

**EPIDEMIOLOGY OF POLYPARASITISM IN COASTAL KENYA:
DETERMINANTS, INTERACTIONS AND HEALTH EFFECTS OF
PLASMODIUM SPECIES AND *SCHISTOSOMA HAEMATOBIIUM* INFECTIONS**

by

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**To my mother, who would be so proud,
my father, who is,
and my daughter, who will be.**

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ABSTRACT

Concurrent infections with multiple parasites are common in human populations inhabiting tropical regions of the world. Although morbidities associated with single parasite infections are well-established, much less is known about the risk factors for co-infection, the epidemiological and biological associations among parasite species, or the related health effects of polyparasitism. This cross-sectional study of *Plasmodium* species and *Schistosoma haematobium* co-infections among people in a rural village of coastal Kenya was conducted to address some of these questions.

Predictors of polyparasitic infections were identified within the social, environmental and spatial context of households. The relative importance of individual-versus household-level factors in predicting parasite infection also was examined using multi-level modeling techniques, thereby providing insights into mechanisms by which socio-economic position (SEP) and other factors might influence disease risk. In addition, evidence for biologically meaningful associations between parasites was evaluated after adjustment for household clustering of individuals. Finally, potential synergistic relationships between these infections and their effects on anemia and stunting in children were assessed.

Results revealed a heavy burden of parasitic infection in this population, especially in children. Intense *Plasmodium* species and *S. haematobium* infections were found to cluster in a subset of children with suggestions of synergistic effects on anemia and stunting. Determinants of heavy infections were age-specific and included household SEP. Individual-level characteristics explained much more of the household-level variation in infection than did household-level variables. Finally, analyses of species-

specific *Plasmodium* infections demonstrated fewer co-infections than expected by chance, suggesting the presence of cross-species interaction.

This research highlights the unacceptable burden of parasitic disease in tropical regions of the world, and suggests that integrated control efforts which consider multiple infections, and which are targeted at school-aged children, should maximize disease reduction under resource-limited conditions.

CHAPTER 1

INTRODUCTION

A substantial proportion of the world's disease burden is caused by pathogenic microbes that lead to severe morbidities and death among millions of individuals. Residents of sub-Saharan Africa carry a disproportionate amount of this disease burden due to combinations of social and environmental conditions that favor parasitic disease transmission. Of the plethora of parasite species endemic to this region, *Plasmodium* spp., *Schistosoma haematobium* and *Wuchereria bancrofti* are among the most well-known due to their associated morbidities. *Plasmodium* spp. infections lead to over 247 million reported cases of malaria each year, causing close to 1 million deaths [1]. Over 90 percent of the malaria deaths occur in African children under the age of 5. Schistosome species infect an estimated 200 million people worldwide, causing severe morbidity in several million [1]. Each year, due to *Schistosoma haematobium* infection, 70 million people suffer from haematuria, 18 million from bladder wall pathology, 10 million from hydronephrosis, and 150,000 people die from kidney failure [2]. Eight-five percent of schistosomiasis cases occur in Africa [3]. *Wuchereria bancrofti*, the parasite responsible for 90 percent of the world's lymphatic filariasis cases, infects 120 million people in various parts of the world, 40 million of whom suffer incapacitating, disfiguring, and stigmatizing morbidity. One third of those affected reside in Africa.

One important risk factor for such parasitic infections is poverty.

Schistosomiasis, malaria and lymphatic filariasis are all considered diseases of poverty because poor social and environmental conditions increase the risk of these diseases [4-6]. Lack of safe water supplies, inadequate sanitation, insufficient access to health care and prohibitive treatment costs all contribute to disease transmission and high morbidities [7, 8]. This is true both on a global scale as well as on smaller micro-scales measured at the village, neighborhood, household or individual level. Additionally, there seems to be a feedback mechanism by which heavy parasitic disease burdens serve to economically disadvantage populations, which in turn increases the risk of infection [9, 10]. Without effective intervention, the gap in economic status and health between heavily infected countries and those without infection will continue to grow.

While the relationship between poverty and parasitic diseases may be clearly established, the mechanisms by which wealth or socioeconomic position (SEP) predicts an individual's or a population's risk of infection require elucidation. Vector-borne disease transmission dynamics are complex and include social, environmental and spatial domains all of which may be influenced by an individual's SEP. Examining disease risk in an individual without considering the context in which transmission occurs increases the risk of individualist fallacy which introduces bias and can lead to faulty inference [11]. The analyses included in this dissertation identify risk factors for multi-parasitic infections within the social, environmental and spatial context of the household. The relative importance of individual- versus household-level factors in predicting parasitic infection is examined, providing insight into potential mechanisms by which SEP and other factors influence transmission and disease incidence. Although similar studies have

been conducted on individual infections [12-14] few have focused on polyparasitism [11, 13]. This dearth of polyparasitism research hinders our ability to accurately assess the disease burden attributable to individual infections and hampers development of informed policy decisions.

A better understanding of polyparasitism is also essential from a clinical, biological and pharmaceutical perspective. Laboratory and preliminary field studies have indicated that effects of attenuation or competition from multiple parasite species within one human host may play an important role in disease dynamics [15-17]. Also, evidence demonstrates that the therapeutic effects of pharmaceutical treatments and vaccines may be reduced by the presence of other parasites [18, 19]. With the massive financial resources being spent on vaccine and pharmaceutical development, these underlying parasitic infections are costly both to economies and to human health.

Finally, research on polyparasitism allows the identification of common risk factors for multiple infections that could help streamline prevention and control efforts. Similarly, concrete examples of the effect of multiple infections on health outcomes might help to motivate intervention. Due to the great burden of disease imposed by parasites in tropical locations, determining the most effective means of intervention has the potential to preserve the health of millions of individuals.

MALARIA

Impact

Malaria is the parasitic disease responsible for the greatest morbidity worldwide. Globally, over 2 billion individuals are at risk for malaria leading to over 250 million cases annually and over 1 million deaths (Figure 1.1). The vast majority of these deaths

occur in children under five years of age in sub-Saharan Africa. Malaria morbidity can involve anemia, hypoglycemia, jaundice, hepatic dysfunction, splenomegaly, pregnancy complications including miscarriage, premature birth, and low birth weight, and pulmonary edema, with cerebral malaria characterized by convulsions and coma [20]. Severe malarial anemia may be responsible for as many deaths as cerebral malaria, with peak incidence occurring in children less than two years of age [20]. Malaria also has severe economic implications, causing endemic countries to lose an average of 0.5-1% of annual GDP [5, 9, 10].

In Kenya, malaria is the leading cause of morbidity and mortality, accounting for 30% of outpatient visits in 1999. Approximately one in five malaria cases required inpatient services. Malaria causes 5% of total deaths in Kenya with an estimated 26,000 children dying annually [21].

Natural History

Malaria is caused by single-celled protozoan parasites of the genus *Plasmodium*. Four species, *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae* infect humans by entering the bloodstream via the bite of an infected female mosquito of the genus *Anopheles*. The greatest worldwide mortality is caused by *P. falciparum* [20] and this species is found throughout tropical Africa, Asia and Latin America. The *P. malariae* species is found worldwide but with a patchy distribution whereas *P. ovale* is endemic mainly in tropical West Africa. Finally, *P. vivax* is found worldwide in tropical regions, as well as in some temperate zones [5, 22].

Upon entry into the human bloodstream, *Plasmodium* spp. parasites migrate to the liver where they multiply exponentially and proceed to infect red blood cells (Figure 1.2).

Gametocytes are formed and are ingested by mosquitoes with blood meals. A reproductive phase occurs inside the mosquito and the resulting sporozoites are transmitted to other humans if the infected mosquito takes another blood meal [5, 22].

Epidemiologic profile

The distribution of *Plasmodium* spp. infections in human populations depends on levels of endemicity. In holoendemic zones, most severe morbidity and mortality occurs in young children who have not acquired the partial immunity accorded to older individuals by repeated infection [9]. Peak infection occurs in children between one and five years of age in these zones and 25% of all-cause mortality in children aged 0-4 years is due to malaria [23, 24]. In hypoendemic zones with occasional introduction of the parasite malaria epidemics can occur with severe morbidity and mortality in all age groups due to lack of immunity [25]. Some areas have seasonal periods of high transmission often following rains.

Sex does not appear to be an important factor in determining risk of infection biologically, with the exception of pregnant women, although gender-specific risk behaviors may influence the distribution of malaria in some populations [23]. Some genetic factors play a role in determining susceptibility to disease, including presence of the hemoglobin S allele, red blood cells negative to Duffy blood group determinants, and deficiency of glucose-6-phosphate dehydrogenase enzyme [23, 25].

Household socioeconomic factors

Although poverty is clearly associated with malaria at the global level, individual- and household-level associations are more difficult to discern. A recent review of the literature found that conclusions depend on the definition of poverty used such that

income expenditure or asset ownership are not associated with malaria incidence, whereas occupation, housing type and rural location do show strong associations with malaria [26]. Evidence relating poverty to uptake of interventions and treatment seeking is more straightforward, with a number of studies showing that poorer groups are less likely to use insecticide-treated nets (ITNs) and chemoprophylaxis or to access private health providers, and are more likely to self-treat than are richer groups [26]. SEP is also likely to affect risk of malaria by determining household location [27] and crowding within a household [28].

Knowledge, attitudes, and practices

Knowledge, attitudes and practices (KAP) of malaria risk factors, transmission, symptoms, and prevention are potentially important factors of disease avoidance behaviors and consequently of disease prevalence. However, knowledge does not always translate into behavior change and the processes by which individuals' knowledge regarding malaria affects behaviors are influenced by other factors such as gender, age and SEP (reviewed in [26]). A recent study in Kenya compared the behavioral and socioeconomic factors associated with malaria prevention in two urban environments. Results showed a significant association between wealth, education and use of mosquito nets in both locations, but associations between use of multiple prevention activities and wealth and education differed between cities [29]. The effect of knowledge on health-seeking behaviors is also likely to vary depending on infrastructural and community realities such as distance to health centers, availability and cost of treatment and quality of available health care [30, 31].

Environmental/Ecologic Factors

Malaria, like most vector-borne diseases, is highly influenced by environmental and ecologic factors due to the habitat requirements of the mosquito vectors species. Perhaps the most important environmental factors in disease transmission are temperature and humidity [25]. Malaria parasites require a minimum temperature for development and mosquito vectors require temperature and humidity conditions that allow a long enough life span to permit parasite development and multiple blood meals. Larval habitats for mosquito vectors are influenced by both climatic factors such as rainfall and temperature, as well as by human factors such as use of irrigation for agriculture, dam construction and other alterations of the landscape that create pooled water.

The household environment must also be considered in this discussion. Housing factors such as the presence of open eaves, covered windows or doors, screens and bednets are important in determining an individual's risk of exposure. Presence of potential breeding sites in the vicinity of the house may also be influenced by human behaviors such as clearing of vegetation, agricultural practices, sanitation practices and household water use behaviors.

Data from entomologic and parasitological studies conducted along the Kenyan coast reveal considerable heterogeneity in distribution of vector species, transmission intensities and importance of rainfall throughout the study area [32, 33]. Densities of the endemic vector species in Kwale District were not influenced by rainfall, whereas one and three month lag effects of rainfall on vector densities were seen in the other districts studied. These results suggest significant environmental heterogeneity in aquatic habitats in the region [32]. Another Kenyan study showed evidence of household environments

influencing mosquito populations in that extensive clustering of mosquitoes was seen at the household level throughout the year [33].

Geographic distribution and spatial modeling

The spatial distribution of *Anopheles* spp. larval habitats and of human domiciles is an important element in predicting patterns of malaria prevalence. Large scale mapping projects have used Landsat imagery along with information on temperature, elevation and precipitation to predict areas of varying levels of malaria transmission. Although important in the development of national or regional prevention and control efforts, such large-scale mapping studies ignore the significant heterogeneity in community level environments that influences individual risk of infection. As mentioned above, studies from coastal Kenya suggest that significant micro-level environmental heterogeneities exist and that they may influence parasite transmission dynamics [32]. Entomologic and parasitologic studies of micro-level spatial distributions should help to locate vector preferences for breeding sites, as well as hotspots of human disease that could be targeted by control and prevention efforts.

SCHISTOSOMIASIS

Impact

Urinary schistosomiasis is a parasitic disease infecting 200 million people worldwide (Figure 1.3). Endemic in 74 countries, 85% of cases occur in sub-Saharan Africa [1]. Schistosomiasis is responsible for nearly two million disability adjusted life-years lost and caused 15,000 deaths in 2002 [6]. Twenty million infected individuals worldwide suffered from severe morbidity and 200,000 died in 2003 [6]. In sub-Saharan Africa, schistosomiasis is second only to malaria in causes of morbidity. Schistosomiasis

is also growing as a public health problem due to exploding population growth and increase in suitable aquatic snail habitats due to construction of hydroelectric dams, agricultural irrigation systems and watering sources for domestic livestock [34].

Nineteen species of schistosomes exist in the genus *Schistosoma* of which five complete normal development in humans. The three major species pathogenic in humans are *S. haematobium*, *S. mansoni*, and *S. japonicum*, with two minor species, *S. mekongi* and *S. intercalatum*, also responsible for schistosomiasis infection over a more restricted distribution [6]. *Schistosoma haematobium* causes cases of urinary schistosomiasis in which pathogenesis affects the urinary tract and transmission occurs via excretion of parasite eggs in the urine. Morbidity includes hematuria, anemia and undernutrition with chronic infections responsible for scarring and dysfunction of the ureters, bladder and kidney [35]. Rare pulmonary complications are reported due to eggs in the lung capillaries as is transverse myelitis from pressure due to response to presence of eggs around the spinal cord. Bladder cancer is responsible for the majority of mortality in urinary schistosomiasis cases, especially in certain predisposed populations [35]. Urinary schistosomiasis-associated morbidity is high due to the chronic nature of the infection but morbidity is also likely to be underestimated due to symptoms that are common to many parasitic infections such as anemia and low growth rates [35].

Infection and disease in the human host is a multi-factorial process mediated by ecologic, social, genetic and immunological factors; however, the intensity of infection seems to be of crucial importance both in transmission and in morbidity [36]. Infection is not randomly distributed in endemic populations, rather is aggregated with heavy infection occurring in only a subset of the population. This phenomenon has important

consequences for the epidemiology, clinical morbidities and transmission dynamics of the disease and knowledge of these parameters will be essential in creating effective intervention and control programs [36].

Natural History

Urinary schistosomiasis is caused by a trematode flatworm *Schistosoma haematobium*. Lifecycles of the parasite require both a human and a snail host (Figure 1.4). Upon human infection juvenile flukes develop in the human liver, pairing as they reach maturity. Paired adults then migrate to the veins around the bladder. Sexual reproduction occurs and eggs must traverse the walls of the bladder, enter the lumen and be voided. They must then reach fresh water where they hatch and release miracidia which are free-living larval forms of the parasite. Continued development then requires a competent snail host (*Bulinus* spp.) in which asexual multiplication will occur and large numbers of cercariae will be produced. Cercariae are released into the water but will die unless they penetrate the skin of an appropriate human host. Upon penetration of the host skin, cercariae transform into schistosomulae, enter the vascular system, and migrate to the liver where the cycle begins again [34].

In coastal Kenya, *S. haematobium* is the sole endemic schistosome species within 100 km of the coast with *S. mansoni* occurring further inland [37]. The *Bulinus africanus* group of snails is responsible for local transmission with *Bulinus nasutus* being the sole snail host in the study region of interest for this research. It has been hypothesized that the lack of *S. mansoni* transmission in the area is due to the intolerance of *S. mansoni*-transmitting snails, *Biomphalaria* spp., to the high coastal temperatures [38].

Epidemiologic profile

Urinary schistosomiasis infection is not evenly distributed among individuals in endemic populations. Typical age profiles show the highest prevalence and the highest intensity of infection in school-aged children [39]. Lower prevalence of infection in adults is explained by lower exposures to infection sites but also by the development of partial immunity in highly endemic areas [35], while the low prevalence in young children is due to lower rates of exposure. Gender profiles of *Schistosoma* spp. infection are more variable across populations due to cultural differences in water use behaviors leading to differing levels of exposure. Research has found sex differentials in infection intensities [14, 40], which is hypothesized to be the result of differential duration and extent of exposure by gendered domestic activities as well as the effect of occupation on exposure [12, 41, 42]. However, other studies in the area have found no evidence of a sex effect on infection prevalence [43].

Schistosomiasis morbidities are also highly prevalent and unevenly distributed by age and sex across endemic populations. A recent study from coastal Kenya found that 51% of participants from a five village area experienced hematuria (blood in urine), 14% had bladder abnormalities and 1.2% had hydronephrosis [40]. Hematuria and bladder abnormality age distributions mimicked the typical prevalence and intensity of infection age distributions peaking in children 10-14 years of age, whereas hydronephrosis displayed a bimodal distribution with a peak in 5-24 year olds and another in those over 45 years of age [40]. In that study, males experienced heavier infections levels and were more likely to experience morbidity than were females [40].

Household Socioeconomic Factors

Socioeconomic factors have long been recognized as playing an important role in the dynamics of schistosomiasis infection. An individual's resources define options for water sources and use, modes and locations of latrines, occupation, access to treatment, location of domicile, and education, all of which influence risk of exposure to infection or re-infection [44]. Empirical research suggests that occupation is highly predictive of prevalence of *Schistosoma* infection [42]. In Egypt, fishermen and boatmen experienced the highest rates of *S. haematobium* and *S. mansoni*, with farmers and farm laborers also experiencing high rates. Domestic servants, factory workers and clerical workers all had lower rates of infection [45]. A Brazilian study similarly revealed high risk of infection, elevated infection intensity and high rates of splenomegaly in manual workers [46]. These findings are not surprising given that these higher risk occupations involve contact with potentially exposed water sources. The relationship between education and risk of schistosomiasis is less clear, with empirical research revealing equivocal findings [42].

The complexity of *Schistosoma* transmission, involving immunologic, environmental, spatial and social factors, highlights the importance of context in any study of infection. The household is proposed as an appropriate context for studying polyparasitism in the present studies. Several researchers have stressed the importance of the household as a setting for studies of disease, as biological, social and environmental transmission factors interact complexly at the household-level [7, 47]. Kloos and colleagues argue that while the household may be an appropriate context for the study of schistosomiasis exposure risk in Brazil, the patterns of water use reported in studies from Africa require a community-based approach to risk assessment [48]. This suggestion was

tested empirically in Chapter 3, in which the relative importance of household risk factors to disease transmission was evaluated. Many authors have discussed the importance of the domicile as a context for schistosomiasis transmission [12, 14, 41, 49]. Studying risk factors for disease at the individual level ignores the sharing of water resources, the partitioning of water-related household activities and the social patterning of behaviors that occur in families. Incorporating household-level measures into studies of schistosomiasis infection allows analyses of relative importance of individual-level versus household-level factors on disease risk.

Knowledge, attitudes, and practices

Similar to socioeconomic factors, KAP regarding individual water contact behaviors and risk of schistosomiasis disease are likely to be influenced by the cultural, geographical, and ecological realities of a given local context. In areas where schistosomiasis morbidity is considered secondary to concerns of hunger, poverty and lack of services, KAP is not likely to predict risk of disease. This phenomenon was observed in Egypt where contact with infected water persisted despite high levels of knowledge regarding schistosomiasis risk and morbidities [50]. Conversely, in a Malawian study, knowledge of disease outcomes and infection control was positively associated with adherence to prevention and control procedures [51]. Alternatively, those with the least knowledge of schistosomiasis may exhibit severe infection avoidance behaviors out of fear of disease, as was reported by one study in the Philippines [52]. Within different cultural and geographic contexts, knowledge of schistosomiasis may have variable effects on risk of disease.

An improved understanding of the role of disease-specific KAP in predicting disease will facilitate decisions of resource allocation in intervention efforts. Some recent studies in Kenya suggest that vertical control programs were more effective than were community mobilization and health education in reducing infections [53]; however, long-term reductions in schistosomiasis disease burdens are likely to require multiple approaches to control efforts, and to rely on community participation.

Environmental and ecologic factors

Environmental and ecologic factors are essential elements of any vector-borne disease transmission system. Schistosomiasis is no exception. The climatic and biologic conditions necessary for this parasite's life-cycle constrain the geographic distribution of disease and provide potential areas for intervention when they are well understood. In the study area of this project, more than 20 years of malacologic data [54] as well as more recent ecologic studies [37, 55] have provided a thorough characterization of vector species, habitat and distribution.

