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THE ROLE OF CO-INFECTION IN THE SPREAD OF HIV IN SUB-SAHARAN AFRICA

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Dr. Brian C. Rymond, Director of Graduate Studies

DISSERTATION

Diego Fernando Cuadros

The Graduate School
University of Kentucky
2011

THE ROLE OF CO-INFECTION IN THE SPREAD OF HIV IN SUB-SAHARAN AFRICA

DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Arts and Sciences
at the University of Kentucky

By

Diego Fernando Cuadros

Lexington, Kentucky

Director: Dr. Gisela Garcia-Ramos, Assistant Professor of Biology
Co-Director: Dr. Philip H. Crowley, Professor of Biology

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ABSTRACT OF DISSERTATION

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ABSTRACT OF DISSERTATION

THE ROLE OF CO-INFECTION IN THE SPREAD OF HIV IN SUB-SAHARAN AFRICA

The cause of the high HIV prevalence in sub-Saharan Africa is incompletely understood, with heterosexual penile-vaginal transmission proposed as the main mechanism. Heterosexual HIV transmission has a very low probability; further, a single estimation of heterosexual probability of HIV transmission fails to reproduce the variation associated with important biological cofactors. In particular, studies of HIV incidence suggest that co-infection with other infectious diseases influence the HIV transmission, and therefore might substantially vary the pattern of the spread of the infection. To assess the effect of co-infection on the spread of HIV, I developed and analyzed several mathematical and statistical models based on published data. The results show that despite the low probability of heterosexual transmission per sexual contact, the inclusion of individual variation generated by transient but repeated increases in HIV viral load associated with co-infections may provide a biological basis for the accelerated spread of HIV in sub-Saharan Africa, and raises the possibility that that the natural history of HIV in sub-Saharan Africa cannot be fully understood if individual variation in infectiousness is neglected.

Co-infection might be a key explanatory variable for the rapid spread of HIV infection in sub-Saharan Africa; in fact, co-infection may be a necessary factor, rather than merely being a contributing factor, in the successful spread and survival of HIV in populations where heterosexual vaginal-penile contact is the main mechanism of transmission. Consequently, broad population based control strategies to decrease infectivity and reduce the incidence of other sexual and parasitic infectious diseases might be effective strategies in diminishing the spread of HIV in sub-Saharan Africa.

KEYWORDS: Co-infection, HIV, Malaria, Sub-Saharan Africa, Mathematical Models.

Diego Fernando Cuadros
Student's Signature

10 November 2011
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THE ROLE OF CO-INFECTION IN THE SPREAD OF HIV IN SUB-SAHARAN AFRICA

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Miguel y Martha, este es el producto de su amor y dedicacion, del esfuerzo y la paciencia que ustedes me han entregado. Maria, sin ti a mi lado todos estos años esto no seria una realidad.

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"*Walk with the dreamers, the believers, the courageous, the cheerful, the planners, the doers, the successful people with their heads in the clouds and their feet on the ground. Let their spirit ignite a fire within you to leave this world better than when you found it...*" (Wilfred Peterson)

In this journey as a graduate student, I have walked with many dreamers and believers that have ignited a fire inside me to pursue my goals, to never give up, and to give all I can possible give. Thank you to all of them.

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Chapter 1 - Introduction

Overview

AIDS was first described in 1981 in homosexual men in North America (Gottlieb et al., 1981) followed by the first report in patients from Central Africa in 1983 (Clumech et al., 1983). Three years later, it was evident that HIV-1 had spread into populations of many countries from sub-Saharan Africa and had become an enormous public health problem (Quinn et al., 1986). Of the estimated 40 million HIV-seropositive patients in 2001, 70% were from sub-Saharan Africa, residence of less than 10% of the human population (UNAIDS/WHO). According to the global HIV/AIDS/STI working group (UNAIDS/WHO), an HIV epidemic is generalized if the prevalence in pregnant women consistently exceeds 1%. Using this definition, in 1999 the HIV epidemic was generalized in most of the countries of sub-Saharan Africa. By contrast, in Asia, the second most affected continent, only three countries had a generalized HIV epidemic (UNAIDS).

The causes of the uncontrollable expansion of the HIV epidemic in most countries from sub-Saharan Africa is incompletely understood (Curran et al., 1988; Hall et al., 2008; Karon et al., 2001). Unlike the HIV epidemic in the US and Europe, which seems concentrated in specific high risk groups such as injection drug users and men who have sex with men (MSM) (Curran et al., 1988; Karon et al., 2001; Orroth et al., 2007), the epidemic in Africa is more widely distributed across the general population. The core risk group concept, the theory that a small group of people with large number of partners and repeated infections can maintain a reservoir of the infection within a population, does not fit in sub-Saharan Africa, where the infection has spread broadly beyond the putative risk groups (Hamilton et al., 2008).

Additionally, the hypothesis that heterosexual penile-vaginal transmission is the main mechanism of HIV transmission in sub-Saharan Africa (Gisselquist and Potterat, 2002; Orroth et al., 2007; Vernazza et al., 1999; Waver et al., 2005) seems inconsistent with the low probability of heterosexual HIV transmission, estimated to be ~ 1/300 per

coital act in low-income countries (Boily et al., 2009; Gisselquist and Potterat, 2003). In particular, the assumption that the high incidence of HIV epidemic among African adults is almost exclusively due to sexual transmission actually requires much higher probabilities of heterosexual transmission in Africa than in the developed world (Boily et al., 2009). These contrasting differences observed among the HIV epidemics from different settings of the world raise a fundamental question: why does HIV prevalence vary so greatly in different locations? Answers to this question would help us to better understand the natural history of HIV and implement appropriate interventions. The identification of biological or behavioral factors that could explain the variation in prevalence of HIV infection may help us to design the more efficient public-health strategies to control the epidemic.

Epidemics caused by the transmission of infectious agents such as HIV are characterized by heterogeneities in the pathogen and host populations, and interactions between them profoundly affect the dynamic of the infection. There are several epidemiologically important sources of variation: disease independent host parameters such as sex, age and contact rates, and disease dependent host parameters such as susceptibility, transmission rate and duration of the infectiousness. Resulting from the possible influence of the population heterogeneity on the transmission efficiency of the virus, individual variation emerges as an important element for understanding the natural history of HIV. Regional factors may affect the spatial spread of the infection, while individual heterogeneities such as behavior may influence the pattern of the spread of the infection within populations. To understand the natural history of HIV, particularly in sub-Saharan Africa, and thus generate the most cost-efficient method to control the HIV epidemic, it becomes fundamental to identify the factors that generate the individual variation on the HIV infectivity in space and time.

Background and motivation

The causes for the extremely high HIV prevalence in sub-Sahara Africa is one of the biggest debates nowadays. Most attempts to explain the epidemic are based in behavioral

components of the population (i.e. number of sexual partners per year, partnership concurrency and commercial sex), yet they have not explained satisfactorily the dynamic of the HIV epidemic in sub-Saharan Africa. Unlike HIV in the US and Europe, which seems concentrated among injection drug users and men who have sex with men (Curran et al., 1988; Hall et al., 2008; Karon et al., 2001), the epidemic in Africa is evenly distributed into the general population, with heterosexual penile-vaginal contact proposed as the main mechanism of transmission (Gisselquis et al., 2002; Orroth et al., 2007; Vernazza et al., 1999; Waver et al., 2005).

The transmission efficiency of the virus and behavioral parameters such as the number of sexual partners per unit of time play a fundamental role in determining the course of the HIV epidemic. For sexually transmitted infections (STI), the contact rates are commonly used as a measure of the variation in infectiousness and susceptibility. With a high rate of partner change, sexual behaviors are assumed to determine how rapidly a STI can spread into the population. Thus host contact rates are used as a measure of individual infectiousness leading to the concept of high-risk (core groups) where most individuals experience low or no transmissions but a few are responsible for most of the transmissions (Bansal et al., 2007). This assumption, however, only captures the variability of the transmission generated by sexual behavior and therefore assumes constant infectivity of HIV across human populations. Based on this premise many studies have focused on the study of sexual behavior to understand the natural history of HIV. These studies have indicated that despite substantial regional variation in the prevalence of multiple partnerships, most people report having only one recent sexual partner. Particularly, surveys and standard measures available for African countries indicated that monogamy or serial monogamy is the dominant pattern (Ferguson et al., 2004; Kapiga and Lugalla, 2002; Lagarde et al., 1996; Wellings et al., 2006).

Additionally, studies attempted to clarify the role of sexual behavior on the pattern of the HIV epidemic from different locations have found that sexual behavior did not appear to explain the observed differences in HIV prevalence (Ferry et al., 2001). These results support the possibility that highly infectious individuals are not only dependent on the contact rate but also may depend on other sources of host variation generated by biological cofactors. Hence, a fixed value for the infectivity of HIV may

ignore important differences associated with biological cofactors such as circumcision and the presence of co-infections with other STIs (Powers et al., 2008).

Studies have indicated that the infectivity of the virus is not constant but may vary with space and time, and demonstrated that the risk of HIV transmission could be strongly correlated with variations in blood viral burden (Corey et al., 2004; Pilcher et al., 2004; Zetola and Klausner, 2007). Furthermore, a wide range of infectious diseases, from STI to parasitic diseases, may affect the HIV viral load triggering an amplificatory effect in the risk of HIV transmission in co-infected individuals (Corey et al., 2004; Freeman et al., 2007; Kublin et al., 2005; Zetola and Klausner, 2007). Thus, biological cofactors such as co-infections may then play an important role to understand the temporal and individual variation on the infectivity of HIV.

The aim of this study was to explore the limitations of the consideration that the existent heterogeneity on HIV infectivity could be explained merely by variations on sexual behavior. Additionally this study wants to show some evidence that disregarding the variation across individuals on the HIV infectivity generated by biological cofactors would fail to describe the natural history of HIV, especially in sub-Saharan Africa. Determining the degree of host heterogeneity in the transmission of the virus and the sources of this variation is especially important: it may help us to understand the contrasting differences on the pattern of the spread of the infection in different locations; consequently it would allow us to identify the characteristics of super-infectious individuals responsible for the majority of transmissions.

This study will be guided by the hypothesis that the inclusion of temporal and individual variation on the infectivity of HIV generated by transient but repeated increases in viral load affects the pattern of the spread of the infection at population level, and could potentially explain the differences on the HIV epidemic between populations. This research was conducted through mathematical and statistical analyses at three specific levels of sources of variation explored in the different chapters: (1) Chapter 2 focused on population level variations generated by the structure of the sexual network and the prevalence of co-infection with other STIs, (2) Chapter 3 and 4 assessed the regional variations generated by the co-infection with environmental diseases such as

malaria, and (3) Chapter 5 and 6 explored the host variation generated by the immune response in presence of co-infections, and methodological issues regarding control interventions based on co-infections. The analyses of published data was focused on describing the possible causes of the differences on HIV prevalence between locations, and the implications of individual variations on the HIV infectivity on the spread of the disease at population level.

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Chapter 2 - Effect of variable transmission rate on the dynamics of HIV in sub-Saharan Africa

Introduction

The cause of the high HIV-1 prevalence in sub-Saharan Africa is incompletely understood (Curran et al., 1988; Hall et al., 2008; Karon et al., 2001). Unlike HIV in the US and Europe, which seems concentrated among injection drug users and men who have sex with men (Curran et al., 1988; Karon et al., 2001; Orroth et al., 2007), the epidemic in Africa is more widely distributed across the general population, with heterosexual penile-vaginal transmission proposed as the main mechanism (Gisselquist and Potterat, 2002; Orroth et al., 2007; Vernazza et al., 1999; Waver et al., 2005).

Mathematical models are powerful tools in epidemiology: they can facilitate understanding of the interplay between the variables that determine the course of infection within an individual and the variables that control the pattern of infections within communities of people. But, mathematical modeling studies that attempt to reproduce the observed HIV epidemic curve in sub-Saharan Africa are often criticized for using per-contact and per-partnership heterosexual transmission efficiencies that are improbably high (Brewer et al., 2007; Deuchert, 2007). For example in the calculation of per-partner rate of transmission, behavioral parameters such as number of sexual partners per year and number of sexual contacts per partner may be overestimated by assuming levels of promiscuity in African societies that are too high (Brewer et al., 2007).

Unlike more traditional epidemiological approaches that focus strictly either on individuals or on populations, sexual networks are based on the dynamics of the sexual links (connections between nodes = between individuals) and the topology of the linkage in a group (Doherty et al., 2005; May and Lloyd, 2001). Sexual networks have multiple advantages for characterizing individual heterogeneity of sexual behavior. This approach to understanding the spread of a sexually transmitted infection (STI) has focused attention on the properties of the frequency distribution of sexual partner number. In sexual networks, partner number is the node degree, the number of sexual links that each

node (individual) has to others (Hamilton et al., 2008). Thus, network studies mainly focus on the distribution of node degree, which can be characterized by the data (Handcock and Holland, 2004).

Network models also focus on other components of the network structure that cannot be described from the observation of individual nodes alone. The degree distribution is only one example of an aggregate statistics obtained by the study of the individual properties within the network. For the calculation of other statistics, such as the level of clustering, it would be necessary to observe larger fragments of the network (Proulx et al., 2005). Clustering measures focus on describing both the connections from focal nodes and the connections made by its neighbors. In particular, high levels of clustering may reduce the rate of spread of an infectious disease (Witten and Poulter, 2007).

The typically high skew of sexual degree distributions has suggested that sexual networks may follow a power law (scale-free) distribution (Lilijeros et al., 2001; Ravasz and Babarasi, 2003). Power law distributions are characterized by many nodes with only one or few connections but also a few nodes with many more connections, generating a high contact variance. The high variance generated in large populations that follow the power law distributions implies that even very low transmission rates are consistent with disease spread (Anderson and May, 1991; May and Lloyd, 2001).

Most of the studies that have attempted to describe sexual behavior in Africa have found that the power law distribution does not adequately fit the data. Instead, fixed rate models such as the negative binomial model, which is a generalization of the Poisson model, appear to fit the degree distribution best (Hamilton et al., 2008; Handcock and Holland, 2004). In the negative binomial model, the propensities of individuals to form connections are estimated from a gamma distribution. This approach, with its lower variance in connectedness among nodes, raises the possibility that the infectivity of HIV may be an important determinant of the epidemic in sub-Saharan Africa (Anderson and May, 1991; Doherty et al., 2005).

Yet this conclusion is inconsistent with the low probability of heterosexual HIV transmission, estimated to be ~ 1/300 per coital act in low-income countries (Boily et al., 2009; Gisselquist and Potterat, 2003). Moreover, studies that attempted to estimate the

probability of HIV transmission per sexual contact have found that the Bernoulli model accurately estimates the per-partner probability of HIV transmission but does not seem to correlate with the number of sex acts and thus fails to estimate the per sexual contact probability of transmission (Padian et al., 1990). It has been suggested that the constant transmission probability in the Bernoulli model may be the problem: variability of infectiousness among individuals and over time, such as may arise from important transmission cofactors, may be essential for a realistic representation of HIV transmission (Kaplan, 1990; Padian et al., 1990; Powers et al., 2008).

Despite the low probability of heterosexual penile-vaginal transmission per sexual contact, some studies have demonstrated that the risk of HIV transmission can be strongly correlated with variation in blood viral burden (Attia et al., 2009; Cohen et al., 2007; Pilcher et al., 2007). The most relevant finding from these studies is that infectiousness can be directly correlated with the concentration of HIV-RNA in blood, which indicates shedding of the virus into genital track secretions.

In a pioneering study attempting to correlate the viral load and the transmission of the virus, Quinn and coworkers (Quinn et al., 2000) measured the HIV-RNA load in the blood of more than 15 000 subjects. They found that the virus was hardly ever transmitted by infected subjects with less than 1500 copies of HIV-RNA/ml, whereas individuals with more than 50 000 copies infected their sexual partners at a rate of 23 per 100 person-years over 30 months.

A similar study conducted with discordant couples for HIV status in Uganda showed the existence of a strong correlation between HIV plasma viral load and HIV transmission rates (Wilson et al., 2008). The Uganda study indicated that a ten-fold increment in viral load could increase the risk of HIV transmission per sexual contact 2.45-fold (95% CI 1.85-3.26). They pointed out that although blood and semen reside in separate biological compartments, blood viral burden can be correlated with viral burden in semen.

Growing evidence suggests the existence of additional biological factors that cause variations in the viral load. The viral set point is actually not constant and may be perturbed by reactivations of the immune system, such as those resulting from the invasion of other pathogens (Bentwich et al., 1995). Changes in the host immune

response may account for variations in the viral load that could make the host more infectious and increase the risk of transmission.

The average African host is usually exposed to numerous bacterial, viral and parasitic infections. Of special importance is the very high prevalence of STIs, particularly genital ulcerations caused by herpes simplex virus type 2 (HSV-2) (Bentwich et al., 1995). The existence of a synergistic relationship between HIV and HSV-2 has been strongly suggested by many observational and biological studies in which HSV-2 has been implicated as a biological cofactor for the acquisition and transmission of HIV (Glynn et al., 2008; Weiss et al., 2001).

The rapid spread of HIV as a sexually transmitted disease is exceeded by that of HSV-2 (O'Farrell, 1999). The prevalence of HSV-2, which may be as high as 75% among women in parts of sub-Saharan Africa (WHO/UNAIDS/LSHTM), has reached a prevalence of up to 90% in HIV-positive persons (Weiss et al., 2001).

While bacterial STIs such as gonorrhea and syphilis, which also amplify the risk of HIV transmission (John-Arne et al., 2001), tend to be concentrated in high risk groups (Brunham and Plummer, 1990), the biological characteristics of HSV-2 allow this virus to be sustainable at high levels in the general population, as observed in sub-Saharan Africa (Corey et al., 2004). Consequently, as the HIV epidemic reaches the general population, the epidemiological overlap between HSV-2 and HIV is considerably larger than any other STI.

The ulcers caused by HSV-2 contain substantial numbers of CD4+ lymphocytes, the target cell for HIV, and therefore are likely to facilitate the acquisition of HIV in HIV-negative individuals (Celum et al., 2004). Additionally, the high levels of HIV-RNA in herpetic lesions from dually infected patients (Schacker et al., 1998) may be explained by studies *in vitro* demonstrating that HSV-2 increases HIV transcription, which supports the higher infectivity in co-infected individuals. Population-based studies have also demonstrated that HIV-RNA levels can increase during active HSV-2 infection (Mole et al., 1997), and suppression of HSV-2 with acyclovir was associated with a measurable decrease on the HIV-RNA levels (Schacker et al., 2002).

The enhanced HIV infectivity caused by HSV-2 co-infection has also been corroborated by population-based studies suggesting a relative risk of three-to five-fold

of HIV transmission from co-infected individuals compared to HSV-2 seronegative persons (Buve et al., 2001; Corey et al., 2004; Gray et al., 2001). These data, which indicate epidemiologic synergy between the two infectious diseases at the population level, suggest that HSV-2 may be playing a key role fueling the HIV epidemic in sub-Saharan Africa (Abu-Raddad et al., 2008).

The activation of the immune system, however, is not only produced by STIs such as HSV-2, syphilis and gonorrhea. Parasitic infections such as helminth infections, leishmaniasis and malaria might produce a strong response from the immune system and consequently generate similar effects on the replication of the virus in HIV co-infected individuals (Bentwich et al., 1995; Korenromp et al., 2005; Kublin et al., 2005; Pisell et al., 2002; Reina et al., 2006). The geographical overlap observed between malaria and HIV infections has suggested a possible interaction influencing HIV transmission in some countries of sub-Saharan Africa. Malaria occurs throughout the tropical world, where it remains one of the most prevalent infectious diseases, with an estimated 300 million cases per year (CDC).

The evidence of an interaction between malaria and HIV comes from various sources. Several *in vitro* studies have found that malaria antigens significantly enhanced HIV-1 replication (Kublin et al., 2005; Pisell et al., 2002; Reina et al., 2006; Xiao et al., 1998). Additionally, population-based studies conducted with HIV-1 infected adults have indicated that the HIV-1 RNA concentration almost doubled between baseline (96 215 copies per ml) and those co-infected with malaria (168 901 copies per ml). The authors concluded that HIV-positive individuals co-infected with malaria had a significantly increased viral load and possibly increased infection transmission (Kublin et al., 2005).

Based on the evidence previously mentioned, this study examines the limitations of the view that the level of the HIV epidemic in sub-Saharan Africa could be explained merely by a constant probability of transmission. We suspected that disregarding the variation across individuals in HIV infectivity would fail to replicate the HIV epidemic observed in a sexual network from sub-Saharan Africa. Instead, we predicted that individual and temporal variations in HIV transmission generated by biological factors such as co-infections with other infectious diseases could explain the severity of the HIV epidemic.

Methods

With the aim of testing the effect of temporal and individual variation on HIV transmission generated by co-infection, we developed a dynamic sexual network model (Witten and Poulter, 2007). Modeling studies of sexual networks have focused primary on the node degree distribution (Bell et al., 2002; Doherty et al., 2005; Hamilton et al., 2008; May and Lloyd, 2001). But the partnership acquisition process relevant to HIV infections is too complex to be adequately captured by a static degree distribution. Other nodal attributes such as gender, age and marital status are also of fundamental importance, as are the dynamics of the linkages themselves. To include these characteristics, we used Monte Carlo simulations to depict a dynamic sexual network with given nodal and structural characteristics, where links between nodes are formed and dissolved according to estimated parameters.

The model incorporates the dynamic of the behavioral components of the population, as well as the dynamics of HIV and the co-infection effect on the HIV transmission caused by other infectious diseases, including HSV-2, gonorrhea, syphilis and malaria, along with the spread of HIV infections caused by commercial sex. We used data from studies in Malawi when available as an example of a generalized HIV epidemic (Helleringer et al., 2007a; Helleringer et al., 2007b; University of Pennsylvania, 2003).

Model structure

A stochastic, individual-based sexual network model was created to simulate disease dynamics using the MATLAB® computing language version 7 (The MathWorks, 1994-2008). The model was divided in two main modules: a behavioral module and an epidemiological module.

Sexual partnerships were assumed to be exclusively heterosexual, and two types of partnerships, distinguished by duration, were considered. The population size remained constant, with individuals maturing into the network to offset those who die or mature out of the network. In accord with the highest resolution of relevant data, a monthly time step was used. With this model, the effects of network structure on disease transmission, relationship type, and co-infection with other infectious diseases were evaluated.

For the estimation of the main parameters of the sexual network, data were used from a study of Malawi by the University of Pennsylvania Population Study Center and called "The Malawi Social Network Project" (University of Pennsylvania, 2003), as well as data from the Demographic and Health Survey (DHS) database from Malawi (National Statistical Office). The study was conducted in three districts of Malawi, and the sampling strategy is explained elsewhere (Helleringer et al., 2007a; Helleringer et al., 2007b). The study focuses on the description of the sexual behavior in the Malawi population, where the more important characteristics such as age distribution, number of sexual partners per year, type of relationship, duration of the relationship and age mixing patterns of marriage were derived. Table A.1 in Appendix A lists the key assumptions of the behavioral module.

Equal numbers of individuals of each sex were created and assigned an age and node degree (maximum number of partners per year). Consequently, individual age was used to determine when individuals should be removed from the sexual network and was the basis for other age-specific traits. The epidemiological module was subdivided into two steps, the spread of the infections, and the progression and recovery of each infection. We selected gonorrhea and syphilis as examples of bacterial STIs concentrated in the high-risk (core) groups based on the amplification effect on HIV transmission, and their relevance in terms of prevalence in the Malawi population (Lule et al., 1994; Mensch et al., 2008). The dynamics of these infections are well known, and the effect of each infection on the transmission of HIV has been determined.

We also included two infections with high prevalence in the general population: herpes simplex virus type 2 (HSV-2) and malaria. The chronic nature of HSV-2 and its relatively high transmission efficiency make it sustainable in the general population. HSV-2 reactivations increase HIV transcription (Golden et al., 1992), which in turn generates an increase in the HIV plasma viral load (Mole et al., 1997) and supports higher HIV infectivity in dually infected individuals (Quinn et al., 2000). The evidence suggests an epidemiologic synergy between both diseases, and HSV-2 has been postulated as the most important STI driving the HIV prevalence in sub-Saharan Africa (Abu-Raddad et al., 2008).

We included malaria as an example of a parasitic infection, given its geographical overlap with HIV and its high prevalence in Malawi. Malaria is endemic in all parts of Malawi and many other countries in sub-Saharan Africa. According to The World Health Organization, 6 million of episodes of malaria occurred in 2006, accounting for about 33% of all outpatient visits in Malawi. In one study malaria, infection was associated with a 78% higher HIV RNA concentration than in malaria-free individuals also infected with HIV (Kublin et al., 2005). Table A.2 in Appendix A lists the key assumptions of the epidemiological module.

A key assumption for the epidemiological module is that the interaction caused by co-infection has only one direction. In other words, we assumed that HIV infection has no effect on the natural history of the other infectious diseases included in the model. This assumption may be seen as an oversimplification, because studies have shown that HIV infection affects the transmission and progression of other infectious diseases such as HSV-2 and malaria. Yet, studies have mainly focused on the impact of co-infection on HIV. As a result, uncertainty about the effect of co-infection on the other diseases is still high.

The core of our model is the spread of HIV infection, through penile-vaginal contact. Before the introduction of HIV infected individuals, the model simulates for several months the dynamic of the other infectious diseases previously mentioned. When an endemic steady state for all infectious diseases is reached (after about 500 monthly time steps), the model introduces HIV infected individuals until the HIV prevalence reaches 1%, which is the prevalence observed in Malawi in 1981 (Crampin et al., 2002).

For our simulation, the algorithm assessed whether the individual infected with HIV has another infectious disease, and if co-infection was present, the HIV transmission probability was increased depending on the amplification factor. Then, the new HIV transmission probability including the amplificatory effect was calculated by

$$T_c = T * cofactor .$$

where T is the stage or sex-specific transmission probability per sexual contact (Table A.2 parameter 1-6). The HIV transmission probability per partnership per month is then calculated using the binomial (Bernoulli) model as

$$T_p = 1 - (1 - T_c)^{c_n},$$

where C_n is the number of sexual contacts the individual has with the partner.

Cofactor values of the STI's included in the model were obtained from population-based estimations expressed as odds ratios and relative risk per sexual contact. For malaria, we assume that the enhancement on the transmission probability per sexual contact depends on the logarithmic (base 10) incremental change in the viral load according to $T_d^{'} = T_d * 2.45^{LogInc}$. The 2.45 factor is the rate ratio increase in transmission probability with each one-log increment in viral load (Quinn et al., 2000); see Kublin et al. (2005) for malaria increment data. Cofactor values included in the model are listed in Table A.2 in Appendix A (parameter 49-53).

When multiple co-infections are present, we assumed a saturation effect of the enhancement on the transmission probability. Thus, when more than one co-infection is present, the transmission probability is amplified only by the highest cofactor. For the special case of HSV-2, the amplification factor is only effective if the HSV-2 infection is reactivated (shedding) (Celum et al., 2004). Therefore, the algorithm not only verifies the presence of HSV-2 co-infection but also its reactivation. On the other hand, HSV-2 not only enhances the transmissibility of HIV but also affects the susceptibility to being infected with HIV (Celum et al., 2004). For this reason, the algorithm verifies if the susceptible receptor is infected with HSV-2 and its reactivation stage. In this case, the transmission probability is also increased by the respective amplification factor (Table A.2 parameter 53 in Appendix A). A detailed description of the methodology can be found in the Appendix A.

Calculation of the epidemiologic synergy

HIV infections caused by co-infection with other infectious diseases may also generate secondary HIV infections, regardless of the presence of co-infection (Abu-Raddad et al., 2008). Therefore, the HIV prevalence measures both the HIV transmissions caused by the direct biological effect of co-infection and the secondary or indirect infections caused by co-infection. We estimated the effect of co-infection on the dynamic of the HIV epidemic in the sexual network by comparing the prevalence for different scenarios: no co-infection, all co-infections (default scenario), no HSV-2 co-infection, and no malaria co-

infection. We also measured the direct effect of co-infection on the HIV incidence by using population attributable fractions (PAF) (Heller et al., 2003; Heller et al., 2002; Rockhill et al., 1998). We estimated the PAF of HIV incidence attributable to all co-infections, HIV-2, malaria, and gonorrhea and syphilis. The PAF is calculated by

$$PAF(t) = \left(1 - \frac{IR_{nocofactor}(t)}{IR_{defaultcofactor}(t)}\right) \text{X } 100\%,$$

where $IR_{nocofactor}(t)$ is the incidence rate of HIV in the different scenarios with the cofactor effect removed at time t, and $IR_{defaultcofactor}(t)$ is the HIV incidence rate with the default cofactor effect at time t (simulation 2) (Abu-Raddad et al., 2008; White et al., 2008).

To identify the epidemiologic synergy at different periods of the HIV epidemic, we calculated the PAF for different time points in a separate set of simulations by removing the cofactor effect on HIV transmission over two years, starting at times (t) 0, 8, 15, and 20 after the introduction of HIV. This allows us to measure of the direct role of co-infection in HIV incidence at each time point (t). Results from all different scenarios are based on means over 200 simulations.

Uncertainty and sensitivity analyses of the key parameters

To conduct uncertainty and sensitivity analyses of the key parameters, we adopted the Latin Hypercube Sampling/Partial Rank Correlation Coefficient (LHS/PRCC) technique (Blower and Dowlatabadi, 1994; Marino et al., 2008). In LHS the estimation of uncertainty for each key parameter is modeled by treating each input parameter as a random variable with a uniform probability distribution function. Upper and lower bounds on these distributions were assigned based upon the available data.

To study the uncertainty of the parameters for the two different modules, we conducted three different uncertainty analyses. For the behavioral module, the simulations did not include the amplification cofactor caused by co-infection. Hence, we used the default probability of HIV transmission T= 0.003, and we performed a LHS of the more important behavioral parameters. For the epidemiological module, the

simulations included the amplification co-factor caused by confection and we conducted a LHS of the cofactor values. In the third uncertainty analysis, we performed a LHS of both the behavioral parameters and the cofactor values. 200 simulations were run for each uncertainty analysis. The variability in the outcome variable (HIV prevalence) was then estimated by simple descriptive statistics. Sensitivity analyses were then performed by calculating PRCCs for each input parameter. The details of these analyses can be found in the Appendix A.

Results

Sexual network

The graphical representation of the degree distribution (i.e. number of sexual partners per year) for both males and females shows that the gamma distribution provides a good fit to the data (Figure A.1). For males, the mean number of sexual partners per year was μ = 2.12, with standard deviation SD = 1.23, and with a scale parameter θ = 1.1 and shape parameter k = 1.9 for the gamma distribution. For females, the mean number of sexual partners per year was μ = 1.84 with standard deviation SD = 1.04, and with scale parameter θ = 0.4 and shape parameter k = 3.8 (Table A.1).

The resulting number of sexual partners per year obtained from the simulations for both males and females seems to agree with the estimated annual degree distribution from Malawi (Figure A.1), the age distribution of the entire population (Figure A.2), and the age distribution of married individuals (Figure A.3). We found, however, that individuals accumulated more long-term relationships (marriages) throughout their entire sexual life than indicated by the data (Figure A.4). This inconsistency could result from a low estimate of the average duration of marriage (~7 years) in the simulations.

Since long-term relationships increase the number of exposures, the risk of transmission or acquisition of the virus also increases. Consequently, the observed discrepancy between the lifetime number of long-term relationships observed in the simulations and the data may generate an overestimation of the HIV prevalence.

Using the number of connections per month (Appendix A, eq. 9) and the number of connections generated by individuals who already have one connection, we found that the frequency of concurrency estimated from the model was 0.3. In other words, 30% of the individuals who had a sexual partner were in at least one concurrent relationship. This value seems to be consistent with some estimates of concurrency from sub-Saharan Africa (Lagarde et al., 2001). See also (Helleringer et al., 2009), where the concurrency estimate was higher, but concurrent relationships were not clearly distinguished from serial monogamy (Kretzschmar et al., 2010).

