, New York: MD Publications.

6. May, J.M., *Studies in disease ecology*. 1961, New York: Hafner Publishing Company, Inc.

7. Dubos, R., *Mirage of health; Utopias, progress, and biological change: world perspectives, v. 22*. 1959, New York, NY: Harper & Brothers Publishers.

8. Audy, J.R., *Health and disease.* Human ecology: an interdisciplinary journal, 1974.

9. Audy, J.R., *Aspects of human behavior interfering with vector control*, in *Meeting of the Advisory Committee on Medical Research*. 1971, Pan American Health Organization: Washington, D.C.

10. Audy, J.R. *Types of human influence on natural foci of disease*. in *Theoretical questions of natural foci of disease: Proceedings of a symposium*. 1965. Prague: Czechoslovak Academy of Science.

11. Audy, J.R., *A biological approach to medical geography.* The British Medical Journal, 1954. **1**(4868): p. 960-962.

12. Meade, M.S. and R. Earickson, *Medical geography*. Vol. 2. 2000, New York: Guilford Press. 501.

13. Glass, G.E., *Update: spatial aspects of epidemiology: the interface with medical geography.* Epidemiologic reviews, 2000. **22**(1): p. 136.

14. Ostfeld, R.R.S., *Spatial epidemiology: an emerging (or re-emerging) discipline.* Trends in ecology & evolution (Amsterdam), 2005. **20**(6): p. 328-336.

15. Elliott, P.P., *Spatial epidemiology: Current approaches and future challenges.* Environmental health perspectives, 2004. **112**(9): p. 998.

16. Cromley, E.E.K., *GIS AND DISEASE.* Annual Review of Public Health, 2003. **24**(1): p. 7-24.

17. *Demographic and health surveys*. Available from: http://www.measuredhs.com.

18. Bureau, U.S.C. *International data base - demographic indicators - Congo (Kinshasa).* [cited 2011 November 2]; Available from: http://www.census.gov/population/international/data/idb/country.php.

19. Unaids, *2008 report on the global AIDS epidemic*. 2008.

20. Gray, R.H., et al., *Increased risk of incident HIV during pregnancy in Rakai, Uganda: a prospective study.* Lancet, 2005. **366**(9492): p. 1182-1188.

21. Dodoo, F.F.N.A., *Urban–rural differences in the socioeconomic deprivation–sexual behavior link in Kenya.* Social science & medicine (1982), 2007. **64**(5): p. 1019-1031.

22. Kalipeni, E.E., *Concern and practice among men about HIV/AIDS in low socioeconomic income areas of Lilongwe, Malawi.* Social science & medicine (1982), 2007. **64**(5): p. 1116-1127.

23. Masanjala, W.W., *The poverty-HIV/AIDS nexus in Africa: A livelihood approach.* Social science & medicine (1982), 2007. **64**(5): p. 1032-1041.

24. Spiegel, P.B., et al., *Prevalence of HIV infection in conflict-affected and displaced people in seven sub-Saharan African countries: a systematic review.* Lancet, 2007. **369**(9580): p. 2187-2195.

25. Anema, A., et al., *Widespread rape does not directly appear to increase the overall HIV prevalence in conflict-affected countries: so now what?* Emerging themes in epidemiology, 2008. **5**(Journal Article): p. 11.

26. Kim, A.A., et al., *HIV infection among internally displaced women and women residing in river populations along the Congo River, Democratic Republic of Congo.* AIDS and behavior, 2009. **13**(5): p. 914-920.

27. Mulanga, C., et al., *Political and socioeconomic instability: how does it affect HIV? A case study in the Democratic Republic of Congo.* AIDS (London, England), 2004. **18**(5): p. 832-834.

28. Unaids, *2009 AIDS Epidemic Update*. 2009.

29. Glynn, J.R., *Why do young women have a much higher prevalence of HIV than young men? A study in Kisumu, Kenya and Ndola, Zambia.* AIDS (London), 2001. **15**(Journal Article): p. S51.