These local studies highlight the variable nature of schistosomiasis transmission in both time and space. Transmission is seasonally influenced by both temperature and rainfall. Temperature affects both the mortality and the fecundity of snail populations, which adds seasonal and diurnal cycles to transmission dynamics [34]. Most importantly, rainfall provides the water necessary for aquatic snail habitats but also causes flooding which may radically alter habitat conditions. Rainfall is also seasonal and prolonged droughts have mortal effects on snail populations despite the ability to survive short dry periods through aestivation [34]. Vector distribution is also variable on a smaller spatial scale with certain zones of water bodies harboring high concentrations of snails while

other zones are snail free [37, 56, 57]. Human risk behaviors also vary with landscape and seasons since water use and excretion behaviors show seasonal variation [41].

Biotic as well as abiotic environmental factors are important in the dynamics of schistosomiasis transmission. Biotic factors influencing vector populations include vegetation, food supplies, and the influence of predators, competitors, and pathogens [34, 37]. A study in the Msambweni area in Kenya found that the presence of *Nymphaea* spp water lilies was highly predictive of a water body being infested with *Bulinus nasutus* snails, whereas *Cyperus exaltatus* sedges were negatively associated with presence of these snails [37]. Possibly important abiotic factors determining microhabitats of snail populations include calcium and electrolyte levels, NaCl levels and pH, as well as the geomorphology of rocks and soil, and water turbidity.

Geographic distribution and spatial modeling

As snail vector populations are unevenly distributed over the landscape so are human populations spatially heterogeneous. The spatial distribution of human domiciles and of water sources is an important piece of information in predicting infection patterns as it has been shown to influence exposure behaviors [44, 48, 55, 58]. A study in a village near to Kingwede, Kenya (the study site of this dissertation research) found evidence of age- and sex-specific case clustering to the east of an infected pond. Given the limited heritability of *S. haematobium* susceptibility [40], this pattern of clustering is most likely due to age and sex differences in water contact behavior [55].

While spatial mapping of infection has gained popularity at the national and regional level for use in risk prediction, micro-level spatial studies are also needed to

improve understanding of transmission dynamics and to pinpoint appropriate targets for control efforts [59, 60].

POLYPARASITISM

Transmission of *S. haematobium*, and *Plasmodium* spp. is widespread in coastal Kenya resulting in high prevalence of urinary schistosomiasis, and malaria in the region. These chronic vector-borne infections share common risk factors and distributions, and may, therefore, interact in the perpetuation of human disease. Further elucidation of shared risk factors and of the potential antagonistic or synergistic relationships between these infections is needed.

Prevalence

Despite the paucity of research on polyparasitism, published research has firmly established the high prevalence of multiple parasitic infections across a range of populations [61-68]. Few studies on polyparasitism, however, explicitly state the prevalence, incidence or intensity of co-infection, choosing instead to focus on either species-specific associations [15, 16, 60, 61, 64, 66, 69-78] or related morbidities [62, 68, 79-88]. Thus, reliable prevalence estimates of *Plasmodium-S. haematobium* co-infection are rare.

The handful of studies that focused on these two infections in Africa did not report prevalence of joint infection [15-17, 74, 89]. Despite the ubiquity of co-infection throughout sub-Saharan Africa, the prevalence of co-infection is likely to vary over endemic regions due to geographic, climatic, and host differences that influence susceptibility and exposure to infection agents.

Risk Factors

The lack of well-designed and adequately-powered polyparasitism studies renders the identification of common risk factors difficult. Social, environmental, and spatial characteristics deserve consideration in such studies due to the importance of context in the transmission dynamics of parasitic diseases. In particular, vector-borne diseases, like malaria and schistosomiasis, are predicted by a complex set of interwoven risk factors determined by hosts, agents, environments and the interactions among them. Some of the most relevant characteristics for this discussion include host factors such as age or sex, socially determined behaviors such as water contact patterns or use of bednets, and household factors such as SEP and location.

Age of hosts is strongly associated with many parasitic diseases, and therefore with co-infections. This is due to both human biology, such as immune responses, and age-specific behaviors that determine exposures to infectious agents. In the case of co-infection with *Plasmodium* and *S. haematobium* parasites, children have higher prevalence than adults for both [67, 68], but the age range experiencing peak prevalence is context-specific. Sex is another host characteristic that may be associated with co-infections. However, little evidence of differences in *Plasmodium* spp. infection between sexes exists, aside from the known increased risk associated with pregnancy [90]. Sex-determined trends in *S. haematobium* infection vary across studies, possibly due to regional and cultural differences in water contact patterns [42, 45, 46, 48, 52, 91, 92]. Another important risk factor for multiple parasitic infections is poverty. Low SEP has been shown to be associated with increased risk of both malaria [26, 77, 93, 94] and schistosomiasis [44, 46, 94, 95]. Mechanisms behind these associations are likely to

include increased exposure to infectious agents due to poor hygiene, poor quality of house construction, lack of access to prevention and treatment of disease, increased susceptibility to infection due to poor nutrition and increased transmission due to crowded households. Finally, common spatial and environmental characteristics may predict distributions of co-infections. For example, the distance of residences to local water bodies has been shown to be predictive of both *Plasmodium* infections [96-98] and *S. haematobium* infections [58, 96, 99]. The focal distribution of *S. haematobium* [55, 56] and increasingly recognized spatial heterogeneity of *Plasmodium* infections [96, 98] demonstrate a need for polyparasitism studies focusing on a small spatial scale [100].

Parasite Associations

Identifying trends in the directions and strengths of associations between pairs of parasite species is a daunting task. The plethora of potential combinations, lack of standardized and sensitive diagnostic tools, differences in outcomes measured (i.e. asymptomatic infection vs. clinical disease vs. infection intensity), as well as nonconformity of study population and study site traits render comparison of results across studies challenging. Unbiased estimates of biologically relevant associations between parasites require an understanding and control of shared individual-level and contextual factors. Few published polyparasitism studies have adequately controlled for such confounding [100]. Nonetheless, the current state of knowledge regarding associations between *Plasmodium* and *S. haematobium* requires brief review.

In general, infection with helminth species has been shown to be associated with higher incidence of malaria, but not necessarily higher prevalence of *Plasmodium* infection [19, 71, 72, 101, 102]. Similarly, helminth infections have been shown to be

associated with increased *Plasmodium* parasitemia [74, 77], increased likelihood of mixed *Plasmodium* infection [103], increased gametocytemia [104] and cerebral malaria [74], although a protective effect between *Ascaris lumbricoides* and cerebral malaria has also been reported [105]. One study in southwest Uganda with low, unstable malaria transmission found no association between helminth infection and malaria incidence [70]. Few studies have focused specifically on associations between *Plasmodium* and *S. haematobium* infections. In those that have, patterns of observed associations have been inconclusive. Lyke and colleagues found evidence of delayed time to first clinical malaria episode in Malian children infected with *S. haematobium* as compared to uninfected children, however this association was only significant for young children [15]. Similarly, Briand and colleagues found that children carrying light *S. haematobium* infections had lower densities of *P. falciparum* than did those who were uninfected [16], indicating a negative association between the two parasites when intensity of infection was considered. Finally, Le Hesran and colleagues found 10.3% of children with malaria to be co-infected with *S. haematobium* compared to 6.9% of controls in a Senegal study, although this difference was not statistically significant [74]. Clearly, further investigation of these associations and their biological implications are warranted.

Morbidities

Research on the health effects of polyparasitic infections is limited. Publications reveal associations between concomitant infections and anemia [62, 81, 106], malnutrition (reviewed in [107]), organ pathology [96, 108], low birthweight [80] and self-reported morbidities [68], although others suggest protective effects of co-infections on malaria morbidity [16, 79, 82, 105]. Few studies have specifically investigated the

joint effects of *Plasmodium* and *S. haematobium* parasites on morbidity [107].

Additionally, most analyses do not evaluate interactions between parasites and their effects on morbidities using an additive scale which can be argued to have more biological relevance than multiplicative interactions [81, 109].

SUMMARY

The research presented in this dissertation is aimed at providing a thorough investigation of the associations, risk factors, and morbidities associated with *S. haematobium* and *Plasmodium* spp. co-infections in a coastal Kenyan village, with a focus on the household as the appropriate spatial scale and context to study these relationships. Fine-scale studies are justified due to the focal nature of transmission, as well as the complex interaction of risk factors that determine disease transmission for these infections.

Identification of common risk factors at different levels, and the relative importance of each in predicting individual and joint infections, is important. Indeed, few multilevel modeling studies have been published that deal with infectious or parasitological diseases [13]. An examination of the relative importance of individual- versus household-level risk factors in predicting infection and co-infection will help streamline intervention efforts to focus on heavily infected individuals, on certain communities or on particular environments. Studying the effects of multiple infections on health outcomes such as anemia and undernutrition will aid policy makers in decisions of resource allocations, and will focus attention on much needed reduction of parasitic disease morbidities in the region.

Obviously, much work is left to be done to clarify our understanding of the spatial distribution, risk factors, and associated health effects of polyparasitism. The enormous global burden of parasitic diseases necessitates careful consideration of concomitant infections in order to optimize limited resources develop effective prevention and control strategies. Accordingly, the research presented in this dissertation has been designed to contribute additional new knowledge toward these important goals.

Figure 1.1: Global distribution of malaria [110].

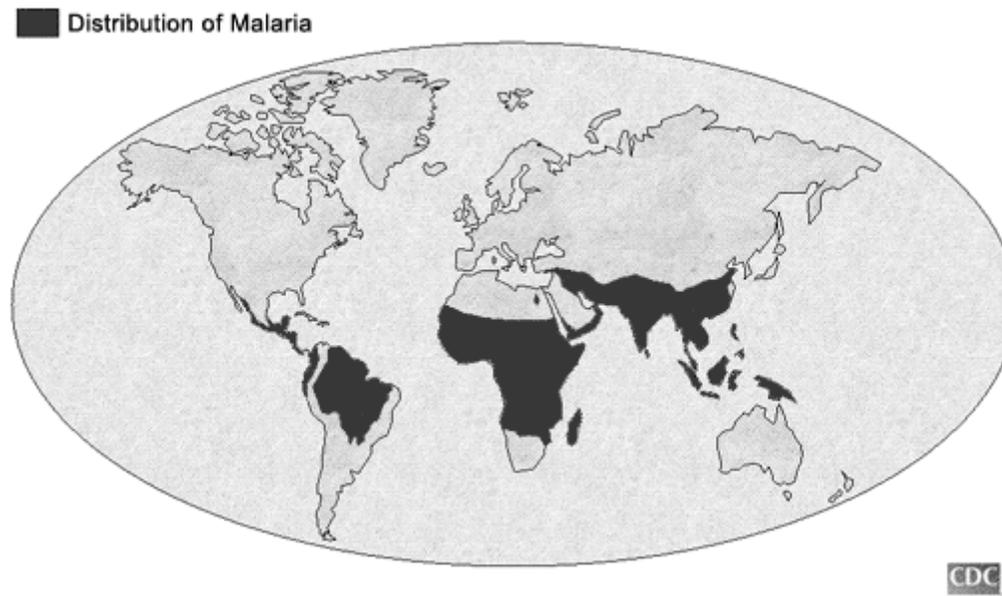


Figure 1.2: Life cycle of *Plasmodium* spp. parasites [111].

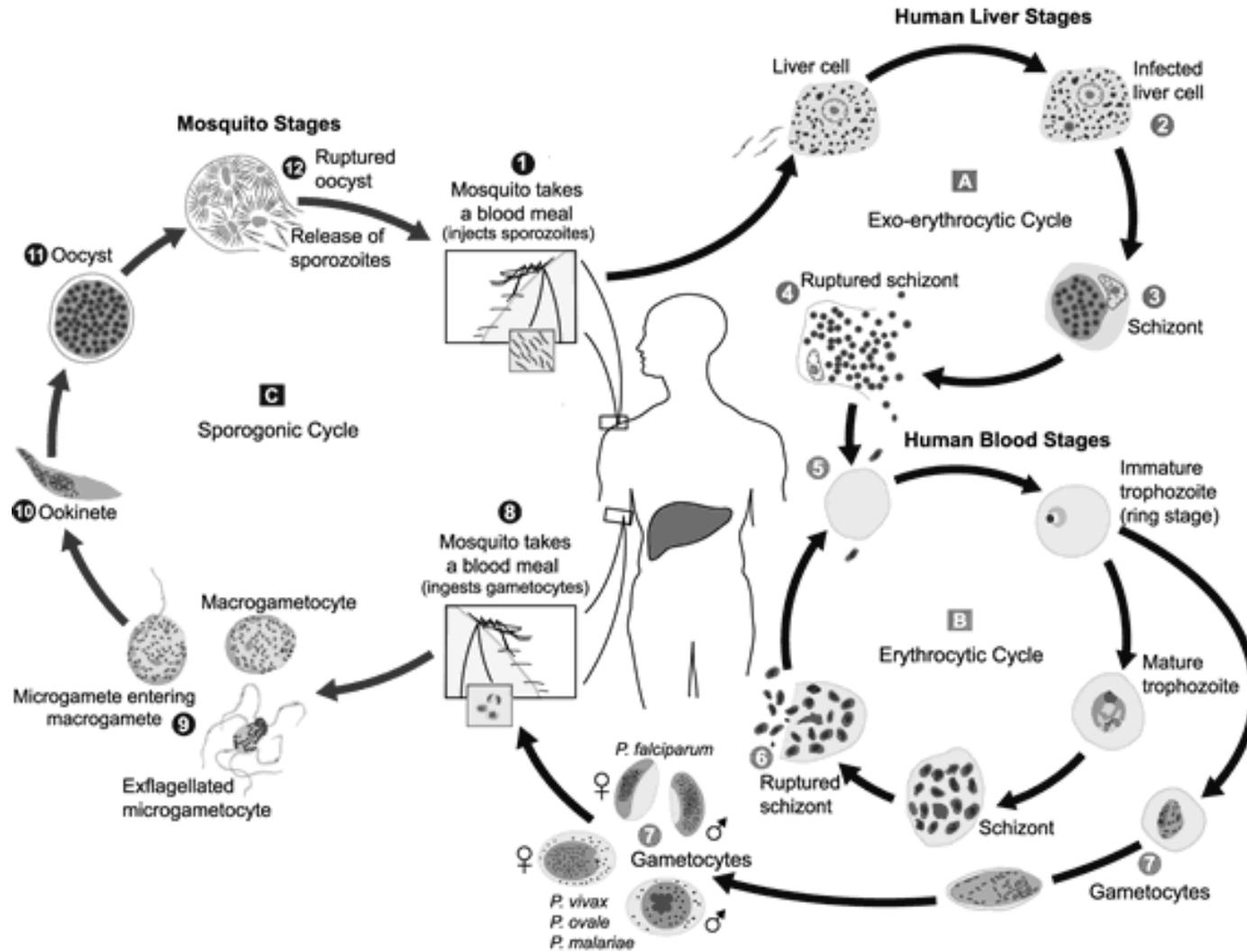


Figure 1.3: Global distribution of schistosomiasis [112].

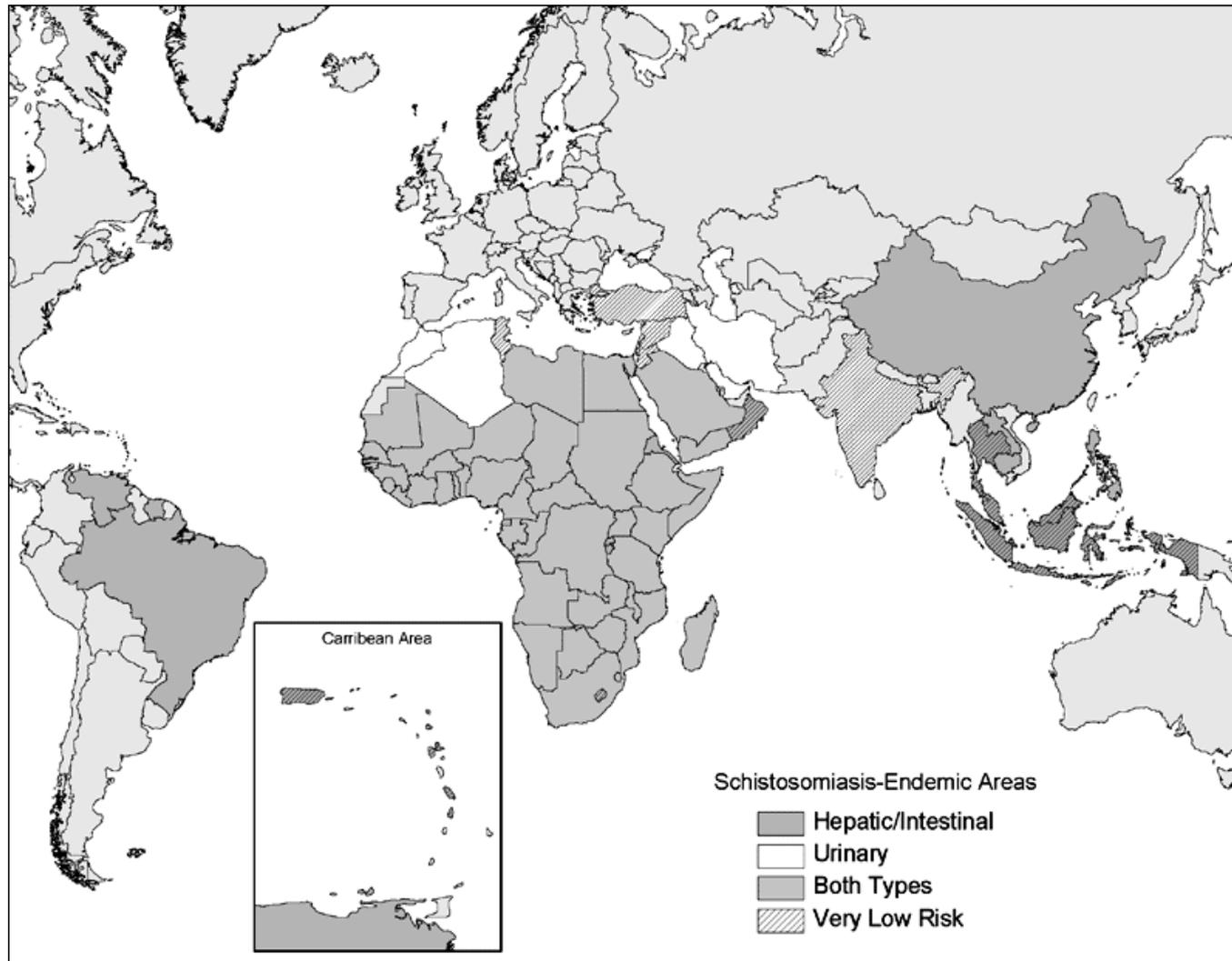
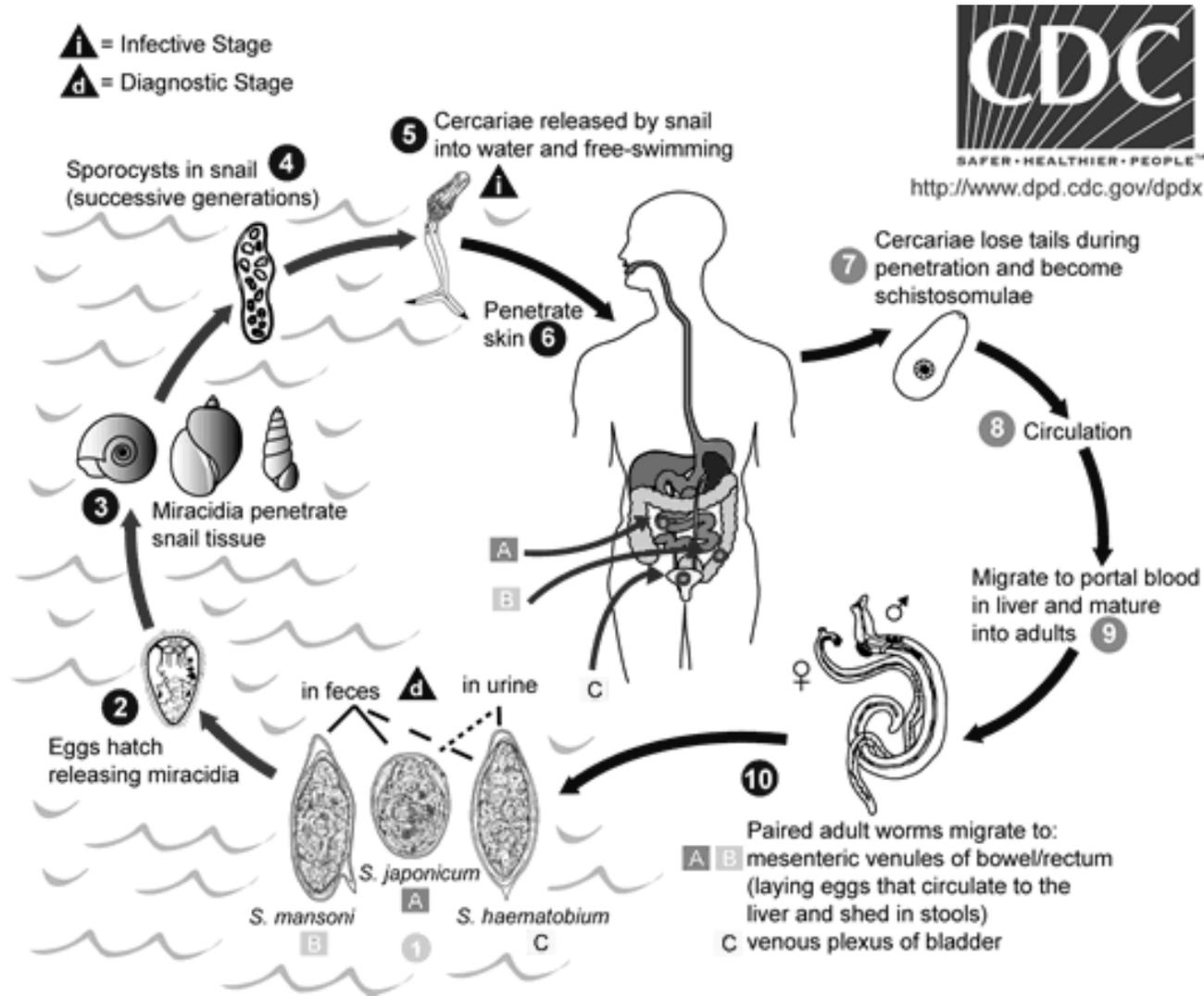


Figure 1.4: Life cycle of *Schistosoma* spp. parasites [113].