Simulation 1: no co-infection

The first simulation, which included the most recent estimate of the male-to-female probability of HIV transmission $T = 0.003$ (Boily et al., 2009), suggests that with this constant probability of HIV transmission the infection does not survive in the population: no epidemic can be generated, and HIV prevalence decreases to extinction (Figure 2.1). On the other hand, with $T = 0.005$ we observed that the infection can persist and reached an endemic steady state at a prevalence equal to the initial prevalence (1%). Finally, with a $T = 0.0068$ we could generate an epidemic curve similar to the HIV epidemic observed in Malawi (Figure 2.1). This value is slightly above the 95% confidence interval estimated for male-to-female probability of HIV transmission (Boily et al., 2009) (see details in Appendix A).

Simulation 2: amplification cofactor

For this simulation, we used the baseline probability of HIV transmission for low income countries $T = 0.003$ (Boily et al., 2009), but we also included the amplification cofactor for HIV transmission generated by co-infection with HSV-2, gonorrhea, syphilis and malaria. The results from this scenario showed that the amplification effect caused by co-infection was sufficient to generate an HIV epidemic curve similar to the curve observed in Malawi (Figure 2.2 B, HIV prevalence estimated for 2005 ~17%).

From our model, we observed that the prevalence for gonorrhea (~2%), syphilis (~4%), HSV-2 (~40%) and malaria prevalence in dry (30%) and rainy (40%) seasons resemble the prevalence observed for these STIs in Malawi and other countries of sub-Saharan Africa (Buvé et al., 2001a; Crampin et al., 2002; Ghani et al., 1998; Holmes et

al., 2004; Marion and Schecter, 1992; Pinkerton and Abramson, 1997; Salomon and Murray, 2001; Zetola and Klausner, 2007) (Figure 2.2 A).

Even when parameter values of the model were obtained independently of the Malawi data, our results resemble the HIV prevalence as well as the age distribution prevalence observed in that country (Figure A.5 A, B). As reported in the data, the female HIV prevalence was higher than the male prevalence; the resulting age distribution of prevalence, however, differs somewhat from the distribution reported for Malawi. In our results, the peak of the prevalence is located at ages 25-29 for both males and females, while the data suggest that the peak is at ages 30-34. The model also overestimates the HIV prevalence at early ages (Figure A.5 A, B).

We compared the simulated age distribution of HSV-2 prevalence with the observed prevalence in Malawi. The age distribution for HSV-2 obtained from the simulation is consistent with the data, but the resulting HSV-2 prevalence is somewhat lower than the prevalence observed in Malawi (Figure A.5 C, D).

Our results also showed that more than 40% of the HIV infections were associated with HSV-2 co-infection (Figure 2.3 A), and more than 70% of the total HIV transmissions were associated with STI or malaria co-infection (Figure 2.3 C). Significantly, these values indicate that transmission was commonly by co-infected individuals, but the results do not estimate the incidence of HIV due to the direct effect of co-infection.

Simulation 3: no HIV-HSV-2 co-infection

Because HSV-2 was the most important infectious disease in terms of co-infected individuals who transmitted the HIV infection (Figure 2.3 A), we simulated the absence of the amplificatory effect generated by HSV-2. For this simulation, the prevalence decreased to ~6% in comparison with the prevalence observed in simulation 2 (Figure 2.4 A).

Simulation 4: no HIV-malaria co-infection

Without the malaria amplification effect, the HIV prevalence declined to ~10%, indicating that malaria contributed ~7% to the overall HIV prevalence (Figure 2.4 B). This result is higher than the result obtained by Abu-Raddad and coworkers (Abu-Raddad

et al., 2006), who simulated the co-infection effect of malaria on HIV infection in Kisumu, Kenya, using a system of differential equations. They found that excess of HIV prevalence caused by malaria was 2.1%.

Population attributable fraction

The results indicated that the proportion of new HIV infections due to all co-infections together was >50% and that this value remained constant over time (Figure 2.5 A). When we obtained the PAF for each infection, however, we observed different patterns throughout the simulation. At the beginning of the HIV epidemic, infections such as gonorrhea and syphilis had the highest PAF (~26%). Over time, their influence on the spread of HIV decreased to a final PAF of 13%, 20 years after the introduction of HIV (Figure 2.5 B). In contrast, common infections in the general population such as malaria and HSV-2 had a low initial PAF (~12%), followed by an increase. In agreement with previous studies (Abu-Raddad et al., 2008; Freeman et al., 2007) we found that the PAF due to HSV-2 increases during the first 10 years and then remained constant after 15 years from HIV introduction at the highest PAF (~30%).

Likewise, the proportion of new HIV infections caused by malaria increased during the early years of the epidemic, and then remained constant after 15 years from HIV introduction, with a PAF of ~ 25% (Figure 2.5 B); this value is in close agreement with previous results for eastern sub-Saharan Africa based on regression analysis (Cuadros et al., 2011b).

Discussion

Our results indicate that a data-supported fixed value for HIV infectivity fails to describe the dynamics of the epidemic. Regardless of the low probability of heterosexual transmission per sexual contact, the inclusion of individual variation in HIV infectivity generated by transient but repeated increases in HIV viral loads associated with co-infections may substantially increase the transmission rate (Cohen et al., 2007).

Our model thus suggests that the HIV epidemic in sub-Saharan Africa may be explained by heterosexual transmission, and supports the hypothesis that variation among individuals and through time caused by biological cofactors such as co-infection may

have triggered the vast HIV epidemic observed in sub-Sahara Africa. The high prevalence of infectious diseases such as malaria and HSV-2 probably provided suitable conditions for the spread of the infection in the general population.

The remarkably high HIV prevalence observed in sub-Saharan Africa may thus reflect the particular environment at the early and mature stages of the epidemic that are unique to this part of the world (Abu-Raddad et al., 2008). These results highlight the possibility that co-infection is a necessary rather than merely a contributing factor in the successful spread and survival of HIV in populations where heterosexual vaginal-penile contact is the main mechanism of transmission.

According to our results, 50% of all new HIV infections throughout the epidemic can be attributed to co-infection with the infectious diseases included in the model. However, we observed opposite time trends in the contribution from two infections with low prevalence in the general population (i.e. decreasing trends for gonorrhea and syphilis), and from infections with high prevalence (i.e. increasing trends for HSV-2 and malaria).

Some similar results have been obtained in previous studies (Abu-Raddad et al., 2008; Freeman et al., 2007; White et al., 2008), but none has documented the pattern in PAF that we observed for malaria throughout the epidemic. Our model is the first to include not only the co-factor effect of other STIs on HIV transmission but also the co-factor effect of a parasitic disease such as malaria in the same simulation. The expected high frequency of malaria-HIV co-infected individuals who transmitted the HIV infection (Figure 2.3 A) raises the possibility that parasitic diseases like malaria with high prevalence in Africa, may be playing a similar role to that STI like HSV-2 in terms of new HIV infections. This similarity should be greatest in populations with a mature HIV epidemic and where both infections overlap geographically. Despite the low co-factor effect on HIV transmission generated by malaria (Table A.2, parameter 5), the high prevalence of this infection may have increased its effect on the HIV incidence as the HIV epidemic invaded the general population.

Our analysis suggest that the synergy among sexually transmitted infections and parasitic infections allowed the HIV epidemic to reach the general population, which may not have been possible without the cofactor effect on HIV transmission generated by co-

infection (Abu-Raddad et al., 2008). This in turn suggests that an HIV epidemic may be mitigated or halted through measures that decrease viral infectivity. The control and treatment of several common infectious diseases could decrease the incidence of HIV over the long-term.

Although interventions aimed at reducing the incidence of STIs have a prominent place in control strategies, some studies have failed to show an impact of STI treatments on HIV incidence (Kamali et al., 2002; Wawer et al., 1999). Some authors have suggested that population differences in sexual behavior, differences in STI prevalence and the stage of the HIV epidemic may explain the poor impact of this control intervention (White et al., 2004). These studies (Kamali et al., 2002; Wawer et al., 1999) commonly focused on STIs with low prevalence in the general population, such as syphilis, trichomoniasis, gonorrhea and chlamydia.

However, as our study and other studies (Baeten et al., 2008; Modjarrad et al., 2008; Schacker et al., 2002) have indicated, infectious diseases present in the general population such as HSV-2 and parasitic diseases have the highest impact on the HIV incidence in mature HIV epidemics. A transmission study conducted to determine whether HSV-2 suppression in HIV/HSV-2 co-infected individuals reduces the risk of HIV transmission indicated that, despite a notorious reduction in the prevalence of genital ulcer diseases generated by HSV-2, and a 0.25 $\log_{10}$ copies/ml reduction in plasma HIV-RNA levels, HSV-2 suppression with acyclovir did not prevent HIV transmission (Celum et al., 2010). In a subsequent work, however, Lingappa and coworkers demonstrated that a 0.74 $\log_{10}$ copies/ml reduction in HIV plasma RNA concentration is necessary to reduce the HIV transmission rate by half (Lingappa et al., 2010). HSV-2 suppression with acyclovir may thus have been insufficient to yield a detectable reduction in HIV transmission risk. They concluded that treatment of co-infections capable of reducing plasma HIV levels by > 0.7 $\log_{10}$ copies/ml might be a valuable tool for suppressing of HIV transmission.

Limitations of the model

The results of this study derive from a simulation model and depend on the validity of the underlying assumptions and parameter magnitudes. Uncertainties about the magnitudes

of these parameters suggest that the conclusions presented here should be interpreted with caution. HIV transmission probability and the effect of behavioral and biological cofactors on HIV transmission require more thorough quantification (Abu-Raddad et al., 2008; Freeman et al., 2007; White et al., 2008), and we hope that our results will help motivate this work.

Additionally, cofactor values are commonly estimated from population-based observations of co-infection status in individuals or couples (Korenromp et al., 2000). In these cases, the association between the transmission of HIV and the presence of an STI is generally expressed in terms of odd ratios, hazard ratios or relative risk per sexual contact (Boily and Anderson, 1996). These estimates, however, can be particularly difficult to interpret as a consequence of multiple potential biases.

To reduce confounding effects resulting from other behavioral and biological risk factors, estimates of cofactor effects are statistically adjusted for the influence of these risk factors. But these analyses may not completely control for the confounding effects because STIs, HIV and other behavioral and biological risk factors may cluster not only in study subjects but also in the unknown partners of the individuals included in the study (Korenromp et al., 2000). Moreover, the confounding generated by the characteristics of the sexual network such as concurrency, mixing patterns and numbers of sexual partners is virtually impossible to control for completely (Korenromp et al., 2000).

On the other hand, the high variation in HIV prevalence produced by the model observed in the uncertainty analysis compromises the accuracy of the model's predictions (Table A.9). The sensitivity analysis indicated that the model is highly sensitive to the behavioral parameters that influenced the per-partner probability of HIV transmission, such as the average duration of casual relationships and the mean number of sexual contacts, and to the biological parameters responsible for the cofactor effect of infections present in the general population, such as malaria and HSV-2 (Table A.10). Thus, efforts focused on more precise estimation of these parameters will improve the accuracy of predictions from models exploring the causes of the HIV epidemic in sub-Saharan Africa.

Lastly, the model assumes that HIV is transmitted exclusively by penile-vaginal heterosexual contact, and thus the model does not include anal intercourse as a mechanism of HIV transmission. Since anal intercourse increases the probability of

transmission (Boily et al., 2009), this type of sexual behavior has been proposed as an important risk factor of HIV transmission. Although many authors claim that heterosexual penile-vaginal contact is the main mechanism of transmission in sub-Saharan Africa, more of information about the frequency of anal intercourse, including men who have sex with men, would allow an evaluation of this possibly important but little-studied mechanism of transmission in the epidemic in sub-Saharan Africa (Smith et al., 2009).

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Figure 2.1 HIV epidemic curves with constant probability of HIV transmission (no co-infection)

At $T = 0.003$ no epidemic can be generated, and HIV prevalence decreases leading to extinction (prevalence estimated for 2010 = 0%)). With a $T = 0.005$ the infection can persist and reached and endemic steady state at a prevalence equal to the initial prevalence (HIV prevalence estimated for 2010 = 1%). At $T = 0.0068$ the epidemic curve generated is similar to the HIV epidemic observed in Malawi (HIV estimated prevalence for 2010 = 10%).

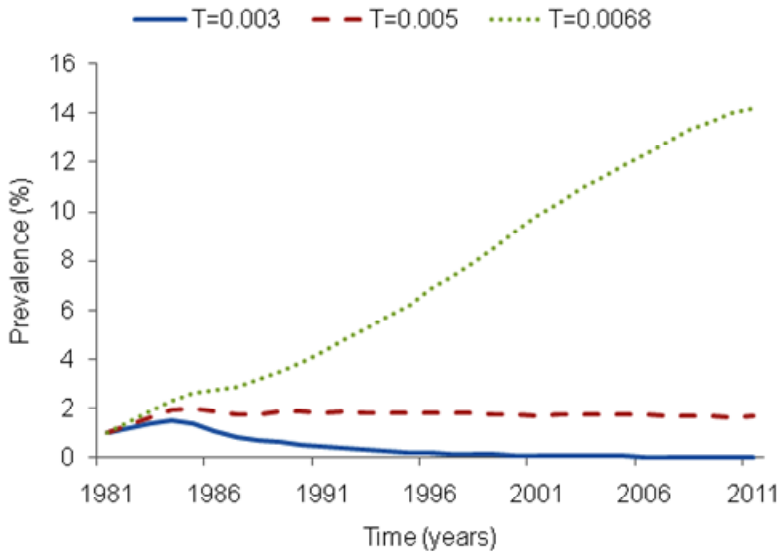


Figure 2.2 Time course of HIV with the inclusion of the amplification effect caused by co-infection (simulation 2)

In (A), the dynamics of HSV-2, syphilis, gonorrhea and malaria were simulated and allowed to approach steady state before the introduction of HIV into the population. Once endemic steady states of these infections were reached, 1% of individuals were infected with HIV, and HIV established in the population. (B) shows in more detail the time course of HIV in Malawi simulated by the inclusion of the amplification effect generated by co-infection (Simulation 2). The measured HIV prevalence from Malawi, for both males and females in general population, was extracted from several studies (USAIDS, 2006).

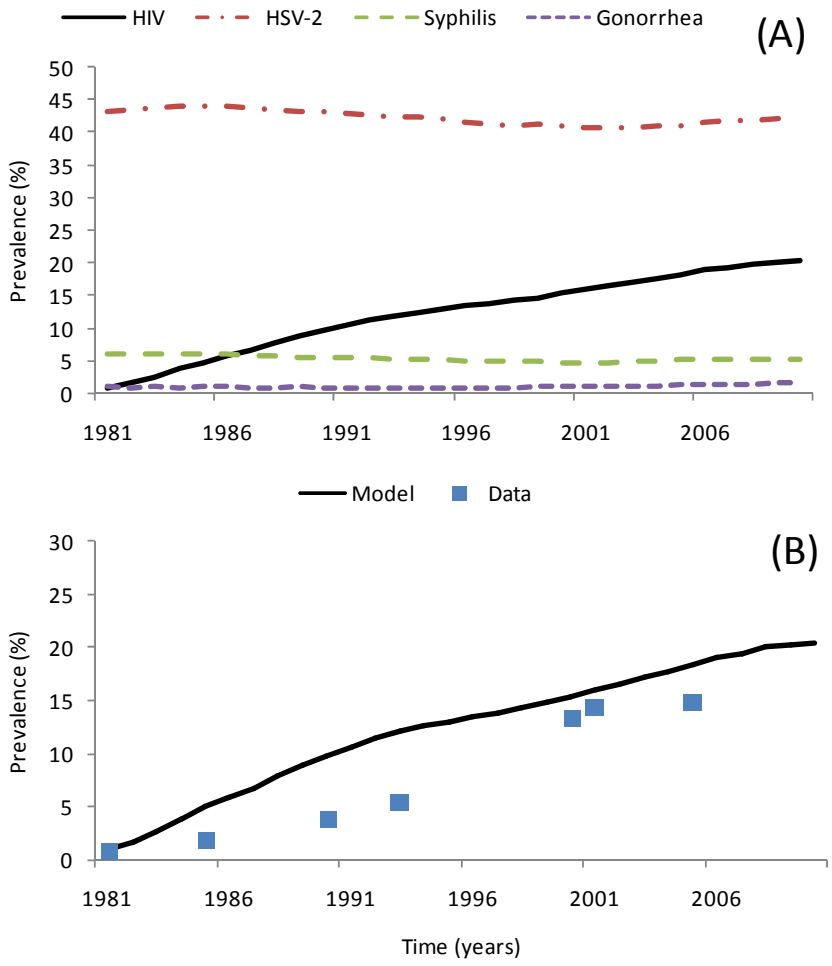


Figure 2.3 Fraction of new infections when the HIV infectious (transmitter) individual had a co-infection (simulation 2)

The model tracks the mechanism of the transmission of the infection. For the simulated period, if an HIV infection takes place, we recorded the presence in the infectious individual of any of the four infectious diseases included in the model, or in the case of HSV-2 due to the enhanced susceptibility caused by HSV-2 infection, we also recorded the presence of this infection on the susceptible individual and identified the cause of the amplification of the transmission caused by co-infection. (A) shows that HSV-2 was the most prevalent infection in co-infected individuals who transmitted the HIV infection, followed by malaria. This result is associated with the high prevalence of both infections in the general population. Grouping all STIs, more than 50% of the HIV infections were associated with an STI (B). More than 70% of the total HIV infections were caused by co-infected individuals (C).

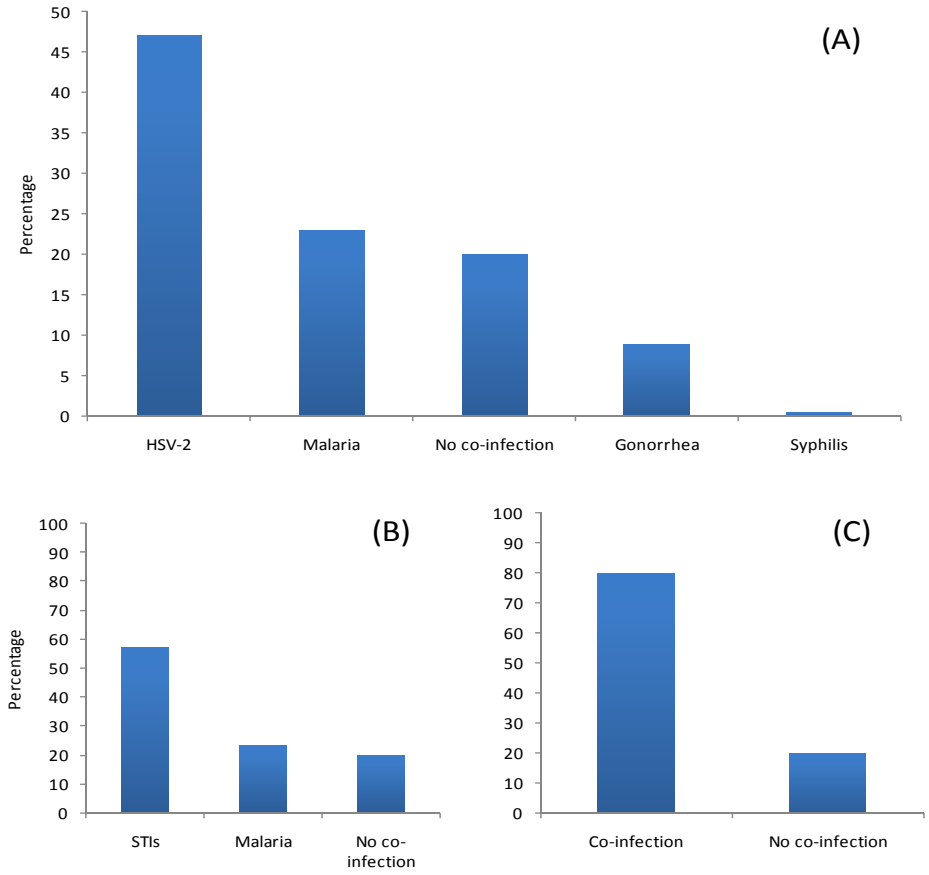


Figure 2.4 Removing the amplification effect on HIV transmission

(A) shows the time course of HIV when HSV-2 does not have an amplification effect on HIV (simulation 3) and the prevalence for 2005 decays to ~6% (C).In the scenario where malaria does not have an amplification effect on HIV transmission (simulation 5), the resulting prevalence decays to ~10% (B).

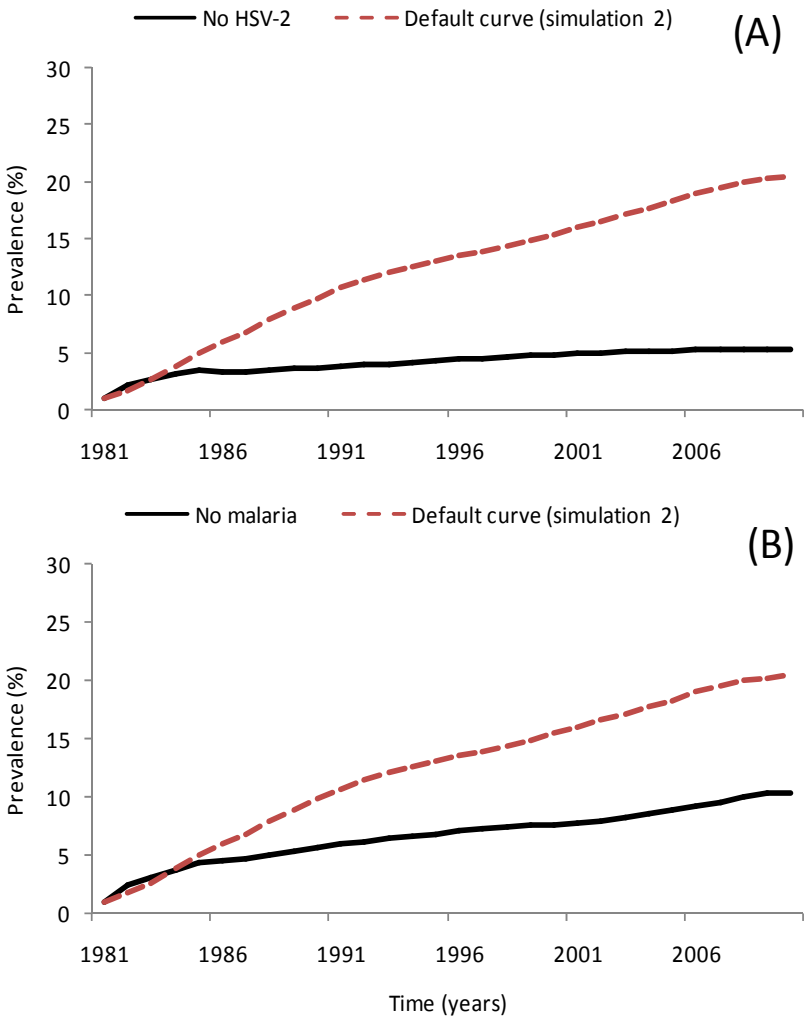
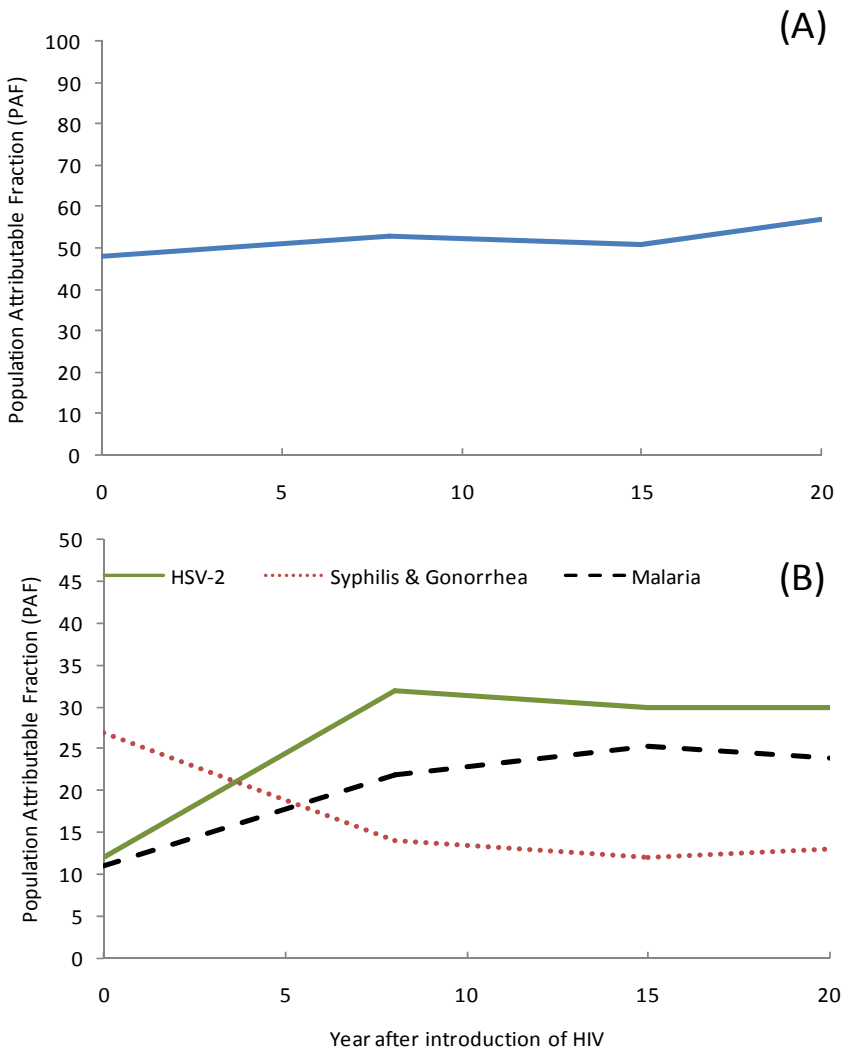


Figure 2.5 Population Attributable Fraction (PAF) due to co-infection

We measured the direct effect of co-infection on the HIV incidence by using the population attributable fractions. (A) The PAF due to co-infection in general, and (B) the PAF due to HSV-2 (solid line), syphilis and gonorrhea (dotted line) and malaria (dashed line).



Chapter 3 - HIV-malaria co-infection: effects of malaria on the prevalence of HIV in East sub-Saharan Africa

Introduction

The heterosexual transmission of HIV is characterized by a low transmission rate, estimated to be ~ 1/300 per coital act in low income countries (Boily et al., 2009; Gisselquist and Potterat, 2003). Despite this low probability of transmission per sexual contact, some studies have demonstrated that the risk of HIV transmission can be strongly correlated with variations in blood viral burden (Attia et al., 2009; Cohen et al., 2007; Pilcher et al., 2007). The viral set point is not constant and may be perturbed by reactivations of the immune system such as those resulting from the invasion of other pathogens (Bentwich et al., 1995).

The average African host is usually exposed to numerous bacterial, viral and parasitic infections. Of special importance is the very high prevalence of sexually transmitted infections (STIs), particularly genital ulcerations caused by herpes simplex virus type 2 (HSV-2) (Bentwich et al., 1995). The ulcers caused by HSV-2 contain substantial numbers of CD4+ lymphocytes, the target cell for HIV, and therefore are likely to facilitate the acquisition of HIV in HIV-negative individuals (Celum et al., 2004). Additionally, the high levels of HIV-RNA in herpetic lesions from dually infected patients (Schacker et al., 1998) may be explained by studies *in vitro* demonstrating that HSV-2 increases HIV transcription, which supports the higher infectivity in co-infected individuals.

The activation of the immune system, however, is not only produced by STIs such as HSV-2, syphilis and gonorrhea. Parasitic infections such as malaria might produce a strong response from the immune system and consequently generate similar effects on the replication of the virus in HIV co-infected individuals (Bentwich et al., 1995; Korenromp et al., 2005; Kublin et al., 2005; Pisell et al., 2002; Reina et al., 2006). The geographical overlap observed between malaria and HIV infections has suggested a

possible interaction influencing HIV transmission in some countries of sub-Saharan Africa. Malaria occurs throughout the tropical world, where it remains one of the most prevalent infectious diseases with an estimated 300 million cases per year (CDC). Approximately 90% of these cases occur in sub-Saharan Africa (Snow et al., 2005). This region has also experienced the greatest burden of HIV-1 infection, with an estimated 25 million infected individuals over the past two decades (WHO). The geographic overlap of these diseases has sparked much interest in their potential interactions (Dolores et al., 2007; Idemyor , 2007; Whitworth and Hewitt, 2005). Several *in vitro* studies have found that malaria antigens significantly enhanced HIV-1 replication (Kublin et al., 2005; Pisell et al., 2002; Reina et al., 2006; Xiao et al., 1998). A population-based study conducted with HIV-1 infected adults in rural Malawi indicated that the HIV-1 RNA concentration almost doubled between baseline (96 215 copies per ml) and those co-infected with malaria (168 901 copies per ml). Researchers estimated that, on average, malaria generates a 0.25 (95% CI 0.11-0.39) log increment on the mean HIV RNA concentration (Kublin et al., 2005). Abu-Raddad and coworkers generated a mathematical model to examine the impact of this increment on the HIV epidemic in Kisimu, Kenya (Abu-Raddad et al., 2006). Using the functional relationship between HIV plasma viral load and transmission probability per coital act, in which a logarithmic increase in viral load is associated with a 2.45-fold increase in transmission probability (Quinn et al., 2000), they demonstrated that the enhancement on the HIV infectivity of co-infected individuals may account for a cumulative 8500 excess HIV infections in Kisumu district.

Despite the evidence about the relationship between malaria and HIV *in vitro* and *in silico*, the causes and implications of this co-infection at the population level are still unclear. One important limitation is the inaccurate documentation of geographical overlap between the two infections in sub-Saharan Africa. Visual analysis of the spatial distribution of each infection may be misleading in the absence of adequate statistical adjustment. Moreover, assessing the impact of the co-infection is impeded by the scarcity of data on co-infection prevalence and consequences in the transmission of HIV infection.

In the present study, we measure the association between malaria and HIV prevalence. To achieve this goal we combined current comprehensive databases for these

two infections, namely the Demographic and Health Survey (DHS) for HIV and the atlas malaria project for malaria, by using Geographical Information Systems (GIS) tools in a representative area of East sub-Saharan Africa. Our hypothesis was that individuals in locations with high prevalence of the vector that transmits malaria, namely the *Plasmodium falciparum* parasite, which is the species responsible for most of the 95% of the malaria cases in the region studied (USAID, 2008a; USAID, 2008b; USAID, 2009), have increased likelihood of being infected with HIV.

Methods

Data

Data were extracted from DHS for 2003 in Kenya (KDHS) (Central Bureau of Statistics, 2003) and Malawi (MDHS) (National Statistical Office), and for 2003-04 in Tanzania (THIS) (TACAIDS, 2005), to cover the population residing in households in these countries. These countries exemplify the HIV epidemic in East sub-Saharan Africa because they present similar HIV epidemic levels and risk factors for HIV infection as the other East sub-Saharan countries (Buvé et al., 2002; Johnson and Way, 2006; Msisha et al., 2008; Orroth et al., 2007), and the malaria prevalence in the region covered by these three countries is heterogeneous for comparisons (Hay et al., 2009). A representative probability sample of almost 10 000 households was selected from the KDHS, 13 664 from the MDHS and 6900 from the THIS survey.

All DHS surveys were based on two-stage sampling of the households. The first stage involved selecting sample points (clusters), 400 clusters in Kenya (129 in urban and 271 in rural areas), 522 clusters in Malawi (64 in urban and 458 in rural areas), and 345 clusters in Tanzania (87 in urban and 258 in rural areas). The global position system (GPS) was used to establish and record the geographic coordinates of each of the DHS clusters (TACAIDS, 2005).