30. Laga, M., *To stem HIV in Africa, prevent transmission to young women.* AIDS (London), 2001. **15**(7): p. 931.

31. Kalipeni, E.E., *HIV/AIDS, gender, agency and empowerment issues in Africa.* Social science & medicine (1982), 2007. **64**(5): p. 1015-1018.

32. Kalipeni, E.E., *HIV/AIDS in women: stigma and gender empowerment in Africa.* Future HIV therapy, 2008. **2**(2): p. 147-153.

33. Serwadda, D., et al., *HIV risk factors in three geographic strata of rural Rakai District, Uganda.* AIDS (London, England), 1992. **6**(9): p. 983-989.

34. Amat-Roze, J.M., et al., - *The shrinking costs of war*. 2009. Available from: http://humansecuritygateway.com.

35. Remy, G., *Geographic distribution of HIV-1 infection in Central Africa: remarkable discontinuities.* Annales de la Societe Belge de Medecine Tropicale, 1993. **73**(2): p. 127-142.

36. Arroyo, M.A., et al., *HIV-1 diversity and prevalence differ between urban and rural areas in the Mbeya region of Tanzania.* AIDS (London, England), 2005. **19**(14): p. 1517-1524.

37. Arroyo, M.A., et al., *Higher HIV-1 incidence and genetic complexity along main roads in Rakai District, Uganda.* Journal of acquired immune deficiency syndromes (1999), 2006. **43**(4): p. 440-445.

38. Coffee, M., M.N. Lurie, and G.P. Garnett, *Modelling the impact of migration on the HIV epidemic in South Africa.* AIDS (London, England), 2007. **21**(3): p. 343-350.

39. Coffee, M.P., et al., *Patterns of movement and risk of HIV infection in rural Zimbabwe.* The Journal of infectious diseases, 2005. **191 Suppl 1**(Journal Article): p. S159-67.

40. Karim, S.S., *Commentary: spatial clustering of HIV infection: providing clues for effective HIV prevention.* International journal of epidemiology, 2009(Journal Article).

41. Gray, R.R.R., *Spatial phylodynamics of HIV-1 epidemic emergence in east Africa.* AIDS (London), 2009. **23**(14): p. F9-F17.

42. Obbo, C., *HIV transmission through social and geographic networks in Uganda.* Social science & medicine (1982), 1993. **36**(7): p. 949-955.

43. Webb, D., *Mapping the AIDS pandemic: geographical progression of HIV in South Africa 1990-93.* Nursing RSA = Verpleging RSA, 1994. **9**(9): p. 20-21.

44. WHO/Afro, *Malaria Country Profiles: Democratic Republic of Congo*. 2002. Available from: http://www.afro.who.int.

45. Guerra, C.A., R.W. Snow, and S.I. Hay, *A global assessment of closed forests, deforestation and malaria risk.* Annals of Tropical Medicine and Parasitology, 2006. **100**(3): p. 189-204.

46. Fontenille, D.D., *Unravelling complexities in human malaria transmission dynamics in Africa through a comprehensive knowledge of vector populations.* Comparative immunology, microbiology and infectious diseases, 2004. **27**(5): p. 357-375.

47. Coetzee, M., M. Craig, and D. Le Sueur, *Distribution of African malaria mosquitoes belonging to the anopheles gambiae complex.* Parasitology Today 2000. **16**(2): p. 74-77.

48. Drakeley, C.J., et al., *Estimating medium- and long-term trends in malaria transmission by using serological markers of malaria exposure.* Proceedings of the National Academy of Sciences of the United States of America, 2005. **102**(14): p. 5108-5113.

49. White, N.J., *Antimalarial drug resistance.* The Journal of clinical investigation, 2004. **113**(8): p. 1084-1092.

50. World Health Organization, *World Malaria Report 2008*.

51. World Health Organization, *World Malaria Report 2008*.

52. Van Herp, M., et al., *Mortality, violence and lack of access to healthcare in the Democratic Republic of Congo.* Disasters, 2003. **27**(2): p. 141-153.