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CHAPTER 2

EVIDENCE OF CLUSTERING OF HEAVY *S. HAEMATOBIIUM* AND *PLASMODIUM* SPP. INFECTIONS AMONG CHILDREN IN COASTAL KENYA.

INTRODUCTION

Parasitic infections account for a large proportion of the burden of disease in Kenya, with far-reaching effects on the nation's health and economy. Malaria, for example, represents 30-50% of all outpatient visits to health facilities, causing more than 20% of deaths in children less than 5 years old (yo) [1]. Other tropical diseases such as urinary schistosomiasis and lymphatic filariasis are important "neglected" causes of morbidity in Kenya as well. Urinary schistosomiasis is estimated to affect roughly one-quarter of the Kenyan population leading to anemia, impaired growth, development and cognition [2] and other adverse outcomes. Lymphatic filariasis also causes severe morbidity including elephantiasis of the limbs and hydrocele, often leading to drastic reductions in quality of life [3-5]. The parasites that cause these three diseases are co-endemic in Coastal Kenya subjecting the local population to a disproportionate disease burden.

Despite the importance of these infections on health outcomes, surprisingly little is known about the distributions, causes and effects of co-infections. At the population level, distribution of co-infections will depend on the distribution of determinants of each infection and on the extent to which these determinants are shared across species. Contextual as well as individual-level determinants should be examined since single

infections have been shown to cluster within households [6-11] as well as within individuals [12]. Comparing and interpreting results of polyparasitism studies is complicated by the difficulty of standardizing the many factors contributing to each infection across study sites. Specific species studied, ranges in endemicity of parasites [13], and age distributions of host populations as well as the highly focal nature of schistosome and filarial infections [14, 15] encumber direct comparisons of research results. More studies are needed in order to elucidate true trends from artifact.

Morbidities are also likely to be compounded in people harboring multiple parasites. For example, co-infections with helminths and *Plasmodium* species have been shown to increase negative health effects, including organomegaly [16], low birth weights [17], and anemia [6, 18] as compared to single infections. The morbidity associated with co-infection also is likely to depend on parasite loads [6, 19, 20] as is seen in single-species *Plasmodium* infections [21, 22] and *S. haematobium* infections [23]. Understanding the complexities of the pathogen-host landscape in settings endemic for multiple human parasites is essential for mitigating morbidities and deserves more attention. Identifying interspecies associations could advance intervention efforts by providing high risk targets that might benefit most from prevention and treatment.

To advance knowledge on polyparasitism, this study examined *Plasmodium* spp., *Schistosoma haematobium*, and *Wuchereria bancrofti* infections and co-infections in a community in coastal south-eastern Kenya. The specific research objectives were to 1) describe the prevalence and intensity of single and multiple species infections, 2) identify individual and household-level predictors of single and multiple infections, and 3)

determine associations between infection prevalence and intensities after controlling for contextual variables.

MATERIALS AND METHODS

Study site and population

This study was conducted during 2006 in Kingwede, a rural village in coastal Kenya located ~50 km south of Mombassa on the major road to Tanzania. Kingwede residents are predominantly subsistence farmers and fishermen of the Digo tribe. Electricity and access to piped water are virtually nonexistent in the village. Pumped, potable water is available at 8 locations in the village. A seasonal stream and ponds are used for washing clothes, bathing and swimming. The study site is bordered on the north by the paved road to Tanzania, on the south by the Indian Ocean (3 km) and comprises a ~6 km sq area. Approval for this study was granted by the University of Michigan Institutional Review Board and the Kenya Medical Research Institute (KEMRI).

Data collection

Household Demography. A complete demographical profile of Kingwede was compiled in December 2005. Interviewers identified every house in the study area and collected information (sex, age, relationship to household head and years of residence) on each person who slept in the house the previous night. Subsequently, every individual ≥ 8 years old (yo) was invited to participate in study. Informed consent was obtained from adults. Parental assent and child consent were obtained for each child.

Parasitologic and clinical information. Participants were asked to give two midday urine samples on two consecutive days and one finger prick blood sample. Height and weight measurements were recorded for participants < 18 yo for use in

anthropometry calculations. Fingerprick blood of participants was tested for hemoglobin levels on site using a blood hemoglobin photometer (HemoCue, Inc, Lake Forest, CA).

Presence of *S. haematobium* eggs was determined by microscopic examination of filtered urine. Two 10 ml aliquots of each urine sample were filtered using 12- μ m pore Nucleopore filters (Nucleopore, Pleasanton, CA) and mounted on microscope slides for examination at the study site [24].

Fingerprick blood (~200 μ L) that had been collected in potassium ethylene-diaminetetraacetic acid (K-EDTA)-coated Vacutainer tubes and stored at -20C was further evaluated for presence and quantity of circulating parasites at Case Western Reserve University in Cleveland, OH. *Plasmodium* spp. was detected in these preparations using polymerase chain reaction/ligase detection reaction fluorescent microsphere-based assay (PCR/LDR-FMA) as previously described (Mehlotra, Kasehagen et al. 2002; McNamara, Thomson et al. 2004; McNamara, Kasehagen et al. 2006). Briefly, DNA was extracted, amplified and added to a multiplex, species-specific ligase detection reaction (LDR) where species-specific primers hybridized to target sequences and are subsequently labeled with oligonucleotide probes with fluorescent capacity. A Bio-Plex array reader (Bio-Rad Laboratories, Hercules, CA) is used for detection of fluorescence in a species-specific manner.

Circulating *W. bancrofti* antigens (CAg) were measured using the TropBio Og4C3 enzyme-linked immunosorbent assay (ELISA) kit [25] according to the manufacturer's instructions (TropBioMed, James Cook University Tropical Biotechnology Ltd., Townsville, Australia). Samples responding with >32 U/ml were considered positive and maximum values were set to 32,000 antigen units [26].

Samples were considered positive if they had a median fluorescent intensity (MFI) greater than 2 standard deviations above those of negative controls (MFI above 205, 260, or 220 for *P. falciparum* (Pf), *P. malariae* (Pm) and *P. ovale* (Po), respectively). Positive *S. haematobium* samples had one or more eggs in at least one of two urine samples. *Wuchereria bancrofti* samples were those with a CAg value >127.5. Individuals positive for any *Plasmodium* spp. and positive for *S. haematobium* were considered co-infected.

Intensity of infection was defined as arithmetic mean eggs per 10mL of urine for *S. haematobium*. For *Plasmodium* spp. infections, a standardized measure of MFI was created. All values below the aforementioned cut-off values were set to zero. Subsequently, the MFI were divided by the maximum observed value to give a range from 0 to 1. This method was used for each *Plasmodium* species. A summed value was then created to represent burden of any *Plasmodium* spp. The same method was used to create standardized *S. haematobium* intensity scores. Finally, a summed pathogen burden score was created using the standardized values for *Plasmodia* and *S. haematobium*. Due to the very low prevalence of *W. bancrofti* (5.8%) in the study population, and the resulting low power to detect associations, co-infections with this parasite were ignored in subsequent analyses.

Ordered categorical variables were also created based on the density distribution of each parasite. Intensities of each infection were divided into five, approximately equal categories based on natural cut-points in the distributions (0-4; no infection to heaviest). Finally, binary variables were created to define heavy infections as follows: *S. haematobium* \geq 100 eggs per 10mL urine [23, 27] (15% of individuals testing positive for

S. haematobium); *Plasmodium* spp. ≥ 0.55 standardized score (selected to include the two most heavily infected categories representing 26.9% of *Plasmodium*-infected individuals); and pathogen burden score ≥ 0.55 (chosen by selecting the two most heavily infected categories representing 30.7% of co-infected individuals).

Individual and Household Questionnaires. Once biological measures were obtained, trained interviewers conducted questionnaires with all willing participants. Separate questionnaires were used for adults and for children. These questionnaires included queries aimed at assessing socio-economic position (SEP), knowledge, attitudes and practices (KAP) regarding malaria and schistosomiasis, mosquito avoidance behaviors, and water contact patterns. Household-level information (e.g. ownership of assets) was extracted from questionnaire responses by the household head or most senior household member, and was applied to other household members in analyses. All questionnaire data relied on self-reporting by participants or extrapolation from head-of-household responses. Other household-level information was collected by interviewers who observed house quality (e.g. roofing and construction material, presence of window screens, etc).

Spatial data. The location of each house entrance (latitude, longitude) was measured using a handheld GPS device (Garmin etrex summit, Olathe, KS). A 1 km resolution IKONOS image of the study area was obtained from GeoEye (www.geoeye.com, Dulles, VA) for use in spatial and environmental analyses. ArcGIS v. 9.1 (ESRI, Redlands, CA, USA) was used to create spatial variables such as distances from each house to water sources.

Data Analysis

All data were double entered in Microsoft Access and analyzed using SAS 9.1 (SAS Institute, Inc, Cary, NC). Outcome variables included binary variables (Y/N) for *Plasmodium* spp., *S. haematobium* and co-infection as well as binary variables (Y/N) for heavy *Plasmodium* spp., heavy *S. haematobium* and heavy co-infection determined as described in the methods section. Covariates of primary interest in these analyses were age, sex, knowledge of malaria (MKAP), knowledge of schistosomiasis (SKAP), individual educational attainment, regular income, use of bednets, night outdoor activities, water contact behaviors, and recent malaria diagnosis. Household-level variables included household socio-economic position (SEP) and distance to the local stream. Malaria and schistosomiasis KAP questions (N=3 for each) were used to create a scale (0-3) based on the number of correct responses. Individual educational attainment was measured by highest class completed. Five categories of educational status were created based on quintiles of distributions. Education was ignored in subsequent analyses due to co-linearity with age. Bednet use was treated as a binary variable and did not distinguish between treated and untreated nets. A binary variable was created to measure effective treatment of malaria with medication in the past month. Water contact behaviors were assessed by combining responses to questionnaire data such that participants reporting swimming, fishing, bathing, washing dishes or washing clothes in water sources potentially infected with *S. haematobium* were considered exposed. An asset index, constructed using principal components factor analysis of questionnaire responses, was used to assess household SEP [28, 29]. Assets measured include electricity, radio, television, bicycle, motor vehicle, land ownership, domestic animals

and toilet. The score also included information on crowding, quality of house construction, and numbers of full and part time workers in a household. Missing data necessary for construction of the household SEP score were first imputed using the IVEware (Imputation and variance estimation software) SAS macro [30]. Each individual was assigned an SEP score corresponding to their household and this household SEP score was treated as a continuous variable.

General estimating equation (GEE) models with exchangeable correlation matrix structures and logistic distributions were used to estimate the association between odds of infection, both single and co-infection, and various predictors. This analytic approach accounts for household clustering in the data and estimates fixed effects of variables averaged across households. To identify individual and household-level predictors of single and multiple infections, all individual and household-level variables significantly associated with infection outcomes at $\alpha = 0.1$ were included in multiple logistic GEE models. Backward elimination, in which the least significant effect as identified by Wald tests is removed from the model stepwise, was then employed to select models best predicting the outcomes. Datasets for this analysis were limited to include only participants with complete biological and questionnaire data devoid of any missing responses. Continuous variables were centered on the grand mean. Similarly, intensity of infection among infected individuals was modeled using a binary outcome of heavy versus light infection for each parasite and for co-infection.

To identify potential synergy or antagonism between infections, potential confounders, identified in the literature as important in predicting both infections, were included in logistic GEE models and the association between the two infections was

assessed. Associations were considered significant at an alpha of 0.05 for the Type 3 likelihood ratio test of the co-infection regression coefficient in the full model, controlling for all other variables. Separate models were analyzed for infection prevalence and for infection intensities (as measured by dichotomous variables for heavy infection among infected individuals). The second infection, treated as an independent variable, was included in infection intensity models as an ordered categorical variable (standardized intensity score) with 5 categories (0-4).

RESULTS

Characteristics of the study population

A total of 1,854 persons 8 years of age and older from 460 households was identified in the study area. Approximately half (935) of these individuals from 310 different households chose to participate in the study by contributing samples for parasitological testing. The proportions of the eligible population contributing parasitological samples for each infection and those contributing questionnaire data varied (Figure 2.1). Complete parasitological and at least partial questionnaire data were received for 766 (41.3%) individuals from 252 (54.8%) households. By further restricting the dataset to those with no missing questionnaire responses for the variables needed in subsequent analyses 561 persons from 226 households remained. This study population represented the total eligible population in its age distribution but adult males were underrepresented (30.8% of participating adults were male, 47.1% of the eligible adult population was male).

When the study population was stratified by age, characteristics of children differed greatly from those of adults (Table 2.1). Among children, about half of the

population was male whereas only 31% of adult participants were male. Adults had higher malaria and schistosomiasis KAP scores than did children and more adults reported having a regular income, using bednets and going outside at night. Since only a few children reported having a regular income, this variable was only used in subsequent analyses of infection in adults. Children were more likely than adults to report water contact with potentially infected water sources. Recent consumption of antimalarials was similar among the age groups, as were household SEP scores and distances of households to the local stream.

Infection prevalence

Results show high prevalence of infections in this population (Table 2.1), with over 75% of participating children carrying *Plasmodium* spp. and over 40% harboring *S. haematobium*. Co-infection was seen in 31.8% of children. In adults, the infection prevalence was lower but still substantial with 34%, 14.5% and 5% carrying *Plasmodium*, *S. haematobium* and co-infection, respectively. In contrast, only 2.4% of children were infected with *W. bancrofti*, with 0.5% carrying triple species infections, whereas 8% of adults had *W. bancrofti* infection with 1.2% concomitantly infected with all three parasites. As these percentages indicate, infection distributions differed significantly between children and adults. Children were much more likely than adults to be infected with *Plasmodium* spp. (OR = 6.56, 4.40-9.78), *S. haematobium* (OR=4.09, 2.77-6.04) and to carry *Plasmodium-S. haematobium* co-infections (OR = 9.28 (5.29-16.27)); however, children were less likely than adults to be infected with *W. bancrofti* (OR=0.30, 0.12-0.75). These differences are also illustrated in Figure 2.2 in which prevalence and intensity for single species and co-infection are shown.

Multivariable GEE models of single and multiple-species infection prevalences are presented for both single infections and multiple infections (Tables 2.2 and 2.3). Results of full and reduced multivariable models are presented stratified by age. In children (Table 2.2), odds of *Plasmodium* spp. infection were higher among those who reported not going outside at night and those with lower household SEP, although the associations were not very strong. In adults, odds of *Plasmodium* spp. infection were inversely associated with household SEP, age and malaria KAP. Odds were also lower in adults who reported recent consumption of antimalarial medication. Adult males had higher odds of infection than did females, but this association was not very strong.

Odds of *S. haematobium* infection in children were higher in those with lower schistosomiasis KAP scores, and those living closer to the local stream. Odds were also higher in children reporting water contact with potentially infected water sources. In adults, an opposite association was seen between *S. haematobium* infection and water contact, with odds decreasing with increasing age.

Finally, the odds of co-infection with *Plasmodium* spp. and *S. haematobium* parasites in children were lower in those using bednets, in those who engaged in outdoor activities at night, and in those living farther from the stream, whereas odds of co-infection were higher in those who reported potentially risky water contact. In adults, odds of co-infection decreased with age and with increasing household SEP.

Infection Intensity

As with infection prevalence, the intensity of infection was much heavier in children than in adults (*Plasmodium* spp. (OR=8.09, 4.41-14.81); *S. haematobium* OR = 3.11, 1.31-7.39). Co-infection intensity was also greater in children (OR=9.21, 2.87-

29.59). When restricting analyses to infected individuals, children were still more likely than adults to harbor heavy *Plasmodium* spp. infections (OR = 3.79, 1.97-7.29) whereas this was not the case for *S. haematobium* or co-infections (Table 2.1). In crude analyses, heavy infections were significantly associated (OR=3.15, 1.26-7.89).

Variables associated with heavy infections among infected individuals were identified for each single infection and for mixed infection (Tables 2.4-2.6). Among *Plasmodium*-infected children, bednet use and intensity of *S. haematobium* co-infection best predicted the odds of heavy *Plasmodium* spp. infection, with both variables associated with increased odds. Among infected adults, outdoor night activity and household distance to the stream appeared to be most important in explaining heavy *Plasmodium* spp. infections. For *S. haematobium*-infected children, bednet use was associated with reduced odds of heavy infection as was increasing household distance to the stream. High household SEP and high intensity of *Plasmodium* spp. co-infection appeared to increase likelihood of harboring intense *S. haematobium* infections. In infected adults, men appeared to have slightly higher odds of heavy *S. haematobium* infection than did women, although this association was not measured very precisely (note the wide confidence interval). Finally, heavy pathogen burdens were much less likely in older co-infected children than in younger co-infected children (OR=0.76, 0.63-0.92, for everyone 1 year in age). For adults, only household SEP remained in the reduced model of pathogen burden, with a marginally strong protective effect.

Co-Infection Associations

Univariate analyses indicated that odds of *Plasmodium* spp. infection were higher in individuals co-infected with *S. haematobium* (OR=1.97, 1.34-2.89). However, this

association disappeared after controlling for shared contextual variables (Tables 2.2 and 2.3). In contrast, the intensities of infection with these two parasites were significantly associated after adjustment for confounders; children with heavy *Plasmodium* infections were more likely than those with light infection to have heavy *S. haematobium* infection controlling for relevant covariates (OR= 1.74, 1.01-3.00). No evidence of association in odds or intensities of these infections was seen in adults (Tables 2.4 and 2.5).

DISCUSSION

This study explicitly measured the joint distributions of important human parasites among residents of a rural village in southeastern Kenya and examined evidence of associations between presence and intensity of pathogens within individuals. The epidemiology of *Plasmodium* spp. and *S. haematobium* infections as well as joint infections was characterized separately for school-aged children and for adults. Within these age groups, between which prevalence of infections varied markedly, the relationships of infections with individual and household-level variables were explored. Factors influencing parasite densities also were identified as were associations between different parasite species.