Adult men (aged 15-59 years) and women (aged 15-49 years) in the selected household were eligible for the survey, with a response rate of 86% and 96% for men

and women in Kenya (Central Bureau of Statistics, 2003) and Malawi (National Statisitcal Office Malawi, 2004), and 91% and 96% for men and women in Tanzania (TACAIDS, 2005). In all households selected, anonymous HIV testing was performed with the consent of all eligible men and women. We restricted the analysis to only sexually active people and after combining all three DHS surveys and excluding missing data, the final sample population for data analysis consisted of 8919 men and 10 816 women.

We used the dichotomous HIV serostatus for each individual as the response outcome (TACAIDS, 2005). The HIV serostatus was identified by collecting drops of blood from each individual for HIV testing with the Vironostika Uniform 2 Ag/AB kit. All samples that tested positive and a random sample of 10% of samples that tested negative on the first ELISA (enzyme-linked immunosorbent assay) test were tested with a second ELISA, the Vironostika Uniform 2 plus O. A confirmatory INOLIA HIV Western blot kit was done for all samples that were still discrepant (Central Bureau of Statistics, 2003; National Statisitcal Office Malawi, 2004; TACAIDS, 2005).

Socio-economic and demographic covariates

To adjust for socioeconomic and demographic factors, we conducted preliminary univariate analyses with a total of 14 socioeconomic and demographic covariates extracted from the DHS of each country. The covariates included age, place of residence (urban or rural), highest educational level, main floor material of the house, main roof material of the house, main wall material of the house, religion, current marital status, ever been tested for AIDS, wealth index, number of sex partners, age at first intercourse, responder have a bednet for sleeping, and responder sleeps under bednet. Covariates with a $P<0.2$ in univariate analysis were included in the final model, which included age, urban or rural residence, education, wealth index, marital status and religion. These covariates have also been identified from previous studies conducted in Tanzania and Kenya as the most relevant covariates for the risk of HIV infection in these countries (Johnson et al., 2006; Msisha et al., 2008).

Education level was evaluated as a categorical variable with four levels: no education, primary education, secondary education and higher education. Wealth index is

an ordinal variable that characterizes standard of living as determined by material possessions. The DHS calculated the living standard of a household based on key assets such as television and bicycles, materials used for housing construction, and types of water access and sanitation facilities. The data were analyzed and weighted by principal component analysis. The resulting asset scores were then used to define wealth quintiles: poorest, poorer, middle, richer, richest. Marital status has been found in many studies as an important risk factor for being HIV-infected (Auvert et al., 2001; Glynn et al., 2001; Nunn et al., 1994). In the current study, marital status was represented by three categories: never married, currently married and formerly married. Religion was also included for its potential confounding effects and was composed of four categories: Moslem, Catholics, Protestants or other Christians, and other religions.

Biological covariates

Previous studies have found that reporting any (STIs during the 12 months previous to the interview was not correlated with being HIV-positive (Johnson et al., 2006; Msisha et al., 2008). For that reason, and with the aim to control for potential confounding by STIs, we included the reported presence of genital ulcerations during the last 12 months previous to the interview. Male circumcision is a well known risk factor for being HIV-positive (Auvert et al., 2001; Gray et al., 2000; Rakwar et al., 1999). Males with a lack of circumcision have nearly twice the risk of being HIV-positive than circumcised males. For the risk analysis performed for males, we included male circumcision as a co-variable.

Malaria covariate

We used the largest and most contemporary spatial database for *Plasmodium falciparum* parasite rate (*Pf*PR) (Hay et al., 2009; Hay et al., 2006). This database contains nearly 9000 distinct community surveys across 78 malaria endemic countries. To create a continuous surface of malaria endemicity, previous research produced a model-based geostatistical procedure in a Bayesian framework to incorporate factors such as the spatial density and location of the data, and the number of people sampled in each survey (Hay et al., 2009). Geostatistical algorithms generate a continuous map by imputing

values at unsampled locations by using a weighted linear combination of the available (neighboring) sample data. One of the most important characteristics of this database is age-standardization for malaria prevalence (the *PfPR* reported by the atlas malaria project is age-standardized to 2-10 years old), which provides a valid estimate of the transmission intensity (Hay et al., 2009). Further details on the malaria data and statistical procedures for mapping it have been described elsewhere (Hay et al., 2009).

To display and extract the *Pf*PR from each DHS cluster we used the program ArcGIS version 9.2 (ESRI, 2004). From each geo-referenced cluster we obtained the *Pf*PR, the value of which was assigned to each individual who belonged to that cluster.

Statistical analysis

We generated unadjusted models for each covariate using a simple logistic regression model. To generate the final adjusted analysis, which included all covariates and accounted for correlated data, generalized linear mixed models (GLMM) stratified by gender (in order to include circumcision as a covariate for men) were then fitted with the cluster as a normally distributed random effect. We included *PfPR* as a continuous variable. In a second analysis, we categorized *Pf*PR by its empirical quartiles. All statistical analyses were conducted using R version 2.11.1 (R. Core Team, 2010).

Adjusted Probability Ratio (PR) parameters were used to compare regions with varying degrees of *Pf*PR, separately for men and women (Rockhill et al., 1998). To calculate PRs, we dichotomized *Pf*PR using its first quartile to define low malaria prevalence. Areas with *Pf*PR higher than the first quartile were defined to have high malaria prevalence.

Results

Overall malaria and HIV prevalence

Results of the 2003-04 DHS from the region studied indicated that the HIV prevalence in responders who have had sex was 6.66% for men and 9.46% for women. The prevalence of HIV peaked for age group 35-39 for men and 30-34 for women (Table 3.1). The mean

*Pf*PR in the region was 0.27, with a standard deviation of 0.18. The quartiles of *Pf*PR led to four groups, namely *Pf*PR less than or equal to 0.10, *Pf*PR between 0.11 to 0.30 , *Pf*PR between 0.31 to 0.42, and *Pf*PR higher than 0.42 (Table 3.1). Areas where the *Pf*PR is 40% or more are identified as areas with high stable transmission, where theory predicts that mixed control interventions need to be considered for the interruption of malaria transmission (Hay et al., 2008). Hay and coworkers (Hay et al., 2009) estimated that in Africa these areas cover 8.5 million Km^2, which contains 345.28 million people at risk.

Individuals (males and females) with higher education and with higher wealth index were primarily distributed in (mainly urban) areas with low *PfPR* (*PfPR* <= 10%), whereas individuals with lower education level and lower wealth index (poorest, poorer) were mainly in rural areas with high *PfPR* (*PfPR* > 42%) (Table 3.2). In areas with low malaria prevalence (*Pf*PR < 0.10), the HIV prevalence was 5.11% for males and 8.27% for females, whereas in areas with high malaria prevalence (*Pf*PR > 0.42) the HIV prevalence was 7.67% for males and 10.73% for females (Table 3.1).

Malaria

With *Pf*PR modeled as a continuous variable, the unadjusted analysis indicated that *Pf*PR was a risk factor for HIV infection in both men ($P < 0.001$) and women ($P = 0.013$). After adjusting for measured socio-economic and biological characteristics the *Pf*PR remained as a risk factor of being infected with HIV in both men and women ($P < 0.001$ for both). After categorizing the *Pf*PR we observed that malaria is positively and monotonically related to HIV-infection (Table 3.3 and 3.4). The adjusted model indicated that men and women who live in areas with high *Pf*PR (>0.42) are nearly twice as likely to be HIV-positive as those who live in areas with low *Pf*PR (<0.10) (men: estimated OR 2.24, 95% CI 1.62-3.12; women: estimated OR 2.44, 95% CI 1.85-3.21).

To perform the probability ratio estimation we created a dichotomous variable for malaria prevalence where low *Pf*PR indicates low risk of being infected with malaria (for individuals who live in areas with a *Pf*PR≤ 0.10) and high *Pf*PR indicates high risk of being infected with malaria (for individuals who live in areas with *Pf*PR >0.10). The

calculated probability ratio attributable to malaria was 27% for males and 29% for females.

Socio-economic characteristics

Men and women formerly married had the highest odds of having HIV compared with individuals never married, (estimated OR 6.27, 95% CI 4.38-8.97) and (estimated OR 6.83, 95% CI 5.39-8.66), respectively in the unadjusted model. Controlling for all other socio-economical and biological risk factors attenuated the estimated odds ratios to 3.15 (95% CI 2.03-4.90) for men (Table 3.3), and 4.20 (95% CI 3.09-5.71) for women (Table 3.4).

As expected (Johnson et al., 2006; Msisha et al., 2008), unadjusted models indicated that men and women living in rural areas had lower odds of being HIV-infected than those living in urban areas, with an estimated OR of 0.52 (95% CI 0.45-0.62) for men and an estimated OR of 0.57 (95% CI 0.5-0.66) for women. In the adjusted analysis the estimated effect remained similar with odds ratio 0.54 (95% CI 0.41-0.70) for men and 0.59 (95% CI 0.46-0.77) for women. In the unadjusted model (Table 3.4), women with a secondary educational level had increased odds of having HIV compared to women with any level of education (estimated OR 1.45, 95% CI 1.15-1.83). Education was not a risk factor for being infected with HIV in adjusted models for women (likelihood ratio $P = 0.12$).

As observed in previous studies (Johnson et al., 2006; Msisha et al., 2008), the wealth index for both men and women is positively correlated with HIV. In unadjusted models, men and women in the highest wealth category were estimated to have 2.64 (95% CI 1.97-3.54) and 2.74 (95% CI 2.19-3.44) times the odds of being HIV infected compared with those in the poorest category. The adjusted model attenuated the estimated odd ratio for men to 2.32 (95% CI 1.58-3.42), but increased the estimated odd ratio for women to 2.92 (95% CI 2.12-4.02).

Biological characteristics

In unadjusted models the estimated OR of having HIV was 2.59 (95% CI 1.76-3.8) for males who had genital ulcerations during the last 12 months (Table 3.3). For women the

odds were considerably higher than for males (estimated OR 3.21, 95% CI 2.94-4.05). This difference was maintained in the adjusted model with 1.99 (95% CI 1.30-3.03) for men and 2.79 (95% CI 2.07-3.76) for women. These results are consistent with previous studies, which have found that the presence of an STI increases the risk of being HIV-positive (Blower and Ma, 2004; Freeman et al., 2006; Glynn et al., 2008b; Nunn et al., 1994; Waver et al., 2005).

Lack of male circumcision has been proposed as one of the most important biological risk factors of being HIV-positive in men (Auvert et al., 2001; Gray et al., 2000; Jewkes et al., 2006; Orroth et al., 2007; Rakwar et al., 1999). In our analysis, the adjusted models indicated that circumcised males had slightly more than half the risk of those who were not circumcised to be HIV-positive (estimated OR 0.62, 95% CI 0.50-0.79).

Discussion

The factors related to high HIV-1 prevalence in sub-Saharan Africa are incompletely recognized. The epidemic in this part of the world is widely distributed across the general population, and heterosexual penile-vaginal mechanism of transmission has been proposed to account for more of the 80% of the new cases (Orroth et al., 2007; Vernazza et al., 1999; Waver et al., 2005). For many years, behavioral factors such as commercial sex, concurrency and promiscuity have been proposed as driving the epidemic in sub-Saharan Africa (Lagarde et al., 2001; Morison et al., 2001). Recent behavioral studies carried out in different populations from Africa, however, have found no significant differences compared to populations in developed countries such as the United States or Europe, and in some cases had no correlation with the risk of HIV infection (Brewer et al., 2007; Lurie et al., 2009; Orroth et al., 2007; Reniers et al., 2010). In addition, a previous cross-sectional population-based study, called the Four City study (Orroth et al., 2007), examined the differences in the spread of HIV in four cities from the western and eastern regions of sub-Saharan Africa and found that differences in sexual behavior could not explain the differences between the HIV epidemics (Ferry et al., 2001; Lagarde et al., 2001; Morison et al., 2001). The authors postulated that biological factors may be

responsible for these differences (Buve et al., 2001). Biological cofactors such as male circumcision and other STIs, especially Herpes Simplex Virus type 2 (HSV-2), thus have been proposed as a plausible explanation for these observed differences (Orroth et al., 2007).

The substantial evidence that sexually transmitted infections, HSV-2 in particular, enhanced the transmission and acquisition of HIV infection contrasts with the scarce information about the cofactor effect of malaria on HIV at the population level. Like HSV-2, malaria contributes to transient viral load increases in co-infected individuals (Kublin et al., 2005; Pisell et al., 2002; Xiao et al., 1998). Due to the methodological difficulties in designing cross-sectional or prospective studies for measuring the significance of malaria on the risk of HIV infection, however, the effect of malaria on the spread of HIV in the population is virtually unknown.

This is the first study to report malaria as a risk factor for concurrent HIV infection at the population level. After adjusting for important socio-economic and biological covariates, we found that the *Pf*PR of the area of residence is positively and monotonically correlated with HIV prevalence (Table 3.3 and 3.4). According to our results, men who live in areas with high *Pf*PR (> 0.42) have at least twice the risk of being HIV-positive (estimated OR 2.24, 95% CI 1.62-3.12) in comparison to men who live in areas with low *Pf*PR (≤ 0.10). As we expected, the risk of malaria infection was not related to gender; thus women who live in areas with high *Pf*PR have nearly the same OR (2.44, 95% CI 1.85-3.21).

The probability ratio attributable to *Pf*PR indicated that malaria may account for ~27% of incidental HIV infections in areas with a *Pf*PR higher than 0.1. This value is similar to the value reported previously by Cuadros and coworkers (2011a), who by computer simulations estimated that malaria might account for more than 20% of new HIV infections in Malawi in 2003. The probability ratio due to malaria resembles the population attributable fraction (PAF) associated with HSV-2, estimated by epidemiological and mathematical models (Abu-Raddad et al., 2008; Blower and Ma, 2004; Freeman et al., 2007). This similarity may be due to the high prevalence of both infections in the general population.

Some studies have shown that curable STIs such as gonorrhea, syphilis or chlamydia have had the biggest impact on HIV transmission at early stages of the HIV epidemic (Freeman et al., 2007; White et al., 2008). These infections generally are present in high-risk groups such as prostitutes or individuals with a high number of sexual partners (Abu-Raddad et al., 2008; White et al., 2008). The effect of curable STIs on HIV has decreased as the HIV epidemic has moved from the core group to the general population. Prevalent infections, such as HSV-2, in the general population may then play the most important role driving the HIV prevalence in populations with a mature HIV epidemic (Abu-Raddad et al., 2008; Cuadros et al., 2011a; White et al., 2008). Although malaria infection has a non-sexual mechanism of transmission, its high prevalence in the general population and its ability to generate an immune activation associated with increases in systemic HIV viral load suggests that malaria may have a similar role to HSV-2 in mature HIV epidemics.

Although our work makes use of the most complete and contemporary databases for both HIV and malaria, our results were based on geographical estimates for *Pf*PR in locations where demographic and health surveys were made. Despite the use of this indirect measure of the possible synergistic interaction between malaria and HIV at the population level, our results suggest that malaria is likely to be a frequent co-infection in HIV infected individuals living in those areas with high malaria prevalence. Hence, we emphasize that malaria might be playing an important role in the present HIV epidemic of these areas. The scenario could be more complicated if we take into account infections such as chronic helminth infections and water borne pathogens that, like malaria, are associated with similar immune activation by inducing viral replication (Bentwich et al., 1995; Lawn and Folks, 2001) and could also be affecting the HIV transmission risk and the natural history of HIV in sub-Saharan Africa.

Our work emphasizes the need for field studies on the interaction among parasitic infections and the risk of HIV infection and on the impact of control interventions. Thus, it would be important to obtain data on individuals who are known to be dually infected with malaria and HIV. Reducing malaria transmission becomes even more important to address in the context of HIV. With the high numbers of cases of HIV and malaria, even

small decreases in malaria prevalence could have an important impact on the relative risk of HIV infection in areas with high malaria prevalence.

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Table 3.1 HIV prevalence by socio-economic and biological characteristics for sexually active people surveyed in Kenya, Malawi and Tanzania

Characteristics	N(%HIV-positive)	
	Women	Men
Age		
15-19	2285(2.67)	2018(1.24)
20-24	2276(9.31)	1578(3.99)
25-29	1939(10.93)	1483(7.75)
30-34	1543(14.32)	1237(9.54)
35-39	1172(12.8)	954(11.53)
40-49	1601(10.43)	1372(10.28)
50-59	-	277(7.94)
Religion		
Moslem	2610(9.00)	1996(5.96)
Catholic	2998(9.71)	2530(7.19)
Protestant/other Christian	4060(9.70)	3222(6.55)
Other	1148(9.97)	1171(7.00)
Place of residence		
Urban	2629(13.47)	2064(10.22)
Rural	8187(8.17)	6855(5.59)
Marital status		
Never married	2434(4.27)	3457(2.37)
Currently married	7137(8.80)	5046(9.06)
Formerly married	1245(23.37)	416(13.22)
Highest educational level		
None	2388(7.79)	947(5.17)
Primary school	6979(9.77)	6218(6.38)
Secondary	1280(10.94)	1497(8.86)
Higher	169(8.88)	275(6.18)
Wealth index		
Poorest	2067(5.27)	1507(4.11)
Poorer	2111(7.01)	1811(4.36)
Middle	2101(8.66)	18.21(5.93)
Richer	2127(12.46)	1863(8.05)
Richest	2410(13.24)	1917(10.17)
Circumcision		
Uncircumcised	-	3423(8.41)
Circumcised	-	5496(5.57)

Genital ulceration		
No genital ulceration last 12 months	10489(8.99)	8708(6.45)
Genital ulceration last 12 months	327(24.46)	211(15.17)
Malaria		
*Pf*PR <= 0.1	2612(8.27)	2113(5.11)
*Pf*PR [0.11-0.3]	2899(9.07)	2480(6.61)
*Pf*PR [0.31-0.42]	2415(9.69)	2071(7.19)
*Pf*PR > 0.42	2890(10.73)	2255(7.67)

Table 3.2 Socio-economic characteristics distribution by different *Pf*PR levels (males and females combined)

Characteristics	Total individuals (% per *Pf*PR category)			
	***PfPR* <=0.1**	***Pf*PR [0.11-0.3]**	***Pf*PR [0.31-0.42]**	***Pf*PR > 0.42**
Age				
15-19	1054(24)	1285(30)	941(22)	1023(24)
20-24	926(24)	1049(27)	895(23)	984(26)
25-29	785(23)	876(26)	809(24)	952(27)
30-34	665(24)	733(26)	634(23)	748(27)
35-39	521(25)	590(27)	480(23)	535(25)
40-49	678(23)	790(27)	665(22)	840(28)
50-59	96(35)	56(20)	62(22)	63(23)
Religion				
Moslem	1130(25)	1253(27)	548(12)	1675(36)
Catholic	1162(22)	1510(27)	1626(29)	1230(22)
Protestant/other Christian	2240(31)	2123(29)	1577(22)	1342(18)
Other	193(8)	493(21)	735(32)	898(39)
Place of residence				
Urban	2202(47)	1172(25)	803(17)	516(11)
Rural	2523(17)	4207(28)	3683(24)	4629(31)
Marital status				
Never married	1784(30)	1767(30)	1159(20)	1181(20)
Currently married	2558(21)	3167(26)	2971(24)	3487(29)
Formerly married	383(23)	445(27)	356(21)	477(29)
Highest educational level				
None	543(16)	901(27)	798(24)	1093(33)
Primary school	2865(22)	3659(27)	3103(24)	3570(27)
Secondary	1008(36)	744(27)	549(20)	458(17)

Higher	309(70)	75(17)	36(8)	24(5)
Wealth index				
Poorest	521(15)	1193(33)	771(22)	1089(30)
Poorer	491(13)	1005(26)	979(25)	1447(36)
Middle	608(16)	1120(28)	1058(27)	1136(29)
Richer	1037(26)	983(25)	1033(26)	937(23)
Richest	2068(48)	1078(25)	645(15)	536(12)

Table 3.3 Unadjusted and adjusted results: men's HIV serostatus according to selected socio-economic and biological characteristics

Indicator	OR(95% CI) Unadjusted	Adjusted
Malaria		
*Pf*PR <= 0.1	1.00	1.00
*Pf*PR [0.11-0.3]	1.31(1.02-1.69)	1.73(1.27-2.35)
*Pf*PR [0.31-0.42]	1.44(1.11-1.86)	1.74(1.25-2.41)
*Pf*PR > 0.42	1.54(1.20-1.98)	2.24(1.62-3.12)
Age		
15-19	1.00	1.00
20-24	3.13(2.08-5.29)	2.54(1.52-4.25)
25-29	6.69(4.32-10.38)	4.14(2.42-4.07)
30-34	8.40(5.42-13.01)	4.84(2.78-8.44)
35-39	10.38(6.68-16.15)	6.58(3.75-11.57)
40-49	9.13(5.93-14.05)	5.66(3.24-9.88)
50-59	6.87(3.82-12.37)	4.67(2.26-9.23)
Religion		
Moslem	1.00	1.00
Catholic	1.22(0.96-1.55)	1.05(0.78-1.40)
Protestant/other Christian	1.11(0.88-1.39)	1.06(0.80-1.41)
Other	1.19(0.89-1.58)	0.93(0.64-1.33)
Place of residence		
Urban	1.00	1.00
Rural	0.52(0.43-0.62)	0.54(0.41-0.70)
Marital status		
Never married	1.00	1.00
Currently married	4.10(3.28-5.21)	1.85(1.31-2.62)
Formerly married	6.27(4.38-8.97)	3.15(2.03-4.90)
Highest educational level		
None	1.00	1.00
Primary school	1.25(0.92-1.69)	1.15(0.83-1.61)
Secondary	1.78(1.26-2.49)	1.34(0.91-1.98)
Higher	1.21(0.68-2.13)	0.68(0.36-1.29)
Wealth index		
Poorest	1.00	1.00
Poorer	1.06(0.76-1.49)	0.94(0.66-1.36)
Middle	1.47(1.07-2.02)	1.34(0.96-1.91)

Richer	2.04(1.51-2.77)	1.94(1.38-2.71)
Richest	2.64(1.97-3.54)	2.32(1.58-3.42)
Circumcision		
No circumcised	1.00	1.00
Circumcised	0.64(0.54-0.75)	0.62(0.50-0.79)
Genital ulceration		
No genital ulceration last 12 months	1.00	1.00
Genital ulceration last 12 months	2.59(1.76-3.8)	1.99(1.30-3.03)

Table 3.4 Unadjusted and adjusted results: women's HIV serostatus according to selected socio-economic and biological characteristics

	OR(95% CI)	
Indicator	**Unadjusted**	**Adjusted**
Malaria		
*Pf*PR <= 0.1	1.00	1.00
*Pf*PR [0.11-0.3]	1.11(0.92-1.34)	1.61(1.23-2.12)
*Pf*PR [0.31-0.42]	1.19(0.98-1.45)	1.68(1.27-2.23)
*Pf*PR > 0.42	1.33(1.11-1.60)	2.44(1.85-3.21)
Age		
15-19	1.00	1.00
20-24	3.75(2.80-5.01)	3.31(2.36-4.65)
25-29	4.47(3.34-5.99)	3.92(2.75-5.59)
30-34	6.10(4.55-8.16)	5.29(3.69-7.58)
35-39	5.35(3.94-7.27)	4.33(2.97-6.32)
40-49	4.24(3.14-5.73)	3.30(2.27-4.80)
Religion		
Moslem	1.00	1.00
Catholic	1.09(0.91-1.30)	1.22(0.97-1.52)
Protestant/other Christian	1.09(0.92-1.29)	1.28(1.03-1.59)
Other	0.99(0.78-1.27)	0.95(0.70-1.29)
Place of residence		
Urban	1.00	1.00
Rural	0.57(0.50-0.66)	0.59(0.46-0.77)
Marital status		
Never married	1.00	1.00
Currently married	2.16(1.75-2.67)	1.22(0.92-1.60)
Formerly married	6.83(5.39-8.66)	4.20(3.09-5.71)
Highest educational level		
None	1.00	1.00
Primary school	1.28(1.08-1.52)	1.16(0.95-1.41)
Secondary	1.45(1.15-1.83)	1.05(0.78-1.42)
Higher	1.15(0.67-2.00)	0.71(0.38-1.35)
Wealth index		
Poorest	1.00	1.00
Poorer	1.36(1.05-1.75)	1.43(1.07-1.89)
Middle	1.70(1.33-2.17)	1.83(1.39-2.43)
Richer	2.56(2.03-3.22)	2.69(2.04-3.56)

Richest	2.74(2.19-3.44)	2.92(2.12-4.02)
Genital ulceration		
No genital ulceration last 12 months	1.00	1.00
Genital ulceration last 12 months	3.21(2.52-4.26)	2.79(2.07-3.76)

Chapter 4 - No evidence of association between HIV and malaria in populations with low HIV prevalence

Introduction

Malaria has been proposed as an important facilitator for the spread of HIV-1 in areas where both infections geographically overlap (Abu-Raddad et al., 2006; Dolores Herrero et al., 2007; Idemyor, 2007; Kublin and Steketee, 2006; Renia et al., 2006). This parasitic infection is one of the most prevalent infectious diseases worldwide. Of the estimated 300 million cases per year, 90% occur in sub-Saharan Africa (Snow et al., 2005). Most of the African continent is considered to have stable malaria transmission, with prevalence that exceed the threshold (40%) above which theory predicts it is unlikely that malaria transmission can be interrupted with insecticide-treated bed nets alone (Hay et al., 2009). This region covers 8.5 million km^2 of the continent, with 354.28 million people at risk. Areas with malaria prevalence between 5% and 40%, the range in which theory predicts that malaria transmission could be reduced with insecticide-treated bed nets, cover 5.6 million km^2 of the continent, with 196.83 million people at risk.

Sub-Saharan Africa has also experienced a severe HIV-1 epidemic, with an estimated 25 million infections over the past two decades (WHO Joint United Nations Programme on HIV/AIDS (UNAIDS)/World Health Organization). The epidemic, however, is not homogeneous, and its spread has been more intense in the southern and eastern part of the continent, with the western region experiencing a slower growing and less severe epidemic. The causes of this difference are not well understood (Buve et al., 2001). In the western part of the continent, the epidemic seems to be more concentrated in the high-risk (core) groups, such as female sexual workers and individuals with high number of sexual partners, while the epidemic is more widely distributed across the general population in countries located in the east and south part of the continent.

In previous work (Cuadros et al., 2011b), we showed that the prevalence of malaria caused by the parasite *Plasmodium falciparum* is associated with HIV-1 infection

in eastern sub-Saharan Africa. Our results indicated that individuals who lived in areas with high *P. falciparum* parasite rate (*Pf*PR > 0.42) were approximately twice as likely to be infected with HIV than individuals who lived in areas with low *P. falciparum* parasite rate (*Pf*PR ≤ 0.10) [men: estimated odds ratio (OR) 2.24, 95% confidence interval (CI) 1.62–3.12; women: estimated OR 2.44, 95% CI 1.85–3.21]. In western sub-Saharan Africa, the pattern of the HIV-1 epidemic is different and less severe, yet the prevalence of malaria is high. To examine the association between malaria and the prevalence of HIV-1 under these conditions, and to measure this association in a region with intermediate HIV-1 prevalence in central Africa, we used the most current and comprehensive database, namely the Demographic and Health Survey (DHS) for HIV-1. We also utilized a new source of data for *P. falciparum* malaria endemicity, the malaria atlas project.

To complement our knowledge of HIV-malaria co-infection, the objective of this work was to assess the relationship between malaria and HIV-1 prevalence in the western and central regions of sub-Saharan Africa. The primary aim was to examine the difference in the likelihood of current infection with HIV between individuals who lived in locations with high transmission intensity of the most common vector that transmits malaria in the region, namely the *P. falciparum* parasite, and individuals who lived in areas with low malaria transmission intensity.

Methods

HIV data

This study refers to HIV-1 exclusively. We considered the following countries from the western part of sub-Saharan Africa: Burkina Faso, Ghana, Guinea, Liberia and Mali. These countries have similar HIV prevalence (between 1% and 2%), and the collective geographic region has heterogeneous prevalence of malaria for comparisons (Hay et al., 2009). We also included Cameroon in a separate analysis to investigate for an association between HIV and malaria in a country with an intermediate HIV prevalence (~5%) and with similar malaria transmission intensity to the western region included in the study.

To cover the population residing in households in these countries, we extracted data from DHS for 2003 in Burkina Faso (EDSBF-III) (Institut National de la Statistique et de la Démographie MdlÉedD) and Ghana (GDHS) (Ghana Statistical Service NMIfMR), for 2004 in Cameroon (EDSC-III) (National Statistical Office aOM (2005) Cameroon Demographic andHealth Survey 2004), for 2005 in Guinea (EDSG-III) (National Statistical Office CG) , for 2006 in Mali (EDSM-IV) (Cellule de Planification et de Statistique Ministère de la Santé DNdlSedlI), and for 2007 in Liberia (LDHS) (Liberia Institute of Statistics and Geo-information Services LISGIS). Methods used in these demographic and health surveys have been described in detail elsewhere (National Statistical Office aOM (2005) Cameroon Demographic andHealth Survey 2004; National Statistical Office CG). Briefly, the surveys used a two-stage cluster sampling technique. The first stage involved selecting sample points (clusters); 400 clusters in Burkina Faso (300 in urban and 100 in rural areas), 412 in Ghana (240 in urban and 172 in rural areas), 297 in Guinea (209 in urban and 88 in rural areas), 408 in Mali (265 in urban and 143 in rural areas), 297 in Liberia (117 in urban and180 in rural areas), and 466 in Cameroon (224 in urban and 242 in rural areas). The global position system (GPS) was used to identify and record the geographic coordinates of each of the DHS clusters.

The second stage used by each DHS included in this study involved the systematic sampling of households from the selected clusters. Adult men (aged 15-59 years) and women (aged 15-49 years) in the selected households were eligible for the survey. In all DHS surveys included in this study, HIV testing was performed using blood samples. The DHS reported that participation in HIV testing was voluntary, and before collecting the samples, each selected participant was asked to provide informed consent to the testing. Further details of the performed HIV test have been described elsewhere (Ghana Statistical Service NMIfMR; National Statistical Office OM (2005) Cameroon Demographic and Health Survey 2004). In summary, HIV testing was conducted in a laboratory by following a standard testing algorithm that uses two HIV enzyme immunosorbent assays based on different antigens. Discordant samples that were positive on the first test were retested with the same enzyme immunosorbent assays. A confirmatory HIV Western blot kit was used for all samples that were still discrepant.

We used the HIV serostatus reported by the DHS for each individual as the dichotomous response outcome and restricted the analysis to only sexually active people. After combining all DHS surveys and excluding missing data, the final sample population for data analysis consisted of 41 064 individuals: 18 385 men and 22 679 women for West sub-Saharan Africa, and 10 088 individuals: 4986 men and 5102 women for Cameroon.

Malaria data

A commonly used index of malaria transmission intensity is the *P. falciparum* parasite rate (*Pf*PR), defined as the proportion of the population found to carry asexual blood-stage parasites. The enhancement of HIV replication generated by infection with malaria, which supports higher HIV infectivity in co-infected individuals (Quinn et al., 2000), is produced by the presence of any parasitaemia, and not only during clinical episodes (Kublin et al., 2005). For that reason, this index of malaria transmission might be a measure for use in the initial assessment of the association between malaria and HIV.

For this study, we used the largest and most contemporary spatial database for the *Pf* PR for children aged 2 to 10 years old (Hay et al., 2006; Hay et al., 2009). This database contains nearly 9000 distinct community surveys across 78 malaria-endemic countries. Many of these surveys, however, reported crude *Pf*PR without stratifying by age. Thus, in previous research (Smith et al., 2007), an algorithm was developed to age-standardize *Pf*PR data. The *Pf*PR in children aged 2-10 years is correlated with the entomological inoculation rate the number of infected bites per person per unit of time) (Hay et al., 2008), and has provided a basis for the most common categorical measures of malaria transmission (Smith et al., 2007). Therefore, the *Pf*PR reported by the malaria atlas project is age-standardized to 2-10 years old (Hay et al., 2009). Since the *Pf*PR in children aged 2-10 years is related to the entomological inoculation rate, which is an estimate of the force of infection, we used this measure as an approximation of the prevalence of malaria infections in the adult population studied. This assumption, however, has some implications on the study results, which are discussed in the limitations of the study.