53. World Health Organization, *Malaria*. 2009.

54. Bloland, P.B. and M. Ettling, *Making malaria-treatment policy in the face of drug resistance.* Annals of Tropical Medicine and Parasitology, 1999. **93**(1): p. 5-23.

55. Hyde, J.E., *Drug-resistant malaria.* Trends in parasitology, 2005. **21**(11): p. 494-498.

56. Wongsrichanalai, C., et al., *Epidemiology of drug-resistant malaria.* The Lancet infectious diseases, 2002. **2**(4): p. 209-218.

57. White, N.J., *Drug resistance in malaria.* British medical bulletin, 1998. **54**(3): p. 703-715.

58. Lindsay, S.W. and W.J. Martens, *Malaria in the African highlands: past, present and future.* Bulletin of the World Health Organization, 1998. **76**(1): p. 33-45.

59. Mouchet, J., et al., *Evolution of malaria in Africa for the past 40 years: impact of climatic and human factors.* Journal of the American Mosquito Control Association, 1998. **14**(2): p. 121-130.

60. Packard, R.M. *Filling in the gaps: documenting the social determinants of malaria*. in *WHO/UCL Conference on the Social Determinants of Health*.

61. Bloland, P. and H. Williams, *Malaria control during mass population movements and natural disasters*. 2003, Washington: The Academic Academies Press.

62. Lindsay, S.W., L. Parson, and C.J. Thomas, *Mapping the ranges and relative abundance of the two principal African malaria vectors, Anopheles gambiae sensu stricto and An. arabiensis, using climate data.* Proceedings.Biological sciences / The Royal Society, 1998. **265**(1399): p. 847-854.

63. Hay, S.I., et al., *The global distribution and population at risk of malaria: past, present, and future.* The Lancet infectious diseases, 2004. **4**(6): p. 327-336.

64. Gemperli, A., et al., *Mapping malaria transmission in West and Central Africa.* Tropical medicine & international health : TM & IH, 2006. **11**(7): p. 1032-1046.

65. Martin, C., et al., *The use of a GIS-based malaria information system for malaria research and control in South Africa.* Health & place, 2002. **8**(4): p. 227-236.

66. Mouchet, J., *Vectors and environmental factors in malaria.* Transfusion clinique et biologique : journal de la Societe francaise de transfusion sanguine, 1999. **6**(1): p. 35-43.

67. Omumbo, J., et al., *Mapping malaria transmission intensity using geographical information systems (GIS): an example from Kenya.* Annals of Tropical Medicine and Parasitology, 1998. **92**(1): p. 7-21.

68. Craig, M.H., R.W. Snow, and D. le Sueur, *A climate-based distribution model of malaria transmission in sub-Saharan Africa.* Parasitology today (Personal ed.), 1999. **15**(3): p. 105-111.

69. Kleinschmidt, I., et al., *A spatial statistical approach to malaria mapping.* International journal of epidemiology, 2000. **29**(2): p. 355-361.

70. Carter, R., K.N. Mendis, and D. Roberts, *Spatial targeting of interventions against malaria.* Bulletin of the World Health Organization, 2000. **78**(12): p. 1401-1411.

71. Tatem, A.J. and S.I. Hay, *Measuring urbanization pattern and extent for malaria research: a review of remote sensing approaches.* Journal of urban health : bulletin of the New York Academy of Medicine, 2004. **81**(3): p. 363-376.

72. Keiser, J., et al., *Urbanization in sub-saharan Africa and implication for malaria control.* The American Journal of Tropical Medicine and Hygiene, 2004. **71**(2 Suppl): p. 118-127.

73. Robert, V., et al., *Malaria transmission in urban sub-Saharan Africa.* American Journal of Tropical Medicine and Hygiene, 2003. **68**(2): p. 169-176.