Residents of this coastal region were heavily burdened with multiple parasitic infections. Co-infection with *Plasmodium* spp. and *S. haematobium* parasites was identified in over 15% of participants and was much more prevalent among children (31.8%) than adults (5.0%) (OR=9.28, 5.29-16.27). The high prevalence of *Plasmodium* spp. and *S. haematobium* infections identified in this study is similar to that in previous reports from the region [31-38]; however, this study adds important and often omitted information on joint distributions of infection presence and intensity. In addition, this

study improves upon past estimates of *Plasmodium* spp. infection prevalence and intensity through the use of more sophisticated diagnostic techniques and advanced statistical methods. Although risk factors for single infections have been described for individuals, few studies have characterized these relationships while controlling for the clustering of individuals in households [10]. Ignoring this spatial and social hierarchy could bias observed associations, assuming that the household context is important in the transmission and acquisition of infection. The role of household-level variables in co-infection with these parasites is addressed in Chapter 3.

Here, analyses were stratified by age for all infection outcomes to account for the strong age effect seen in bivariate analyses. In general, children had much higher prevalence of all infections (except *W. bancrofti*) than did adults (Table 2.1 and Figure 2.2). This age association is similar to that reported in endemic regions between age and clinical malaria [39] and between age and *S. haematobium* infection [40]. Higher prevalence at younger ages may be due to unmeasured immunological factors governing susceptibility to both infections [41-43].

***Plasmodium* spp. Infection:**

In children, the final multivariable model that explained the most variation in infection prevalence, while remaining parsimonious, adjusted for outdoor night activity and household SEP. Interestingly, children reporting outdoor night activity were less likely to be infected than those not reporting this behavior. This may be evidence of the highly endophilic nature of the predominant local vectors, *A. gambiae* and *A. funestus* [44, 45]. Indoor occupation was found to be a risk factor for malaria in adults in Ugandan highlands [46]. Children from households reporting higher SEP were less

likely to carry infection than those from lower SEP households, as expected. Household SEP is hypothesized to affect infection through the ability to reduce exposure to vectors (purchase of bednets, window screens, good quality house construction, etc) and to treat any apparent infection (access health care and purchase medication). Since inclusion of such intermediaries (bednet use, house construction, recent malaria diagnosis) in analysis did not negate the effects of household SEP on infection, other explanations must be considered. Individuals from households with lower SEP may suffer from poor nutrition which is known to affect susceptibility to infection [47] and which was not measured in this study. In addition, those with lower SEP may be more likely to harbor other parasites which increase susceptibility to infection with *Plasmodium* parasites. Our analyses examined co-infection with *S. haematobium* (discussed below) but did not measure soil transmitted helminthes (STH) which have been shown to affect *Plasmodium* spp. infection [reviewed in 10], nor did we test for HIV.

In adults, *Plasmodium* spp. infection was associated with age, sex, recent consumption of antimalarials, malaria KAP, and household SEP. Infection prevalence decreased with age in adults. This may be evidence of reduced exposure to parasites in the elderly, or possibly to more complete immunological protection due to repeated exposures. Adult males experienced more infection than adult females which is a bit surprising since pregnant women are known to more susceptible to *Plasmodium* infections than non-pregnant women or men [17, 48, 49]. However, this study did not test for pregnancy so the importance of this factor in the observed sex effect could not be determined. Differential odds of infection between men and women also may be mediated by differences in exposures to infectious vectors, although we attempted to

account for this by including mosquito exposure variables in the analyses. Recent consumption of antimalarial medication reduced the likelihood of infection in adults, as would be expected, suggesting minimal local drug resistance. Higher malaria KAP scores among adults also were associated with lower prevalence of infection suggesting a protective effect of targeted health education. As was seen among children, adults from households reporting higher SEP had reduced infection.

These results provide support for the importance of household-level analyses; as such measures of SEP were independently associated with *Plasmodium* spp. infection. That SEP remained in the models, while measures of individual exposures and behaviors (such as bednet use) did not, argues for the integration of poverty reduction with malaria prevention efforts. Somi and others [50] provide support for this finding as well, and further suggest that the link between economics and *Plasmodium* infection is bidirectional and exists at both the household and country levels [51].

The reduced multivariable models of heavy *Plasmodium* infections among infected individuals isolated factors that may be important targets for decreasing malaria morbidity. Among children, bednet use and intensity of *S. haematobium* co-infection were associated with increased odds of heavy infection. While the direction of association with bednet use and *Plasmodium* intensity may be surprising due to the current focus on insecticide treated nets (ITNs) as an effective malaria prevention tool [52, 53], several plausible explanations exist. Bednet use was not associated with reduced prevalence in this population, so an association with infection intensity might not be expected. Data on bednet use were gathered through self-reporting and may not be reliable. In addition, no information was collected on duration, consistency, or

correctness of use. Insecticide-treated nets were not differentiated from untreated nets, nor were nets examined for other indicators of quality or effectiveness (such as holes). Improper use of bednets could lead to increased exposure to vectors. Perhaps bednets are used more often by sick individuals, a possibility that cannot be examined with our data.

Among infected adults, outdoor night activity and household distance to the stream appeared to be most important in explaining heavy infections. These variables might influence infection intensity by affecting mosquito biting rates. Adults who spend more time outdoors during peak transmission hours may be exposed to fewer infectious bites by endophilic mosquitoes thereby reducing parasite loads. Similarly, those who live closer to the stream could have more intense exposure to infectious vectors if the stream serves as a breeding site for competent *Anopheles* mosquitoes. Supporting this hypothesis, a recent study found stream pools to be a source of Anopheline larvae in this region [54].

***S. haematobium* infection:**

Among children, the reduced multivariable model included schistosomiasis KAP, water contact behaviors and distance of household to the local stream as important predictors of infection. Likelihood of infection decreased with increasing schistosomiasis KAP and with increasing distance of households to the stream. These results indicate that health education and environmental control efforts may be effective means of disease reduction. Children reporting water contact with potentially infected water sources had higher odds of infection than did those without such water contact. The importance of water contact patterns in determining *S. haematobium* infection is well supported in the literature [14, 55-57]. In adults, prevalence of infection was lower among older age

groups and among those reporting water contacts. These results suggest that water contact behaviors might be important determinants of infection in children and young adults, but not in older individuals. In fact, the negative association seen in adults suggests a protective effect of water contact, perhaps due to acquired immunity among those repeatedly exposed to *Schistosoma* parasites [58].

A relationship was also found between water contact behaviors and household location. Children living closer to the stream were more likely to report risky water exposures, whereas the opposite was true for adults. Adult exposure to infected water is perhaps more likely to occur at the seasonal ponds located outside the study site. It has been reported that local residents are willing to travel long distances to wash clothes in these ponds to avoid the hard water from the village water pumps [59]. Other studies have shown household distance to water sources as predictive of schistosomiasis [58, 60, 61]. Our results suggest that disease prevention efforts focused on reducing exposures should target youth.

For children infected with *S. haematobium*, heavy egg loads more often occurred among those using bednets, with high SEP, living close to the stream, and having heavy *Plasmodium* co-infection. In adults, *S. haematobium* infected males were more likely to harbor heavy infections than were females, but this estimate may not be accurate due to the low prevalence.

Co-infection of *Plasmodium* spp. and *S. haematobium*:

The limited existing literature on co-infection makes it difficult to hypothesize which factors should be most strongly predictive. We found that variables associated with both single species infections were important in modeling co-infection prevalence.

In children, the reduced multivariable model for co-infection included bednet use, outdoor night activity, water contact and household distance to the stream. Bednet use and outdoor night activity were associated with lower prevalence of co-infection, hypothetically through a reduction of exposure to *Plasmodium*-infected mosquitoes. The effectiveness of bednets in reducing *Plasmodium* infection is well-established [52, 62]; however, our single infection analyses did not show evidence of this association. The apparent protective effect of bednets on co-infection may be driven more by the relationship between bednet use and infection intensities (bednet use is negatively associated with *Plasmodium* spp. intensity in children and with *S. haematobium* intensity in children and co-infected children are more likely to harbor heavy infections with both parasites than are single or uninfected children). The associations of outdoor night activity and water contact with co-infection are likely significant due to the relationship between these variables and single species-infections. Household location was shown to be associated with *S. haematobium* infection in children in single infection analyses which could drive the association with co-infection, but a relationship with *Plasmodium* spp. also could play a role. Other studies have shown distance to water sources as predictive of malaria [58, 63, 64].

In adults, the only variables in the final model of co-infection were age and household SEP. Older adults had lower prevalence of co-infection as did those with higher household SEP. This observed age effect was also seen in both single infection models. Elderly people might be expected to have lower prevalence of co-infections due to reduced exposure to mosquito vectors or risky water contact, or possibly to a composite of immunological history conferring protection against new infections.

Among co-infected children, high pathogen burdens were concentrated in younger individuals. Again, this is probably due to unmeasured immunological factors since younger children are more likely to have naïve immune responses. In co-infected adults, low household SEP was associated with higher pathogen burdens. This finding reinforces literature on malaria and schistosomiasis as diseases of poverty [51, 65-67] and suggests that poorer households are likely to suffer from greater parasite-associated morbidity.

Our study used a novel approach to measuring parasite intensities by creating an overall, non-ordinal pathogen burden score that reflects a summed, standardized measure of independent infection intensities. While this measure may provide a simplified snapshot of the distribution of parasite density within individuals and within the community, the complexity of the human immune system makes it difficult to translate pathogen burden scores into practical strategies for disease reduction. For example, as discussed in a recent review [68], acute *S. haematobium* infection causes a Th1 response which could aid in attacking *Plasmodium* parasites but could also cause an inflammatory reaction associated with increased severity of malaria morbidity. In contrast, chronic *S. haematobium* infections cause a shift in immune response to type Th2 which may inhibit the Th1 response necessary for controlling *Plasmodium* parasitemia ultimately leading to more prevalent but mild malaria. Thus, the temporality of infection and the history of exposure to parasites are likely to play an important role in co-infection morbidity. Table 2.7 summarizes the observed associations between variables and odds of single and multiple parasite infection (presence/absence as well as intensity).

Identifying co-infection associations

Finally, we examined whether the observed prevalence of co-infection was higher than expected by chance, thus indicating potential biological interaction between infections. Age-stratified, multivariable GEE analyses of *Plasmodium* spp.-*S. haematobium* co-infection prevalence revealed no evidence of association between infections after adjustment for relevant SEP, behavioral and environmental variables (Tables 2.2 and 2.3). A significant association was seen, however, in analyses of infection intensity, in which heavy *S. haematobium* infections and heavy *Plasmodium* infections clustered in a subset of infected children but not in adults (Tables 2.4 and 2.5).

The apparent lack of association between these two parasite species using prevalence data has been supported by previous research [34]. However, comparability of our results is limited by differences in outcome measures (clinical disease instead of asymptomatic infection) [7] and helminth species of interest (most other co-infection studies investigate a variety of STH species instead of focusing solely on *S. haematobium*) [reviewed in 10]. Since STH parasites have different modes of transmission from *S. haematobium* we would expect to see a difference in important predictor variables, although the immunological arguments for biological interaction could still apply.

Our findings of positive associations between the intensities of the two parasites contribute to the ongoing debate on this topic. While several studies have reported opposite findings (i.e. lower *P. falciparum* intensities in children harboring light *S. haematobium* infections vis-à-vis those not co-infected [37, 38]), other studies support our findings of positive associations (reviewed in [13]). The complex immunological

milieu governing infection profiles complicates interpretation of polyparasitism study results. Differentiation between studies of co-infection and those of co-morbidity may be preferable to this end. To illustrate, *P. falciparum* has been shown to protect against *S. haematobium* infection by promoting protective antibody development but *Plasmodium* infection can also increase inflammatory factors associated with morbidity [68, 69]. The relevance of each immunological pathway likely depends on local epidemiologic setting (endemicity of each parasite, frequency of other important co-morbidities, nutritional status of the community, etc.).

Our study provided no evidence of association between likelihood of infection with these two parasite species. After controlling for individual- and household-level variables, prevalence of co-infection was consistent with what would be expected based on distributions of single infections. Within co-infected children, however, the intensities of the two infections appear to be positively correlated, such that heavy infections clustered within a few individuals. Since these analyses controlled for contextual risk factors and spatial aggregation of individuals, the observed associations probably are biological. However, unmeasured variables may have been confounding.

Although our study applied novel methodological techniques to address an important gap in scientific knowledge of polyparasitism, conclusions must be constrained by relevant limitations. For example, data were collected cross-sectionally which prevents evaluation of causality. In addition, the generalizability of our results may be limited for various reasons. First, this study used different diagnostic techniques than those used in most other polyparasitism investigations. We posit that since identification of *Plasmodium* spp. infection via PCR is far more sensitive than light microscopy

techniques, our results improved estimates of co-infection. Secondly, comparison of results with other studies is limited by differences in age ranges of participants. Our study excluded children under 8 yo, those who are most likely to harbor *Plasmodium* spp. parasites. Since age was found to be a very important predictor of infection prevalence and intensities, conclusions should be limited to the appropriate age group. Third, conclusions from these data should be to the study region due to the focal nature of *S. haematobium* and *W. bancrofti* infection [14, 15, 70]. Results are also seasonally specific due to natural seasonal cycles in transmission dynamics of each infection [71, 72]. Multi-year climatic variation such as the recent drought in the area will also influence the generalizability of our findings.

Finally, the potential for unmeasured confounders must be considered. This study did not control for other infections that could affect susceptibility to, or intensity of *Plasmodium* spp. or *S. haematobium*, such as STH or HIV. Although STH are likely to be ubiquitous, *S. mansoni* is not endemic to this region and HIV rates are fairly low (7% prevalence in rural Kenya and 7.9% prevalence in Coast province overall, [73]). Such infections also could have effects on immune responses thereby influencing the *S. haematobium-Plasmodium* spp. associations. Other potentially important unmeasured confounders include nutritional status and genetic factors. The analyses conducted based on marginal models should account for attenuate any genetic effects shared within families as they account for clustering of individuals within households. Furthermore, a study of *S. haematobium* from the same geographic region concluded that heritability in host susceptibility is low and unlikely to play a major role in determining individual risk for infection [74]. Potential confounding effects of nutritional status should not be large

assuming similar access to nutrition within households. This also would be partially accounted for by the inclusion of household SEP variables.

Despite these limitations, our study provides a thorough characterization of the burden of infection by important parasitic infections in southern coastal Kenya, using sensitive diagnostic techniques. The analytic methodologies employed account for the hierarchical structure of the data (clustering of individuals in households) thereby reducing bias. This investigation also reduces bias by including and controlling for measures of infection exposures and behaviors in analyses. Few other studies have taken such precautions [10]. Finally, we have improved upon existing co-infection studies by using general estimating equations to account for non-independence of infections.

Our results support the current trends in disease prevention and treatment efforts to focus on school-aged children, age showing strong associations with infection prevalence and intensities. An important caveat would be that the high levels of asymptomatic *Plasmodium* spp. infections seen in adults could be an important reservoir of infection abetting continued transmission in children and should therefore not be ignored in intervention efforts. In addition, spatial location of households was shown to have significant associations with *S. haematobium* infection and with co-infection in children, and on *Plasmodium* spp. infection intensity in adults, even after controlling for individual-level variables. Similarly, associations between household SEP and co-infection prevalence and intensity were found. Relying solely on school-based interventions might result in missing important reservoirs of infections identifiable by household characteristics such as house location and household SEP.

Figure 2.1: Selection of study population.

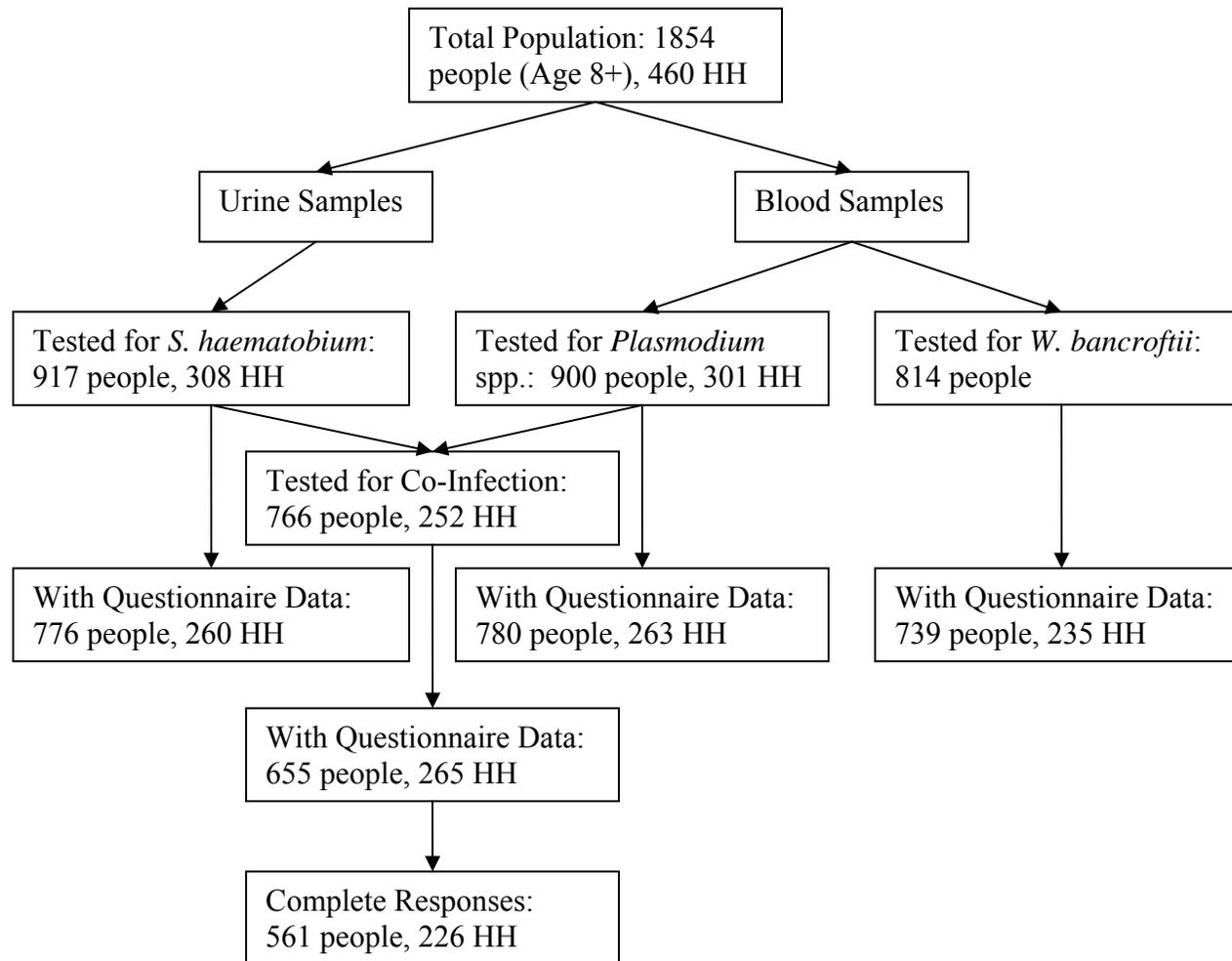


Figure 2.2: Distribution of infection prevalence and intensity by age category.