To create a continuous surface of malaria endemicity, previous research (Hay et al., 2009) produced a model-based geostatistical map using a Bayesian framework to incorporate factors such as the spatial density and location of the data, and the number of people sampled in each survey. Geostatistical algorithms generate a continuous map by interpolating values at unsampled locations using a weighted linear combination of the available (neighboring) sample data. Further details on the malaria data and statistical procedures for generating the map have been described elsewhere (Hay et al., 2009). To display and extract the *Pf*PR from each DHS cluster, we used the program ArcGIS version 9.2 (ESRI, 2004). From each geo-referenced cluster, we obtained the *Pf*PR and assigned its value to each individual who belonged to that cluster.

Socio-economic and biological covariates

Socio-economic and biological covariates of potential importance were investigated for inclusion into the final model. To select the covariates to be included in the final model, we first conducted preliminary unadjusted bivariate analyses from a pool of 16 socio-economic and biological variables: age, place of residence (urban or rural), highest educational level, main floor material of the house, main roof material of the house, main wall material of the house, religion, current marital status, previous AIDS testing, wealth index, number of sexual partners in the last year, age at first intercourse, presence of bed net for sleeping, use of a bed net for sleeping, male circumcision, and reported presence of genital ulceration during the last month. Covariates of a priori importance with $P<0.2$ in the unadjusted analyses were included in the final multivariable model relating HIV and malaria.

Several factors were included as categorical variables. Marital status was comprised of three categories: never married, currently married and formerly married. Wealth index is an ordinal variable that characterizes standard of living as determined by material possessions. The DHS calculated the living standard of a household based on relevant assets such as television and bicycles, materials used for housing construction, and the availability of amenities such as electricity and source of drinking water. The resulting asset scores, constructed using principal component analysis, were then used to define wealth quintiles: poorest, poorer, middle, richer, richest (National Statistical Office

aOM (2005) Cameroon Demographic and Health Survey 2004; National Statistical Office CG). Religion was subdivided into four categories: Muslim, Catholic, traditional religion, and other religions. Education level was included as a categorical variable with four levels: no education, primary education, secondary education and higher education.

Statistical analysis

Data analysis used generalized linear mixed models (GLMM), specifically logistic regression models with normally distributed random cluster effects to include covariates and account for correlated data among individuals within the same cluster. We first conducted unadjusted analyses for each covariate. We then generated an adjusted model, where the *Pf*PR was categorized into two transmission intensity levels, namely areas with stable malaria transmission (*Pf*PR > 0.46), which corresponds to areas with *Pf*PR higher than the first quartile, and areas with *Pf*PR $\leq$ 0.46). We analyzed the data from the western region and Cameroon separately. All statistical analyses were conducted using R version 2.11.1 (R.Core Team, 2010).

Results

West sub-Saharan Africa

The HIV prevalence in responders included in the study for western sub-Saharan Africa was 1.53%: 1.15% for men and 1.80% for women (Table 4.1). The mean *Pf*PR in the region was 0.52, with a standard deviation of 0.12. In areas with stable malaria transmission (*Pf*PR > 0.46) the HIV prevalence was 1.48%, whereas in areas with *Pf*PR $\leq$ 0.46 the HIV prevalence was 1.58% (Table 4.1).

The final model included age, gender, urban or rural residence, education, religion, wealth index, marital status and the presence of genital ulcerations. Male circumcision was not significantly associated with being HIV-positive in men (unadjusted OR 0.63, 95% 0.31-1.26). A similar result has been reported by other studies (De Walque et al., 2006; Mishra et al., 2007), and might be a consequence of the extremely high prevalence of male circumcision in the area of study. We estimated that

94% of the male individuals from the western region and 95% of the male individuals from Cameroon were circumcised. For that reason, we combined the data for men and women into a single analysis, and included gender to adjust for confounding in the final models.

The results indicated no evidence that *Pf*PR predicted HIV infection in western sub-Saharan Africa, where we observed no statistical difference in the odds of being HIV-positive between individuals who lived in areas with stable malaria transmission (*Pf*PR > 0.46) and individuals who lived in areas with *Pf*PR ≤ 0.46 (estimated OR 1.14, 95% CI 0.86-1.50). Other categorizations of *Pf*PR, such as grouping by quartiles, gave the same qualitative conclusions. Men have almost half the likelihood of being HIV seropositive compared to women (estimated OR 0.63, 95% CI 0.51-0.78), which could be connected to the protective effect of male circumcision for HIV acquisition. As observed in eastern sub-Saharan Africa (Cuadros et al., 2011b), age was found to be a strongly associated with HIV infection. The HIV prevalence peaked at age group 30-34 years, and these individuals had higher odds of being HIV seropositive compared with the age group 15-19 years (estimated OR 4.11, 95% CI 2.60-6.51).

As expected and in agreement with other studies (Johnson et al., 2006; Msisha et al., 2008), the adjusted model indicated that individuals living in rural areas had lower odds of being HIV-positive compared to those living in urban areas, with an estimated OR of 0.58 (95% CI 0.43-0.78). Unadjusted analysis indicated that reported genital ulcerations during the last 12 months increased the likelihood of current HIV infection (estimated OR 1.65, 95% CI 1.21-2.26). The presence of genital ulcerations, however, was not a significant factor after adjusting for the other variables. Following the same pattern observed in the eastern part of the continent, being currently married was not a significant factor compared with individuals that had never been married (estimated OR 1.17 95% CI 0.85-1.63), whereas individuals formerly married had higher odds of having HIV compared with individuals never married (estimated OR 2.55, 95% CI 1.73-3.77).

Among the socio-economic covariates considered, the lone discrepancy between the current study and results obtained from our previous study of eastern sub-Saharan Africa (Cuadros et al., 2011b) was the relationship between wealth index and HIV. While

in the eastern part of the continent the wealth index was found to be positively and monotonically associated with HIV, where individuals in the richest category had the highest odds of being HIV-positive compared with individuals in the poorest category (Cuadros et al., 2011b; Johnson et al., 2006; Msisha et al., 2008), we observed no significant association between wealth index and current HIV status in western sub-Saharan Africa (Table 4.2).

Cameroon

The general HIV prevalence in Cameroon was 5.4%: 4.0% for men and 6.7% for women (Table 4.1). The mean *Pf*PR in this country was 0.44, slightly lower than the *Pf*PR estimated for the western region. In areas with stable malaria transmission (*Pf*PR > 0.46) the HIV prevalence was 6.4%, whereas in areas with *Pf*PR ≤ 0.46 the HIV prevalence was 4.1% (Table 4.1).

The adjusted analysis indicated that *Pf*PR predicted HIV infection in Cameroon and suggested that individuals who lived in areas with stable malaria transmission had increased odds of being HIV-positive compared to individuals who lived in areas with *Pf*PR ≤ 0.46 (estimated OR 1.56, 95% CI 1.23-2.00).The difference in magnitude of the association between *Pf*PR and current HIV status for Cameroon and west sub-Saharan Africa was also identified in an analysis that included an interaction term between *Pf*PR and region (western sub-Saharan or Cameroon) in an adjusted model. The significant interaction term ($P = 0.04$) indicated that the association between HIV and the *Pf*PR was different among these regions.

Unlike the countries from the western region included in the study, higher wealth index was associated with higher odds of being HIV seropositive compared to individuals in the poorest category (Table 4.3), resembling the relationship between wealth index and HIV observed in East sub-Saharan Africa. Contrary to the results from the western and the eastern parts of Africa, the adjusted model indicated that living in urban areas did not increase the odds of being HIV-positive compared to rural areas (estimated OR 0.76, 95% CI 0.57-1.01).

Discussion

In contrast to the observations from eastern sub-Saharan Africa, the results from this study suggest the absence of evidence for an association between malaria transmission intensity and current infection with HIV in western sub-Saharan Africa. The lower HIV prevalence in the western region compared to the eastern region of the continent might be a contributing factor for this difference. Unlike the eastern region, where the HIV epidemic is present in the general population, the low HIV prevalence observed in western countries might indicate that the HIV epidemic is concentrated in high-risk groups.

Our collective work provides evidence that the epidemiology of co-infection is not a simple additive relationship. The effect of co-infection on the spread of HIV might be influenced by other external factors such as the stage of the epidemic, individual and community behavior, and the HIV prevalence in the population, among others. Mathematical models have suggested that the number of HIV infections attributable directly to co-infection, estimated as the population attributable fraction, depends on the stage of the HIV epidemic and the epidemiology of the parasite involved in the co-infection (Abu-Raddad et al., 2008; Cuadros et al., 2011a; Freeman et al., 2007; Orroth et al., 2007). These models indicate that co-infections with sexually transmitted infections prevalent in high-risk groups, such as syphilis, trichomoniasis, gonorrhea and chlamydia, have the highest impact on the spread of HIV when the epidemic is concentrated in these subgroups. On the other hand, infections that are highly prevalent in the general population, such as herpes simplex virus type 2 and malaria, are more important contributors to fueling the spread of HIV infection when the epidemic has invaded the general population.

The results from this study and our previous work are thus consistent with the results obtained from mathematical models and indicate that co-occurrence of HIV and infections present in the general population, like malaria, might not play an important role in the spread of HIV in populations where its prevalence is low. Malaria apparently acts as a facilitator and fuels the spread of HIV when the epidemic is present in the general population but it is not the cause of the invasion of HIV in the general population.

This hypothesis is supported by the results obtained from data for Cameroon, where regardless of the similar *Pf*PR and prevalence of male circumcision compared to the studied western region, the higher HIV prevalence in Cameroon was observed together with a significant association between HIV and malaria.

In agreement with other studies (Auvert et al., 2001b; Buve et al., 2001b), we found no difference in the socio-economic and demographic cofactors for HIV infection between the eastern and western regions of sub-Saharan Africa. Circumcision, however, was not significantly associated with HIV infection for men in our study. Circumcision is almost universal in western sub-Saharan Africa (Auvert et al., 2001a), where 94% of the male individuals included in our study were circumcised. In contrast, only 62% of the male individuals included in the study for the eastern region were circumcised. This difference has been proposed as a key factor for the difference between both epidemics (Auvert et al., 2001a; Auvert et al., 2001b). Circumcision is a cultural practice, and therefore it is plausible to hypothesize that the current prevalence of circumcision might be similar to the prevalence at the early stage of the HIV epidemic in sub-Saharan Africa. Consequently, the high rate of male circumcision observed in western sub-Saharan Africa could have generated a protective effect that prevented the invasion of the epidemic to the general population.

The results from Cameroon, however, did not support this hypothesis. Despite the extent of male circumcision (95%), the higher HIV prevalence indicates the likely presence of an HIV epidemic in the general population, which in turn might have governed the interaction between HIV and malaria that is suggested by our results. Thus, the factors (biological and behavioral) that trigger the movement of an HIV epidemic from high-risk groups to the general population remain indeterminate. The identification of these factors may give us a better understanding of the geographical differences observed in the HIV epidemic and a better comprehension of the complex co-infection relationship between HIV and other parasites.

The present work as well as our previous study (Cuadros et al., 2011b) indicate that the presence of a parasite influencing the transmission of HIV, even when the parasite is highly prevalent in the population, does not fully explain the interaction and

the outcome of co-infection on the spread of HIV. Other factors such as the HIV prevalence and the distribution of the infection in the population may dictate the role of co-infection in fueling the epidemic.

Our work highlights the importance of identifying factors for the implementation of effective control interventions focused on co-infection. The effect of co-infection might not be the same in different populations, and control strategies will not necessarily have the same impact in each population. Understanding the epidemiological effects of co-infection and the relevant factors involved in this relationship is a prerequisite to developing accurate and effective recommendations for population-level control strategies.

Limitations of the study

Although our study makes use of the most complete and contemporary databases for both HIV and malaria, it is important to emphasize that our results were based on geographical estimates of *Pf*PR in children aged 2 to 10 years old in locations where DHS surveys were made, and thus we estimated the effect of malaria on the prevalence of HIV based on indirect measures of malaria transmission intensity. Therefore, the results obtained in this study depend on the quality of these secondary data and on the methodology implemented in our and in previous analyses. Consequently, they should be interpreted with caution.

Furthermore, it is important to highlight that our estimates are based on prevalence data for HIV. Since HIV prevalence is highly influenced by other independent factors, such as the stage and distribution of the epidemic in the population, this epidemiological measure might not be the most appropriate for evaluating the interaction between both infections. We used this measure based on the availability and quality of the data derived from different DHS surveys. Other epidemiological estimates such as HIV incidence, however, would be a more appropriate measure for direct estimation of the association between incident HIV and malaria. Therefore, our collective work represents a preliminary step to elucidate the role of malaria on the HIV epidemic in sub-Saharan Africa, and highlights the necessity of more appropriate data for understanding the malaria-HIV relationship in order to implement effective control interventions.

On the other hand, the *Pf*PR values used in our study were obtained by mathematical algorithms that standardized the malaria transmission intensity in children aged 2-10 years (Hay et al., 2008). Whereas this estimation is a widely accepted approximation of the malaria transmission intensity in a specific region (Smith et al., 2007), it is important to note that most HIV infections occur in the adult population. Repetitive malaria infections during childhood generate partial immunity to the infection and thus decrease the prevalence of clinical malaria in adults, especially in areas where malaria is endemic (Kamya et al., 2006). Although the *Pf*PR in children aged 2-10 has been widely accepted as a measure of the transmission intensity, this variable is only an approximation to the malariological index. Furthermore, the relationship between this variable and the transmission intensity in the adult population is still not well defined, and most likely the *Pf*PR in adults would be somewhat different. For that reason, it is important to note that other malariological measures, such as the entomological inoculation rate or the prevalence of malaria in the adult population, would be alternative ways to investigate for the association between the co-infection studied here. These alternative data, however, are scarce, whereas the database for malaria used here allowed us to obtain the proxy data necessary for performing the analysis in this study. Moreover, we expect that the geographic distribution of the intensity of malaria transmission remains the same for all populations (children and adults), in which case the geographic variability of the *Pf*PR used here represents a useful approximation for the comparisons made in this study.

Additionally, the relationship between malaria and HIV is bilateral, where HIV infection might alter the natural history of malaria (and vice versa), especially in areas with high HIV prevalence and unstable malaria transmission (Grimwade et al., 2004; Korenromp et al., 2005; Whitworth et al., 2000). In our study, however, we focused on the association between an ecological proxy of malaria infection and current HIV infection rather than the potential effect of HIV on malaria. Since the region studied is characterized by an endemic malaria epidemic (Hay et al., 2006) and low prevalence of HIV, we expect that this assumption would not drastically affect the outcome of our study.

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Table 4.1 HIV prevalence by socio-economic and biological characteristics in western sub-Saharan Africa and Cameroon

Characteristics	Western Africa N (% HIV-positive)	Cameroon N (% HIV-positive)
Malaria		
*Pf*PR ≤ 0.46	10 207 (1.58)	4871 (4.1)
*Pf*PR > 0.46	30 857 (1.48)	5217 (6.4)
Gender		
Female	22 768 (1.80)	5102 (6.7)
Male	18 296(1.15)	4986 (4.0)
Age		
15-19	8690 (0.52)	2443 (1.4)
20-24	6844 (1.18)	1940 (5.0)
25-29	6006 (2.00)	1582(8.2)
30-34	5247 (2.19)	1245 (8.5)
35-39	5009 (2.15)	966 (8.3)
40-49	7614 (1.60)	1476 (5.6)
50-59	1654 (1.75)	436 (2.0)
Place of residence		
Urban	13 589 (2.25)	4887(6.6)
Rural	27 475 (1.16)	5201(4.3)
Marital status		
Never married	11 575 (0.83)	3160 (2.7)
Currently married	27 349 (1.60)	6013 (5.8)
Formerly married	2140 (4.06)	915 (12.0)
Religion		
Muslim	19 884 (1.31)	1628 (4.70)
Christian	12 755 (1.65)	4051 (5.60)
Traditional religion	388 (0.25)	271 (1.85)
Other	8037 (1.87)	4138 (5.58)
Wealth index		
Poorest	8469 (1.02)	1569 (2.9)
Poorer	8235 (1.25)	1819 (3.2)
Middle	8219 (1.27)	2264 (6.0)
Richer	7937 (1.88)	2189 (7.0)
Richest	8204 (2.17)	2247 (6.7)
Highest educational level		
None	21 502 (1.30)	1504 (3.6)

Primary school	8460 (1.45)	4007 (5.7)
Secondary	10 268 (1.94)	4217 (5.9)
Higher	834 (1.99)	360 (4.4)
Genital ulceration		
No genital ulceration last 12 months	40 644 (1.42)	9843 (5.5)
Genital ulceration last 12 months	420 (2.22)	245 (9.7)
Male circumcision		
No circumcised	17 364 (1.79)	253 (1.59)
Circumcised	1021 (1.31)	4733 (4.18)

Table 4.2 Unadjusted and adjusted results from western sub-Saharan Africa: HIV serostatus according to selected socio-economic and biological characteristics

Indicator	OR (95% CI) Unadjusted	Adjusted
Malaria		
*Pf*PR ≤ 0.46	1.00	1.00
*Pf*PR > 0.46	0.95 (0.74-1.21)	1.14 (0.86-1.50)
Gender		
Female	1.00	1.00
Male	0.64 (0.53-0.77)	0.63 (0.51-0.78)
Age		
15-19	1.00	1.00
20-24	2.39 (1.58-3.62)	2.12 (1.38-3.25)
25-29	4.18 (2.82-6.17)	3.66 (2.35-5.68)
30-34	4.68 (3.16-6.94)	4.11 (2.60-6.51)
35-39	4.62 (3.10-6.87)	4.18 (2.62-6.67)
40-49	3.46 (2.34-5.12)	3.14 (1.97-4.99)
50-59	3.91 (2.28-6.72)	5.30 (2.84-9.88)
Place of residence		
Urban	1.00	1.00
Rural	0.48 (0.39-0.60)	0.58 (0.43-0.78)
Marital status		
Never married	1.00	1.00
Currently married	2.19 (1.70-2.82)	1.17 (0.85-1.63)
Formerly married	5.45 (3.90-7.62)	2.55 (1.73-3.77)
Religion		
Muslim	1.00	1.00
Christian	1.28 (1.01-1.62)	1.11 (0.86-1.46)
Traditional religion	0.24 (0.02-2.45)	0.28 (0.02-2.87)
Other	1.39 (1.07-1.80)	1.24 (0.94-1.63)

Wealth index		
Poorest	1.00	1.00
Poorer	1.20 (0.85-1.69)	1.15 (0.82-1.62)
Middle	1.21 (0.86-1.70)	1.10 (0.78-1.56)
Richer	1.81 (1.31-2.51)	1.40 (0.97-2.01)
Richest	2.01 (1.45-2.77)	1.38 (0.92-2.08)
Highest educational level		
None	1.00	1.00
Primary school	1.03 (0.80-1.32)	1.21 (0.93-1.57)
Secondary	1.33 (1.07-1.66)	1.38 (1.06-1.79)
Higher	1.31 (0.74-2.29)	1.01 (0.56-1.82)
Genital ulceration		
No genital ulceration last 12 months	1.00	1.00
Genital ulceration last 12 months	1.65 (1.21-2.26)	1.27 (0.93-1.76)

Table 4.3 Unadjusted and adjusted results from Cameroon: HIV serostatus according to selected socio-economic and biological characteristics

Indicator	OR (95% CI) Unadjusted	Adjusted
Malaria		
*Pf*PR ≤ 0.46	1.00	1.00
*Pf*PR > 0.46	1.64 (1.29-2.07)	1.56 (1.23-2.00)
Gender		
Female	1.00	1.00
Male	0.55 (0.46-0.67)	0.58 (0.47-0.71)
Age		
15-19	1.00	1.00
20-24	4.05 (2.67-6.13)	3.59 (2.33-5.52)
25-29	6.65 (4.42-9.98)	5.86 (3.76-9.14)
30-34	7.07 (4.66-10.71)	6.07 (3.82-9.63)
35-39	6.45 (4.18-9.95)	5.65 (3.48-9.15)
40-49	4.58 (2.99-7.02)	3.98 (2.46-6.43)
50-59	1.36 (0.60-3.10)	1.72 (0.73-4.09)
Place of residence		
Urban	1.00	1.00
Rural	0.63 (0.50-0.78)	0.76 (0.57-1.01)
Marital status		
Never married	1.00	1.00
Currently married	2.41 (1.86-3.13)	1.17 (0.86-1.60)
Formerly married	5.39 (3.95-7.37)	2.66 (1.87-3.79)
Religion		
Muslim	1.00	1.00
Christian	1.17 (0.86-1.59)	0.94 (0.67-1.30)
Traditional religion	0.45 (0.17-1.20)	0.51 (0.19-1.37)
Other	1.14 (0.84-1.55)	0.92 (0.66-1.27)
Wealth index		
Poorest	1.00	1.00
Poorer	0.99 (0.64-1.51)	0.88 (0.57-1.36)
Middle	1.94 (1.33-2.83)	1.66 (1.12-2.47)
Richer	2.26 (1.55-3.29)	1.90 (1.24-2.93)
Richest	2.09 (1.43-3.06)	1.70 (1.06-2.93)
Highest educational level		
None	1.00	1.00

Primary school	1.44 (1.03-2.02)	1.33 (0.91-1.94)
Secondary	1.45 (1.03-2.04)	1.24 (0.82-1.86)
Higher	1.05 (0.57-1.96)	0.7 (0.37-1.43)
Genital ulceration		
No genital ulceration last 12 months	1.00	1.00
Genital ulceration last 12 months	1.81 (1.14-2.88)	1.39 (0.87-2.22)

Chapter 5 - Heterogeneity in the HIV infectivity generated by co-infection: insights from a mathematical model

Introduction

At present, the role of viral genetic factors in the HIV epidemic is poorly understood. Many different HIV genetic subtypes circulate worldwide as well as their recombinant forms (Saathoff et al., 2010). Moreover, there is a substantial variation in the length of the asymptomatic (chronic) phase of HIV infection, ranging from few months to many years. This variation has been correlated with the so-called set point viral load (spVL), defined as the measure of the HIV RNA concentration in blood during the chronic stage of the infection, representing the quasi-steady state equilibrium between virus production and clearance (Perelson et al., 1996; Stafford et al., 2000).

The functional differences and the epidemic significance of these variations are still unclear (Geretti, 2006). Some studies, however, have revealed potential differences in the replicative capacity among different virus genotypes (Åsjö et al., 1986; Ayele et al., 2010; Betts et al., 2001; Blaak et al., 1998; Hu et al., 2001; Kivelä et al., 2005). As a result, it has been suggested that if viral factors can affect the efficiency of viral replication and can be preserved from one infection to the next, this would imply a significant role of viral genetic factors determining the spVL (Hollingsworth et al., 2010).

Recent studies conducted to assess this hypothesis have evidenced that virus characteristics might indeed explain a significant proportion of the variation in the spVL observed among individuals. They demonstrated a correlation of the spVL between transmitting couples and suggested that the spVL might be a heritable factor. Even a weak correlation between spVL of individuals in transmitting couples might correspond to a large estimate for the role of viral genetic factors (Alizon et al., 2010; Fraser and Hollingsworth, 2010; Hecht et al., 2010). This result has profound implications for important virus-related processes in which the spVL is a key determinant, such as the progression and transmission of the infection. The evidence suggests a strong correlation

between HIV plasma viral load (VL) and HIV transmission rates (Quinn et al., 2000; Wilson et al., 2008). It has been estimated that a ten-fold increment in VL almost doubles the risk of HIV transmission per sexual contact (Quinn et al., 2000). Consequently, if viral genetic factors influence the concentration of the virus during the chronic stage of the infection, these viral characteristics might indeed play an important role on the transmission of the infection.

The spVL, however, is not in an absolute equilibrium state; it is frequently perturbed by immune activations generated by the invasion of other pathogens, resulting in a significant amplification of the VL. Changes in the host immune response induced by concomitant infections may account for increments in the VL that could make the host more infectious and increase the risk of transmission of the virus during the chronic stage of the infection (McLeans and Nowak, 1992; Modjarrad et al., 2008; Modjarrad and Vermund, 2010). The amplification of the VL generated by co-infection may be an important contributor to the successful transmission of the infection; especially in areas were the immune system is constantly challenged by multiple infections, such as in sub-Saharan Africa (Barnabas et al., 2011; Bentwich et al., 1995; Lawn, 2004).

Furthermore, there is substantial heterogeneity in the effect of co-infection at the individual and population levels. Within individuals, the co-infection induced increment of the VL varies greatly (Kublin et al., 2005; Ostrowski et al., 1998; Rosok et al., 1996). Likewise, the effect of co-infection at the population level depends on several behavioral and epidemiological factors related to the stage and the distribution of the HIV epidemic within the population. Co-infections with sexually transmitted infections (STI) such as gonorrhea and chlamydia would have the highest impact on the transmission of the virus in populations where the epidemic is concentrated in the high risk (core) groups. Conversely, infections prevalent in the general population such as malaria and herpes simplex virus type 2 (HSV-2) would be important contributors to the spread of the infection when the epidemic has invaded the general population (Abu-Raddad et al., 2008; Cuadros et al., 2011a).

Based on the biological evidence previously discussed, I suspect that viral factors might contribute to the variation of the effect of co-infection at host and population level. Here, taking a simple modeling approach, I examined how viral factors that dictate the

spVL can alter the dynamics of the virus population in presence of co-infection and can introduce heterogeneity among individuals. I expect that viruses with high replicative capacity that sustain elevated spVL would also generate larger increments in the VL in response to host immune activations induced by co-infection. In contrast, viruses with poor capacity to infect target cells and replicate would generate lower spVL and consequently lower VL increments in response of co-infection. Since the transmission efficiency of the virus among individuals is associated with viral burden, highly infectious individuals would have viral characteristics that maximize the increment of the VL in presence of co-infection. This result might have important implications at both the individual and the population levels.

To assess this hypothesis, I generated a mathematical model that describes the dynamics of HIV and a concomitant pathogen within the host to evaluate the heterogeneity of viral replicative capacity on the spVL and the increment on the VL in response of co-infection. Additionally, I evaluated the effects of these variations at population level.

Methods

I developed a deterministic differential equation compartment model based on two independent models that describe the dynamics of HIV and another parasite, namely malaria.

HIV model

The basic model of HIV dynamics implemented here has three variables: T the population sizes of uninfected cells, T^* infected cells, and V free virus particles (Callaway and Perelson, 2002; Stafford et al., 2000). These quantities denote the total abundance of the given population per milliliter (ml) of blood, and their dynamic is described by the following system of differential equations:

$$\frac{dT}{dt} = s - (d_t + k_v V)T$$
$$\frac{dT^*}{dt} = k_v VT - \delta T^*$$
$$\frac{dV}{dt} = N\delta T^* - cV$$

,

where *s* represents the production rate of activated immune (CD4+ T) cells, d_t the natural death rate of activated immune cells, k_v the HIV infection rate of activated immune cells, δ death rate of HIV infected cells, *N* the HIV production rate by an infected cell, and *c* the HIV removal rate. The magnitudes of these parameters are summarized in Table 5.1. From this system of differential equations it is possible to determine the concentration of the virus population at equilibrium, which might be associated with the spVL in the chronic stage of the infection, and is described by the following equation (Callaway and Perelson, 2002):

$$\bar{V} = \frac{Ns}{\delta c} - \frac{d_t}{k_v}.$$

This equation shows that virus concentration at equilibrium may depend on virus factors that determine its replicative capacity: the infection rate of immune cells (k_v) and the production rate by infected cells (*N*). This result has been used by other authors to estimate these parameters using least squares parameter estimation techniques (Perelson and Nelson, 1999; Stafford et al., 2000). Since these parameters represent intrinsic characteristics of the virus, and their variation generates different spVL, I focused on evaluating the effect of such variations on the VL burden generated by the presence of a concomitant pathogen.

Malaria model

Malaria was selected as the concomitant pathogen based on substantial biological and epidemiological evidence suggesting an important malaria-HIV interaction, especially in areas where the infections overlap geographically. Several studies have demonstrated the

increment of VL in individuals with acute malaria (Barnabas et al., 2011; Chen et al., 2003; Hoffman et al., 1999; Kublin et al., 2005). Likewise, the risk of HIV infection is higher in areas with high malaria prevalence (Cuadros et al., 2011b), and malaria has been proposed as an important facilitator for the spread of HIV in sub-Saharan Africa (Abu-Raddad et al., 2006; Cuadros et al., 2011a).

To introduce malaria into the system, I implemented a minimal model developed earlier (Anderson et al., 1989) and used by several authors (Hellriegel, 1992; Mideo et al., 2008; Mugisha et al., 2008; Thibodeaux, 2010) to study the population dynamics of X uninfected red blood cells, Y infected red blood cells, M free merozoites, and T_m malaria activated immune cells. The system is described by the following system of differential equations:

$$\begin{aligned}
\frac{dX}{dt} &= \Delta - \mu_x X - k_m MX \\
\frac{dY}{dt} &= k_m MX - (\mu_1 + \mu_y)Y - \mu_c Y T_m \\
\frac{dM}{dt} &= r\mu_1 Y - k_m MX - (\mu_m + \mu_h T_m)M \\
\frac{dT_m}{dt} &= \varepsilon + \left[\lambda_y Y + \lambda_m M\right] T_m - d_t T_m
\end{aligned} \quad ,$$

where Δ stands for the rate of red blood cell production, μ_x the natural death rate of uninfected red blood cells, k_m the infection rate of red blood cells by merozoites, μ_1 the differentiation rate of merozoites, μ_y natural death rate of infected red blood cells, μ_c clearance rate of infected red blood cells due to the immune system, μ_m natural death rate of free merozoites, μ_h death rate of merozoites by contact with immune cells, r merozoite production per infected cell, ε rate of immune cell production, λ_y proliferation rate of immune cells in response to infected red blood cells, and λ_m proliferation rate of immune cells in response to merozoites. The parameter values are summarized in Table 5.1.

Within-host co-infection model

As previously discussed, co-infection activates host immunity, which in turn enhances HIV replication (Modjarrad and Vermund, 2010). This phenomenon has been suggested to be a direct consequence of T helper cell (CD4+ T) activation that consequently raises the number of target cells susceptible to virus infection and replication (Obrien et al., 1995). This increment in the pool of susceptible target cells has been observed in reaction to different agents that induce an immune response such as influenza vaccine (Staprans, 1995), tetanus immunization (Cheynier et al., 1994; Ostrowski et al., 1998), and malaria (Hoffman et al., 1999; Xiao et al., 1998). This biological argument was used to link both models in a single co-infection model. This approach was also used by Jones and Perelson (2002) to model the effect of vaccination on chronically HIV positive persons and the effect of opportunistic infections on patients treated with antiretroviral therapy (Jones and Perelson, 2005). But this is the first model to include the dynamics of more than one infection simultaneously to evaluate the effect of a concomitant infection on the dynamics of HIV. It is important to notice, however, that the immune stimulation that promotes HIV replication is multifactorial, and cell proliferation is only one of these mechanisms. Malaria also stimulates HIV transcription in other ways such as the activation of viral transcription via cytokines (Xiao et al., 1998). Therefore, the model presented here is a simplification of the effect of co-infection on the virus population and only describes one of the possible mechanisms in which parasites might affect the immune pathway that could alter the replication of the virus.