74. Stoltzfus, R.J., *Defining iron-deficiency anemia in public health terms: a time for reflection.* Journal of Nutrition, 2001. **131**(2): p. 565S-567.

75. Ngnie-Teta, I.I., *Multilevel modelling of sociodemographic predictors of various levels of anaemia among women in Mali.* Public health nutrition, 2009. **12**(09): p. 1462.

76. Ngnie-Teta, I.I., O. Receveur, and B. Kuate-Defo, *Risk factors for moderate to severe anemia among children in Benin and Mali: Insights from a multilevel analysis.* Food and Nutrition Bulletin, 2007. **28**(1): p. 76-88.

77. Conway, G. and S. Sechler, *Helping Africa feed itself.* Science, 2000. **289**(5485): p. 1685.

78. Guyatt, H.L., *The epidemiology and burden of Plasmodium falciparum-related anemia among pregnant women in sub-Saharan Africa.* The American Journal of Tropical Medicine and Hygiene, 2001. **64**(1 suppl): p. 36.

79. van Eijk, A.M., et al., *Malaria and human immunodeficiency virus infection as risk factors for anemia in infants in Kisumu, western Kenya.* American Journal of Tropical Medicine and Hygiene, 2002. **67**(1): p. 44-53.

80. Ter Kuile, F.O., et al., T*he burden of co-infection with human immunodeficiency virus type 1 and malaria in pregnant women in sub-Saharan Africa.* American Journal of Tropical Medicine and Hygiene, 2004. **71**(2_suppl): p. 41-54.

81. Diez-Roux, A.A.V., *Multilevel analysis in public health research.* Annual Review of Public Health, 2000. **21**(1): p. 171-192.

82. Duncan, C., *Context, composition and heterogeneity: using multilevel models in health research.* Social science & medicine (1982), 1998. **46**(1): p. 97.

83. Diez Roux, A.V., *A glossary for multilevel analysis.* Journal of epidemiology and community health, 2002. **56**(8): p. 588-594.

84. World Health Organization. *Democratic Republic of the Congo:sSummary country profile for HIV/AIDS treatment scale-up*. 2005; Available from: http://www.who.int/publications.

85. Tanser, F.C. and D. Le Sueur, *The application of geographical information systems to important public health problems in Africa.* International journal of health geographics, 2002. **1**(1): p. 4.

86. Busgeeth, K. and U. Rivett, *The use of a spatial information system in the management of HIV/AIDS in South Africa.* International journal of health geographics, 2004. **3**(1): p. 13.

87. Chamla, D.D., et al., *Geographical information system and access to HIV testing, treatment and prevention of mother-to-child transmission in conflict affected Northern Uganda.* Conflict and health, 2007. **1**(Journal Article): p. 12.

88. Vanmeulebrouk, B., et al., *Open source GIS for HIV/AIDS management.* International journal of health geographics, 2008. **7**(Journal Article): p. 53.

89. Tanser, F., et al., *Localized spatial clustering of HIV infections in a widely disseminated rural South African epidemic.* International journal of epidemiology, 2009(Journal Article).

90. Amat-Roze, J.M., *Geographic inequalities in HIV infection and AIDS in sub-saharan Africa.* Social science & medicine (1982), 1993. **36**(10): p. 1247-1256.

91. MacPhail, C.C., *Relative risk of HIV infection among young men and women in a South African township.* International Journal of STD & AIDS, 2002. **13**(5): p. 331-342.

92. Zierler, S.S., *Reframing women's risk:social inequalities and HIV infection.* Annual Review of Public Health, 1997. **18**(1): p. 401-436.

93. Berkley, S., *AIDS and HIV infection in Uganda-are more women infected than men?* AIDS (London), 1990. **4**(12): p. 1237.

94. Pisani, E., *AIDS into the 21st century: some critical considerations.* Reproductive Health Matters, 2000. **8**(15): p. 63-76.