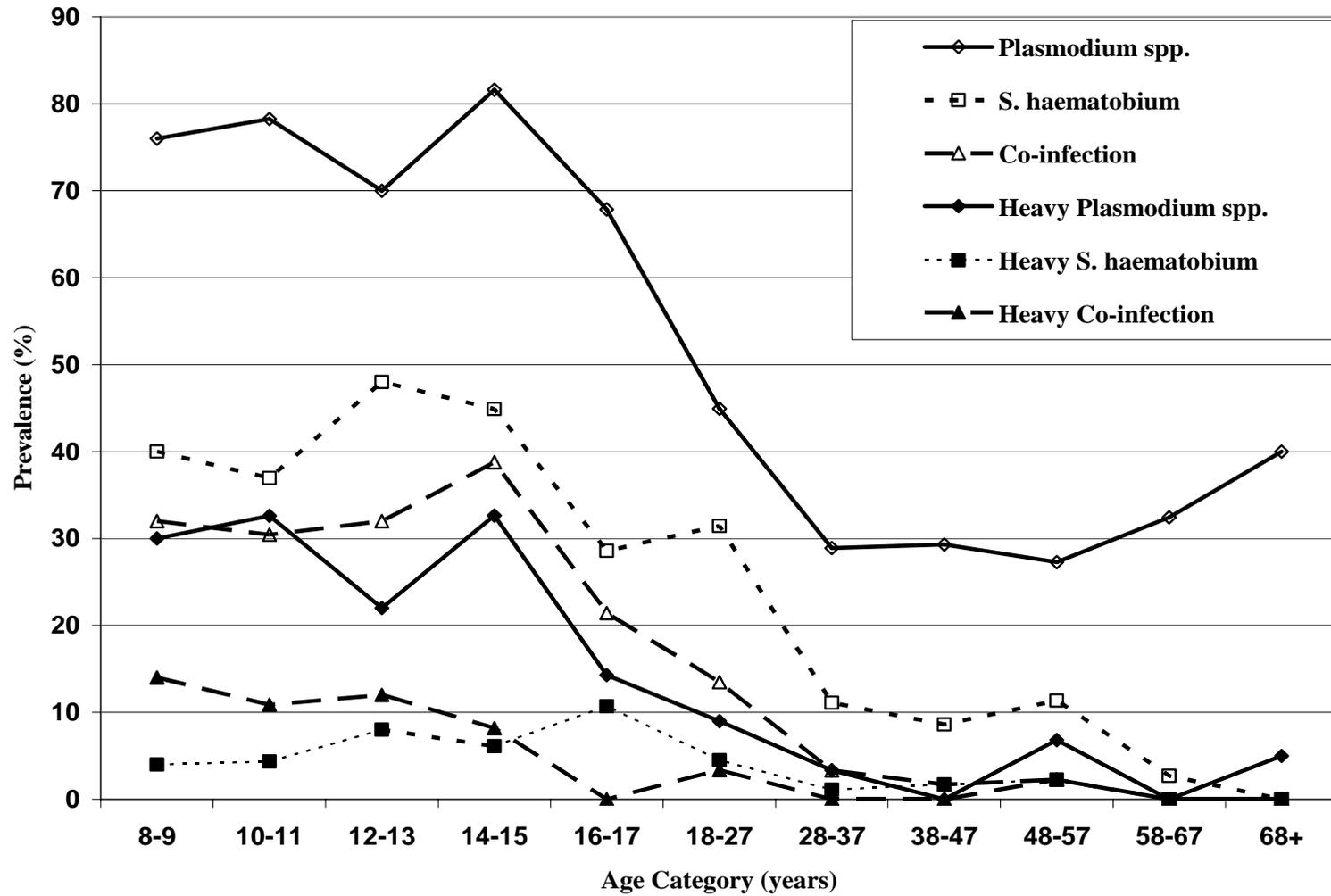


Table 2.1: Age stratified characteristics of the study population.

Social and Demographic Characteristics	Overall (n=561)	Children (n=223)†	Adults (n=338)†	Odds Ratio (children/adults)	95% CI	p-value
Age (years)	28.7 (18.3)	12.1 (2.7)	39.6 (15.8)	--	--	--
Male	38.5	50.2	30.8	2.32	1.69-3.20	<0.0001
Bednet use	55.1	48.0	59.8	0.62	0.45-0.84	0.002
Recent antimalarial treatment	59.7	56.5	61.8	0.81	0.57-1.15	0.2325
Outdoor night activity	67.9	49.8	79.9	0.25	0.17-0.36	<0.0001
Malaria KAP (0-3)	2.5 (0.8)	2.19 (1.01)	2.77 (0.55)	0.56	0.48-0.65	<0.0001
Schistosomiasis KAP (0-3)	1.8 (1.2)	1.35 (1.22)	2.12 (1.04)	0.46	0.38-0.56	<0.0001
Water contact	41.4	49.3	36.1	1.71	1.19-2.45	0.0036
Regular income	51.8	1.79	84.9	0.003	0.001- 0.01	<0.0001
Household SEP score (-1.4-4.1)	0.14 (1.05)	0.19 (1.06)	0.11 (1.04)	1.09	0.92-1.28	0.3172
Household distance to stream (km)	0.66 (0.40)	0.69 (0.37)	0.64 (0.41)	1.04	0.98-1.11	0.1957
Infection Characteristics						
<i>Plasmodium</i> spp (P)	50.4	75.3	34.0	6.56	4.40-9.78	<0.0001
<i>S. haematobium</i> (S)	25.0	40.8	14.5	4.09	2.77-6.04	<0.0001
<i>W. bancrofti</i> (W) (n=534)	5.8	2.4	8.0	0.30	0.12-0.75	0.0106
P-S	15.7	31.8	5.0	9.28	5.29-16.27	<0.0001
P-S-W (n=534)	0.9	0.5	1.2	--	--	--
Heavy <i>Plasmodium</i> spp.(in positives)	26.9	36.3	13.0	3.79	1.97-7.29	<0.0001
Heavy <i>S. haematobium</i> (in positives)	15.0	15.4	14.3	1.11	0.45-2.76	0.8249
Heavy co-infection (in co-infected)	30.7	32.4	23.5	1.91	0.46-7.97	0.3744
<i>Plasmodium</i> spp. standardized	0.167 (0.266)	0.31 (0.32)	0.07 (0.16)	--	--	--
<i>S. haematobium</i> mean eggs/10mL	1.44 (7.51)	20.8 (59.2)	10.2 (83.7)	--	--	--
<i>S. haematobium</i> standardized	0.010 (0.054)	0.015 (0.04)	0.01 (0.06)	--	--	--
Co-infection standardized	0.151 (0.219)	0.27 (0.26)	0.07 (0.14)	--	--	--

† Percent of study population reporting characteristic for dichotomous variables and mean (standard deviation) for continuous variables.

Table 2.2: Full and reduced logistic GEE models of odds of infection in children aged 8-17. Values in the table represent odds ratios and 95% confidence intervals associated with each independent variable controlling for the other variables in the model.

Independent Variables	<i>Plasmodium spp.</i>		<i>S. haematobium</i>		Co-infection	
	Full	Reduced	Full	Reduced	Full	Reduced
Age†	0.99 (0.87-1.12)		1.09 (0.95-1.24)		1.01 (0.87-1.17)	
Male	0.93 (0.52-1.68)		1.37 (0.78-2.42)		1.54 (0.77-3.09)	
Bednet use	1.00 (0.52-1.91)				0.60 (0.31-1.16)	0.58 (0.32-1.03)
Recent malaria treatment	1.03 (0.56-1.89)				0.83 (0.41-1.65)	
Outdoor night activity	0.55 (0.29-1.04)	0.55 (0.29-1.03)	0.62 (0.34-1.13)		0.52 (0.27-0.99)	0.55 (0.31-0.99)
Malaria KAP	1.03 (0.72-1.47)					
Schistosomiasis KAP			0.71 (0.51-0.99)	0.74 (0.58-0.95)	0.99 (0.72-1.37)	
Water contact			2.35 (1.18-4.67)	2.12 (1.14-3.94)	1.69 (0.89-3.18)	1.71 (0.95-3.08)
HH SEP	0.76 (0.53-1.07)	0.76 (0.54-1.05)	0.83 (0.61-1.15)		0.82 (0.58-1.16)	
HH distance to stream	0.89 (0.39-2.04)		0.33 (0.14-0.78)	0.36 (0.15-0.84)	0.33 (0.13-0.84)	0.32 (0.13-0.82)
<i>S. haematobium</i> infection	0.99 (0.56-1.73)					
<i>Plasmodium spp.</i> infection			1.14 (0.63-2.08)			

KAP = knowledge, attitudes and practices; HH = household; SEP = socio-economic position.

† Grand mean centered among children included in this analysis.

Table 2.3: Full and reduced logistic GEE models of odds of infection in adults aged 18-86. Values in the table represent odds ratios and 95% confidence intervals associated with each independent variable controlling for the other variables in the model.

Independent Variables	<i>Plasmodium spp.</i>		<i>S. haematobium</i>		Co-infection	
	Full	Reduced	Full	Reduced	Full	Reduced
Age †	0.98 (0.96-0.99)	0.98 (0.96-0.99)	0.93 (0.90-0.96)	0.93 (0.91-0.96)	0.89 (0.81-0.96)	0.89 (0.82-0.97)
Male	1.85 (1.03-3.34)	1.67 (0.95-2.94)	1.33 (0.61-2.88)		1.71 (0.51-5.71)	
Bednet use	0.65 (0.38-1.14)				0.92 (0.28-2.99)	
Recent malaria treatment	0.59 (0.36-0.97)	0.56 (0.35-0.91)			0.53 (0.17-1.67)	
Outdoor night activity	1.00 (0.54-1.86)		0.65 (0.29-1.46)		0.59 (0.17-1.99)	
Malaria KAP	0.54 (0.35-0.81)	0.50 (0.33-0.76)				
Schistosomiasis KAP			1.07 (0.73-1.56)		1.06 (0.58-1.93)	
Water contact			0.45 (0.23-0.91)	0.47 (0.24-0.90)	0.67 (0.21-2.15)	
Regular income	0.72 (0.39-1.35)		1.28 (0.49-3.35)		0.72 (0.20-2.52)	
HH SEP	0.76 (0.59-0.98)	0.70 (0.55-0.90)	0.86 (0.64-1.17)		0.50 (0.28-0.88)	0.61 (0.36-1.01)
HH distance to stream	0.62 (0.32-1.22)		1.23 (0.53-2.83)		1.58 (0.33-10.01)	
<i>S. haematobium</i> infection	0.90 (0.46-1.78)					
<i>Plasmodium spp.</i> infection			0.91 (0.45-1.85)			

KAP = knowledge, attitudes and practices; HH = household; SEP = socio-economic position.

† Grand mean centered among adults included in this analysis.

Table 2.4: Age-stratified full and reduced logistic GEE models of odds of heavy *Plasmodium* spp. infections among infected individuals. Values in the table represent OR and 95% CI.

Independent Variables	Heavy <i>Plasmodium</i> spp.			
	Children (8-17)		Adults (18+)	
	Full	Reduced	Full	Reduced
Age†	0.94 (0.78-1.10)		0.98 (0.94-1.03)	
Male	0.89 (0.46-1.71)		0.68 (0.23-1.97)	
Bednet use	1.78 (0.90-3.51)	1.75 (0.92-3.34)	1.76 (0.46-6.76)	
Recent malaria treatment	0.72 (0.36-1.47)		0.59 (0.17-2.00)	
Night activity	0.83 (0.40-1.72)		0.21 (0.06-0.82)	0.26 (0.08-0.93)
Malaria KAP	0.92 (0.64-1.32)		1.22 (0.35-4.29)	
Regular income	---		2.02 (0.59-6.93)	
HH SEP	1.17 (0.85-1.61)		1.43 (0.91-2.52)	
HH distance to stream	0.50 (0.18-1.37)		0.20 (0.04-1.11)	0.27 (0.07-1.02)
<i>S. haematobium</i> intensity‡	1.35 (0.93-1.98)	1.39 (0.99-1.97)	1.16 (0.53-2.56)	

KAP = knowledge, attitudes and practices; HH = household; SEP = socio-economic position.

† Grand mean centered among relevant population (children or adults) included in this analysis.

‡ Ordinal variable with values 0-4 representing increasing intensity. Categories based on distribution of infection intensities in the study population.

Table 2.5: Age-stratified full and reduced logistic GEE models of odds of heavy *S. haematobium* infections among infected individuals. Values in the table represent OR and 95% CI.

Independent Variables	Heavy <i>S. haematobium</i>			
	Children (8-17)		Adults (18+)	
	Full	Reduced	Full	Reduced
Age†	1.04 (0.77-1.40)		0.96 (0.87-1.06)	
Male	1.34 (0.30-6.04)		4.19 (0.53-33.48)	3.95 (0.93-16.78)
Bednet use	0.14 (0.01-1.37)	0.14 (0.03-0.61)		
Recent malaria treatment	2.31 (0.41-13.21)			
Night activity	0.95 (0.07-13.75)			
Schistosomiasis KAP	1.41 (0.53-3.78)		0.70 (0.31-1.58)	
Water contact	0.52 (0.03-8.32)		0.59 (0.09-3.98)	
HH SEP	2.28 (1.15-4.52)	1.97 (1.37-2.85)	0.87 (0.30-2.55)	
HH distance to stream	0.02 (0.001-0.33)	0.04 (0.01-0.17)	2.11 (0.73-6.04)	
<i>Plasmodium</i> spp. intensity‡	1.60 (0.97-2.64)	1.74 (1.01-3.00)	1.05 (0.36-3.06)	

KAP = knowledge, attitudes and practices; HH = household; SEP = socio-economic position.

† Grand mean centered among relevant population (children or adults) included in this analysis.

‡ Ordinal variable with values 0-4 representing increasing intensity. Categories based on distribution of infection intensities in the study population.

Table 2.6: Age-stratified full and reduced logistic GEE models of heavy pathogen burden in individuals co-infected with *Plasmodium* spp. and *S. haematobium* parasites.

Independent Variables	Children (8-17)		Adults (18+)	
	Full	Reduced	Full	Reduced
Age†	0.76 (0.58-1.00)	0.76 (0.63-0.92)	1.09 (0.96-1.23)	
Male	1.75 (0.50-6.04)			
Bednet use	3.56 (1.17-10.82)			
Recent malaria treatment	1.15 (0.39-3.42)			
Outdoor night activity	1.26 (0.40-3.98)			
Malaria KAP	0.75 (0.38-1.50)			
Water contact	0.49 (0.08-2.96)		0.47 (0.01-17.03)	
HH SEP	0.89 (0.49-1.62)		0.54 (0.21-1.42)	0.36 (0.12-1.08)
HH distance to stream	1.27 (0.18-9.17)		0.14 (0.01-1.76)	

KAP = knowledge, attitudes and practices; HH = household; SEP = socio-economic position.

† Grand mean centered among relevant population (children or adults) included in this analysis.

Table 2.7: Summary of observed associations between variables of interest and infections from logistic, multivariable GEE models.

Independent Variables	<i>Plasmodium spp.</i>				<i>S. haematobium</i>				Co-infection			
	Children		Adults		Children		Adults		Children		Adults	
	I	HI	I	HI	I	HI	I	HI	I	HI	I	HI
Age†			-				-			-	-	
Male			+					+				
Bednet use		+				-			-			
Recent malaria treatment			-									
Outdoor night activity	-			-					-			
Malaria KAP			-									
Schistosomiasis KAP					-							
Water contact					+		-		+			
HH SEP	-		-			+					-	-
HH distance to stream				-	-	-			-			
<i>S. haematobium</i> intensity‡		+										
<i>Plasmodium spp.</i> intensity‡						+						

I = infection (Y/N); HI = heavy infection (Y/N); “+” = positive association; “-” = negative association; KAP = knowledge, attitudes and practices; HH = household; SEP = socio-economic position.

† Grand mean centered among relevant population (children or adults) included in this analysis.

‡ Ordinal variable with values 0-4 representing increasing intensity. Categories based on distribution of infection intensities in the study population.

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CHAPTER 3

THE HOUSEHOLD CONTEXT OF POLYPARASITIC INFECTION: HOUSEHOLD-LEVEL VARIABLES EXPLAIN LITTLE OF THE VARIANCE IN *PLASMODIUM* SPP., *S. HAEMATOBIIUM* AND MULTIPLE SPECIES INFECTIONS.

INTRODUCTION

Infectious diseases account for a large proportion of the global burden of disease. In low-income countries, the top four causes of disability adjusted life years lost (DALYs) are diseases with infectious origins [1]. Parasites are one important cause of infectious disease, and are widely prevalent in the developing world. For example, over 150 million people were estimated to carry heavy intensity intestinal nematodes in 2004 [1], making these infections among the most prevalent of global health problems. Carriage of multiple parasitic infections is increasingly recognized as the norm rather than the exception in the developing world [2]. Malaria is one of the most important such diseases, with 247 million cases reported annually causing roughly one million deaths [1]. Over 90 percent of all malaria deaths in children less than 5 years of age occur in Africa. Schistosomiasis, another important and often underestimated cause of morbidity, affects an estimated 200 million people worldwide and causes severe morbidity in several million [1]. A recent assessment attributed 70 million cases of haematuria, 18 million of bladder wall pathology, 10 million of hydronephrosis, and 150,000 cases of kidney failure deaths to *S. haematobium* infection [3]. Eighty-five percent of schistosomiasis cases occur in Africa [4]. *Plasmodium* spp. and *S. haematobium* parasites are co-endemic

in many areas of the world, including coastal Kenya, thereby subjecting local populations to a disproportionate disease burden.

Transmission of parasitic infections is governed by several factors related to hosts, agents, environments and other characteristics of susceptible populations. Age is one host factor that has been found to play an important role in transmission dynamics. School-aged children constitute a high-risk group for acquiring helminth infections and associated morbidities [5-8]. Malaria prevalence and incidence is typically highest in infants and pre-school aged children [9, 10]; however, recent advances in diagnostic techniques have shown older children and adults to be important reservoirs of asymptomatic infection (see Chapter 2) [11, 12]. The risk of school-aged children acquiring multiple parasitic infections may be elevated in children with compromised immune systems, poor nutrition, or co-morbidities such as HIV [7, 13-15]. Individual behaviors such as care seeking and hygiene practices [16-25] also are important. In addition, contextual factors such as location of residence or physical features of the household may contribute to risk of infection [25-30]. Many of these social and environmental factors may be influenced by an individual's or a household's socio-economic position (SEP) [18, 25, 31-33]. Investigations of multi-species infections in children constitute a key target in prevention, control and elimination efforts due to the high prevalence of infection in this population and the related potential for cost-effective interventions. In particular, the global public health community has recently been leaning towards implementation of integrated, school-based programs [5, 34, 35]. Because children are at high risk for severe infection-related morbidity with long-lasting

consequences for individual health, polyparasitism research should prioritize children over other populations.

Children are a particularly high-risk population for infection with helminthes [7, 8], and *Plasmodium* spp. [8, 9, 36](also see Chapter 2) and for associated morbidity [3, 8, 9, 37-45](see Chapter 5). Furthermore, transmission patterns of these infections vary widely among households within communities [8, 31, 46-49], and between individuals in the same household [50]. Risk factors underlying these patterns exist at both the individual and the household level. Understanding such interactions should improve intervention design. Illustrating this point, several recent reviews have noted a need for improved control of contextual variables in polyparasitism studies [8, 31, 51]. Of the few investigations that have examined effects of contextual variables on polyparasitism, none, to our knowledge, has explicitly quantified the relative contributions of host- and household-level factors in determining likelihood of joint infection with *Plasmodium* spp. and *S. haematobium* parasites. In fact, few studies have used these multi-level techniques in examination of any parasite combination. Therefore, the primary goal of this study was to integrate information on parasite distributions, household-level factors (e.g. SEP and local environmental conditions), and individual characteristics among residents of a village in coastal southeastern Kenya, and to examine the relative importance of contextual variables on *Plasmodium* spp. and *S. haematobium* (P-S) co-infections.

METHODS

Study site and population

This cross-sectional study was conducted during 2006 in Kingwede, a rural village in coastal Kenya located ~50 km south of Mombassa on the major road to

Tanzania. The study site is bordered on the north by a paved road to Tanzania, on the south by the Indian Ocean (3 km) and comprises a ~6 km sq area. Kingwede residents are predominantly subsistence farmers and fishermen of the Digo tribe. Electricity and access to piped water are virtually nonexistent in the village. Pumped, potable water is available at eight locations in the village. A seasonal stream and ponds are used for washing clothes, bathing and swimming. Approval for this study was granted by the University of Michigan Institutional Review Board and the Kenya Medical Research Institute (KEMRI).

Data sources and collection

Household Demography. A complete demographical profile of Kingwede was compiled in December 2005. Interviewers identified every house in the study area and collected information (sex, age, relationship to household head and years of residence) on each person who slept in the house the previous night. Subsequently, every individual ≥ 8 years old (yo) was invited to participate in study. Informed consent was obtained from adults. Parental assent and child consent were obtained for each child.

Parasitologic and clinical information. Participants were asked to give two midday urine samples on two consecutive days and one finger prick blood sample. Height and weight measurements were recorded for participants ≤ 18 yo for use in anthropometry calculations. Fingerprick blood of participants was tested for hemoglobin levels on site using a blood hemoglobin photometer (HemoCue, Inc, Lake Forest, CA).

Presence of *S. haematobium* eggs was determined by microscopic examination of filtered urine. Two 10 ml aliquots of each urine sample were filtered using 12- μ m pore

Nucleopore filters (Nucleopore, Pleasanton, CA) and mounted on microscope slides for examination at the study site [52].