For the complete model of co-infection, the two models were linked by describing the mechanism in which malaria stimulates an immune response in terms of activation and proliferation of malaria activated immune cells (T_m), which in turn will become available to HIV infection. The model includes a maximum number of immune cells per ml of blood ($T_{\max}$) (Perelson et al., 1993); thus, the immune cell population will grow in a logistic fashion. After linking the two models, the dynamic of the HIV-malaria co-infection is described by the following system of differential equations:

$$\frac{dX}{dt} = \Lambda - \mu_x X - k_m MX$$
$$\frac{dY}{dt} = k_m MX - (\mu_1 + \mu_y)Y - \mu_c YT_m$$
$$\frac{dM}{dt} = r\mu_1 Y - k_m MX - (\mu_m + \mu_h T_m)M$$
$$\frac{dT_m}{dt} = \varepsilon + \left[\lambda_y Y + \lambda_m M\right]\left[1 - \frac{T_m + T + T^*}{T_{\max}}\right]T_m - (d_t + k_{Tm}V)T_m$$
$$\frac{dT}{dt} = s - (d_t + k_v V)T$$
$$\frac{dT^*}{dt} = k_v VT + K_{Tm} VT_m - \delta T^*$$
$$\frac{dV}{dt} = N\delta T^* - cV$$

.

I estimated the effect of the HIV infection rate (k_v) and production rate (*N*) on the increment of the VL induced by co-infection by conducting several numerical simulations of the model evaluated over the bi-dimensional parameter space generated by the range parameter values of 1.5×10^{-6} to 2×10^{-5}/day for the HIV infection rate (k_v), and 20 to 300 virions/cell for the HIV production rate (*N*). Due to the uncertainty associated with HIV infection rate of immune cells produced by the presence of malaria (k_{Tm}), I explored four different values for this parameter, which were proportional to the values used for the HIV infection rate (k_v): $k_{Tm} = k_v$, $k_{Tm} = k_v/10$, $k_{Tm} = k_v/20$, and $k_{Tm} = k_v/30$. The simulations started with the introduction of HIV alone; when the dynamics of the virus reached a steady state equilibrium (the spVL), malaria was introduced into the system (day t = 600). I recorded the VL immediately before the introduction of malaria, which represented the value for the spVL, and the following 30 days after the introduction of malaria. The values recorded during these 30 days were then averaged to estimate the increment on the VL generated by co-infection.

To compare spVL and the increment on the VL induced by co-infection, I sampled the parameter space evaluated to generate different combinations of the two parameters evaluated, k_v and *N*. These combinations were then used to calculate the spVL

and its corresponding increment on the VL induced by co-infection. Lastly, a geometric per capita growth rate of the virus was estimated for each combination of parameters.

Effect of co-infection at the population level

Understanding how concomitant infections could influence between-host transmission and epidemic dynamics represents a major challenge in epidemiology. The scenario is even more complicated when possible viral genetic differences might introduce heterogeneity into the outcome of co-infection. For that reason, using the within-host model described previously, I attempted to evaluate the effect of virus heterogeneity on transient co-infection-induced amplification of the VL at population level.

To estimate the effect of co-infection on the spread of HIV at population level, I implemented a standard deterministic population model constructed by Abu-Raddad and coworkers (Abu-Raddad et al., 2008; Abu-Raddad et al., 2006). The model is a deterministic compartment model that stratifies the population into compartments according to HIV sero-status and stage of HIV infection, and sexual-risk activity group. Details of the model are described elsewhere (Abu-Raddad et al., 2008; Abu-Raddad et al., 2006), and a short description of the model is included in the Appendix B.

For this model, the functional relationship between VL and the probability of transmission per sexual contact was used as the within-host and between-host interface. VL is one of the principal determinants of heterosexual HIV transmission (Fideli et al., 2001; Gray et al., 2001; Operskalski et al., 1997; Pedraza et al., 1999). It has been estimated that each one $\log_{10}$ increase in the VL doubles the per coital-act probability of HIV transmission (Lingappa et al., 2010; Quinn et al., 2000). Consequently, transient co-infection-induced increments in VL could potentially enhance between-host transmission (Abu-Raddad et al., 2006; Cattadori et al., 2007).

The model assumes that the effect of the VL on the infectiousness follows the empirical relationship between VL and transmission probability per sexual contact as observed initially by Quinn and colleagues (Quinn et al., 2000):
$p^{HIV}_{I(i)\to S(j)} = 2.45^{\log(vlH/vlB)}\, p^{HIV}$, where $p^{HIV}_{I(i)\to S(j)}$ stands for the probability of transmission per sexual contact for an infected individual from the risk group (i) to a

susceptible individual from the risk group (j), p^{HIV} is the baseline probability of transmission per sexual contact in the chronic stage of the infection, vlH is the amplified VL, and vlB is the baseline VL. The baseline probability of transmission per sexual contact in chronic stage was assumed to be $p^{HIV} = 0.0008$, with a VL of $vlB = 15\,000$ virion copies/ml (Quinn et al., 2000). Thus, as an example of the calculations performed, at VL = 33 478 virion copies/ml, the probability of transmission per sexual contact in the chronic stage is $p^{HIV}_{I(i)\rightarrow S(j)} = 2.45^{\log(33478/15000)} * 0.0008 = 0.00109$. Finally, to account for the potential effect of the VL on the HIV disease progression to AIDS, each $\log_{10}$ rise in the VL increases the rate of HIV progression to the latest stage of the infection by twofold (Modjarrad et al., 2008).

The spVL and its corresponding co-infection induced increment on the VL for each combination of parameters evaluated previously were used to compare the spread of HIV in two different populations: one population free of co-infection, in which the probability of transmission per sexual contact was estimated at the spVL, and the corresponding population affected by co-infection, in which the probability of transmission was estimated by including the increment on the VL induced by the concomitant infection with malaria. To calculate the probability of transmission in the population affected by co-infection, it was assumed that each individual has, in average, one episode of malaria infection per year. Thus, the mean VL per year was the average of the spVL during 11 months and the increased VL for the month of the co-infection. The model was also evaluated assuming two and four episodes per year.

The system was evaluated at the endemic equilibrium (see appendix B). After the equilibrium solution was found through convergent successive approximations, the total prevalence and incidence were calculated. Finally, to evaluate the effect of co-infection under different viral replicative capacities, the direct effect of co-infection on the HIV incidence was measured by using population attributable fractions (PAF) calculated as:

$$PAF = \left(1 - \frac{IR_{vlsp}}{IR_{Hvl}}\right) \times 100\%,$$

where IR_{vlsp} is the incidence rate of HIV with the spVL, and IR_{Hvl} is the HIV incidence rate with the heightened VL in presence of co-infection for each combination of parameters k_v and N as defined previously.

Results

Within-host model

In general, the within host co-infection model indicated that small values for the two parameters evaluated, the HIV production rate by infected cell (N) and the HIV infection rate (k_v), produced small increments on the VL when dual infection with HIV and malaria is present. Conversely, the increment on the VL increased as both parameters increased (Figure 5.1). Different values for k_{Tm}, however, generated different patterns. When $k_{Tm} = k_v$, the differences on the increment of the VL in response to co-infection were determined by the variation on the HIV production rate per infected cell (N) (Figure 5.1 A). The highest VL amplification was produced at a very low HIV infection rate ($k_v = 1 \times 10^{-6}$) and high HIV production rate per infected cell (N= 300). Contrary to expectation, when k_v increases, the increment in the VL generated by co-infection decreases. This behavior is derived from the ability of the virus to infect new target cells activated by malaria. At high k_v, the virus rapidly infects the new target cells impeding the proliferation of these immune cells. Consequently, this overexploitation of the new target cells by the virus at early stages of co-infection prevents an effective immune response to control malaria. This scenario seems to be unrealistic since individuals infected with HIV are able to have an immune response and cell proliferation in presence of concomitant infections (Wyler, 1976; Chougnet et al., 1992). In addition, the increment on the VL in individuals dually infected with HIV and another pathogen is associated with an increment on the CD4+ T cells (Chen et al, 2003; Hoffman et al., 1999).

On the other hand, when the k_{Tm} was a fraction of the k_v, the increment of both parameters, the HIV production rate per infected cell and the HIV infection rate, induced larger increments in the VL in response of co-infection. This result suggests that large HIV infection rates might affect the availability of the new target cells for infection by the virus by diminishing the potential cell proliferation. When $k_{Tm} = k_v / 10$ the effect of the overexploitation of target cells by the virus is still evident at high HIV infection rate (starting from $k_v = 1 \times 10^5$) (Figure 5.1 B). This effect is diluted when $k_{Tm} = k_v / 20$ (Figure 5.1 C), and apparently, the target cell concentration stabilizes with no substantial changes at smaller fractions of k_v (Figure 5.1 D). For that reason, and to include possible differences on the HIV infection rate generated by the availability of the new target cells for HIV infection, the remaining results were derived assuming $k_{Tm} = k_v / 20$.

Figure 5.2 illustrates the dynamics of the virus according to the variation of the virus parameters used. The combination of parameters generating low spVL were almost unable to produce an increment on the VL when malaria was introduced into the system (Figure 5.2 A, B, and C). Conversely, larger spVL might be associated with more efficient viral replicative capacity that generated much larger increments in the VL in response to co-infection (Figure 5.2 D, E, and F). After the introduction of malaria, there was a transient increment on virus concentration followed by a peak and successive decay to the original spVL. These viral dynamics in response to immune stimulations have been observed previously *in vivo* (Staprans et al., 1995; Xiao et al., 1998) and replicated by other mathematical models (Jones and Perelson, 2002; Jones and Perelson, 2005).

On average, VL peaked after one week and returned to baseline levels four weeks after the immune stimulation, consistent with other studies in which a large increase in the VL was observed during acute malaria and after influenza vaccine (Kublin et al., 2005; Staprans et al., 1995). The average increment in the VL generated by co-infection estimated from the 30 days recorded during the co-infection episode ranged from 500 to 800 000 virion copies/ml, variation consistent with the ranges estimated from studies designed to evaluate the effect of malaria on the VL of HIV-positive individuals in Africa (Hoffman et al., 1999; Kublin et al., 2005).

The simulations performed using the samples from the parameter space evaluated when $k_{Tm} = k_v / 20$ illustrate in more detail the positive correlation between the spVL and the increment on the VL induced by co-infection. Viral genotypes with poor replicative capacity that produce low spVL were not able to respond to increments in the activated target cells induced by the presence of concomitant infections. Consequently, the co-infection related increment in the VL generated by these viruses was fairly small. At lower spVL, the relationship between the increment on the virus concentration and the spVL increased exponentially, suggesting that viral genotypes with potent replicative capacity that were able to produce elevated spVL were also associated with large co-infection induced increments in the VL. The pattern followed a logistic function, suggesting a saturation effect at very high spVL (Figure 5.3 A). Additionally, the high per capita growth rate observed at large spVL might reflect the high replication capacity of these viruses that were able to effectively respond to increments in the target cell population (Figure 5.3 B).

Between-host model

The functional relationship between the viral load and the probability of transmission per sexual contact was used to link the viral replicative capacity and the effect of co-infection at the population level. As expected, and in accordance with the immunological model, the PAF analysis indicated that co-infection would have a negligible impact in populations where the circulating virus has low replicative capacity, reflected in low spVL. Conversely, the impact of co-infection would increase as the spVL of the population increased, reaching a maximum effect at spVL around 1×10^5 (Figure 5.4). This result indicates that viruses with high replicative capacity that are able to maintain elevated spVL would have a substantial impact on the spread of the infection in terms of new HIV infections directly caused by co-infection. This result was consistent regardless of the average number of co-infection episodes per year. In addition, the number of co-infection episodes has almost no impact on the PAF at low spVL; even four episodes were not able to produce a PAF larger than 1% when the spVL is lower than 2×10^6.

Discussion

In this work, I explored factors that could introduce heterogeneity in the effect of co-infection at individual and population levels. I postulated that if the HIV replication capacity was an important determinant of the spVL, it would also determine the effect of co-infection on the dynamics of the virus. Here, using a simple mathematical model to describe the dynamics of HIV at host level, it was possible to determine that the variation in the viral replication capacity described by two parameters, the HIV infection rate (k_v) and the HIV production by infected cell (N), generated different spVL. Moreover, the influence of the heterogeneity in these parameters on the increment of the VL in response of co-infection, evaluated using a within-host mathematical model of HIV-malaria co-infection, indicated that the viral replication capacity would strongly influence the induced increment of the VL when a concomitant infection is introduced in the system.

In addition, the model suggests the existence of differences in the HIV infectivity rate for new target cells generated by co-infection. Infections such as malaria generate localized immune response occurring in specific organs such as the spleen (Chougnet et al., 1992).This infection also produces a decrease in peripheral T cells consequence of a sequestration of these cells to specific lymphoid organs (Wyler, 1976). These characteristics might generate a compartmentalization of the system in which not all new target cells would be available to the virus. Consequently, the HIV infection rate for the new target cells generated by co-infection might be a fraction of the HIV infection rate for non-specific immune cells (k_v), as suggested by the model. Poor virus replicative capacity, associated with low spVL, generated modest increments on the VL in response to co-infection. Regardless of the co-infection induced proliferation of target cells, the low replicative capacity of these viruses prevented an effective viral response to the newly available activated target cells. Conversely, viruses with more efficient replicative capacity, reflected in high spVL, were able to produce much stronger increments on the VL when the population of target cells were incremented. Regardless of the introduction of new target cells to the system, the effect of co-infection could mainly depend on the ability of the virus to efficiently exploit the temporal rise of the target cell population. This in consequence would generate a considerable increment on the VL when the

immune system of the host is stimulated by the presence of a concomitant pathogen. This result might indicate that viral factors could play an important role not only in determining the spVL but also in driving several virus-related processes such as the increment of the VL induced by co-infections.

This result would have important implications for the identification of highly infectious individuals. HIV-positive persons that sustain high spVL might therefore be able to produce large increments in virus concentration in response to immune stimulations and consequently become much more infectious individuals, compared to those maintaining low spVL, even in presence of the same concomitant pathogen. This argument was then evaluated at the population level by linking the results from the within-host model with a population model using the functional relationship between viral load and probability of HIV transmission per sexual contact. I generated several virtual populations with different viral replicative capacities that sustained different mean spVL, in which the direct effect of co-infection on the incidence of HIV was then evaluated. The results from these simulations were consistent with the results from the host level model and raise the possibility that co-infection might play a substantial role in the spread of HIV in populations where the circulating virus maintains elevated spVL. Conversely, co-infection would not be an important driver of the epidemic in populations with low spVL.

This work underscores the possibility that viral factors might have an important impact on the role of co-infection on the spread of HIV. Therefore, understanding their variation would be a key element in the design of control interventions to prevent HIV transmission. To pursue this goal, however, it would be necessary to evaluate the results from the theoretical model proposed here with experimental data. These kinds of studies should be conducted in HIV positive individuals in the chronic stage of the infection with similar CD4+ T cell count, and whose spVL have being well established. Their immune systems can be challenged with the application of previously used antigen immune stimulants such as tetanus toxoid vaccine (Ostrowski et al., 1998) or influenza vaccine (Obrien et al., 1995; Rosok et al., 1996). The VL should then be measured frequently (preferably daily) over a considerable period of time (at least one month), to accurately delineate the dynamics of the virus during co-infection.

I emphasize that this work relies entirely on a hypothetical relationship between viral genotypic factors and the spVL. As noted previously, regardless of the evidence about the relationship between viral genetic factors and the spVL (Alizon et al., 2010; Fraser and Hollingsworth, 2010; Hecht et al., 2010), the evidence about the role of virus genotype on the natural history of HIV is controversial (Hemelaar et al., 2006; Thomson et al., 2002), and the evidence about differences in VL among populations is limited (Geretti, 2006; Morison et al., 2001b). Therefore, our study indicates the importance of conducting such studies that focus on estimating the spVL to elucidate possible differences at population level. Such studies would help us better understand the extensive variation on the HIV epidemic.

As suggested by the model, biological differences could alter the effect of co-infection and underscore the importance of identifying these factors for the implementation of effective control interventions focused on co-infection. The effect of co-infection might not be the same among individuals and populations, and control strategies will not necessarily have the same impact in each population. Understanding the role of viral genetic factors on co-infection becomes an important element for developing accurate and effective recommendations for population-level control strategies.

Limitations of the model

This study is a theoretical exercise aimed to explore further implications of the variation of viral genetic factors on the natural history of HIV. To achieve this goal, we developed a within-host co-infection model based on two well known and extensively used mathematical models describing the within-host dynamics of HIV and malaria. These two models were linked by the activation of the immune cells induced by the concomitant infection with malaria, increasing the target cell population for HIV infection.

This mechanism, however, is only one of the several mechanisms in which the invasion of another pathogen stimulates the replication of HIV (Chen et al., 2003; Copeland et al., 1996; Pisel et al., 2002; Staprans et al., 1995). The interaction between HIV and concomitant pathogens appears to be multi-factorial at cellular and immunological levels (Copeland et al., 1996). For example, the transcriptional signaling

used by lymphocyte cells to regulate cell functioning is also used by HIV to regulate virus production. During co-infection, cytokines [small proteins secreted by specific cells of the immune system used for local signals between cells during response to infection (Nathanson et al., 2002)] might enhance their susceptibility to HIV infection and stimulate production of viruses for weeks to months without significant cytopathic effects (Kedzierska et al., 2003; Vicenzi et al., 1997).

Moreover, other infections might have additional mechanisms in which they could affect the transmission of HIV. For example, the genital reactivations of HSV-2 generate local immune activations in genital ulcerations that might increment HIV shedding in the genital tracks (McClelland et al., 2002). This STI infection not only affects the risk of transmission but also increases the risk of acquisition in HIV-negative individuals, characteristic that considerably affects the mode in which HSV-2 influences the pattern of HIV transmission at the population level (Abu-Raddad et al., 2008; Freeman et al., 2006). The differences in which other infections besides malaria interact with HIV are not considered in this study, and they might alter the results observed at the population level.

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Table 5.1 Co-infection within-host model parameter values

Parameter	Value	Reference
Rate of red blood cell production (Λ)	$2.5*10^{8}$ cells/day/ml	(Saul, 1998; Thibodeaux, 2010; Tumwiine et al., 2008)
Natural death rate of uninfected red blood cells (μ_x)	0.0083/day	(Anderson et al., 1989; Tumwiine et al., 2008)
Infection rate of red blood cells by merozoites (k_m)	$2.5*10^{-10}$/day	(Anderson et al., 1989; Tumwiine et al., 2008)
Differentiation rate of merozoite (μ_1)	0.5/day	(Anderson et al., 1989; Tumwiine et al., 2008)
Natural death rate of infected blood cells (μ_y)	0.025/day	(Anderson et al., 1989; Tumwiine et al., 2008)
Clearance rate of infected red blood cells due to the immune system (μ_c)	$1*10^{-8}$/cell day	(Thibodeaux, 2010)
Natural death rate of free merozoites (μ_m)	48/day	(Anderson et al., 1989; Tumwiine et al., 2008)
Death rate of merozoites by contact with immune cells (μ_h)	$1*10^{-8}$/day	(Anderson et al., 1989; Tumwiine et al., 2008)
Merozoite production per infected red blood cell (r)	16	(Anderson et al., 1989; Tumwiine et al., 2008)
Rate of immune cells production (malaria specific) (ε)	0.0001/cell day	(Hellriegel, 1992)
Proliferation rate of immune cells in response to infected red blood cells	$2*10^{-8}$/cell day	(Thibodeaux, 2010)

(λ_y)		
Proliferation rate of immune cells in response to merozoites (λ_m)	$3*10^{-8}$/ cell day	(Thibodeaux, 2010)
Maximum number of activated immune cells per ml of blood (T_{max})	$1.5*10^{6}$/day	(Perelson et al., 1993)
HIV infection rate for nonspecific immune cell (k_v)	$[0.5\times 10^{-6}$ - $2\times 10^{-5}]$/day	Representative value
Production rate of immune cells (s)	$1*10^{4}$ cells/ml/day	(Callaway and Perelson, 2002)
Natural death rate of immune cells (d_t)	0.01/day	(Mohri et al., 1998)
Death rate of HIV infected cells (δ)	0.7/day	(Perelson et al., 1997)
HIV production rate by an infected cell (N)	[20 - 296] virions/cell	Representative value
HIV removal rate (c)	23/day	(Ramratnam et al., 1999)

Figure 5.1 Increment on the VL induced by co-infection using different values for the parameters k_v and *N*

The effect of the heterogeneity of the HIV infection rate (k_v) and production rate (*N*) on the increment of the VL generated by co-infection was evaluated using the bidimensional parameter space generated by the range parameter values of 1×10^{-6} to 2×10^{-5}/day for the HIV infection rate (k_v), and 20 to 300 virions/cell for the HIV production rate (*N*). I explored different values for k_{Tm}, in (A) $k_{Tm}=k_v$; (B) $k_{Tm}=k_v/10$; (C) $k_{Tm}=k_v/20$; and (D) $k_{Tm}=k_v/30$.

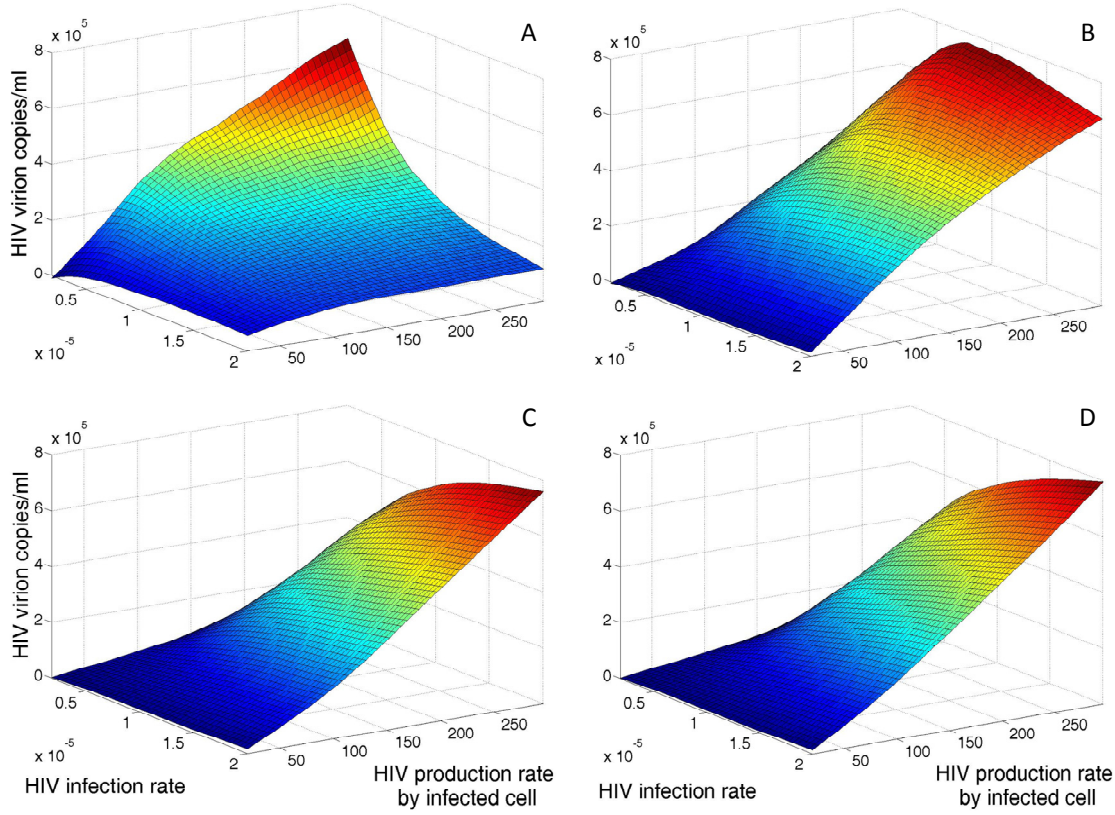


Figure 5.2 Time course of the VL in presence of co-infection with different set of parameters k_v and N

Figures illustrate the HIV dynamics from day 550, to day 800. In (A) $k_v = 2\times10^{-6}$ and $N = 50$; (B) $k_v = 5\times10^{-6}$ and $N = 120$; (C) $k_v = 6\times10^{-6}$ and $N = 170$; (D) $k_v = 9\times10^{-6}$ and $N = 220$, (E) $k_v = 14\times10^{-6}$ and $N = 260$; (F) $k_v = 18\times10^{-5}$ and $N = 280$.

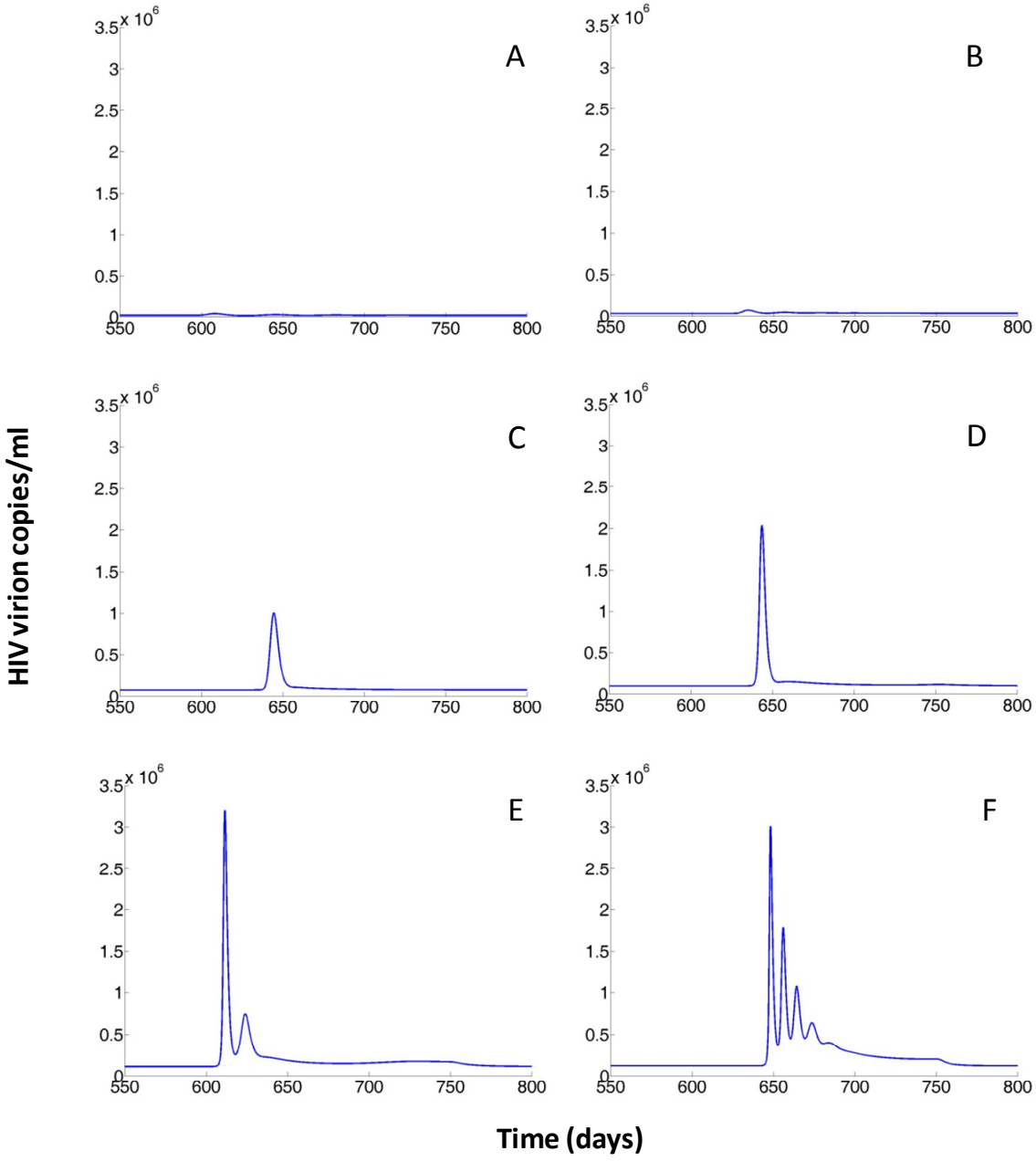


Figure 5.3 Correlation between the spVL and the increment on the VL in response to co-infection

Model estimations are indicated in blue dots and triangles; the black lines represent the logistic fitted line. In (A) the results suggest that viruses with replicative capacity that produce low spVL are not able to generate considerable increments on the VL. Conversely, viruses with potent replicative capacity that are able to produce elevated spVL would generate much larger increments on the VL. The pattern follows a logistic fit, which suggests a saturation effect at very high spVL. In (B) the geometric per capita rate of growth was estimated for each spVL evaluated. Large spVL are accompanied with high per capita rate of growth, which might reflect the high replicative capacity of the virus.

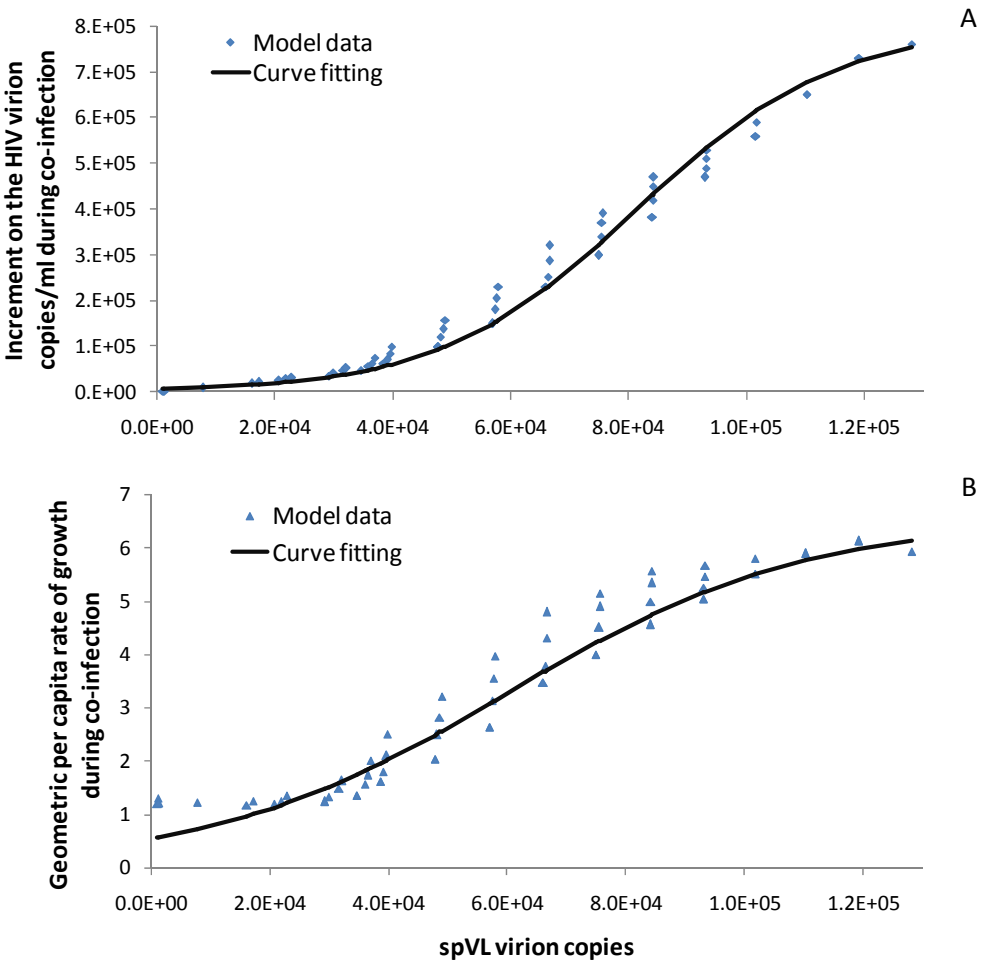
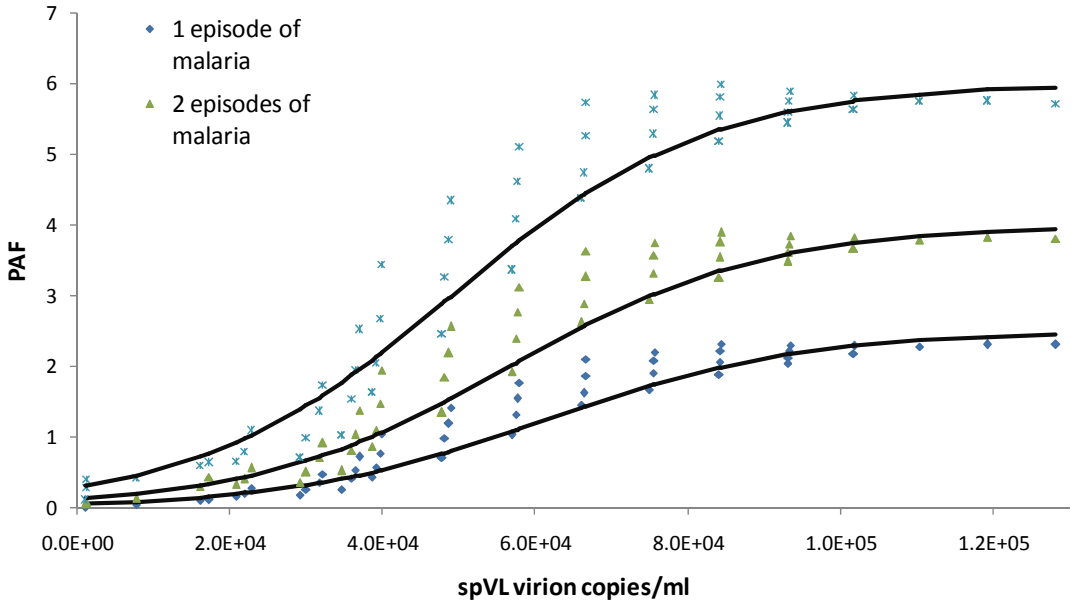


Figure 5.4 Correlation between the spVL and the PAF

The between-host model indicated that co-infection would have a negligible impact in populations where the circulating virus has low replicative capacity reflected in low spVL. Conversely, the impact of co-infection would increase as the spVL of the population increases, until it reaches a maximum effect at spVL around 1×10^5.