95. Raleigh, C. and H. Hegre. *Introducing ACLED: an Armed Conflict Location and Event Dataset'*. in *Disaggregating the Study of Civil War and Transnational Violence*: University of California Institute of Global Conflict and Cooperation.

96. *Gridded Population of the World*. 2008.

97. Burnham, K.P. and D.R. Anderson, *Model selection and multimodel inference : a practical information-theoretic approach*. Vol. 2. 2002, New York: Springer. 488.

98. Maman, S.S., *HIV-Positive Women Report More Lifetime Partner Violence: Findings From a Voluntary Counseling and Testing Clinic in Dar es Salaam, Tanzania.* American journal of public health (1971), 2002. **92**(8): p. 1331-1337.

99. Gillespie, S.S., *Is poverty or wealth driving HIV transmission?* AIDS (London), 2007. **21**(suppl 7): p. S5-S16.

100. Kimuna, S.R., *Wealth and extramarital sex among men in Zambia.* International family planning perspectives, 2005. **31**(2): p. 83.

101. Gauthier, J.M. and L. Guepin. *DRC Snapshot - Roads in the DRC*. 2006 4/30/2009]; Available from: http://www.unjlc.org/drc.

102. Laga, M., R. Matendo, and A. Buve, *Chapter 11: The Situation in the Cradle of AIDS: Congo and Central Africa*, in *Public health aspects of HIV/AIDS in low and middle income countries*, D. Celentano and C. Beyrer, Editors. 2008, Springer: New York. p. 196.

103. *World Health Organization Global Health Observatory*. Available from: http://www.who.int/gho/database/en/.

104. *Roll Back Malaria*. Available from: http://www.rbm.who.int/

105. Molineaux, L., *Nature's experiment: what implications for malaria prevention?* Lancet, 1997. **349**(9066): p. 1636-1637.

106. Mwenesi, H.A., *Social science research in malaria prevention, management and control in the last two decades: an overview.* Acta Tropica, 2005. **95**(3): p. 292.

107. Barnes, K.I., et al., *Effect of artemether-lumefantrine policy and improved vector control on malaria burden in KwaZulu-Natal, South Africa.* PLoS medicine, 2005. **2**(11): p. e330.

108. Bhattarai, A., et al., *Impact of artemisinin-based combination therapy and insecticide-treated nets on malaria burden in Zanzibar.* PLoS medicine, 2007. **4**(11): p. e309.

109. Ceesay, S.J., et al., *Changes in malaria indices between 1999 and 2007 in The Gambia: a retrospective analysis.* Lancet, 2008. **372**(9649): p. 1545-1554.

110. Fegan, G.W., et al., *Effect of expanded insecticide-treated bednet coverage on child survival in rural Kenya: a longitudinal study.* Lancet, 2007. **370**(9592): p. 1035-1039.

111. Nyarango, P.M., et al., *A steep decline of malaria morbidity and mortality trends in Eritrea between 2000 and 2004: the effect of combination of control methods.* Malaria journal, 2006. **5**(Journal Article): p. 33.

112. Okiro, E.A., et al., *The decline in paediatric malaria admissions on the coast of Kenya.* Malaria journal, 2007. **6**(Journal Article): p. 151.

113. O'Meara, W.P., et al., *Effect of a fall in malaria transmission on morbidity and mortality in Kilifi, Kenya.* Lancet, 2008. **372**(9649): p. 1555-1562.

114. Kleinschmidt, I., et al., *Marked increase in child survival after four years of intensive malaria control.* The American Journal of Tropical Medicine and Hygiene, 2009. **80**(6): p. 882-888.

115. Teklehaimanot, H.D., et al., *Malaria in Sao Tome and principe: on the brink of elimination after three years of effective antimalarial measures.* The American Journal of Tropical Medicine and Hygiene, 2009. **80**(1): p. 133-140.

116. Tatem, A.J., et al., *Human population, urban settlement patterns and their impact on Plasmodium falciparum malaria endemicity.* Malaria journal, 2008. **7**(Journal Article): p. 218.