Fingerprick blood (~200 μ L) that had been collected in potassium ethylene-diaminetetraacetic acid (K-EDTA)-coated Vacutainer tubes and stored at -20C was further evaluated for presence and quantity of circulating parasites at Case Western Reserve University in Cleveland, OH. *Plasmodium* spp. was detected in these preparations using polymerase chain reaction/ligase detection reaction fluorescent microsphere-based assay (PCR/LDR-FMA) as previously described (Mehlotra, Kasehagen et al. 2002; McNamara, Thomson et al. 2004; McNamara, Kasehagen et al. 2006). Briefly, DNA was extracted, amplified and added to a multiplex, species-specific ligase detection reaction (LDR) where species-specific primers hybridized to target sequences and are subsequently labeled with oligonucleotide probes with fluorescent capacity. A Bio-Plex array reader (Bio-Rad Laboratories, Hercules, CA) was used for detection of fluorescence in a species-specific manner.

Samples were considered positive if they had a median fluorescent intensity (MFI) greater than two standard deviations above those of negative controls (MFI above 205, 260, or 220 for *P. falciparum* (Pf), *P. malariae* (Pm) and *P. ovale* (Po), respectively). Positive *S. haematobium* samples had one or more eggs in at least one of two urine samples. Individuals positive for any *Plasmodium* spp. and positive for *S. haematobium* were considered P-S co-infected.

Intensity of infection was defined as arithmetic mean eggs per 10mL of urine for *S. haematobium*. For *Plasmodium* spp. infections, a standardized measure of MFI was created. All values below the aforementioned cut-off values were set to zero.

Subsequently, the MFI were divided by the maximum observed value to give a range from 0 to 1. This method was used for each *Plasmodium* species. A summed value was then created to represent burden of any *Plasmodium* spp. The same method was used to create standardized *S. haematobium* intensity scores. Finally, a summed pathogen burden score was created using the standardized values for *Plasmodium* and *S. haematobium*. Due to non-normal linear distributions, ordered categorical variables were created based on the density distribution of each parasite. Intensities of each infection were divided into five categories based on distributions (0-4; no infection to heaviest).

Individual and Household Questionnaires. Once biological samples were obtained, trained interviewers administered questionnaires to all willing participants. Separate questionnaires were used for adults and for children. These questionnaires included queries aimed at assessing socio-economic position (SEP), knowledge, attitudes and practices (KAP) regarding malaria and schistosomiasis, mosquito avoidance behaviors, and water contact patterns. Household-level information (e.g. ownership of assets) was extracted from questionnaire responses by the household head or senior household member, and was applied to other household members in analyses. All questionnaire data relied on self-reporting by participants or extrapolation from head-of-household responses. Other household-level information was collected by interviewers who observed house quality (e.g. roofing and construction material, presence of window screens, etc).

Spatial data. The location of each house entrance (latitude, longitude) was measured using a handheld GPS device (Garmin etrex summit, Olathe, KS). A 1 km resolution IKONOS image of the study area was obtained from GeoEye

(www.geoeye.com, Dulles, VA) for use in spatial and environmental analyses. ArcGIS v. 9.1 (ESRI, Redlands, CA) was used to create spatial variables such as distances from each house to water sources.

Data Analysis

All data were double entered in Microsoft Access and analyzed using SAS 9.1 (SAS Institute, Inc, Cary, NC). Outcome variables included binary variables (Y/N) for *Plasmodium* spp., *S. haematobium* and co-infection and ordinal categorical variables measuring intensity of *Plasmodium* spp., *S. haematobium* and co-infection as described in the methods section. Covariates of primary interest in these analyses were age, sex, knowledge of malaria (MKAP), knowledge of schistosomiasis (SKAP), individual educational attainment, regular income, use of bednets, night outdoor activities, water contact behaviors, and recent malaria diagnosis. Household-level variables included household socio-economic position (SEP) and distance to the local stream. Malaria and schistosomiasis KAP questions (N=3 for each) were used to create a scale (0-3) based on the number of correct responses. Individual educational attainment was measured by highest class completed. Five categories of educational status were created based on quintiles of distributions. Years of education was ignored in subsequent analyses due to co-linearity with age. Bednet use was treated as a binary variable and did not distinguish between treated and untreated nets. A binary variable was created to characterize use of medication in the past month that is recognized as effective against malaria. Water contact behaviors were assessed by combining responses to questionnaire data. Participants reporting swimming, fishing, bathing, washing dishes or washing clothes in water sources potentially infected with *S. haematobium* were considered exposed. An

asset index, constructed using principal components factor analysis of questionnaire responses was used to assess household SEP [53, 54]. Assets measured include electricity, radio, television, bicycle, motor vehicle, land ownership, domestic animals and toilet. The score also included information on crowding, quality of house construction, and number of full and part time workers in a household. Missing data necessary for construction of the household SEP score were first imputed using the IVEware (Imputation and variance estimation software) SAS macro [55]. Each individual was assigned an SEP score corresponding to their household and this household SEP score was treated as a continuous variable.

To assess whether infections were clustered by household we compared -2 log likelihood values obtained from empty, multilevel, random effects models utilizing adaptive Gaussian quadrature and a dual quasi-Newton optimization algorithm with those obtained from similar models without random effects terms (equivalent to standard logistic regression models). Likelihood test ratios were evaluated for significance using a chi-square distribution and an alpha of 0.05. Small p-values indicated household clustering of infections and suggesting it appropriate to use multilevel models over simple logistic models for further analyses.

Next, the relative importance of individual-level predictors versus household factors on household-level variation in odds of infection was assessed by comparing household variance across logistic mixed effects models. The choice of variables included in these analyses was determined using general estimating equations (GEE) and is presented elsewhere (Chapter 2). Individual- and household-level variables found to have significant associations with odds of species-specific single- and co-infections were

included in analyses. Due to the strong association of age with infection outcomes, age-stratified analyses were conducted (children 8-17 yo; adults ≥ 18 yo).

Several methods were applied to evaluate partitioning of variance in these data. First, we applied the latent variable method suggested by Merlo and colleagues [56] in which the individual level variance is converted to the logistic scale for comparability with the household-level variance. This method assumes that the propensity for infection is a continuous latent variable and only those individuals whose propensity crosses a threshold will become infected as defined by our binary infection variable. In this case,

$$V_I = \pi^2/3 = 3.29, \text{ and} \quad (1)$$

$$ICC = V_H / (V_H + 3.29). \quad (2)$$

where V_I is the individual-level variance in infection prevalence, V_H is the household-level variance, and ICC is the Intra-Class Correlation. ICC values were calculated for empty models, models with only individual-level variables, those with only household-level variables and full models with both individual and household-level variables. Unfortunately, V_I in logistic distributions is dependent on the prevalence of the outcome, a characteristic which limits comparability of ICC estimates across models.

Median odds ratios (MOR) were calculated as described by Merlo and colleagues [56] in order to obtain estimates of variance partitioning unbiased by infection prevalence values. MOR were estimated for the same models as above using the equation:

$$MOR = \exp(\sqrt{2 \times V_H} \times 0.6745) \quad (3)$$

where 0.6745 is the 75th percentile of the cumulative distribution function of the normal distribution with mean 0 and variance 1. The MOR is defined as the median value of the odds ratio between the household at highest risk of infection and that at lowest risk when households are chosen at random. In this case, the MOR quantifies the extent to which individual probability of infection is determined by household. The MOR, unlike the ICC, is independent of the prevalence of infection and is interpretable on the odds ratio scale, a characteristic that facilitates comparison with fixed effects. When MOR = 1.0, there are no differences between households in probability of infection; conversely, when the MOR is large, household-level factors are relevant in describing and understanding individual-level variations in infection risk.

Next, we applied two techniques to improve upon estimates of association between household-level variables and odds of infection. Eighty percent interval odds ratios (IOR-80) were calculated as described by Merlo and colleagues [56]. The IOR-80 is defined as the interval, centered on the median of the distribution, that comprises 80% of the values of the OR representing household-level variable association with infection outcome. It is estimated by combining information on the fixed effect of a household level variable with the variability observed across households:

$$\mathbf{IOR}_{\text{lower}} = \mathbf{exp}(\beta + \sqrt{(2 \times V_H)} \times (-1.2816)) \quad (4)$$

$$\mathbf{IOR}_{\text{upper}} = \mathbf{exp}(\beta + \sqrt{(2 \times V_H)} \times (1.2816)) \quad (5)$$

where β is the regression coefficient for the household level variable, and -1.2816 and 1.2816 represent the 10th and 90th percentiles of the normal distribution with mean=0 and variance=1. Whereas the usual OR compares odds of infection across levels of household variables, this measure fails to consider the variability in the outcome across households. The IOR-80 incorporates the magnitude of between-household variations into estimates of household-level effects on infection. If the interval is small, the residual household-level variance is small; if the interval is large, a large amount of household-level variance remains unexplained by the model. Intervals containing the value of 1 indicate a negligible effect of the household-level variable under investigation as compared to residual household-level variation. This method was applied to all models with household-level variables.

The Sorting Out Index (SOI) was also used to assess the relative importance of the household-level variables as compared to the residual household-level variation [56]. The SOI represents the percentage of OR (of the household-level variable) with values >1, or if the household level variable is negatively associated with the outcome, the percentage of OR with values <1:

$$\text{SOI} = \Phi \left[\frac{|\beta|}{\sqrt{2 \times V_H}} \right] \quad (6)$$

where Φ is the cumulative distribution function for the normal distribution with mean zero and variance one and β is the regression coefficient for the household level variable. SOI values range from 50%, in which the residual variance is high and the household-

level variable is irrelevant, to 100% in which the residual variance is negligible and the household-level variable is important.

Lastly, we calculated Proportional Change in Variance (PCV) comparing null models to models with more variables as follows:

$$\text{PCV} = ((V_N - V_F) / V_N) \times 100 \quad (7)$$

where V_N is the variance of the model with the fewest terms and V_F is variance of the model with the most terms.

Finally, linear mixed models were used to investigate the relative effect of individual and household-level variables on household-level variation in infection intensities. Again, the variables to be included in these models were chosen based on GEE analyses of species-specific infection intensities from Chapter 2. Variance partitioning is straightforward for normally distributed data, since individual-level and household-level variances are easily obtained and can be directly compared between models. Likelihood ratios (LR) were estimated for each infection in null models without random effects and compared to LR of mixed models (containing fixed and random effects) to assess model fit. Large differences in LR between models signify significant household-level clustering of infection intensities and the analytic superiority of mixed models to fixed models. All LR tests performed had one degree of freedom since only random intercept models were tested. ICCs were calculated as follows:

$$\text{ICC} = V_H / (V_H + V_I) \quad (8)$$

to evaluate the relative importance of household-level variance in infection intensities in null models, those with only individual-level predictors, those with only household-level variables and in full models. In addition, estimates of PCV between models were calculated as specified in Equation 7.

RESULTS

Characteristics of the study population:

This population has been described in detail elsewhere (see Chapter 2). In brief, a total of 1,854 persons 8 years of age and older from 460 households was identified in the study area. Approximately half (935) of these individuals from 310 different households chose to participate in the study by contributing samples for parasitological testing. The proportions of the eligible population contributing parasitological samples for each infection and those contributing questionnaire data are shown in the previous chapter (Figure 2.1). Complete parasitological and at least partial questionnaire data were received for 766 (41.3%) individuals from 252 (54.8%) households. By further restricting the dataset to those with no missing questionnaire responses for the variables needed in subsequent analyses 561 persons from 226 households remained.

Characteristics of the study population categorized by age are presented in Chapter 2 (Table 2.1), and show that children differ in many ways from adults. Among children, about half of the population was male whereas only 31% of adult participants were male. Adults had higher malaria and schistosomiasis KAP than did children and more adults reported having a regular income, using bednets and going outside at night. Children were more likely than adults to report water contact with potentially infected

water sources. Reports of recent consumption of antimalarials were similar among the age groups, as were household SEP scores and distances of households to the local stream.

Infection prevalence

More than three-quarters of participating children were infected with *Plasmodium* spp. and over 40% harbored *S. haematobium* (Table 2.1, Figure 2.2). Co-infection was seen in 31.8% of children. Among adults, infection prevalences were lower but still substantial, with 34.0%, 14.5% and 5.0% carrying *Plasmodium* spp., *S. haematobium*, or a P-S co-infection, respectively. These prevalences indicate that infection distributions differed significantly between children and adults. Children were much more likely than adults to be infected with *Plasmodium* spp. (OR = 6.56, 4.40-9.78), *S. haematobium* (OR=4.09, 2.77-6.04) and to carry P-S co-infections (OR = 9.28 (5.29-16.27)).

Spatial heterogeneity

Significant within-household clustering of single- and co-infections was seen for children (Table 3.1). Among adults, only co-infections were significantly clustered by household, although *Plasmodium* spp. infections were marginally clustered.

Role of risk factors in household clustering

These analyses addressed the partitioning of variance in infection prevalence, examining the relative importance of household-level and individual-level variables on household clustering of *Plasmodium* spp., *S. haematobium* and P-S co-infection (Table 3.2). For *Plasmodium* spp. infection among children, adding individual-level variables (outdoor night activities) to a null random effects model only slightly decreased the random variance (PCV = -6.4%), ICC and MOR values. Adding household SEP to a

children-only individual-level model decreased household infection variance by an additional 2.6%. MOR values reinforced the conclusion that *Plasmodium* infections were clustered within households but the degree of clustering did not vary based on the fixed effect variables measured (MOR values similar between models). IOR-80 results showed large intervals containing the null value for both household-only and full models, indicating considerable residual variation in *Plasmodium* infection unaccounted for by household SEP and a small role of household SEP in explaining household-level clustering of infection in children. The SOI estimate indicated that 57.4 % of odds ratio comparisons between children from high SEP households to those from low SEP households were below 1.0 in the full model. Since an SOI of 50% suggests large household-level residual variance and an irrelevant household-level variable, while an SOI of 100% indicates the opposite, these findings suggest that household SEP has relatively weak explanatory power when modeling household clustering of *Plasmodium* species infection.

Among adults, clustering of *Plasmodium* infections could be largely explained by the distribution of individual-level characteristics. Household-level infection variance decreased by >30% when adult individual-level variables (age, sex, antimalarial use, and malaria KAP) were added to a null, random effects model. Including household SEP in this adult individual-level model explained an additional 18.3% of the household-level variation in infection. MOR values in adults were much lower than for children, indicating less clustering among adults than among children. IOR-80 intervals were much narrower for adults than for children, but still included the null value. Finally, SOI estimates indicated that 71.2% of odds ratio comparisons in the IOR-80 were below 1.0

in the full model, indicating moderately strong associations of household SEP with household clustering of *Plasmodium* infection in adults.

For *S. haematobium* infection among children, trends from variance partitioning analyses were quite different than those for *Plasmodium* (Table 3.2b). Household-level random variance, ICC values and MOR values were all higher for models that included individual-level variables than in the null model. This could indicate reverse confounding across levels in which individual-level variables, strongly associated with *S. haematobium* infection, increased household variance in odds of infection by the nature of household distribution of these variables. Adding the relevant household-level variable (distance of household to the stream) to a model with only individual-level variables explained an additional 6.8% of household infection variance; however, the corresponding change in ICC between these two models (19.1% to 18.0 %) was negligible. The IOR-80 interval was much narrower than those derived from *Plasmodium* models but still contained the null value. The SOI value for the full model was low relatively high (84.7%), indicating that household distance to the stream was predictive of *S. haematobium* infection clustering at the household level. In adults, no household-level variables were significantly associated with odds of infection so variance partitioning analyses were not possible.

Analyses of variance partitioning of P-S co-infection among children showed similar but attenuated patterns to those discussed above for *Plasmodium* infection (Table 3.2c). Individual-level variables (bednet use, outdoor night activity and water contact) explained 25.0% of the household variation in infection. Adding household distance to stream to the model of individual-level variables explained an additional 10.1% in

household co-infection variance and decreased ICC by 3.8% (from 19.1% to 15.3%), indicating that household location explained a fair proportion of infection clustering. Narrow IOR-80 intervals confirm these results; however, the null value was included in these intervals implying that household location is only marginally predictive of household clustering of infection. An SOI value of 87.7% suggests that household distance to stream is fairly important as compared to residual household variations in *S. haematobium* infection. Among adults, the small number of co-infected individuals led to large household variance estimates. As seen with *S. haematobium* infection in adults, addition of individual-level variables increased household infection variance. Adding household distance to the stream to an individual-level model explained 3.4% of the household-level variance in co-infection. ICC, MOR, IOR-80 and SOI values were not useful for interpretation in this case.

Infection Intensity

As was seen with infection prevalence, heavy infections in children were much more likely than adults for *Plasmodium* spp. ((OR=8.09, (4.41-14.81)), *S. haematobium* (OR = 3.11 (1.31, 7.39)), and P-S co-infection (OR=9.21 (2.87-29.59)) (data not shown). Restricting analyses to infected individuals, children were still more likely than adults to harbor heavy *Plasmodium* spp. infections ((OR = 3.79 (1.97, 7.29)), whereas this was no longer true for *S. haematobium* or co-infections (Table 2.1).

Spatial heterogeneity

Overall, infection intensities were clustered by household (Table 3.3). Global null model likelihood ratio tests compared the fit of each model with that of a model sharing the same fixed effects but without a random variance term. Results of these test as well

as ICC values revealed significant household clustering of *Plasmodium* infection intensity and of P-S co-infection intensity in children ($X^2 = 10.87, 6.82$; ICC = 28.6%, 24.0%, respectively); however, *S. haematobium* intensity was not clustered ($X^2 = 2.15$, ICC = 12.5%). Similar trends were seen in infection intensities among adults ($X^2 = 7.04, 1.06$ and 18.19 ; ICC = 25.9%, 12.3%, and 46.0% for *Plasmodium*, *S. haematobium* and P-S co-infection, respectively).

Our last set of analyses addressed the partitioning of variance in infection intensity, examining the relative importance of household-level and individual-level variables on household clustering of heavy infection. These analyses were not conducted with *S. haematobium* intensity since this outcome appears to be homogeneously distributed at the household level (Table 3.3). Looking more specifically at the relative effects of household and individual-level factors on variation in infection intensities, PCV analyses revealed interesting trends (Table 3.4). Adding household SEP to a model of *Plasmodium* infection intensity in children only explained 0.02% of the household clustering whereas adding household-level variables (HHSEP and distance of household to the stream) explained 12.7% of household clustering of this outcome among adults. A reverse trend was seen for intensity of co-infections; 17.2% of the household clustering of mixed infection intensity among children could be attributed to household SEP, whereas among adults this proportion was only 0.7%.

DISCUSSION

Importance of household-level variables on clustering of infections

The household has been promoted as the appropriate context in which to study transmission and prevention of parasitic infections such as *S. haematobium* and

Plasmodium spp. [31, 46, 49]. Members of households share genes and, therefore, genetically disposed susceptibility and resistance to infections [57], as well as behaviors that affect exposure to pathogens (water use, hygiene, bednet use) [16, 20, 21, 23, 25, 58-62]. Households also share environmental risks defined by the spatial location and physical conditions of residence (proximity to insect breeding sites, access to latrines or potable water, adequate barriers to vectors such as closed eaves and window screens)[26, 29, 30]. Thus, analyses of parasitic infections must account for determinants of infection likely to be shared by members of households in order to avoid biased results [31]. Further insight can be gained by analyses of variance partitioning that examine the extent to which the household context versus individual characteristics is important in explaining household clustering of infections.

Our results indicated that although household factors explored in these analyses were significantly associated with single- and co-infections in individuals, they explained relatively little of the clustering of infections as compared to individual-level factors. This pattern was seen in both children and adults but to differing degrees. ICC estimates designed to examine variance partitioning between levels indicated that a fair amount of the variation in odds of *Plasmodium* spp., *S. haematobium*, and P-S co-infection in children was due to household-level variables (35.9%, 18.0%, and 15.3%, respectively for full models). In adults, however, these measures were not very informative due to lack of significant household-level predictors or unstable variance estimates. In addition, ICC values for binary outcomes have limited practical, use since they are influenced by the underlying prevalence of infection and cannot be compared across models.

Median odds ratios also supported the conclusion of significant household clustering of infection in children, with full model values greater than 1.0 (3.65, 2.25 and 2.09 for *Plasmodium* spp., *S. haematobium* and co-infection, respectively). This was not true for adults, as MOR values suggested a minimal role for household clustering in explaining *Plasmodium* distribution. Interval odds ratios for relevant household-level variables investigated for each infection all contained the null value, thus indicating that these variables explained relatively little of the household-level variation in infection. In other words, a significant amount of household-level variation in infection remained unmeasured. The age differences in the relevance of household variables in explaining odds of infection were reinforced by sorting out index results. With a range of 50% (irrelevance) to 100% (total relevance) full-model SOI values were 57.4%, 84.7% and 87.7%, in children for *Plasmodium* spp., *S. haematobium* and confection, respectively and 71.2 and 51.4% in adults for *Plasmodium* spp., and confection, respectively.