Chapter 6 - HIV prevention randomized clinical trials: quantitative and analytical insights on the failure to measure efficacy

Introduction

HIV infection remains as one of the major public health problems especially in sub-Saharan Africa (UNAIDS/WHO, 2009). During the nearly 30 years of research on HIV, the improved understanding of the determinants of HIV transmission has identified key candidate interventions to prevent HIV infection. More than 40 randomized controlled trials (RCT) were designed to measure the efficacy of interventions on HIV incidence ranging from HIV vaccines to behavioral interventions and male circumcision (Padian et al., 2010). Despite the solid foundation on observational epidemiological evidence, almost 90% of the RCTs failed to measure a statistically significant efficacy against HIV incidence (Padian et al., 2010). The reasons behind the failure of most RCTs to measure efficacy, despite the robust scientific evidence supporting the intervention concept, continue to be poorly understood.

The disappointing results of these trials after massive scientific field work and cost exceeding often tens of millions of dollars per RCT has generated a flurry of scientific research to elucidate plausible explanations for these failures (Cohen et al., 2011; Hayes et al., 2010a; Hayes et al., 2010b; Padian et al., 2010). In this article, we explore further factors that may explain the outcome of such trials by conducting the RCTs *in silico* thereby simulating the actual RCTs and investigating the drivers of their failures.

We focused on the Partners in Prevention HSV/HIV Transmission Study RCT, which was designed to test the efficacy of acyclovir suppressive therapy for herpes simplex virus type 2 (HSV-2) on reducing HIV transmission (Celum et al., 2010). This study found that, despite a significant reduction in the mean plasma HIV concentration and in the genital ulcerations in the acyclovir group, there was no significant effect of

acyclovir on the incidence of HIV. For comparisons, we also generated a model that simulates the effect of male circumcision on the risk of HIV infection. We chose this kind of intervention based on the positive results obtained from three RCTs designed to test the efficacy of male circumcision on the prevention of HIV infection (Hayes et al., 2010b), and the accurate estimation of its effect based on substantial amount of observational data (Weiss et al., 2000). We particularly focused on the RCT study conducted in Rakai, Uganda (Gray et al., 2007). Using a mechanistic mathematical model and RCT simulation, we assessed the drivers and implications of these findings.

Methodology

Simulation of RCTs is a growing area of interest in different fields including HIV prevention (Desai et al., 1999). In pharmacology, simulation of RCTs is a well-established procedure to evaluate alternative trial designs, test hypotheses, and interpret trial outcome (Holford et al., 2000; Karlsson and Sheiner, 1993; Sheiner and Steimer, 2000). The greatest benefit of RCT simulation is the ability to conduct a trial thousands of times *in silico*, and under variable spectrum of assumptions, at very limited cost versus the highly costly and demanding implementation of a single trial in the field. In this article, we developed a Monte-Carlo approach based in the Partners in Prevention HSV/HIV Transmission Study RCT (Celum et al., 2010), and the Rakai circumcision RCT study (Gray et al., 2007). We generated a population of virtual individuals and simulated the two different interventions proposed in these clinical trials. For this *in silico* experiment, the unit of replication was an individual RCT trial.

Partners in prevention trial simulation

To simulate the Partners in Prevention trial, the model generates a population of individuals and simulates the administration of acyclovir suppressive therapy as an intervention in an RCT trial just as the clinical trial. The total number of individuals simulated in each realization (which corresponded to an individual RCT trial) was 3400 (1700 in the control group and 1700 in the acyclovir intervention group). To match the number of HIV sero-conversions within the control group in the Partners in Prevention

trial, the probability of HIV transmission per sexual contact was fixed at $p_{un-treat}$ = 0.0012; nicely in unison with the values estimated from the state of the art empirical measures for this parameter of 0.0012 in the cohort of the Rakai Study (Gray et al., 2001; Wawer et al., 2005) and 0.0011 in the cohort of the Partners in Prevention Study (Celum et al., 2010).

The core of our simulation is the calculation of the effect of acyclovir on the reduction of HIV transmission per sexual contact. Acyclovir acts to reduce HIV plasma viral load, and subsequently HIV transmission probability per coital act. We assumed that the effect on reducing HIV infectiousness is according to the empirical relationship between HIV plasma viral load and HIV transmission probability per coital act as observed initially by Quinn and colleagues (Quinn et al., 2000), and affirmed recently in the Partners in Prevention trial (Lingappa et al., 2010): $p_{treat} = p_{un-treat} \times 2.45^{\log_{10}(vl)}$. Here $p_{un-treat}$ is HIV transmission probability per coital act in absence of intervention (control group), which was fixed at 0.0012, p_{treat} is HIV transmission per coital act in presence of intervention (intervention group), and $\log_{10}(vl)$ is the logarithmic (base 10) reduction in HIV plasma viral load with the intervention. The Partners in Prevention RCT reported an HIV plasma viral load reduction of $0.25 \log_{10}$ (95% CI 0.22-0.29) (Celum et al., 2010) implying 20.1% reduction in the transmission probability per coital act with the intervention $p_{treat} = 0.0010$. If the HIV plasma viral load reduction was $0.75 \log_{10}$, as in the higher dose acyclovir proof-of-concept studies, the reduction in the transmission probability per coital act with the intervention is 48.9% leading to $p_{treat} = 0.0006$.

The number of coital acts per susceptible individual per month was randomly chosen from a gamma distribution parameterized by the data of the Partners in Prevention trial (Lingappa et al., 2009) (Table 6.1). The coital acts are then divided into condom protected acts with efficacy of 80%, based on a systematic review of condom efficacy (Weller and Davis, 2001), and un-protected coital acts. The fraction of acts that are condom protected is 93% based on the Partners in Prevention data. Using these data, the number of un-protected exposures per month per susceptible individual to HIV infection is then estimated by drawing from a binomial distribution, where the number of events

indicates the number coital acts per month per susceptible individual, and a probability of unprotected coital exposures of $93\%(1.00-0.80)+7\%=26\%$.

For this simulation, the total number of individuals simulated in each realization (which corresponded to an individual RCT trial) was 3400 (1700 in the control group and 1700 in the acyclovir intervention group). The 2-year follow-up of the study was simulated in 24 monthly time steps. Each time step (month), and for each unprotected sexual contact, the simulation draw a random number from an uniform distribution to decide if the sero-negative individual remains uninfected or if the individual becomes HIV infected. If a transmission occurs, the time is recorded to perform a log-rank survival analysis. Figure 6.1 illustrated the algoritm implemented for the Partners in prevention RCT simulation.

Circumcision trial simulation

To simulate the Rakai RCT study conducted to assess the effect of male circumcision on the prevention of HIV infection, we estimated the probability of HIV transmission per month based on the number of cases per 100 persons per year. The Rakai study reported 1.33 new infections per 100 persons-year, which gave us a baseline probability of HIV transmission per month, p_m = 0.0008. We assumed that circumcision decreases the risk of infection by 60% (Auvert et al., 2005; Bailey et al., 2007; Gray et al., 2007; Weiss et al., 2000); therefore the probability of HIV transmission per month for individuals allocated in the intervention group was $p_{m-treat}$ = 0.00032. We allocated 2522 individuals in the control group and 2474 individuals in the intervention group. The model simulates the HIV transmission every month over a period of 24 months.

To measure the efficacy of the interventions, we performed two statistical analyses for each replication: log-rank survival analysis and relative risk (RR) estimation. The power of a specific RCT design was then determined from the number of trials that rejected the null hypothesis. All results were based on means of 1000 repetitions. Figure 6.2 ilustrate an example of a survival curve obtained from the tree scenarios implemented.

We designed three distinct scenarios for the RCT simulations. In the first scenario, we assumed an effect size based on the $\log_{10}$ reduction in HIV plasma viral load as reported in the actual Partners in Prevention trial ($0.25\ \log_{10}$). In the second scenario, we assumed a larger reduction in HIV plasma viral load of $0.75\ \log_{10}$ consistent with a stronger dosage of acyclovir. In the third scenario, we simulated the male circumcision RCT assuming a 60% efficacy for the intervention as measured in the three male circumcision trials to date (Auvert et al., 2005; Bailey et al., 2007; Gray et al., 2007).

We generated a population of virtual individuals and simulated the two different interventions proposed in these clinical trials. For this *in silico* experiment, the unit of replication was an individual RCT trial. To measure the efficacy of the intervention (acyclovir or male circumcision), we performed two statistical analyses for each individual trial realization: log-rank survival analysis and relative risk (RR) estimation. The power of the RCT to yield a statistically significant result was then determined as the fraction of realizations that rejected the null hypothesis (Burton et al., 2006). All reported results in this article were based on 1000 realizations of the model.

We designed three distinct scenarios for the RCT simulations. In the first scenario, we assumed an effect size based on the $\log_{10}$ reduction in HIV plasma viral load generated by a dosage of acyclovir of 400 mg twice a day, and reported in the actual Partners in Prevention trial ($0.25\ \log_{10}$). In the second scenario, we assumed a larger reduction in HIV plasma viral load of $0.75\ \log_{10}$ consistent with a stronger dosage of acyclovir. In the third scenario, we simulated the male circumcision RCT. We also assessed the effect of the number of unprotected coital exposures on the RR estimation and statistical power of the RCT.

Results

Table 6.2 summarises the results from all simulations.

Scenario1: Partners in prevention trial with 0.25 log10 reduction in HIV plasma viral load

T In this scenario, the mean number of HIV sero-conversions was 35 in the intervention group and 44 in the control group (Figure 6.3 A) leading to a mean RR of 0.79 (95% CI: 0.51-1.25), where the CI represents the mean of the upper and lower bounds estimated from the 1000 realizations of the model. The log-rank test indicated that 14% of the RCT realizations showed statistically significant efficacy for the intervention, whereas the RR analysis indicated that 16% of the realizations were statistically significant. The RR distribution (Figure 6.3 B) displayed wide variation in RR outcome across the 1000 realizations, ranging from RR point estimates of 0.45 to 1.6 (Figure 6.3 B).

Scenario 2: Partners in Prevention trial with 0.75 log10 reduction in HIV plasma viral load

In this scenario, mean number of HIV sero-conversions was 22 in the intervention group and 44 in the control group (Figure 6.3 C) leading to a mean RR of 0.51 (95% CI: 0.31-0.85). The log-rank test indicated that 75% of the RCT realizations showed statistically significant efficacy for the intervention, whereas the RR analysis indicated that 77% of the realizations were statistically significant. The RR distribution (Figure 6.3 D) displayed wide variation in RR outcome across the 1000 realizations, ranging from RR point estimates of 0.2 to 1.1.

Scenario 3: Rakai male circumcision trial

In this scenario, the mean number of HIV sero-conversions was 19 in the intervention group and 48 in the control group (Figure 6.3 E) leading to a mean RR of 0.41 (95% CI: 0.26-0.64). The log-rank test indicated that 94% of the RCT realizations showed statistically significant efficacy for the intervention, whereas the RR analysis indicated that 95% of the realizations were statistically significant. The RR distribution (Figure 6.3 F) displayed wide variation in RR outcome across the 1000 realizations, ranging from RR point estimates of 0.15 to 0.9.

Scenario 4: Optimal trial design

We estimated an optimal trial design to detect significant differences between the intervention and control arm. We used the same parameters used in simulation 1 (Table 6.1), but the population and the time of the follow-up were increased five and four times, respectively. Thus, the total number of individuals simulated was 20 000 (10 000 in the control group and 10 000 in the acyclovir group) and 96 time steps (months). For this scenario, both the log-rank test and the RR analysis indicated that 99% of the repetitions showed statistical differences. The mean HIV incidence was 778 in the intervention arm and 966 in the control arm (Figure 6.4), and the mean RR was 0.80 (95% CI 0.76-0.88).

Data Effect of the number of unprotected coital exposures on the RR estimation

We explored the effect of the number of unprotected coital exposures on the RR by varying the number of unprotected exposures from five up to 200 for the total duration of follow-up. We did so for both Scenario 1 and Scenario 3 as described above. The results indicated that few unprotected sexual contacts per partnership generate a broad CI, which then shrinks as the number of sexual contacts increases (Figure 6.5), evidencing the dependence of the level of statistical significance on the number of unprotected sexual contacts.

Discussion

It has been suggested that acyclovir is insufficiently effective to produce a reduction in HIV plasma viral load necessary to generate an effect on HIV transmission (Hayes et al., 2010b). As a result, acyclovir therapy has been discredited as an alternative control intervention for HIV prevention (Cohen et al., 2011; Hayes et al., 2010b). The reduction on the plasma concentration of HIV, however, suggests that acyclovir (in a dosage of 400 mg twice a day) may be able to produce in average a 20% reduction on the risk of HIV transmission. This information, complemented with the computer simulation of the trial, indicated that the RCT designed by the Partners in Prevention team could not be expected to detect a significant reduction of the incidence of HIV in response to acyclovir.

We observed that only 14% of the realizations of the simulation obtained statistically significant results, and evidenced the low power of the study to detect any significant efficacy of acyclovir. Moreover, despite a reduction on the relative risk of HIV transmission in the intervention group by 0.79 (95% CI: 0.51-1.25), the wide CI indicated a high variation in the outcome of the simulation, which was consistent with the findings of the study (hazard ratio with acyclovir 0.92; 95% CI: 0.60-1.41) (Celum et al., 2010).

Based on the effect of acyclovir dosage used and other parameters estimated from the trial, our model indicated that a sample size of ~20 000 couples with a follow-up time of ~8 years would be necessary to provide enough power to detect a statistically significant effect (Figure 6.4). However, the modest clinical significance of 20% reduction on the HIV incidence might avert the implementation of such expensive and logistically intensive clinical trial. Conversely, our results suggest that higher dosage of acyclovir (or other antiviral drugs that generates a larger reduction on the mean concentration of plasma HIV viral load) might generate the clinical efficacy desired (Schacker et al., 2002), and indeed, increase the likelihood of success of the clinical trial.

Additionally, the Partners in Prevention team calculated the power of the study based on an incidence of 4.0 per 100 persons-years in the control group, whereas the incidence observed was 1.8 per persons-year. Like the expected effect size of the intervention, the event rates assumed also play an important role in the calculation of the statistical power (Schulz and Grimes, 2005). Our results suggest that the small number of exposures to the infection (unprotected sexual contacts), which was associated with high frequency of condom use, might be an important reason for the low observed incidence and consequently, the failure on the detection of significant effect.

For the Partners in Prevention trial, a minimum number of sexual contacts would be necessary to observe a statistical effect of the intervention. Despite the reduction on the RR of HIV transmission generated by acyclovir, it would not be possible to detect a significant effect on the reduction of the HIV transmission with fewer than 100 unprotected sexual contacts (Figure 6.5 A). Assuming a high rate of condom use (80% of protected sexual contacts), which (in agreement with ethical issues) is the norm in this

kind of studies (Hayes et al., 1997), gives an average of 19 unprotected sexual contacts per partnership during the entire study. According to our analysis, this number of exposures to the infection is far too low to detect a significant effect of the intervention, and cannot provide the basis for an evaluation of the effectiveness of acyclovir.

Conversely, the more effective the intervention the smaller the number of unprotected sexual contacts required to detect a significant difference. Due to the strong effect of male circumcision in reducing the hazard of HIV infection (60% reduction), even with a small number of unprotected sexual contacts, it would be possible to observe a significant effect of the intervention (Figure 6.5 B). This may explain why all RCT studies designed to test the effect of circumcision have shown significant results, and it is consistent with the high power estimated from the simulation of the Rakai study, where 94% of the realizations showed statistically significant results. The estimated mean RR of 0.41 (95% CI 0.26-0.64) nicely agrees with the estimation from the Rakai study (Kaplan-Meier risk ratio 0.40; 95% CI 0.23-0.70) (Gray et al., 2007), and suggests a high accuracy of the mechanistic approach implemented here.

Our work highlights the risk of designing clinical trials to evaluate interventions to prevent HIV infections solely based on results from sample size calculations and statistical power estimations. Standard formulas used to calculate the sample size of an RCT are unlikely to capture the entire complexity generated by several factors that might play an important role in the transmission of HIV. Key parameters such as frequency of condom use, number of exposures to the infection, duration of the follow-up, measured efficacy of the intervention based on biological data, adherence to the intervention, among others, could be evaluated by the use of computer simulations to observe their influence in the range of outcomes, and to design the most appropriate clinical trial.

The implementation of these virtual trials (i.e. computer simulations) that synthesize the mechanistic process of the intervention (and the system in which it is implemented) would help us to evaluate the design and performance of future HIV prevention trials. It will help to succed in implementing costly and logisitcally intensive clinical trials, and more importantly, it will avert confusing evidence on the effectivenes of potential interventions.

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Table 6.1 Key parameters used in the RCT simulations

Parameter	Value	Reference
Time of the follow-up	24 months	(Celum et al., 2010)
Average number of sexual contacts per month	4 (95% CI 2 - 8)	(Lingappa et al., 2009)
Percentage of unprotected sexual contacts per month	26%	(Myer et al., 2002)
Number of individuals		
Scenario 1		(Celum et al., 2010)
Control group	1700	
Intervention group	1700	
Scenario 2		(Celum et al., 2010)
Control group	1700	
Intervention group	1700	
Scenario 3		(Gray et al., 2007)
Control group	2522	
Intervention group	2474	
Probability of HIV transmission in the control group		
Scenario 1	0.0012 per sex act	(Celum et al., 2010)
Scenario 2	0.0012 per sex act	(Celum et al., 2010)
Scenario 3	0.0008 per month	(Gray et al., 2007)
Reduction on the probability of HIV transmission in the intervention group		
Scenario 1	20.1%	(Celum et al., 2010; Quinn et al., 2000)
Scenario 2	48.9%	(Celum et al., 2010; Quinn et al., 2000)
Scenario 3	60%	(Gray et al., 2007; Weiss et al., 2008)

Table 6.2 Summary of the RCT simulations from the different scenarios

Result	Value
Mean HIV incidence rate per 100 persons/year	
Scenario 1	
Control group	1.29
Intervention group	1.03
Scenario 2	
Control group	1.29
Intervention group	0.65
Scenario 3	
Control group	0.95
Intervention group	0.38
Percentage of simulations with significant results	
Scenario 1	
Log-rank test	14%
RR analysis	16%
Scenario 2	
Log-rank test	75%
RR analysis	77%
Scenario 3	
Log-rank test	94%
RR analysis	95%
Relative risk estimation	RR (95% CI)
Scenario 1	0.79 (0.51-1.25)
Scenario 2	0.51 (0.31-0.85)
Scenario 3	0.41 (0.26-0.64)

Figure 6.1 Flow diagram of the algorithm implemented to simulate the Partners in Prevention HSV/HIV transmission study RCT

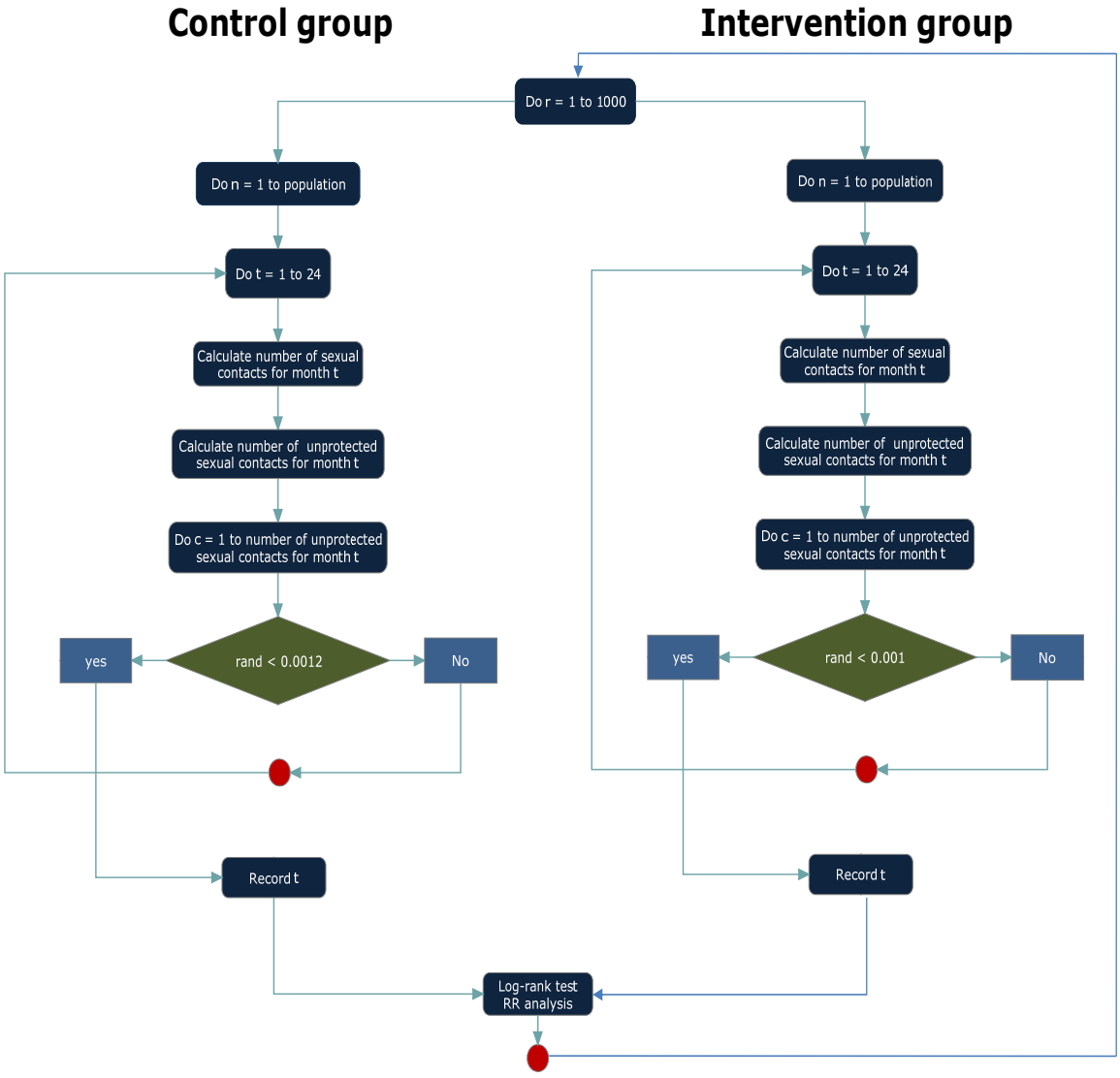


Figure 6.2 Example of the survival curves estimated from a single realization

In (A) 20% reduction on the probability of HIV transmission per sexual contact (scenario 1); (B) 50% reduction (scenario 2); and (C) male circumcision (scenario 3).

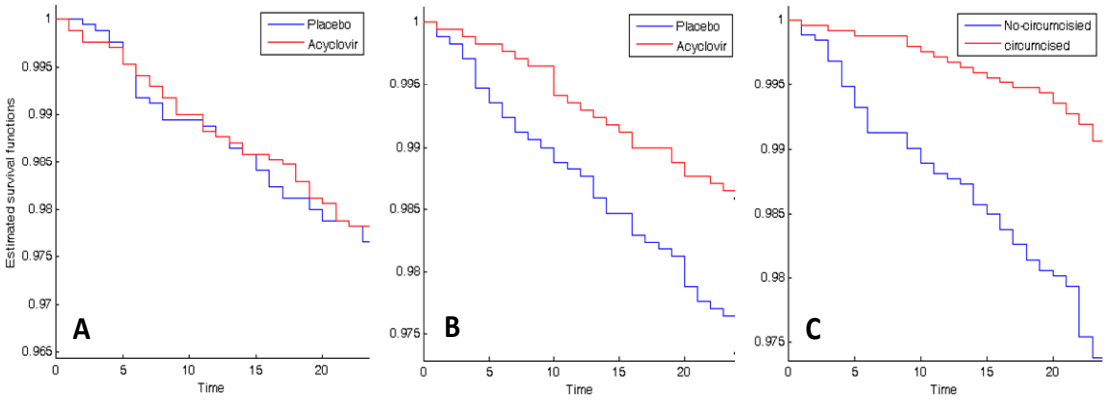


Figure 6.3 Number of HIV infections and RR distribution for the different scenarios In (A) 20% reduction on the probability of HIV transmission per sexual contact (scenario 1), distribution of the number of HIV infections for intervention group (red bars), and control group (blue bars); (B) its corresponding RR distribution, in this simulation for a number of realizations, the RR exceeded one implying that despite the actual reduction in the probability of HIV transmission per coital act, it is possible just because of stochastic statistical nature, to observe higher HIV incidence in the intervention group compared to the control group; (C) 50% reduction (scenario 2), and (D) its corresponding RR distribution; (E) male circumcision (scenario 3), and (F) its corresponding RR distribution.

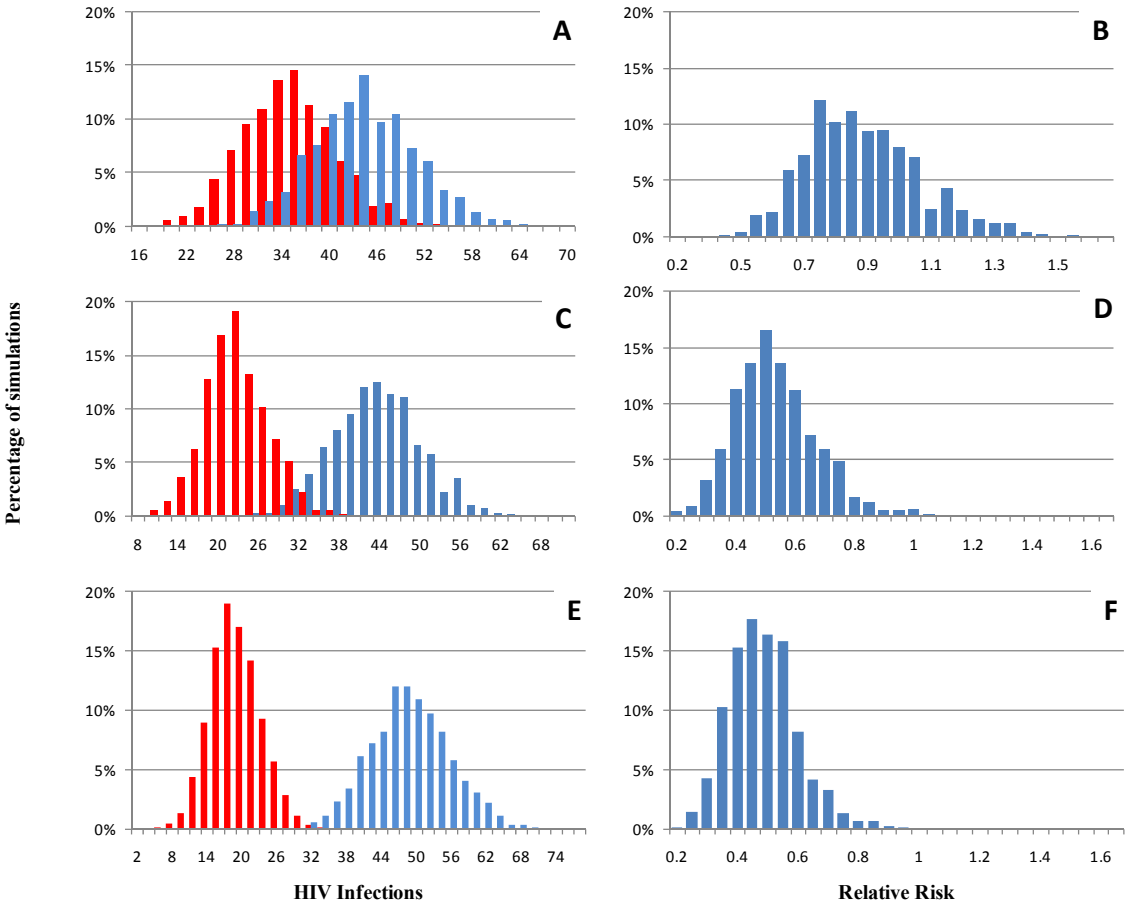


Figure 6.4 HIV incidence distribution for the optimal RCT designed to evaluate acyclovir

In (A) distribution of the HIV incidence for intervention arm (red bars), and control arm (blue bars); (B) its corresponding RR distribution.