117. Hay, S.I. and A.J. Tatem, *Remote sensing of malaria in urban areas: two scales, two problems.* The American Journal of Tropical Medicine and Hygiene, 2005. **72**(6): p. 655-6; author reply 656-7.

118. Hay, S.I., et al., *Urbanization, malaria transmission and disease burden in Africa.* Nature reviews.Microbiology, 2005. **3**(1): p. 81-90.

119. Omumbo, J.A., et al., *The influence of urbanisation on measures of Plasmodium falciparum infection prevalence in East Africa.* Acta Tropica, 2005. **93**(1): p. 11-21.

120. Akhtar, R., A.K. Dutt, and V. Wadhwa, *Malaria resurgence in urban India: lessons from health planning strategies*, in *Malaria in South Asia*, R. Akhtar, A.K. Dutt, and V. Wadhwa, Editors. 2010, Springer: New York. p. 141-156.

121. Akhtar, R., A.K. Dutt, and V. Wadhwa, *The dynamics of urban malaria in India: an update*, in *Malaria in South Asia*, R. Akhtar, A.K. Dutt, and V. Wadhwa, Editors. 2010, Springer: New York.

122. *The Shrinking Costs of War*. 2009, Simon Fraser University.

123. Hay, S.I., et al., *Estimating the global clinical burden of Plasmodium falciparum malaria in 2007.* PLoS medicine, 2010. **7**(6): p. e1000290.

124. Taylor, S.M., et al., *Spatial and molecular epidemiology of malaria in the Democratic Republic of Congo, 2007.* PLoS medicine, 2010(Journal Article).

125. Taylor, S.S.M., *High-throughput pooling and real-time PCR-based strategy for malaria detection.* Journal of clinical microbiology, 2010. **48**(2): p. 512-519.

126. Culleton, R.L., et al., *Failure to detect Plasmodium vivax in West and Central Africa by PCR species typing.* Malaria journal, 2008. **7**(174).

127. . in *Disaggregating the study of civil war and transnational violence*. 2005. San Diego, CA.

128. *FAO Africover*. Available from: http://www.africover.org/.

129. Fotheringham, A.S., M.E. Charlton, and C. Brundson, *Geographically weighted regression: a natural evolution of the expansion method for spatial data analysis.* Environment and Planning A, 1998. **30**(Journal Article): p. 1905.

130. World Health Organization Department of Gender, Women and Health, *Gender, health and malaria*. 2007. Available from: http://www.who.int/gender/documents/gender_health_malaria.pdf.

131. Robert, S.A., *Community-level socioeconomic status effects on adult health.* Journal of health and social behavior, 1998. **39**(1): p. 18.

132. Diez Roux, A.D., *Investigating neighborhood and area effects on health.* American journal of public health (1971), 2001. **91**(11): p. 1783-1789.

133. Diez Roux, A.V., *Estimating neighborhood health effects: the challenges of causal inference in a complex world.* Social science & medicine (1982), 2004. **58**(10): p. 1953-1960.

134. D'Alessandro, U., et al., *A comparison of the efficacy of insecticide-treated and untreated bed nets in preventing malaria in Gambian children.* Transactions of the Royal Society of Tropical Medicine and Hygiene, 1995. **89**(Journal Article): p. 596.

135. Gayer, M., *Conflict and emerging infectious diseases.* Emerging infectious diseases, 2007. **13**(11): p. 1625.

136. Berrang Ford, L., *Civil conflict and sleeping sickness in Africa in general and Uganda in particular.* Conflict and health, 2007. **1**(Journal Article): p. 6.

137. Martens, P., *Malaria on the move: human population movement and malaria transmission.* Emerging infectious diseases, 2000. **6**(2): p. 103.

138. Owusu-Agyei, S., et al., *Molecular epidemiology of Plasmodium falciparum infections among asymptomatic inhabitants of a holoendemic malarious area in northern Ghana.* Tropical medicine & international health : TM & IH, 2002. **7**(5): p. 421-428.