Infection intensities similarly did not appear to be greatly affected by household context. Intensity of *S. haematobium* infection was not significantly clustered at the household level. *Plasmodium* spp. infection intensity clustered by household but household-level variables explained a negligible proportion of household variance in infection intensity in children and only 12.7% in adults. Overall pathogen burden also clustered by household, but household-level variables had very little effect on household variance of pathogen burden in adults and explained only 17.2% of this variance among children.

Our results indicate that a greater proportion of household-level variance in odds of *Plasmodium* infection was due to household-level variables in children than in adults

but that the inverse was true when intensity of infection was the outcome. In comparison, a study set in the Ugandan highlands found that distance of residence to mosquito breeding sites explained much more of the household-level variation in infection risk in adults than in children [36]. Since infection intensity is an outcome more comparable to risk of clinical malaria (measured in the Ugandan study[36]) than asymptomatic infection, that study offers some support of our findings. Comparisons must be made with caution due to differences in study design and environmental features of the study sites (cohort vs. cross-sectional, epidemic malaria transmission vs. holoendemic). However, our results highlight the importance of age-specific analyses.

Although formal examinations of variance partitioning are rare in polyparasitism investigations, several studies focusing on different combinations of infections have been published. Pullan and colleagues [32] studied the relative effects of individual-level variables and household and environmental variables on odds of co-infection with *Necator americanus* and *Schistosoma mansoni* in Brazil, finding that SEP-determined household and environmental variables explained a considerable amount of household clustering of infection. However, as in our study, the household-level variables only explained a small fraction of the between-household variation in co-infection. Another study of *S. mansoni* [17] estimated that shared residence accounted for 22-28% of the variance in individual faecal egg count, concluding that a substantial proportion of variation in infection intensity must be due to genetic factors or other unmeasured variables.

We conclude that, despite significant infection clustering at the household-level, household-level variables are not very strong predictors of this clustering. This may be,

in part, due to the small spatial scale of the study area and the relative homogeneity of household level variables such as SEP across our study population. Other household factors such as household construction (open eaves, holes in wall, screened windows, etc.) and private access to piped water could play a role in determining risk of infection over a larger spatial scale in which greater heterogeneity in cultural and economic factors exist. Our study was limited to one village in which virtually all houses had open eaves and lacked access to piped water, limiting variation in these household-level exposures to evaluate possible effects. Alternatively, our findings could indicate that unmeasured behavioral, immunological or genetic factors affect infection distribution at the household level. Since infections were clearly clustered in households, and infection determinants are likely to differ by locale on a small spatial scale, future research should not discount the importance of household-level variables. However, our results provided no evidence that the current focus of integrated intervention efforts on school-aged children should be modified.

Table 3.1: Evidence of household clustering of infection.

	Children			Adults		
	<i>Plasmodium</i> spp.	<i>S. haematobium</i>	P-S Co-Infection	<i>Plasmodium</i> spp.	<i>S. haematobium</i>	P-S Co-Infection
Household-level variance (p-value)	2.02 (0.0046)	0.54 (0.0264)	0.88 (0.0101)	0.34 (0.0677)	0.11 (0.7797)	133.63 (0.0017)
Log Likelihood Ratio X^2 (p-value)	8.1 (0.0044)	1.3 (0.2542)	3.7 (0.0544)	1.1 (0.2943)	0	31.2 (<0.0001)
ICC*	38.04%	14.09%	21.16%	9.47%	3.31%	97.60%
MOR**	3.88	2.02	2.45	1.75	1.38	61492

*ICC= Intra-Class Correlation: $ICC = V_H / (V_H + 3.29)$ where V_H is the household-level variance in infection intensity and 3.29 estimates the residual, individual-level variance in infection intensity ($V_I = \pi^2/3 = 3.29$). The ICC represents the percent of total variation in odds of infection that can be attributed to household-level clustering.

**MOR= Median Odds Ratio: the median value of the odds ratio between the household at highest risk of infection and that at lowest risk when households are chosen at random. This estimate is not reliant on infection prevalence and is therefore preferable to the ICC as a measure of clustering.

Table 3.2a: Age-stratified mixed effects logistic models of *Plasmodium* spp. infection.

	<i>Plasmodium</i> spp.	Model 1: Null	Model 2a: Individual	Model 3: Full
Children				
Fixed Effects	Outdoor night activity		0.47 (0.21-1.07)	0.45 (0.20-1.03)
	HHSEP			0.70 (0.45-1.08)
	IOR-80†			(0.06-8.18)
	SOI‡			57.4%
Random Effects	HH-level variance (p-value)	2.02 (0.0046)	1.89 (0.0053)	1.84 (0.0069)
	PCV**		-6.4%	-2.6%
	-2LR	241.0	237.6	234.8
	Adjusted ICC*	38.0%	36.6%	35.9%
	MOR***	3.88	3.72	3.65
Adults				
Fixed Effects	Age (yrs)		0.97 (0.96-1.00)	0.97 (0.96-0.99)
	Male		1.59 (0.93-2.74)	1.83 (1.05-3.17)
	Recent antimalarials		0.55 (0.33-0.91)	0.55 (0.33-0.91)
	Malaria KAP		0.48 (0.30-0.77)	0.47 (0.29-0.76)
	HHSEP			0.71 (0.54-0.93)
	IOR-80†			(0.32-1.51)
	SOI‡			71.2%
Random Effects	HH-level variance (p-value)	0.3442 (0.0677)	0.2307 (0.2160)	0.1884 (0.3092)
	PCV**		-33.0%	-18.3%
	-2LR	432.3	412.9	403.9
	Adjusted ICC*	9.5%	6.6%	5.4%
	MOR***	1.75	1.58	1.51

Table 3.2b: Age-stratified mixed effects logistic models of *S. haematobium* infection.

	<i>S. haematobium</i>	Model 1: Null	Model 2: Individual	Model 3: Full
Children				
Fixed Effects	Water contact		2.71 (1.33-5.51)	2.34 (1.15-4.81)
	Schistosomiasis KAP		0.74 (0.55-0.99)	0.71 (0.53-0.96)
	HH distance to stream			0.29 (0.10-0.82)
	IOR-80†			0.06-1.37
	SOI‡			84.7%
Random Effects	HH-level variance (p-value)	0.5398 (0.0264)	0.7755 (0.0160)	0.7230 (0.0187)
	PCV**		43.7%	-6.8%
	-2LR	299.3	288.7	282.5
	Adjusted ICC*	14.1%	19.1%	18.0%
	MOR***	2.02	2.32	2.25
Adults				
Fixed Effects	Age		0.92 (0.89-0.97)	
	Water contact		0.37 (0.14-1.02)	
Random Effects	HH-level variance (p-value)	0.1126 (0.7797)	0.1475 (0.1304)	
	PCV**		23.66%	
	-2LR	279.8	247.9	
	Adjusted ICC*	3.3%	28.6%	
	MOR***	1.38	2.99	

Table 3.2c: Age-stratified mixed effects logistic models of *Plasmodium-S. haematobium* co-infection.

	P-S Co-Infection	Model 1: Null	Model 2: Individual	Model 3: Full
Children				
Fixed Effects	Bednet		0.53 (0.27-1.05)	0.53 (0.26-1.04)
	Outdoor night activity		0.53 (0.25-1.11)	0.49 (0.23-1.04)
	Water contact		2.20 (1.05-4.63)	1.91 (0.90-4.03)
	HH distance to stream			0.28 (0.10-0.83)
	IOR-80† SOI‡			0.70-1.14 87.7%
Random Effects	HH-level variance (p-value)	0.8828 (0.0101)	0.6619 (0.0333)	0.5952 (0.0443)
	PCV**		-25.0%	-10.1%
	-2LR	275.3	266.7	260.6
	Adjusted ICC*	21.3%	19.1%	15.3%
	MOR***	2.45	2.32	2.09
Adults				
Fixed Effects	Age		0.44 (0.25-0.76)	0.44 (0.25-0.77)
	HH SEP			0.20 (0.03-31.47)
	IOR-80†			(0, infinity)
	SOI‡			51.4%
Random Effects	HH-level variance (p-value)	133.63 (0.0017)	1022.11 (0.0289)	988.97 (0.0310)
	PCV**		664.9%	-3.4%
	-2LR	103.6	73.9	73.2
	Adjusted ICC*	97.6%	99.7%	99.7%
	MOR***	61492	Infinity	Infinity

HH = household; SEP = socio-economic position; KAP = knowledge, attitudes and practice

*ICC= Intra-Class Correlation: $ICC = V_H / (V_H + 3.29)$ where V_H is the household-level variance in infection intensity and 3.29 estimates the residual, individual-level variance in infection intensity ($V_1 = \pi^2/3 = 3.29$). The ICC represents the percent of total variation in odds of infection that can be attributed to household-level clustering.

**PCV=Proportional Change in Variance at the household level: $PCV = ((V_N - V_F) / V_N) \times 100$ where V_N is the household-level variance of the model with the fewest terms and V_F is the household-level variance of the model with the most terms. Here, we compare the null model with an individual-level model and an individual-level model with the full model including household-level terms.

***MOR= Median Odds Ratio: the median value of the odds ratio between the household at highest risk of infection and that at lowest risk when households are chosen at random. This estimate is not reliant on infection prevalence and is therefore preferable to the ICC as a measure of clustering.

†IOR-80 = 80% Interval Odds Ratio: the interval centered on the median of the distribution that comprises 80% of the values of the OR representing household-level variable association with infection outcome.

‡SOI = Sorting Out Index: an index depicting the extent to which a household-level variable is of importance as compared to residual household variations. Ranges from 50%-100%.

Table 3.3: Evidence of household clustering of infection intensities.

	Children			Adults		
	<i>Plasmodium spp.</i>	<i>S. haematobium</i>	Co-Infection	<i>Plasmodium spp.</i>	<i>S. haematobium</i>	Co-Infection
Null Model Likelihood Test X² (p-value)	10.87 (0.0010)	2.15 (0.1427)	6.82 (0.0090)	7.04 (0.0080)	1.06 (0.3036)	18.19 (<0.0001)
HH variance	0.4640	0.1029	0.3319	0.2194	0.04657	0.1091
Residual variance	1.1610	0.7239	1.0489	0.6292	0.3332	0.1281
ICC	28.6%	12.5%	24.0%	25.9%	12.3%	46.0%

HH = household

ICC = Intra-Class Correlation = $V_H / (V_H + V_I)$ where V_H is the household-level variance in infection intensity and V_I is the residual, individual-level variance in infection intensity. The ICC represents the percent of total variation in infection intensities that can be attributed to household-level clustering.

Table 3.4a: Age-stratified mixed effects linear models for *Plasmodium* spp. infection intensity.

	Plasmodium spp.	Model 1: Null	Model 2: Individual	Model 3: Full
Children				
Fixed Effects*	Bednet		0.1803 (0.3069)	0.2489 (0.1742)
	Outdoor night activity		-0.4775 (0.0048)	-0.4959 (0.0034)
	HHSEP		--	-0.1352 (0.1636)
Random Effects	Null Model -2LR	10.87 (0.0010)	12.51 (0.0004)	12.64 (0.0004)
	HH-level variance (p-value)	0.4640	0.5032 (0.1710)	0.5031 (0.1700)
	Individual-level variance (p-value)	1.1610	1.0913 (0.1514)	1.0851 (0.1501)
	ICC**	28.6%	31.6%	31.7%
	PCV _H ***		8.4%	-0.02%
Adults				
Fixed Effects*	Age (yrs)		-0.00691 (0.0426)	-0.00695 (0.0395)
	Male		0.1405 (0.1875)	0.1862 (0.0814)
	Recent antimalarial use		-0.1886 (0.0503)	-0.1587 (0.0987)
	Malaria KAP		-0.2779 (0.0058)	-0.2557 (0.0109)
	HHSEP			-0.1450 (0.0097)
	HH distance to stream			-0.2298 (0.0859)
Random Effects	Null Model -2LR	7.04 (0.0080)	5.35 (0.0208)	4.48 (0.0344)
	HH-level variance (p-value)	0.2194	0.1956 (0.08719)	0.1707 (0.08379)
	Individual-level variance (p-value)	0.6292	0.6204 (0.08400)	0.6200 (0.08296)
	ICC**	25.9%	24.0%	21.6%
	PCV _H ***		-10.8%	-12.7%

Table 3.4b: Age-stratified mixed effects linear models for *Plasmodium-S. haematobium* co-infection intensity.

	Co-Infection	Model 1: Null	Model 2: Individual	Model 3: Full
Children				
Fixed Effects*	Bednet		0.2464 (0.1501)	0.2694 (0.0749)
	Outdoor night activity		-0.2360 (0.1539)	-0.2831 (0.0686)
	HHSEP		--	-0.5506 (0.0191)
Random Effects	Null Model -2LR	6.82 (0.0090)	6.10 (0.0135)	4.47 (0.0344)
	HH-level variance (p-value)	0.3319	0.3158 (0.1468)	0.2615 (0.1412)
	Individual-level variance (p-value)	1.0489	1.0430 (0.1463)	1.0583 (0.1477)
	ICC**	24.0%	23.2%	19.8%
	PCV _H ***		-4.9%	-17.2%
Adults				
Fixed Effects*	Age		-0.00575 (0.0004)	-0.00572 (0.0004)
	Bednet		-0.07124 (0.1892)	-0.05590 (0.3225)
	Recent antimalarial use		-0.04628 (0.3318)	-0.04686 (0.3263)
	Water contact		-0.06128 (0.2558)	-0.06060 (0.2614)
	HH SEP		--	-0.02949 (0.3411)
Random Effects	Null Model -2LR	18.19 (<0.0001)	20.01 (<0.0001)	19.02 (<0.0001)
	HH-level variance (p-value)	0.1091	0.1119 (0.02464)	0.1111 (0.02487)
	Individual-level variance (p-value)	0.1281	0.1202 (0.01682)	0.1208 (0.01700)
	ICC**	46.0%	48.2%	47.9%
	PCV _H ***		2.6%	-0.7%

HH = household; SEP = socio-economic position; KAP = knowledge, attitudes and practice

* Fixed effects are presented as β regression coefficient and p-value. ** ICC = Intra-Class Correlation = $V_H / (V_H + V_I)$ where V_H is the household-level variance in infection intensity and V_I is the residual, individual-level variance in infection intensity. The ICC represents the percent of total variation in infection intensities that can be attributed to household-level clustering.

***PCV_H=Proportional Change in Variance at the household level: $PCV = ((V_N - V_F) / V_N) \times 100$ where V_N is the household-level variance of the initial model and V_F is the household-level variance of the full model including more terms. Here we compare null models with individual-level models and individual-level models with full models.

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CHAPTER 4

PATTERNS OF SPECIES-SPECIFIC PLASMODIUM INFECTIONS AND ASSOCIATIONS IN KINGWEDE, KENYA.

INTRODUCTION

Mixed-species *Plasmodium* infections were first studied over a century ago, yet knowledge of their prevalence, distribution and predictors remains limited. Although traditional light microscopy is still used in some clinical contexts, various genetic and antigenic methods now permit more accurate differentiation of strain and multi-species infections. These evolving diagnostic techniques are just now beginning to explore the ecology and epidemiology of natural *Plasmodium* spp. infections, the complexity of multi-species dynamics, heterogeneities in affected human populations, and immunological responses to infection.

Geographic distributions of the four human *Plasmodium* species sometimes overlap, with different combinations of co-infection occurring in different parts of the world. Simultaneous co-infection with all four species [*P. falciparum* (Pf), *P. vivax* (Pv), *P. malariae* (Pm) and *P. ovale* (Po)] has been reported [1-3] but the true prevalence and significance of mixed-species infection is unknown. Light microscopy, previously considered the gold standard for malaria diagnosis, has limited sensitivity to detect light infections, often fails to identify mixed infections, and has varying accuracy and reliability that depends on technician expertise [4, 5]. Consequently, most malaria studies are likely to underestimate the true prevalence of single and mixed *Plasmodium*

infections and consequently to misrepresent species-specific interactions. More sensitive and specific diagnostic tools such as polymerase chain reaction (PCR)/sequence-specific oligonucleotide probe hybridization have increased prevalence estimates of both single and multi-species *Plasmodium* infections [2, 5-9], and have allowed for improved studies of co-infection patterns and dynamics.

Analytic and statistical issues also complicate interpretation of existing multiple-species *Plasmodium* research. Generally accepted patterns of *Plasmodium* spp infection (an excess of Pf-Pm-Po co-infections in areas endemic for these species, a deficit of Pf-Pv-Pm co-infections elsewhere) are based on studies that compare observed and expected frequencies of co-infections in study populations comprised of infected and uninfected individuals [10]. Several important, but infrequently discussed, assumptions are being made in using this sort of data analysis to make inference regarding the immunology of mixed-species *Plasmodium* infections. First, exposure to infection is assumed to be universal and thus, protection against infection is assumed to result entirely from acquired immunity. This assumption has been disputed in a recent study in which protection from clinical disease was found to be conferred from acquired immunity as well as from reduced exposure to *Plasmodium* parasites [11]. The researchers concluded that individuals with minimal parasite exposure should be excluded from analyses when inferences regarding species-specific associations and related immune factors are of interest [11]. The second assumption made is that species-specific infections are independent. This assumption is problematic since *Plasmodium* species share common vectors and have other contextual risk factors in common as well. In addition, human susceptibility to *Plasmodium* infection is not species-specific [12]. Finally, there has

been widespread lack of control for spatial, ecologic and social factors that influence frequency and distribution of mixed infections which could bias conclusions [13].

Understanding of the epidemiology of mixed *Plasmodium* infections is also complicated by contradictory results of clinical studies. For example, Pv and Pm have been shown to protect against severe Pf malaria in some studies [13, 14], but exacerbated disease due to co-infections also has been observed [15-17]. These apparent contradictions may be due to differences in infection intensities which have been shown to play a role in regulating malaria morbidities [18, 19]. Complex immune regulation is also likely. For example, Bruce and colleagues hypothesized a mechanism of species-transcending density-dependent regulation of malaria infection based on evidence of Pv attenuating Pf parasitemia [20, 21].

These diagnostic and analytic difficulties limit understanding of basic malaria epidemiology and need to be addressed in order to create a foundation for more complex research questions. A better understanding of species-specific distributions, predictors and associations are needed to investigate the effects of mixed species infection on malaria morbidity, to develop effective anti-malarial drugs and vaccines, and to improve intervention and control efforts. Accordingly, this cross-sectional study applied advanced diagnostic and analytic techniques to explore distributions of species-specific infections and to identify associated risk factors in a coastal Kenyan village. The objectives were to apply PCR methods to describe prevalence and intensities of single and mixed *Plasmodium* infections and associated risk factors, to examine the effects of infection intensities on mixed species infections, and to identify potential synergistic or antagonistic co-species associations.

METHODS

Study site and population

This study was conducted during May-July, 2006 in Kingwede, Kenya, a rural coastal village located ~50 km south of Mombassa on the major road to Tanzania. Kingwede residents are predominantly subsistence farmers and fisherman of the Digo tribe. Electricity and piped water are virtually nonexistent in the village, although pumped, potable water is available at 8 locations. A seasonal stream and ponds are used for washing clothes, bathing and swimming. The study site is bordered on the north by the paved road to Tanzania, on the south by the Indian Ocean (3 km) and comprises a ~6 km sq area. Approval for this study was granted by the Institutional Review Boards of The University of Michigan and the Kenya Medical Research Institute (KEMRI).

Data collection

Household Demography. A complete demographic profile of Kingwede was compiled in December 2005. Interviewers identified every house in the study area and collected information (sex, age, relationship to household head and years of residence) on each person who slept in the house the previous night. Subsequently, every individual ≥ 8 years old (yo) was invited to participate in study. Informed consent was obtained from adults. Parental assent and child consent were obtained for each child.