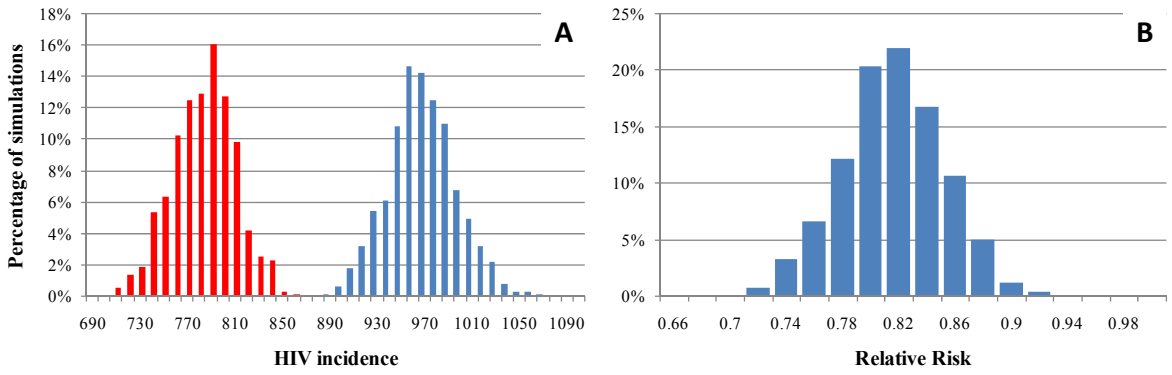
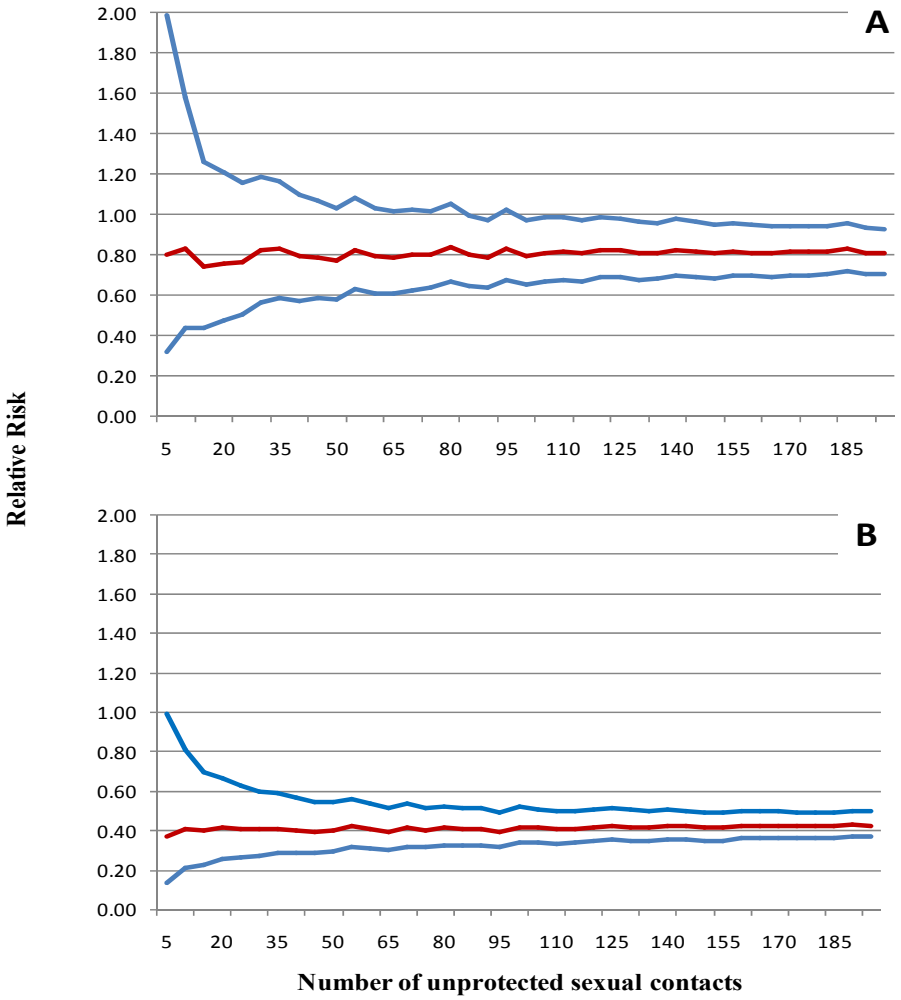


Figure 6.5 Effect of the number of unprotected sexual contacts on the RR estimation
The red line represents the RR trends and the blue lines its corresponding CI for (A) assuming 0.25 $\log_{10}$ reduction on the HIV plasma viral load, and (B) male circumcision. While the point estimate for the RR in Scenario 1 (acyclovir) was 0.80, the confidence interval around this estimate varied from 0.70-0.93 at 200 un-protected coital exposures to 0.32-1.99 at five un-protected coital exposures. Similarly, for Scenario 3 (circumcision), while the point estimate for the RR was 0.4, the confidence interval around this estimate varied from 0.37-0.50 at 200 un-protected coital exposures to 0.14-0.99 at five un-protected coital exposures.



Chapter 7 - Conclusions

This work aimed to explore the limitations of the notion that the existent heterogeneity of the HIV epidemic could be explained merely by variations on sexual behavior. Instead, the inclusion of temporal and individual variation on the HIV infectiveness generated by co-infection might affect the pattern of the spread of the epidemic at population level, and could potentially explain the differences on the HIV epidemic among populations.

Co-infection has been consistently proposed as one of the main factors fueling the spread of HIV in sub-Saharan Africa. Here, I investigated the role of co-infection on the spread of HIV in this part of the world. Using different methodological approaches, I conducted statistical and mathematical analyses to assess the relationship between different infections and HIV in sub-Saharan Africa. This study was based on the hypothesis that the natural history of HIV in sub-Saharan Africa cannot be fully understood if individual and temporal variation in infectiousness is neglected. A single value for HIV infectivity would fail to describe the dynamics of the epidemic, and regardless of the low probability of heterosexual transmission per sexual contact, the inclusion of temporal and individual variation generated by transient increases in HIV viral loads associated with co-infections might provide a biological basis for the accelerated spread of HIV in sub-Saharan Africa.

The results here suggest that the HIV epidemic in sub-Saharan Africa can be explained by heterosexual transmission, and that the amplification effect caused by biological cofactors such as co-infection may have triggered the vast HIV epidemic observed in sub-Sahara Africa. The high prevalence of infectious diseases such as malaria and HSV-2 in sub-Saharan Africa probably provided suitable conditions for the spread of the infection. The remarkably high HIV prevalence observed in sub-Saharan Africa probably reflects the particularly suitable environment for the epidemic that is unique to this part of the world. These results highlight the possibility that co-infection might be a necessary rather than merely a contributing factor in the successful spread and survival of HIV in populations where heterosexual vaginal-penile contact is the main

mechanism of transmission, such as in sub-Saharan Africa. Because most studies attempting to identify the infectivity of HIV have not explicitly accounted for important cofactors, population average estimations cannot capture the variation in infectivity associated with these cofactors.

This study emphasize the need for additional research focused on the infectivity of HIV and underscore the importance of including cofactor effects in HIV infectivity studies and epidemic models. Here, the results evidence the necessity for more and better quality data to assess the role of co-infection in the spread of HIV. The results discussed in chapter 3 and 4 indicate the possible relationship between malaria and HIV in areas where both infections geographically overlap. This result, however, was derived using indirect measures of the effect of malaria on the risk of HIV infection, and several potential confounders such as the impact of HIV on the prevalence of malaria were not taken into account. Therefore, it would be imperative to compare these results with field studies designed to estimate the impact of malaria on the incidence of HIV. Currently, active and efficient control interventions to reduce malaria infections have been notoriously successful in countries such as Malawi, Kenya and Zimbabwe. This event is generating a convenient scenario that would allow the study of the direct impact of reductions on malaria prevalence in the incidence of HIV.

Likewise, results from chapter 5 evidence the priority to continue investigating the role of viral genetic factors on the transmission of the virus. Despite the controversy and skepticism surrounding this topic, the mathematical model developed here suggests some potential implications resulted from the combination of biological factors. In this chapter, several experiments were proposed to elucidate the potential interactions that could be useful for understanding the differences observed among HIV epidemics.

Cofactor-enhanced infectivity of the virus might be a key determinant of the HIV epidemic in sub-Sahara Africa. This suggests that an HIV epidemic may be mitigated or halted through individual measures that decrease viral infectivity. On the other hand, whereas limiting the number of sexual partners may be an effective strategy for reducing an individual's risk of HIV transmission; this strategy is unlikely to prevent an epidemic. The control and treatment of several common infectious diseases would decrease the

incidence of HIV over the long-term. The public health implications of our results are clear: broad population-based control strategies to decrease infectivity such as condom distribution, antiretroviral therapies, circumcision and especially control strategies for the reduction of other sexual and parasitic infectious diseases, may therefore be effective in reducing the spread of HIV and could be an effective strategy against the progression of the epidemic in sub-Saharan Africa.

Several studies have been conducted to evaluate the efficacy of these kinds of interventions on the reduction of HIV incidence. Unexpectedly, and in contrast with the epidemiological evidence discussed in this study, the majority of the studies have shown no significant effect of co-infection treatment on the incidence of HIV. Chapter 6 was devoted to explore the possible causes for the failure of such operational intensive and costly studies. The results from this chapter evidenced the methodological failures from these studies that prevented the detection of significant effects of control interventions focused on co-infection. The treatment of co-infections might indeed be an effective control intervention; the implementation of studies aimed to evaluate the efficacy of these interventions, however, need more thorough design, in which computer simulations might be a powerful tool assessing the role of several unexpected behavioral an biological factors on the final outcome of the study.

In summary, the general findings from this study have direct implications on the design of mathematical models attempting to replicate the epidemic curve observed in sub-Saharan Africa, as well as control interventions focusing on decreasing the incidence of HIV. If highly infectious individuals can be identified, then the efficiency of control measures could be greatly increased. The realization of these kinds of targeted interventions, however, requires a better understanding of factors determining individual infectiousness such as co-infections.

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Appendix A - Supplementary methods and results Chapter 2

Supplementary methods

Sexual partnerships were assumed to be exclusively heterosexual, and two types of partnerships, distinguished by duration, were considered. The population was assumed closed and stable, with only maturation and mortality into and out of the sexual network. The population size remained constant, with individuals maturing into the network to offset those who die or mature out of the network. In accord with the highest resolution of relevant data, a monthly time step was used. With this model, the effects of network structure on disease transmission, relationship type, and co-infection with other infectious diseases were evaluated.

Behavioral module

Building individuals

In the model, equal numbers of individuals of each sex were created and assigned an age and node degree (maximum number of partners per year). The initial age was assigned with a gamma probability distribution, with a range between 15 to 60 years old (Table A.1, parameters 5-8).

Consequently, individual age was used to determine when individuals should be removed from the sexual network, and was the basis for other age-specific traits.

We adopted a likelihood framework to estimate model parameters of the annual degree node distribution by using the number of sexual partners reported in the Malawi study for both males and females. For both males and females, the reported number of sexual partners follows a gamma distribution:

$$f(n) = n^{k-1}\frac{e^{-n/\theta}}{\Gamma(k)\theta^{k}}, \quad (1)$$

where the annual degree distribution is defined as the frequency of node degrees ***n*** in the network, with shape parameter *k* and scale parameter θ.

Because the time step of the simulation is one month, and with the aim of preserving the degree distribution of the network, we estimated the monthly probability of generating a partnership according to the degree node for each individual, and we calculated the maximum number of connections that should be generated in the network each month. We assumed that casual partnerships were temporally independent, began at uniformly distributed random times throughout the year, and lasted for a set duration, *d*. Further, as marriages generally last more than one year, we assumed that if an individual was married, the relationship did not end during any observation year.

Thus, the expected number of casual partnerships within a year, c_y, is the expected number of marital partnerships, m_y, subtracted from the expected number of total partnerships, n_y or

$$c_y = n_y - m_y. \quad (2)$$

P_m, the probability that a casual partnership recorded within the year, would also be recorded within one particular month of the year given by

$$p_m = \frac{1+d}{12+d}. \quad (3)$$

Here a partnership is recorded within the observation month if its center point falls within an interval of length *1+d* centered on the middle of the month, and a partnership is recorded within the observation year if its center point falls within an interval of length *12+d.* Because yearly casual partnerships are assumed to be temporally independent, the expected number of monthly partnerships, n_m, for an individual with n_y yearly partnerships is the expected number of yearly casual partnerships, plus the expected number of yearly marital partnerships, m_y.

$$n_m = c_y p_m + m_y = \frac{(n_y - m_y)(1+d)}{12+d} + m_y. \quad (4)$$

The vector n_m was used for estimating the probability of generating a connection according to the node degree of each individual.

For calculating the maximum number of connections that should be generated each month, we assumed that the expected numbers of yearly casual partnerships are

Poisson distributed; therefore, for each n_m, the probabilities that the expected c_y partnerships generate n_m casual partnerships during the month, are found according to

$$p(c_y, n_m) = \frac{{c_y}^{n_m} e^{-c_y}}{n_m!}, \quad (5)$$

evaluated from $n_m = 0$ to n_{max}. These probabilities are then weighted by the yearly degree frequencies, correcting for the fraction of individuals that are married, and summing over partnership numbers. The probabilities for casual partnerships, weighted by the node degree distribution $f_y(n)$ are

$$p_c(c_y, n_m) = p(c_y, n_m) * (1-m) * f_y(n_m) . \quad (6)$$

The probabilities for marital partnerships, weighted by $f_y(n)$ are

$$p_m(c_y, n_m) = p(c_y, n_m) * m * f_y(n_m) . \quad (7)$$

The probabilities for all partnerships, weighted by $f_y(n)$ are

$$p_t(c_y, n_m) = p_c(c_y, n_m) + p_m(c_y, n_m) . \quad (8)$$

The total monthly partnership frequencies are then

$$f_t(n_m) = \sum_{c_y=1}^{c_{\max}} p_t(c_y, n_m) . \quad (9)$$

Each frequency includes both males and females; therefore we divided it by two to obtain the maximum number of connections that should be generated in one month.

Forming initial partnerships

We allowed for two types of relationships; casual relationships, which lasted on average 6 months (Table A.1 parameter 14), and marriage (long-term relationship), which lasted more than 1 year. Partnership formation occurs in two steps. First, the age mixing pattern of marriage is generated by estimating the probability of generating a marriage using a gamma probability distribution (eq 1) according to age and gender (Table A.1, parameter 9-12). This step generates the age mixing pattern of marriage. Then marriages are generated until the fraction of the married population estimated for Malawi is reached (Table A.1, parameter 13). Casual partnerships are then formed with the remaining available individuals (married or single) according to their node degree, and then these available individuals are randomly connected until the monthly number of connections f_y (eq 9) is reached.

Dynamic of the network

Partnerships are broken and reformed with data-determined probabilities each month; thus, with the aim of keeping the yearly degree distribution, individuals may sometimes have zero partnerships in a particular month. For Malawi, the probability of ending a marital relationship follows an exponential distribution as a function of the duration of the marriage *X*, with an average duration of marriage of 6.92 years. Hence, each marriage has a probability of ending in each time step according to an exponential cumulative probability function with a scale parameter λ (eq 10). Casual partnerships break up following the same function but at a higher rate λ (Table A.1, parameter 14):

$$f(x) = 1 - e^{-\lambda x}. \quad (10)$$

As the sexual network represents only individuals engaging in sexual activity, population dynamics consist of maturation into and out of the sexual network by "mortality" related to age or HIV. At a maximum age of 60, individuals are assumed to no longer be sexually active and are removed from the sexual network and replaced with a new individual of age 15. This new individual has the same sex and node degree as the removed individual, so that the sex ratio and network structure is maintained. Individuals who have broken a relationship and new individuals who have replaced deceased individuals or those who have matured out of the sexual network are reconnected by the same process used to make the initial connections.

Epidemiological model

The epidemiological module was subdivided into two steps, the spread of the infections, and the progression and recovery of each infection. We assumed that HIV transmission is caused by penile-vaginal heterosexual contact exclusively. We selected gonorrhea, syphilis and herpes simplex virus type-2 (HSV-2) as the infections to be simulated in the sexual network, based on the amplification effect on HIV transmission and their relevance in terms of prevalence in the Malawi population. The dynamics of these STI's are well known, and the effect of each infection on the transmission of HIV has been determined.

A key assumption for the epidemiological module is that the interaction caused by co-infection has only one direction. In other words, we assumed that HIV infection has

no effect on the natural history of the other infectious diseases included in the model. This assumption may be seen as an oversimplification due to the fact that studies have shown that HIV infection affects the transmission and progression of other infectious diseases such as HSV-2 and malaria. Yet, studies have mainly focused on the impact of co-infection on HIV. As a result, uncertainty about the effect of co-infection in the direction of the other diseases is still high.

Spread of the infections

For the spread of the STI's, the probability of disease transmission per partnership per month depends on the number of sexual contacts per partnership per month and the transmission probability per sexual contact for each STI. The number of sexual contacts per partnership per month is assumed to be a function of the number of partnerships of the infected sex only. This function is defined as:

$$c_n = c_1 N^{-0.75} \quad , \quad (11)$$

where C_1 is the number of sexual contacts per month for individuals having one partnership according to age (Table A.2 parameter 7-10) , and N is the number of partnerships the individual has in the specific month. This function is consistent with individuals with more partnerships having more sex but keeps the number of sexual contacts per month realistic.

For each infection, we obtained the estimated amplification factor on the probability of HIV transmission per sexual contact. For our simulation, the algorithm assessed whether the individual infected with HIV has another infectious disease, and if co-infection was present, the HIV transmission probability was increased depending on the amplification cofactor (Table A.2 parameter 49-53). Then, the new HIV transmission probability including the amplificatory effect was calculated by

$$T_c = T * cofactor \, . \quad (12)$$

where T is the stage or sex-specific transmission probability per sexual contact (Table A.2 parameter 1-6). When multiple co-infections are present, we assumed a saturation effect on the enhancement on the transmission probability. Thus, when more than one co-infection is present, the transmission probability is amplified only by the highest cofactor. For the special case of HSV-2, the amplification factor is only effective if the HSV-2

infection is reactivated (shedding) (Celum et al., 2004). Therefore the algorithm not only verifies the presence of HSV-2 co-infection but also its reactivation. On the other hand, HSV-2 not only enhances the transmissibility of HIV but also affects the susceptibility to being infected with HIV (Celum et al., 2004). For this reason, the algorithm verifies if the susceptible receptor is infected with HSV-2 and its reactivated stage. In this case, the transmission probability is also increased by the respective amplification factor (Table A.1 parameter 53).

The transmission probability per partnership per month is then calculated using the binomial (Bernoulli) model as

$$T_p = 1-(1-T_c)^{c_n}, \quad (13)$$

where T_p is the stage or sex-specific transmission probability per sexual contact (Table A.2 parameter 1-6) and C_n is the number of sexual contacts the individual has with the partner (eq 11). The probability of condom use is represented by a gamma probability function (eq 1), depending on age (Table A.2 parameter 31, 32). If a condom is used, then the probability of transmission of the STI is reduced 94% (Table A.2, parameter 33):

$$T_{condom} = T_p * 0.06 \quad . \quad (14)$$

For the spread of Malaria infection, we used the daily Entomological Inoculation Rate (EIR), defined as the number of infected bites that a person receives per day, for both rainy and dry seasons estimated for Malawi, as well as the malaria transmission efficiency for an infected bite. Each individual was exposed to a malaria infection by a probability of transmission:

$$T_{malaria} = \text{EIR} * \text{malaria transmission efficiency} \quad . \quad (15)$$

Because $T_{malaria}$ is calculated based on daily EIR, while the time step of the simulation is the month, we calculated the monthly probability of malaria infection, $TM_{malaria}$, using the Bernoulli model:

$$TM_{malaria} = 1-(1-T_{malaria})^{30} \quad . \quad (16)$$

In order to include the effect of partial immunity generated by multiple malaria infections, each new infection is recorded and then used for calculating the susceptibility of a new infection using the model proposed by Gu and coworkers (Gu et al., 2003):

$$S_{malaria} = \frac{1}{1 + \frac{N_{\inf}^{\theta}}{c_1}}, \quad (17)$$

where N_{inf} is the number of malaria infections an individual has had in his/her life; θ and C_1 are constants. After calculating the susceptibility coefficient, the new probability of malaria infection $TM'_{malaria}$ becomes

$$TM'_{malaria} = TM_{malaria} * S_{malaria} \ . \ (18)$$

The core of our model is the spread of HIV infection. Before the introduction of HIV infected individuals, the model simulates for several years the dynamic of the other infectious diseases previously mentioned. When an endemic steady state for all infectious diseases is reached (after about 500 time steps), the model introduces HIV infected individuals until the HIV prevalence reaches 1%, which is the prevalence observed in Malawi in 1981 (Crampin et al., 2002).

The HIV transmission probability per month was calculated in the same way as it was calculated for the other STI's using the Bernoulli model (eq 12), as was the probability of condom use and its effect on the probability of transmission. However, in the spread of HIV the amplification effect caused by co-infection was taken into account.

Circumcision is known to affect the dynamics of HIV and has been proposed as one of the explanatory variables for contrasting differences in prevalence among countries of sub- Sahara Africa. The probability of HIV transmission from infected female to uninfected male is approximately halved by circumcision.

$$T_{circum} = T_t / 2 \ . \quad (19)$$

Circumcision is included in our model at the beginning of the simulation, where a percentage of males estimated for Malawi (Table A.2 parameter 62) are randomly selected from the male population and identified as circumcised males.

HIV transmission caused by commercial sex

The data available show that males do not report sexual contacts with prostitutes as sexual partners; as a result, the degree node distribution estimated for males does not take into account sexual contacts with prostitutes.(Ferry et al., 2001; Helleringer et al., 2007a; Morison et al., 2001) However, because commercial sex has been considered an important risk factor for acquiring HIV infection, we include this mechanism of HIV transmission in the model.

Data from another study in Cameroon, Kenya and Zambia, called the four cities study, was used to parameterize the spread of HIV infections caused by sexual contacts with prostitutes (Ferry et al., 2001; Morison et al., 2001). In our model, commercial sex is a simple generalized linear equation for calculating the probability that a male gets any of the STI's included in our simulation, including HIV. We assumed that in average 8% of the male population have six sexual contacts with a prostitute per year.

In our model, commercial sex is a simple generalized linear equation for calculating the probability that a male gets any of the STI's included in our simulation, including HIV. The model is a mass action Bernoulli model, where the probability of transmission depends on the actual prevalence of the STI into the population. For HIV, because prostitutes are cataloged as a core group, and the prevalence is usually higher compared to the total population, we assumed that the HIV prevalence in prostitutes is five times the actual HIV prevalence in the population. Thus, the probability of transmission caused by sexual contact with a female sexual worker (FSW) is calculated as:

$$I_{fsw}(t) = 1 - \{(1 - p_{fsw}(t)) + [p_{fsw} * (1 - T_d)^n]\}, \quad (20)$$

where $p_{fsw}(t)$ is five times the HIV prevalence of the total population at time t, Td is the probability of female to male transmission in chronic stage of HIV infection (Table A.2 parameter 21) and $\boldsymbol{n}$ is the number of sexual contacts that a male individual has with the prostitute in a month (Table A.2 parameter 60). Every month, a number of male individuals (Table A.2 parameter 54) are chosen randomly and become infected via commercial sex with a probability I_{FSW}. Because our goal was to measure the impact of co-infection on the spread of HIV infection, we allowed to prostitutes to have infections with other STIs according to the prevalence of the STI reported for prostitutes. Hence, the

baseline HIV transmission probability was amplified according to the presence of co-infection.

Progression of the infections

The model simulates the progression of each infection. Gonorrhea is the simplest one, where each month infected individuals have a constant probability of recovery from the infection, with an average duration of the infection of ten weeks (Table A.2 parameter 30). An individual that recovers from gonorrhea infection becomes susceptible again.

Syphilis infection has a more complex progression divided in two main stages. The primary stage of the infection is the first six months, characterized by a high probability of transmission for both sexes and the highest amplificatory effect on HIV transmission (Table A.2 parameter 34, 36). After this initial six-month stage, the infection remains latent for eight years. The latent stage is characterized by a low probability of transmission and no amplification effect on HIV transmission (Table A.2 parameter 35-37). We assume that individuals infected with syphilis receive treatment; therefore, after the latent stage, individuals recover from the infection and become susceptible again.

HSV-2 infection has the most complex dynamics. A newly infected individual enters a chronic stage of the infection lasting 10 years. During this interval, there are about four reactivations of the infection (shedding) per year for non HIV co-infected individuals, and six episodes per year for co-infected individuals (Table A.2 parameter 25,26). These reactivation periods last 15 days and produce the amplificatory effect of HSV co-infection on both the transmission and acquisition of HIV (Table A.2 parameter 27). Hence, for our simulation, only co-infected individuals with HSV-2 in shedding episodes have amplified the transmission and acquisition of HIV.

In contrast to the other STI's included in the simulation, individuals infected with HSV-2 do not recover from the infections; but after the 10-year chronic stage, individuals do not transmit the HSV-2 infection, and there is no cofactor effect on HIV transmission or acquisition (Table A.2 parameter 23, 24).

For malaria, infected individuals recover at a constant rate, with an average duration of the infection of two months ($R_{malaria}$). However, with the aim of including the effect of partial immunity generated by multiple infections, we used the equation

proposed by Gu and coworkers (2003), to calculate a recovery coefficient $r_{malaria}$, which decreases the duration of the infection:

$$r_{malaria} = \frac{1}{1 + \frac{N_{\inf}^{\rho}}{c_2}}. \quad (23)$$

Here N_{inf} is the cumulative lifetime number of the individual's malaria infections; $\boldsymbol{\rho}$ and $\boldsymbol{C_2}$ are constants. After calculating the susceptibility coefficient, the new probability of recovery from malaria infections $R'_{malaria}$ becomes

$$R'_{malaria} = R_{malaria} * r_{malaria} . \quad (24)$$

In order to maintain the concept of partial immunity, the recovery coefficient was truncated at 20 malaria infections with a minimum value of 0.5.

Individuals infected with HIV have a probability of developing AIDS calculated by a Weibull cumulative probability function depending of the duration of the infection $\boldsymbol{x}$, with scale parameter λ, and shape parameter k (Table A.2, parameters 11,12):

$$f(x) = 1 - e^{-(x/\lambda)^k}. \quad (25)$$

We assumed that individuals who develop AIDS are no longer spreaders of the infection; they leave the sexual network and are replaced by new individuals in the same way that was explained previously for “mortality”.

Supplementary results

Parameters included in our model are estimated from available published biological and behavioral data. Due to the variability generated by the different data collections and analyses, all estimations are associated with some degree of uncertainty. Analyses were conducted using Latin Hypercube Sampling and Partial Rank Correlation Coefficient (LHS/PRCC) through Monte Carlo sampling (Blower and Dowlatabadi, 1994; Marino et al., 2008) from specific ranges using the uniform probability distribution function (pdf) for 100 runs of the model.

In the absence of published studies about the distribution and the range of most of the parameters, ranges were chosen to be as large as possible and represent plausible values for these parameters given the empirical evidence. These choices represent the most influential parameters in our formalism as seen in multiple analyses of the model and there are not precise estimates for them.

We conducted three different analyses where we explored the uncertainty and sensitivity of each module, behavioral and epidemiological, separately, and then we explored the uncertainty and sensitivity of the entire model.

Uncertainty and sensitivity analyses of the behavioral module

For the uncertainty and sensitivity analyses of the key parameters of the behavioral module, we set the default probability of HIV transmission $T = 0.003$, but we did not include the cofactor effect caused by co-infection. We sampled seven parameters: the minimum and maximum age of individuals included in the sexual network, the mean number of sexual contacts per month (assuming the same for all ages), the mean durations of casual relationships, the proportion of the married population, the proportion of the male population that has sex with prostitutes per month, and the number of sexual contacts that a male has with a prostitute in a month. The specific range for the sampling of each parameter is specified in Table A.3.

Descriptive statistics for the uncertainty analysis indicated that the variation generated by the uncertainty of the parameters sampled is fairly low (Table A.3). On average, all possible combinations of the parameters sampled did not produce an epidemic. The 95th percentile, however, indicated that with some combination of the behavioral parameters and without the inclusion of co-infection, 5% of the simulations generated a HIV close to 5%.

The sensitivity analysis revealed that the average number of sexual contacts per month and the mean duration of casual relationships were the only statistical significant parameters; in other words, these parameters may explain most of the variation observed among simulations (Table A.5). The number of sexual contacts and the duration of the relationships are parameters related to the HIV transmission risk per partnership. According to the binomial model for the calculation of the per-partner probability of HIV

transmission, increasing the duration of the relationship or the number of sexual contacts will increase the risk of HIV transmission. We observed that simulations with mean duration of casual relationship longer than 8 months and with 12 sexual contacts per month were able to produce an HIV epidemic higher than 6%.

Uncertainty and sensitivity analyses of the epidemiological module

For the uncertainty and sensitivity analyses of the key parameters in the epidemiological module we included the cofactor effect caused by co-infection. We sampled from the range of the cofactor values of the four infectious diseases included in the model, gonorrhea, syphilis, HSV-2 and malaria (Table A.6).

The results from this analysis indicated that the variation generated by the uncertainty of the cofactors is higher than the variation generated by the uncertainty on the behavioral parameters (Table A.7). The first empirical quartile indicated that at least in 75% of the simulations the HIV prevalence was higher than 10%. The sensitivity analysis indicated that infections with a short infectivity period but with high cofactor such as gonorrhea and syphilis, and the cofactor for infections present in the general population such as malaria, are the parameters contributing to the variation observed in the model (Table A.8).

Uncertainty and sensitivity analyses of the complete model

To explore the uncertainty and sensitivity of the complete model to the key behavioral and biological parameters, we performed an analysis that combined the ranges previously mentioned for both modules and also the proportion of male population with circumcision [lower bound = 0.2; upper bound =0.5].

The combination of the uncertainty of the parameters of both modules increased the imprecision of the model. One important result from this analysis was that, in contrast with the uncertainty analysis for the epidemiologic module, the combination of both modules produced some combinations of behavioral parameters that did not allow the generation of an HIV epidemic. Thus, even with the inclusion of the cofactor effect, the results indicated that in 25% of the simulations an epidemic could not be produced (Table A.9).

The results from the sensitivity analysis of the complete model were similar to the result of each module separately. The PRCCs showed that as in the behavioral module, the model was highly sensitive to the average duration of a casual relationship and the mean number of sexual contacts per month (Table A.10). We observed that in simulations with an average duration of casual relationships of 2 months the HIV prevalence was less than 1%, whereas in simulations with an average duration of casual relationship longer than 4 months, the HIV prevalence was higher than 2%.

These results indicated the relevance of parameters that measure the risk of HIV transmission per partnership. Clearly, an increment in the duration of a relationship as well as the number of sexual contacts significantly increased the probability of HIV transmission and therefore, the HIV prevalence. Due to the methodological difficulties for measuring these parameters (especially the mean duration of a casual relationship), they are highly imprecise. Our model indicated however, the relevance of these parameters on epidemiological models.

Efforts focused on increasing the precision of their estimation will improve the accuracy of predictions from mathematical and computational models. On the other hand, the cofactors for infectious diseases present in the general population such as malaria and the enhanced HIV transmission caused by HSV-2 infection were the most influent epidemiological parameters.

Limitations of the model

Although the sexual network simulated allows for link dynamics, the degree distribution of the nodes and the node degree of each individual is maintained constant throughout the entire simulation. This assumption may be unrealistic, since the degree distribution of sexual networks on human populations might change over time. The node degree distribution of the network was estimated from data collected in 2003, and thus this degree distribution might not correspond to the node degree distribution of the sexual network in 1980. Intervention programs focused on preventing promiscuity and changes in behavior resulting from the HIV epidemic itself could decrease the number of sexual partners that a person has in a year. Consequently, the behavioral data for 2003 could

underestimate the node degree distribution of the network at the beginning of the epidemic.

The static degree distribution also has an effect at an individual level. For simplification, and given the lack of data, we assume that each individual has the same node degree (number of sexual partners per year) during his/her entire sexual life. Although the degree distribution of the network may be relatively stable for a long period of time, nodes (individuals) may change their degree over time. For example, an individual may start having many sexual partners; but marriage, possible infections with other STIs, and the HIV epidemic itself may generate a behavioral change in the individual, resulting in a lower number of sexual partners or even monogamy. This decision-making may be an important process in the network, where the inclusion of simulation tools, such as artificial intelligence, may be an interesting approach to simulating such behavioral changes.

We also assumed no control interventions for the other STIs and malaria, and therefore the prevalence of these infections remained constant over the simulation. This assumption may appear unrealistic since important efforts to control malaria and STIs in sub-Saharan Africa have been effective in reducing their prevalence; consequently, the effects of these diseases on HIV may have been decreasing in recent years.