139. *Mapping Malaria Risk in Africa / Atlas du Risque de la Malaria en Afrique*. http://www.mara.org.za/.

140. *Malaria Atlas Project*. http://www.map.ox.ac.uk/.

141. Who, *Worldwide prevalence of anaemia 1993–2005: WHO Global Database on Anaemia*. 2008.

142. Scholl, T., *Anemia and iron deficiency anemia: compilation of data on pregnancy outcome.* The American Journal of clinical nutrition, 1994. **59**(2).

143. Palupi, L., *Effective community intervention to improve hemoglobin status in preschoolers receiving once-weekly iron supplementation.* The American Journal of clinical nutrition, 1997. **65**(4).

144. Meade, M.S., *Medical geography as human ecology: the dimension of population movement.* The Geographical Review, 1977. **67**(Journal Article): p. 379-393.

145. Meade, M. and M. Emch, *Medical geography*. 3 ed. 2010, New York, NY: The Guilford Press.

146. Mayer, J.D. and M. Meade, *A reformed medical geography reconsidered.* The Professional Geographer, 1994. **46**(Journal Article): p. 103-106.

147. Mayer, J.D., *The political ecology of disease as one new focus for medical geography.* Progress in Human Geography, 1996. **20**(4): p. 441-456.

148. Mayer, J.D., *Geography, ecology and emerging infectious diseases.* Social Science and Medicine, 2000. **50**(Journal Article): p. 937-952.

149. Emch, M., *Diarrheal disease risk in Matlab, Bangladesh.* Social Science & Medicine, 1999. **49**(4): p. 519-530.

150. Meade, M., *Geographic analysis of disease and care.* Annual Review of Public Health, 1986. **7**: p. 313-335.

151. Learmonth, A.T.A., *Disease ecology: An introduction to ecological medical geography*. 1988, Oxford, UK and New York, NY: Basil Blackwell.

152. Gesler, W.M., S.T. Bird, and S.A. Oljeski, *Disease ecology and a reformist alternative: the case of infant mortality.* Social Science & Medicine, 1997. **44**(5): p. 657-671.

153. Ayoya, M.A., et al., *Determinants of anemia among pregnant women in Mali.* Food and Nutrition Bulletin, 2006. **27**(1): p. 3-11.

154. Pasricba, S.R., et al., *Anemia, iron deficiency, meat consumption, and hookworm infection in women of reproductive age in northwest Vietnam.* American Journal of Tropical Medicine and Hygiene, 2008. **78**(3): p. 375-381.

155. Lartey, A., *Maternal and child nutrition in sub-Saharan Africa: challenges and interventions.* Proceedings of the Nutrition Society, 2008. **67**(01): p. 105-108.

156. Thomson, C.A., et al., *Nutrient intake and anemia risk in the Women's Health Initiative Observational Study.* Journal of the American Dietetic Association, 2011. **111**(4): p. 532-541.

157. Rogerson, S.J., et al., *Malaria and anemia in antenatal women in Blantyre, Malawi: a twelve-month survey.* American Journal of Tropical Medicine and Hygiene, 2000. **62**(3): p. 335-340.

158. Levine, A.M., et al., *Prevalence and correlates of anemia in a large cohort of HIV-infected women: women's interagency HIV study*. Journal of Acquired Immune Deficiency Syndromes 2001. **26**(1): p. 28-35.

159. Antelman, G., et al., *Nutritional factors and infectious disease contribute to anemia among pregnant women with human immunodeficiency virus in Tanzania.* Journal of Nutrition, 2000. **130**(8): p. 1950-1957.

160. May, J.M., *The ecology of malnutrition in middle Africa*. 1965, New York: Hafner Publishing Company.

161. *Global Land Cover Network*. 2008. http://www.glcn.org/.

162. United States Department of Agriculture. *Nutrient data laboratory*. 2011 [cited 2011 November 4]; Available from: www.nal.usda.gov.