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Table A.1 Parameters used in the behavioral module of the model

Parameter	Value	Reference
Number of sexual partners per year		
Gamma distribution (males)		Derived from (University of Pennsylvania, 2003)
1. scale	1.1	
2. shape	1.9	
Gamma distribution (females)		Derived from (University of Pennsylvania, 2003)
3. scale	3.8	
4. shape	0.4	
Initial condition age distribution		
Gamma distribution (males)		Derived from (National Statistical Office)
5. scale	2.24	
6. shape	17.32	
Gamma distribution (females)		Derived from (National Statistical Office)
7. scale	2.46	
8. shape	13.5	
Marriage age distribution		
Gamma distribution (males)		Derived from (University of Pennsylvania, 2003)
9. scale	2.25	
10. shape	17.23	
Gamma distribution (females)		Derived from (University of Pennsylvania, 2003)
11. scale	2.38	
12. shape	13.85	
13. Percentage of married population	63%	Derived from (University of Pennsylvania, 2003)
14. Duration of casual relationship	6 months	Derived from (University of Pennsylvania, 2003)
Separation probability (exponential distribution)		
15. scale	0.15	Derived from (University of Pennsylvania, 2003)

Table A.2 Parameters used in the epidemiological module of the model

Parameter	Value	Reference
HIV probability of transmission per sexual contact (low income countries)		(Boily et al., 2009)
1. Acute male-female	0.0276	
2. Chronic male-female	0.003	
3. Advanced male-female	0.0219	
4. Acute female-male	0.03496	
5. Chronic female-male	0.0038	
6. Advanced female-male	0.02774	
Number of sexual contacts per month (by age)		(Gray et al., 2001)
7. 15-20	11	
8. 21-30	9	
9. 31-40	9	
10. 41-60	7	
Initial HIV infection age distribution (gamma)		Derived from (National Statistical Office)
11. Scale	8	
12. Shape	5	
HIV progression (Weibull cumulative probability function)		(Marion and Schecter, 1992; Salomon and Murray, 2001)
13. Scale	5	
14. Shape	4	
HSV-2 probability of transmission per sexual contact		(Abu-Raddad et al., 2008; Freeman et al., 2007)
Primary infection		
15. Male to female	0.3	
16. Female to male	0.15	
Early stage		
17. Male to female	0.01	
18. Female to male	0.005	
Chronic stage		
19. Male to female	0.005	
20. Female to male	0.003	
HSV-2 stage duration		(Abu-Raddad et al.,

		2008; Freeman et al., 2007)
21. Primary infection	3 weeks	
22. Early stage	3 months	
23. Chronic stage	10 years	
24. Latent stage	lifetime	
HSV shedding frequency per year		(Abu-Raddad et al., 2008)
25. In HIV positive individual	6 per year	
26. In HIV negative individual	4 per year	
27. Shedding during reactivation	15 days	
Gonorrhea probability of transmission		(Buvé et al., 2001; Orroth et al., 2007)
28. Male to female	0.23	
29. Female to male	0.13	
30. Gonorrhea duration of infection	3 months	(Buvé et al., 2001; Ghani et al., 1998; Orroth et al., 2007)
Condom use by age (Gamma distribution)		Derived from (National Statistical Office)
31. Scale	14.1	
32. Shape	1.78	
33. Condom reduction of transmission	94%	(Holmes et al., 2004; Pinkerton and Abramson, 1997)
Syphilis probability of transmission		(Buvé et al., 2001; Orroth et al., 2007; Oxman et al., 1996; Pourbohloul et al., 2003)
34. Early stage male to female	0.175	
35. Latent stage male to female	0.018	
36. Early stage female to male	0.088	
37. Latent stage female to male	0.009	
Syphilis stage duration		(Buvé et al., 2001; Orroth et al., 2007; Oxman et al., 1996; Pourbohlou et al.,

		2003)
38. Duration early stage	6 months	
39. Duration latent stage	8 years	
Malaria Entomological Inoculation Rate (EIR)		Derived from (Doherty et al., 2005)
40. Rainy season	1.2	
41. Dry season	0.6	
42. Duration of rainy season	6 months	(Aron and May, 1982; Smith et al., 2006)
43. Malaria transmission efficiency	0.026	
Malaria susceptibility coefficients		
44. Alpha	2	(Gu et al., 2003)
45. N1	200	
Malaria recovery coefficients		(Gu et al., 2003)
46. Beta	2	
47. N2	400	
48. Time of malaria parasite clearance	2 months	(Anderson et al., 1991; Kublin et al., 2005)
Amplificatory factor in HIV transmission		
49. Gonorrhea	3	(Celentano et al., 1996; Galvin and Cohen, 2004; Kapiga and Lugalla, 1998; Kassler et al., 1994; Laga et al., 1993; Sexton et al., 2005)
50. Syphilis	2.5	(Deschamps et al., 1996; Otten et al., 1994; Rakwar et al., 1999; Sexton et al., 2005)
51. Malaria	1.25	Derived from (Abu-Raddad et al., 2006; Kublin et al., 2005; Quinn et al., 2000)
52. HSV-2	3	(Abu-Raddad et al.,

		2008; Blower and Ma, 2004; Celum et al., 2004; Corey et al., 2004; Freeman et al., 2007; Sexton et al., 2005; Weiss et al., 2001)
53. Enhanced susceptibility to HIV in HSV-2 infected individual	3	(Abu-Raddad et al., 2008; Blower and Ma., 2004; Celum et al., 2004; Corey et al., 2004; Freeman et al., 2007a; Sexton et al., 2005; Weiss et al., 2001)
Commercial sex parameters		
54. Proportion of male population having sex with prostitutes per month	0.0066 (8% in a year)	(Ferry et al., 2001; Morison et al., 2001; Nzila et al., 1991)
55. Gonorrhea prevalence in prostitutes	23%	(Morison et al., 2001; Nzila et al., 1991)
56. Syphilis prevalence in prostitutes	16%	(Morison et al., 2001; Nzila et al., 1991)
57. HSV prevalence in prostitutes	90%	(Ferry et al., 2001; Morison et al., 2001; Nzila et al., 1991)
58. Percentage of circumcised males	31%	Derived from (National Statistical Office)
59. HIV reduction in probability of transmission in circumcised males	2 times	(Auvert et al., 2001; Baeten et al., 2005)
60. Number of sexual contacts a male individual has with a prostitute in a month	6	Derived from (Morison et al., 2001)

Table A.3 Parameters sampled and their range for the uncertainty analysis for behavioral module

Parameter	Range of uniform pdf	Reference
Minimum age	[14-18]	Derived from (National Statistical Office)
Maximum age	[55-62]	Derived from (National Statistical Office)
Number of sexual contacts per month	[6-14]	Representative assumption
Duration of casual relationship (months)	[3-9]	Representative assumption
Proportion of married population	[0.5-0.7]	Derived from (National Statistical Office)
Proportion of male population having sex with prostitutes per month	[0.005-0.01]	Representative assumption
Number of sexual contacts a male individual has with a prostitute in a month	[1-20]	Representative assumption

Table A.4 Descriptive statistics from the uncertainty analysis for the behavioral module in the absence of co-infections

Descriptive Statistics	HIV prevalence
Mean	0.87
Median	0
Standard Deviation	2.07
25th percentile	0
95th percentile	4.95

Table A.5 Partial rank correlation coefficients of the parameters sampled from the behavioral module

Parameter	PRCC
Minimum age	0.02
Maximum age	0.07
Number of sexual contacts per month	0.49***
Duration of casual relationship	0.53***
Proportion of married population	0.04
Proportion of male population having sex with prostitutes per month	0.01
Number of sexual contacts a male individual has with a prostitute in a month	0.04

The results are significant at the 0.05 level (*), the 0.01 level (**) or the 0.001 level (***).

Table A.6 Parameters sampled and their range for the uncertainty analysis for the epidemiological module

Parameter	Range of uniform pdf	Reference
Amplificatory factor in HIV transmission		
Gonorrhea	[1.1-9]	(Celentano et al., 1996; Galvin and Cohen, 2004; Kapiga and Lugalla, 1998; Kassler et al., 1994; Laga et al., 1993)
Syphilis	[1.4-10]	(Deschamps et al., 1996; Otten et al., 1994; Rakwar et al., 1999)
Malaria	[1.1-1.6]	Derived from (Kublin et al., 2005; Quinn et al., 2000)
Enhanced transmission to HIV in HSV-2 co-infected individual	[2-5]	(Abu-Raddad et al., 2008; Blower and Ma., 2004; Celum et al., 2004; Corey et al., 2004; Freeman et al., 2007a; Weiss et al., 2001)
Enhanced susceptibility to HIV in HSV-2 infected individual	[2-10]	(Abu-Raddad et al., 2008; Blower et al., 2004; Celum et al., 2004; Corey et al., 2004; Freeman et al., 2007a; Weiss et al., 2001)

Table A.7 Descriptive statistics from the uncertainty analysis for the epidemiological module

Descriptive Statistics	HIV prevalence
Mean	23.44
Median	22.83
Standard Deviation	7.35
25th percentile	17.02
95th percentile	32.80

Table A.8 First Table in Chapter 1

Parameter	PRCC
Amplificatory factor in HIV transmission	
Gonorrhea	0.42**
Syphilis	0.46**
Malaria	0.75***
Enhanced transmission to HIV in HSV-2 co-infected individual	0.04
Enhanced susceptibility to HIV in HSV-2 infected individual	0.29

The results are significant at the 0.05 level (*), the 0.01 level (**) or the 0.001 level (***).

Table A.9 Descriptive statistics from the uncertainty analysis for the complete module

Descriptive Statistics	HIV prevalence
Mean	14.03
Median	7.12
Standard Deviation	16.02
25th percentile	0.23
95th percentile	45.06

Table A.10 Partial rank correlation coefficients of the parameters sampled from the complete module

Parameter	PRCC
Minimum age	-0.04
Maximum age	0.09
Number of sexual contacts per month	0.45***
Duration of casual relationship	0.79***
Proportion of married population	-0.10
Proportion of male population having sex with prostitutes per month	0.068
Circumcision	-0.17
Amplificatory factor in HIV transmission	
Gonorrhea	-0.03
Syphilis	-0.31
Malaria	0.23**
Enhanced transmission to HIV in HSV-2 co-infected individual	0.31**
Enhanced susceptibility to HIV in HSV-2 infected individual	0.10

The results are significant at the 0.05 level (*), the 0.01 level (**) or the 0.001 level (***).

Figure A.1 Annual degree distribution of the number of sexual partners per year
Males: (A) Estimated for Malawi (University of Pennsylvania, 2003) (B) resulting from the simulations; and for females: (C) Estimated for Malawi (University of Pennsylvania, 2003) (D) resulted from the simulations. Red lines represent the fitted gamma distribution. The histograms represent the distribution of the data.

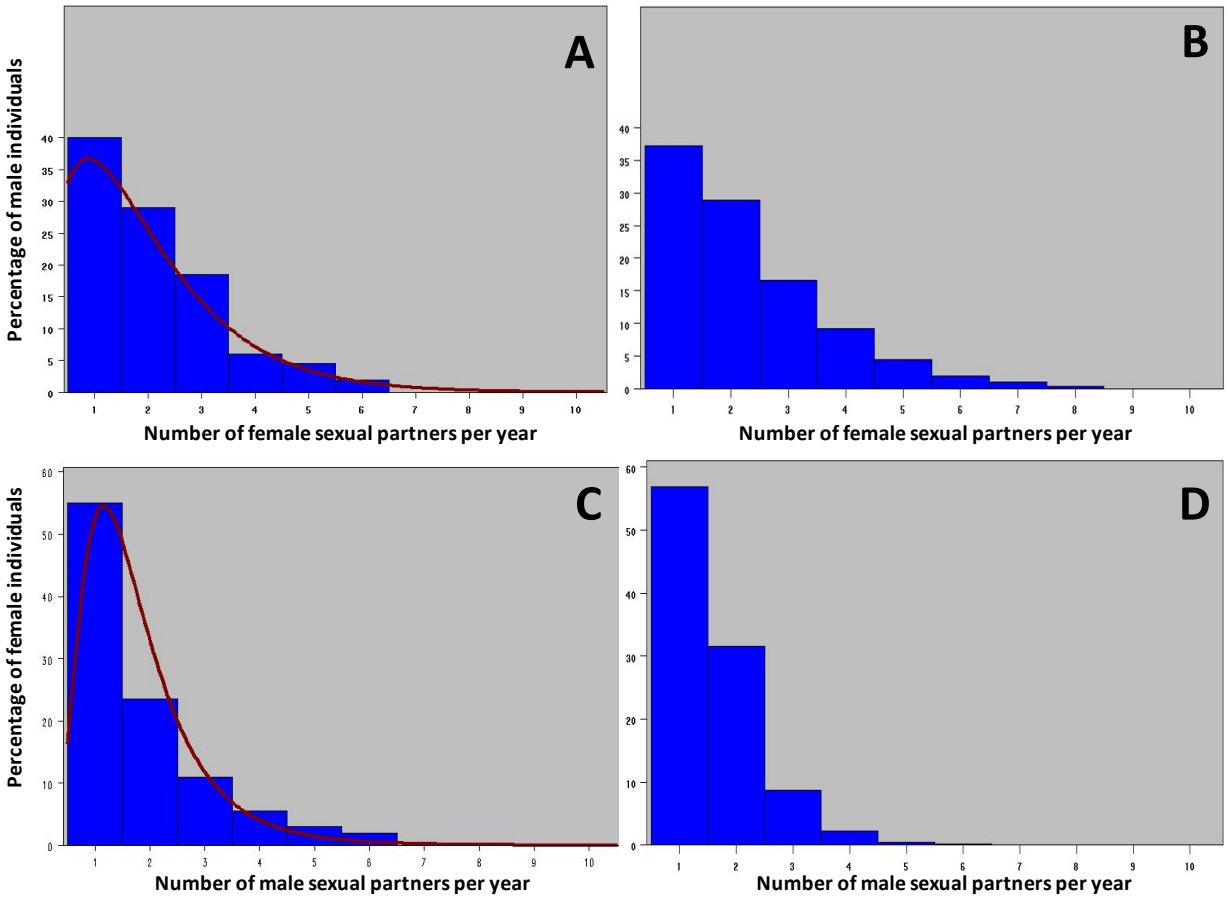


Figure A.2 Age distribution of males and females combined

(A) Age distribution of sexual active population estimated for Malawi in 2003 (National Statistical Office) and (B) Age distribution of sexual active population resulting from the simulation for 2003. Red lines represent the fitted gamma distribution. The histograms represent the distribution of the data.

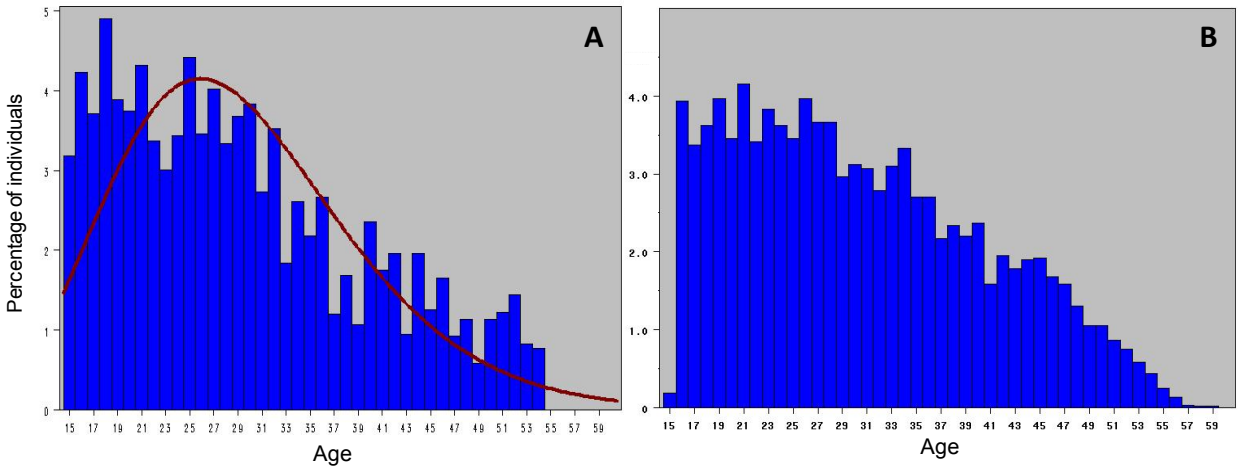


Figure A.3 Marriage distribution according to age

(A) Female age distribution estimated from Malawi in 2003 (National Statistical Office) and (B) the age distribution from the simulation estimated for 2003. (C) Male age distribution estimated from Malawi in 2003 (National Statistical Office) and (D) the age distribution from the simulation estimated for 2003. Red lines represent the fitted gamma distribution. The histograms represent the distribution of the data.

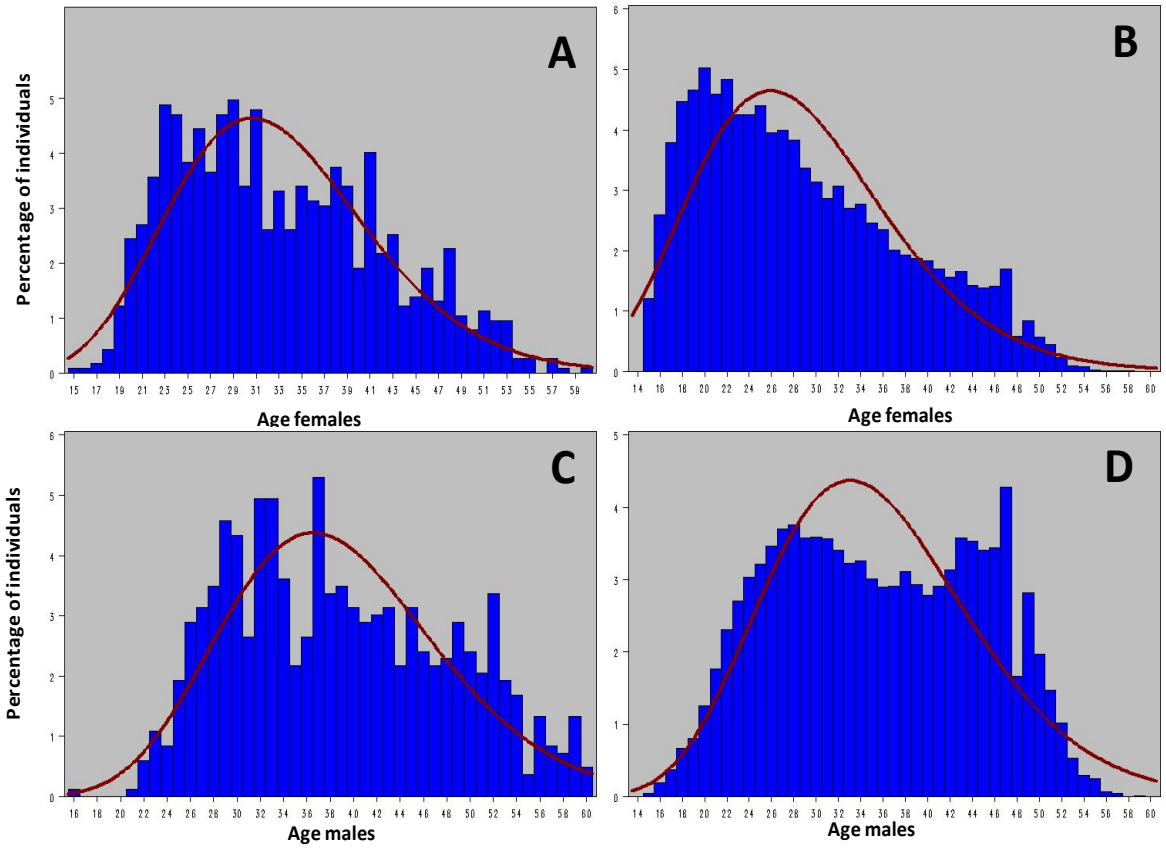


Figure A.4 Number of long-term relationship (marriages) during the entire sexual life an individual male or female

(A) Derived from Malawi in 2003 (National Statistical Office) and (B) derivate from the simulations.

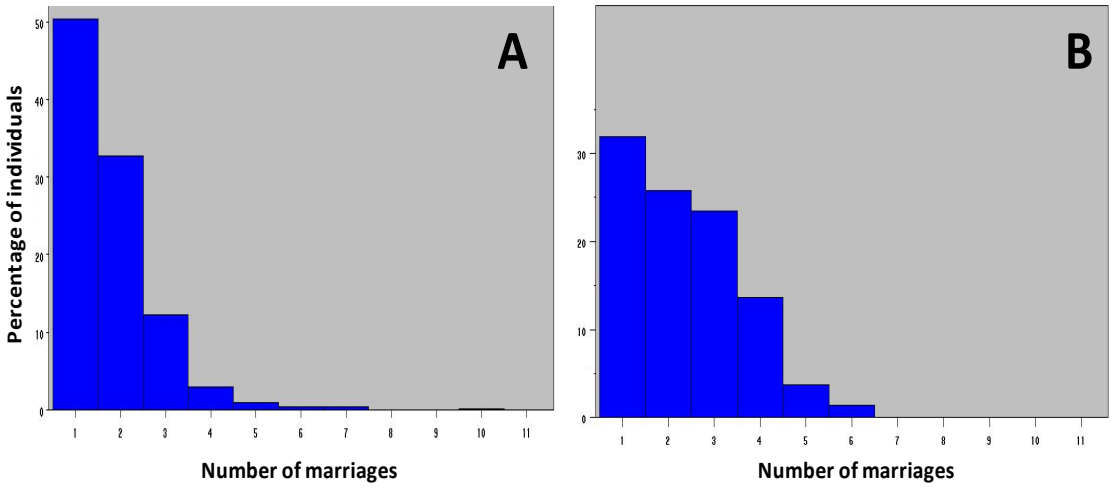
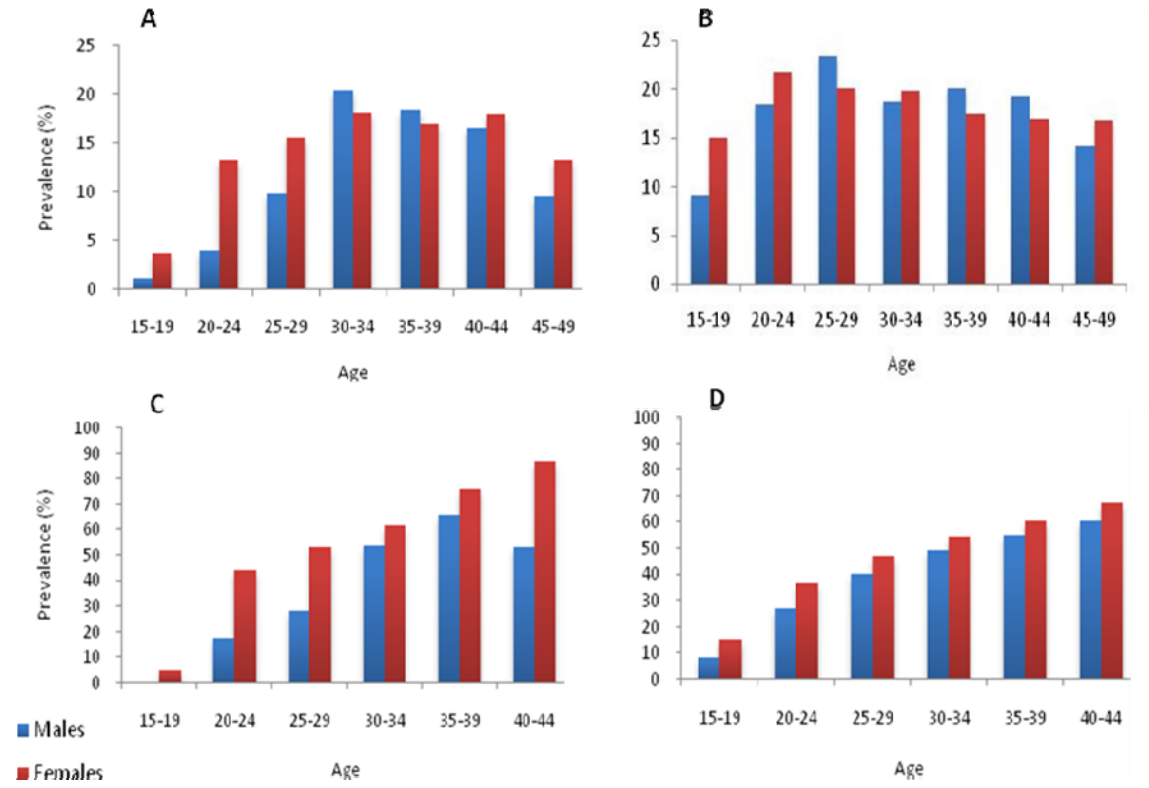


Figure A.5 Age-distribution of the HIV prevalence

(A) the observed data from Malawi in 2004 (Glynn, 2008), (B) the resulting HIV prevalence distribution from Simulation 2 for the same year. (C) The observed age-distribution data from Malawi for HSV-2 in 2004 (Glynn, 2008), and (D) the HSV-2 age-distribution prevalence from Simulation 2 for the same year.



Appendix B - Supplementary methods Chapter 5

Mathematical Approach of the Spread of HIV at Population Level

To evaluate the effect of co-infection at population level, I used a standard deterministic population model constructed by Abu-Raddad and coworkers (Abu-Raddad et al., 2008; Abu-Raddad et al., 2006). The model is a deterministic compartment model, which stratifies the population into compartments according to HIV sero-status and stage of HIV infection, and sexual-risk activity group,

$$\begin{aligned}
\frac{dS(i)}{dt} &= \mu_p N_0(i) - \mu_p S(i) - \Lambda_i S(i) \\
\frac{dI_1(i)}{dt} &= \Lambda_i S(i) - \mu_p I_1(i) - \omega_1 I_1(i) \\
\frac{dI_2(i)}{dt} &= \omega_1 I_1(i) - \mu_p I_2(i) - \omega_2 I_2(i) \\
\frac{dI_3(i)}{dt} &= \omega_2 I_2(i) - \mu_p I_3(i) - \omega_3 I_3(i)
\end{aligned} \quad .$$

The index i stands for an i-sexual risk population where $i = 1, 2, 3, 4$ represent the low, low to intermediate, intermediate to high, and high risk groups, respectively. $S(i)$ is the susceptible population, and $I_\alpha(i)$ is the HIV infected populations. The index α indicates the stage of HIV pathogenesis, where $\alpha = 1, 2, 3$ stand for acute, chronic, and late stages respectively. $N_0(i)$ is the initial population size, of each i-risk group.

The progression of HIV is described by ω_1, the rate of progression from acute to chronic stage, ω_2, the rate from chronic to late stage, and ω_3, the rate of HIV/AIDS disease mortality. μ_p is the death rate. The rates Λ_i are the HIV forces of infection (hazard rates of infection) experienced by the susceptible populations given by:

$$\Lambda_i = \rho_{S(i)} \times \sum_{j=1,2,3,4} \left(\sum_{\alpha'=1,2,3} t_{I_{\alpha'}(j) \to S(i)} \Psi(i,j) \frac{\rho_{I_{\alpha'}(j)} I_{\alpha'}(j)}{\rho_{S(j)} S(j) + \sum_{\alpha''=1,2,3} \rho_{I_{\alpha''}(j)} I_{\alpha''}(j)} \right) \quad .$$

Here, $\rho_{X(i)}$ describes the new sexual partner acquisition rate for each population $X(i)$. The expression $\Psi(i,j)$ represents the mixing among the four risk groups. It estimates the probability that an individual in risk group i would choose a partner in risk group j (Garnett and Anderson, 1993) and is described by:

$$\Psi(i,j) = e\delta_{i,j} + (1-e)\frac{\rho_{s(j)}S(j)\sum_{\alpha'=1,2,3}\rho_{I_{\alpha'}(j)}I_{\alpha'}(j)}{\sum_{k=1,2,3,4}\left(\rho_{s(k)}S(k)+\sum_{\alpha'=1,2,3}\rho_{I_{\alpha'}(k)}I_{\alpha'}(k)\right)}.$$

Here, $\delta_{i,j}$ is the identity matrix and the parameter $e \in [0,1]$ measures the degree of assortativeness in the mixing. For example, at the extreme $e=0$, the mixing in the population is fully proportional, while at the other extreme $e=1$, individuals mix in assortative fashion exclusively.

The parameter t_{I_α} represents the HIV transmission probability per partnership in a partnership between a member of the susceptible population $S(j)$ and a member of the HIV infected population $I_\alpha(i)$. This value is calculated using the binomial model

$$t_{I_\alpha} = 1-(1-p^{HIV}_{I_\alpha(i)\to S(j)})^{n_{I_\alpha(i)\leftrightarrow S(j)}\tau_{I_\alpha(i)\leftrightarrow S(j)}},$$

which includes the HIV transmission probability per sexual contact per HIV stage ($p^{HIV}_{I_\alpha(i)\to S(j)}$), the frequency of sexual contacts per HIV stage in this partnership ($n_{I_\alpha(i)\leftrightarrow S(j)}$), and the duration of the partnership ($\tau_{I_\alpha(i)\leftrightarrow S(j)}$). Assumptions for parameter values are summarized on Table B.1.

I focused on the study of the endemic equilibrium solutions of the system, which yields the following expressions for the population variables in terms of the force of infection and other parameters:

$$\overline{S_i} = \frac{\mu N_{0,i}}{\mu + \Lambda_i}$$

$$\overline{I_{i,1}} = \frac{\mu N_{0,i}}{\mu + \omega_1}\left[\frac{\Lambda_i}{\mu + \Lambda_i}\right]$$

$$\overline{I_{i,2}} = \frac{\mu \omega_1 N_{0,i}}{(\mu + \omega_1)(\mu + \omega_2)}\left[\frac{\Lambda_i}{\mu + \Lambda_i}\right]$$

$$\overline{I_{i,3}} = \frac{\mu \omega_1 \omega_2 N_{0,i}}{(\mu + \omega_1)(\mu + \omega_2)(\mu + \omega_3)}\left[\frac{\Lambda_i}{\mu + \Lambda_i}\right].$$

The equilibrium solution was found through convergent successive approximations. Once the force of infection per risk group was determined, I calculated the total prevalence and incidence using the different values for the $p^{HIV}_{I(i)\to S(j)}$ with and without co-infection.

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Table B.1 Co-infection between-host parameter values

Parameter	Value	Reference
HIV transmission probability per coital act per stage of infection (p_i^{HIV})		
Acute stage	0.036	(Hollingsworth et al., 2008; Pinkerton, 2008; Wawer et al., 2005)
Chronic stage	0.0008	(Wawer et al., 2005)
Late stage	0.0042	(Wawer et al., 2005)
Duration of each HIV stage ($1/\omega_i$)		
Acute stage	49 days	(Pinkerton, 2008; Wawer et al., 2005)
Chronic stage	9.0 years	(Morgan and Whitworth, 2001; UNAIDS, 2007; UNAIDS/WHO)
Late stage	2.0 years	(Wawer et al., 2005)
Frequency of coital acts per HIV stage ($n_{I_\alpha(i)\to S(j)}$)		
Acute stage	10.6/month	(Wawer et al., 2005)
Chronic stage	11.0/month	(Wawer et al., 2005)
Late stage	7.1/month	(Wawer et al., 2005)
Fraction of the initial population size in each risk group		
Low risk	65%	(Ferry et al., 2001)
Low to intermediate risk	23%	(Ferry et al., 2001)
Low to high risk	9.5%	(Ferry et al., 2001)
High risk	2.5%	(Morison et al., 2001a)
Net sexual partner acquisition rate ($\rho_{X(i)}$)		
Low risk	0.095 partners/year	(Abu-Raddad et al., 2008)

Low to intermediate risk	1.30 partners/year	(Abu-Raddad et al., 2008)
Low to high risk	5.46 partners/year	(Abu-Raddad et al., 2008)
High risk	35.78 partners/year	(Abu-Raddad et al., 2008)
Duration of sexual partnership ($\tau_{Y_a(i)\leftrightarrow S(j)}$)		
Low risk with low risk	48 months	(Abu-Raddad et al., 2008)
Low risk with low to intermediate risk	36 months	(Abu-Raddad et al., 2008)
Low risk with intermediate to high risk	24 months	(Abu-Raddad et al., 2008)
Low risk with high risk	12 months	(Abu-Raddad et al., 2008)
Low to intermediate risk with low to intermediate risk	24 months	(Abu-Raddad et al., 2008)
Low to intermediate risk with intermediate to high risk	12 months	(Abu-Raddad et al., 2008)
Low to intermediate risk with high risk	6 months	(Abu-Raddad et al., 2008)
Intermediate to high risk with intermediate to high risk	6 months	(Abu-Raddad et al., 2008)
Intermediate to high risk with high risk	1 months	(Abu-Raddad et al., 2008)
High risk with high risk	1 week	(Abu-Raddad et al., 2008)
Duration of sexual lifespan ($1/\mu$)	35 years	(Abu-Raddad et al., 2008)
Degree of assortativenes (e)	0.224	(Abu-Raddad et al., 2006; Buve, 2001a)

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