Parasitologic and clinical information. Height and weight measurements were recorded for participants ≤ 18 yo for use in anthropometry calculations. One fingerprick blood sample was taken from each participant and collected in potassium ethylene-diaminetetraacetic acid (K-EDTA)-coated Vacutainer tubes. This blood was tested for hemoglobin levels on site using a blood hemoglobin photometer (HemoCue, Inc, Lake

Forest, CA). The remainder (~200 μ L) was stored at -20C and sent to Case Western Reserve University in Cleveland, OH. Circulating *Plasmodium* spp. parasites were detected in these preparations using polymerase chain reaction/ligase detection reaction fluorescent microsphere-based assay (PCR/LDR-FMA) as previously described [6, 22, 23]. Briefly, DNA was extracted, amplified and added to a multiplex, species-specific ligase detection reaction (LDR) where species-specific primers hybridized to target sequences and are subsequently labeled with oligonucleotide probes with fluorescent capacity. A Bio-Plex array reader (Bio-Rad Laboratories, Hercules, CA) is used for detection of fluorescence in a species-specific manner. Samples were considered positive if they had a median fluorescent intensity (MFI) greater than 2 standard deviations above those of negative controls (MFI above 205, 260, or 220 for Pf, Pm and Po, respectively).

Due to non-linear LDR-FMA fluorescent signal distributions, each infection, single, mixed or any, were divided into four categories of infection intensity based on empiric infection-specific MFI distributions (except for Po which had 3 categories). These measures of intensity were restricted to infected individuals. In addition, binary variables were created to represent heavy infection by combining the two most intense categories of infection into one (heavy) and the least intense categories into another (light/none).

Individual and Household Questionnaires. Once biological measures were obtained, trained interviewers conducted questionnaires with all willing participants. Separate questionnaires were used for adults and for children. These questionnaires included queries aimed at assessing socio-economic position (SEP), knowledge, attitudes and practices (KAP) regarding malaria and schistosomiasis, mosquito avoidance

behaviors, and water contact patterns. Household-level information (e.g. ownership of assets) was extracted from questionnaire responses by the household head or most senior household member, and was applied to other household members in analyses. All questionnaire data relied on self-reporting by participants or extrapolation from head-of-household responses. Other household-level information was collected by interviewers who observed house quality (e.g. roofing and construction material, presence of window screens, etc).

Spatial data. The location of each house entrance (latitude, longitude) was measured using a handheld GPS device (Garmin etrex summit, Olathe, KS). A 1 km resolution IKONOS image of the study area was obtained from GeoEye (www.geoeye.com, Dulles, VA) for use in spatial and environmental analyses. ArcGIS v. 9.1 (ESRI, Redlands, CA, USA) was used to create spatial variables such as distances from each house to water sources.

Data Analysis

All data were double entered in Microsoft Access and analyzed using SAS 9.1 (SAS Institute, Inc, Cary, NC). Outcome variables included binary measures for infection with any *Plasmodium* spp., for species-specific infection status (Pf, Pm, Po) and for mixed infections (any combination of two or more *Plasmodium* species). Binary measures of intense infections, as described in the methods section, were also modeled (any *Plasmodium* spp., Pf, Pm, Po, and mixed infections). Covariates of primary interest in these analyses were age, sex, knowledge of malaria (MKAP), individual educational attainment, regular income, use of bednets, night outdoor activities, and recent malaria diagnosis. Household-level variables included household SEP and distance to the local

stream. Malaria KAP questions (N=3) were used to create a scale (0-3) based on the number of correct responses. Individual educational attainment was measured by highest class completed. Five categories of educational status were created based on quintiles of distributions. Education was ignored in subsequent analyses due to co-linearity with age. Bednet use was treated as a binary variable and did not distinguish between treated and untreated nets. A binary variable was created to measure effective treatment of malaria with medication in the past month. An asset index, constructed using principal components factor analysis of questionnaire responses, was used to assess household SEP [24, 25]. Assets measured include electricity, radio, television, bicycle, motor vehicle, land ownership, domestic animals and toilet. The score also included information on crowding, quality of house construction, and numbers of full and part time workers in a household. Missing data necessary for construction of the household SEP score were first imputed using the IVEware (Imputation and variance estimation software) SAS macro [26]. Each individual was assigned an SEP score corresponding to their household and this household SEP score was treated as a continuous variable.

General estimating equation (GEE) models with exchangeable correlation matrix structures and logistic distributions were used to estimate the association between odds of infection and various predictors. This analytic approach accounts for household clustering in the data and estimates fixed effects of variables averaged across households. To identify individual and household-level predictors of species-specific and of mixed species infections, all relevant individual and household-level variables were included in multiple logistic GEE models and backward selection was then employed to select models best predicting the outcomes. Datasets for this analysis were limited to include

only participants with complete biological and questionnaire data devoid of any missing responses. Continuous variables were centered on the grand mean. Similarly, associations between *Plasmodium* spp. intensity and various predictors were examined among those testing positive for infection using a categorical outcome as described above (0-4) using linear GEE models.

To identify potential synergy or antagonism between species-specific *Plasmodium* infections, the association between the each pair of species-specific infections was assessed after adjusting for age, recent antimalarial treatment, household SEP, and intensity of *S. haematobium* co-infection in logistic GEE models. Associations were considered significant at an alpha of 0.05 for the Type 3 likelihood ratio test of the *Plasmodium* species-specific co-infection regression coefficient. These analyses were restricted to *Plasmodium* infected individuals in order to eliminate confounding by exposure status as discussed in the introduction [27].

RESULTS

Characteristics of the study population

A total of 1,854 persons 8 years of age and older from 460 households was identified in the study area. The average age of respondents was 28.7 years, ranging from 8 to 86 yo, with about and 60% female and roughly half between 8 yo and 18 yo. Approximately half (935) of these individuals from 310 different households chose to participate in the study by contributing samples for parasitological testing. The proportions of the eligible population contributing parasitological samples for each infection and those contributing questionnaire data varied (Figure 4.1). Blood samples were analyzed for *Plasmodium* spp. for 900 individuals from 301 households. Complete

data (biological samples and questionnaire data) were obtained for 560 individuals from 226 households (Figure 4.1).

Social and behavioral risk factors are characterized by age group in Table 4.1, but are summarized for all participants as follows. The average highest educational level attained was primary school. Fifty-two percent of the study population reported having a regular income. Over half of participants reported bednet use. The majority of the population (68.2%) reported outdoor night-time activity. Participants responded correctly, on average, to 2.5 of 3 questions regarding malaria prevention, transmission and symptoms. Interestingly, recent treatment with effective anti-malaria medication following malaria diagnosis in the past month was reported by over half of the population (60.1%). At the household level, the average SEP score was 0.097 (range -1.25 to 3.66). (A difference of 1.0 in SEP score corresponds to 1 standard deviation (SD) in SEP as calculated using one value per household. A one unit difference in SD in SEP corresponds to a difference of approximately 1.5-2 assets.) The average household distance to the local stream was 692 m (range 57m to 2.1 km).

Infection prevalence and intensity

Over half (50.7%) of participants were infected with one or more species of *Plasmodium*, including Pf (45.5%), Pm (15.1%), Po (4.0%) and mixed *Plasmodium spp.* infections (13.0%) (Table 4.1). *Plasmodium vivax* was not identified in this study population. Of all participants infected with at least one *Plasmodium spp.* 89.7% harbored Pf, 29.8% had Pm, 7.9% were infected with Po, and 25.7% were positive with more than one species. Frequencies of infection in the study population are depicted in Figure 4.2. Mean intensities of infection for *Plasmodium spp.*, standardized as explained

in the methods section, were 0.14 for any species, 0.14 for *P. falciparum*, 0.03 for *P. malariae*, and 0.01 for *P. ovale* when calculated for the entire study population.

Considering only *Plasmodium* spp. positive cases, mean intensities were higher (*Plasmodium* spp=0.36, Pf=0.27, Pm=0.06, Po=0.02). Mean intensity for mixed species infections was 0.44. Overall, 15.07% of *Plasmodium* infections were considered heavy, whereas 23.3% of Pf, 19.5% of Pm and 30.4% of Po infections and 32% of mixed infections were heavy.

Analyses first examined the distribution and determinants of infection with any *Plasmodium* spp., measured as a dichotomous outcome (Table 4.2). Many of the explanatory variables were significantly associated with infection in bivariate analyses. Children ≤ 18 yo were 6.55 times more likely than were adults to carry *Plasmodium* parasites (95%CI = 4.4-9.7). Males were slightly more likely than females to be infected and individuals with intense *S. haematobium* infections were also more frequently infected with *Plasmodium* parasites. Likelihood of infection decreased significantly with greater bednet use, malaria KAP, reported outdoor night activity, recent antimalarial treatment, having a regular income, and with increasing household SEP scores. Due to the strong non-linear association of age with *Plasmodium* infection (Figure 4.3), multivariable data analyses were conducted separately for adults and for children. Results showed conserved negative associations between household SEP and infection for both adults and children. While no other variables were significantly associated with infection in children, increasing malaria KAP and a recent history of antimalarial treatment were associated with decreased odds of *Plasmodium* infection in adults.

Similar analyses were conducted for species-specific infections as well as for mixed infections. In bivariate analyses (Table 4.1), children were significantly more likely to carry infection with all species than were adults (OR range from 2.7 for Po to 6.2 for Pf). In multivariable analyses in children (Tables 4.3 and 4.4), reduced models revealed only marginally significant associations between measured variables and *Plasmodium* infections. Increasing household SEP was associated with reduced odds of Pf. Recent antimalarial treatment was associated with decreased odds of Pm and of mixed species infections. Finally, more intense *S. haematobium* co-infections were related to greater odds of both Po and of mixed species infections. In adults, multivariable models showed that Pf infection odds were lower in people with recent malaria treatment, with higher household SEP, and residing at greater distances from the local stream. Odds of Pm infection were diminished in those who reported malaria treatment or earning a regular income. Recent anti-malarial medication was only marginally associated with decreased Po infection but strongly with decreased mixed species infections odds.

Analyses of infection intensities show that *Plasmodium* infections were more intense in both children and adults with mixed-species infections compared to single species infections (Table 4.5) after adjusting for other covariates. In multivariable models, mean infection intensity was also higher in children using bednets but was lower in those reporting outdoor night activity. In adults, mixed-species infections was the only variable significantly associated with infection intensity.

Finally, multivariable GEE models were created to test whether infection with one parasite was associated with greater or lesser likelihood of having the other, adjusting for

confounders and for clustering of individuals within households (Table 4.6). These models were restricted to *Plasmodium*-positive individuals in order to eliminate contextual effects of exposure on likelihood of co-infection, and were stratified by age (adults ≥ 18 vs. children 8-17). Models controlled for recent malaria treatment, household SEP, intensity of *S. haematobium* infection, all *Plasmodium* species co-infections, and a mean-centered linear variable for age, since these were the factors that predicted co-infection in at least one combination of species-specific infection profiles. Controlling for these variables, Pf infection was much lower than expected by chance in Pm-infected individuals as compared Pm-uninfected, both for children (OR=0.19; 0.06, 0.63) and for adults (OR=0.01; 0.00, 0.14). The same pattern was observed for Pf among Po-infected vs. uninfected children (OR=0.04; 0.01, 0.17) and adults (OR= 0.02; 0.00, 0.52). Models of Pf infection in adults were reduced in order to achieve convergence while conserving Pm and Po as covariates. Odds of Pm infection were significantly lower in Pf-positive children (OR=0.19; 0.05, 0.70 and adults (OR=0.01; 0.00, 0.08), but no significant effect of Po co-infection was observed (children: OR=0.40; 0.07, 2.36 and adults OR=0.05; 0.00, 2.22). Odds of Po infection were lower in Pf positive children (OR=0.04; 0.01, 0.24) but not in adults (OR=0.34; 0.07, 1.61). Pm did not significantly affect the odds of Po in children (OR=0.35; 0.05, 2.38) and models of Po infection in adults would not converge when Pm was added as a covariate.

DISCUSSION

Mixed-species *Plasmodium* infections were prevalent in this study population. Thirteen percent of people carried more than one species of *Plasmodium* parasites, with over 25% of *Plasmodium*-infected individuals harboring multiple species. While Pm was

present in 29.8% of all infections and Po in 7.9%, Pf was by far the most prevalent parasite (45.5% of single 100% of mixed infections). Other studies using PCR diagnostic techniques have reported similarly high prevalence estimates and have detected Po infection where it had not previously been found [9, 28]. Comparisons of PCR diagnosed infection to traditional microscopy diagnosed infection have repeatedly reported significant increases in species-specific and mixed *Plasmodium* infections [4-6, 8, 29, 30], demonstrating the superior sensitivity of this diagnostic technique.

The age distribution of overall *Plasmodium* infection prevalence followed patterns commonly seen in clinical studies, with children more likely to be infected than adults (Figure 4.3) [31]. Although the existence of an association between age and *Plasmodium* spp. infection is well established in the literature, the use of new diagnostic techniques such as PCR, is changing our understanding of the dynamics of this relationship. For example, estimates of infection prevalence using PCR show peaks in older age groups than do estimates derived from microscopy, highlighting the importance of asymptomatic infection in adolescents and adults in estimates of overall infection burden [5, 29]. Additionally, age-infection trends have been shown to vary by species with Po and Pm peaking in older age groups than Pf [5, 29]. Since our study population was restricted to children aged 8 and older and Plasmodium infections are most prevalent in children under 5, we were not able to examine these species-specific trends in the relevant age groups.

Decreasing prevalence with age is purported to be due to acquired immunity against malaria in individuals with chronic exposures [32, 33]. Acquired immunity is thought to arise from two causal processes that occur in individuals with chronic

exposures: anti-parasite and anti-disease immunity [6, 34, 35]. The observed age-infection associations in our data suggest that anti-parasite immunity is lower in children as compared to adults; thus, parasite exposures are more likely to lead to infection. This trend was shown for all *Plasmodium* species. The age range of our study population implies that effective anti-parasite immunity does not develop until late in childhood. Alternatively, these findings could simply result from greater exposure to parasites in children leading to more infection. Analyses controlled for various environmental and behavioral variables in an attempt to address the effect of variable exposure status; however, confounding by unmeasured variables is always a possibility. In addition, analyses restricted to infected individuals showed that infected children were more likely than infected adults to harbor multiple *Plasmodium* species (Table 4.1), providing further evidence that immune factors and not differential exposures are responsible for the observed age patterns of infection, as was found in a recent field study in Kenya [27].

Age differences in infection intensities might also be expected since young children experience the most malaria morbidity and mortality and since malarial disease severity is often correlated with levels of parasitemia [18, 19]. While our data did show that children had more intense infections and more mixed species infections than did adults, and while mixed species infections were more intense than single species infections, this association did not vary by age (Table 4.1). This finding could support the theory of species transcending density-dependent immune regulation of *Plasmodium* species proposed by Bruce and colleagues [20, 21] in which parasite densities are limited by a maximal threshold value regardless of the species composition of infection. In contrast to our findings, a recent study from Malawi found lower or equivalent parasite

densities in mixed infections compared to single species infections in children, with results varying by region [28]. However, results of other studies are more consistent with ours with higher intensities found in mixed infections [36]. One explanation for the discrepant results is differences in measurement of infection intensity. We measured intensity through median fluorescent intensity results from PCR tests, in lieu of standard quantification of parasitemia via microscopy. Although work of other researchers has shown good correlation of MFI values with parasitemia, the relationship between the two is not linear toward the upper and lower limits [5, 9, 37]. Although use of MFI to estimate infection intensity is not perfect, standard microscopy techniques have limited sensitivity, especially in the case of light, asymptomatic infections [4, 23].

Aside from age, other variables were also identified as potential predictors of mixed *Plasmodium* infection in this population (Table 4.3). Stratified, multivariable analyses found mixed species infections to be associated with recent malaria treatment in both children and adults, with treatment decreasing the odds of infection. While this association was of marginal significance in children, it was strongly significant in adults. Similar relationships were seen with anti-malarial medication and species-specific infections in adults and with Pm infection in children. Similar results have been reported elsewhere [28]. It is unsurprising that reported anti-malarial medication reduced *Plasmodium* infections; however, it is worrisome that so many adults and children (~60%) took medication during the month preceding the usual beginning of peak transmission. Either this population is overmedicating, a phenomenon that contributes to drug resistance, or transmission is less seasonal than previously thought.

Odds of mixed *Plasmodium* infection were also seen to increase with increasing intensity of *S. haematobium* infection in children. Since analyses controlled for contextual variables such as household SEP and clustering of individuals in households, this finding lends support to an immunological explanation of co-infection rather than a social explanation of shared environmental risk factors. Potential immunological relationships between these two parasite genera are discussed in chapter 2.

Surprisingly, no association was seen between bednet use and odds of *Plasmodium* infection in age-stratified multivariable models. The effectiveness of bednets in preventing *Plasmodium* spp. infection and malarial disease has been well established [38, 39]. Other studies of mixed species *Plasmodium* infections have found bednets to be associated with lower odds of infection and with fewer mixed species infections [28]. The lack of association seen in this study may be due to high coverage; over 50% of participants reported sleeping under bednets. Bednets have been shown to confer protection at a community level, even in nonusers, in areas with high coverage [38, 40, 41]. Alternatively, it is possible that bednets are more widely used by individuals experiencing malarial symptoms. The cross-sectional nature of our study and lack of clinical measures prevent further elucidation of this relationship.

We also examined species-specific associations between *Plasmodium* infections and various predictors stratified by age. We hypothesized that similar associations would be seen across parasite species due to common vectors and the small spatial scale of the study site. Indeed, recent malarial treatment appeared to be strongly associated with infection, regardless of species. Contrary to our hypothesis, other variables showed species-specific associations: Intense *S. haematobium* co-infections were associated with

increased odds of Po infection in children but were not associated with Pf or Pm infection; high household SEP was associated with higher odds of Pf infection in both children and adults but not with Pf or Pm infection; and adults residing in households closer to the stream had higher odds of Pf infection than those located further from the stream. These species-specific trends may indicate species-specific transmission efficiencies of the locally endemic competent mosquito vectors for which we do not have data. Alternatively, these trends could be artifacts of small sample sizes within age and species specific strata in our analyses.

Among *Plasmodium*-infected individuals, variables most strongly associated with infection intensity were also identified. In children, more intense infections were observed in bednet users, in those staying inside residences at night and in those harboring mixed-species infections. In adults, mixed-species infection was the only predictor. Since intensity of *Plasmodium* spp. infections is generally correlated with morbidity, these results suggest potential targets for intervention efforts. Residual spraying of insecticides may be warranted in this area due to the strong association between intense infections and nighttime behaviors in children indicating the importance of the highly endophilic, anthropophilic local mosquito vectors in parasite transmission [42, 43]. Improvements in bednet use (insecticide treatment, repair of holes, proper use) may also be implicated.

Analyses of species-specific interactions revealed that Pf-Pm co-infections occurred much less frequently than by chance, contradicting most published literature [5, 6, 8, 28, 44]. This may be due to methodological differences in analyses. We restricted our analyses to *Plasmodium*-infected individuals in an attempt to eliminate the effects of

non-independence of species-specific infections. Since local mosquito species are competent for multiple parasite species, infection with one *Plasmodium* species implies potential exposure to all species. Similarly, human hosts may be characterized by susceptibilities to infection that are not specific to species, such as poor nutrition, or compromised immune systems. These factors would promote co-infection and would lead to higher prevalence of co-infection than what would be expected if species-specific infections were independent. Most other published analyses on this topic make the assumption of non-independence, ignoring the importance of shared contextual risk factors [29] and host susceptibilities across species [12], then drawing conclusions about the implications of their findings at the individual level (e.g.. excess co-infections implies no heterologous immunity, etc.). By restricting analyses to *Plasmodium*-infected individuals, we reduced the effects of differential exposure to vectors since every infected individual must have been exposed to an infected vector. Similarly, differences in host susceptibility to infection should have been reduced by this restriction. Support for restriction of analyses to infected individuals is found in a recent study of immunity to febrile malaria in which researchers found evidence that protection from infection was due to reduced exposure to parasites instead of immunity [27].

Analyses limited to infected individuals revealed trends counter to those commonly seen in field studies of mixed infections. Instead of an excess of mixed-species infections, Pf-Pm co-infections and Pf-Po co-infections were much less common among *Plasmodium*-infected individuals than would be expected by chance (Table 4.6). This finding lends support to hypotheses of heterologous immunity in which infection with one *Plasmodium* species confers some cross-species protection (originally suggested

by [15]). Experimental and clinical studies also support the theory of cross-species immunity (reviewed in [45]. That results from field studies remain inconclusive may be due to the analytic issues discussed above.

Results from this research reinforce the importance of using modern diagnostic tools and sophisticated analytic methods to improve understanding of mixed species *Plasmodium* infections. Our results improve upon previous reports because they can be interpreted at the individual level without confounding by shared contextual risk factors or susceptibilities. This information should help to target future malaria interventions, while providing insights useful to development of anti-malarial pharmaceuticals and vaccines.

Figure 4.1: A flow-chart of participation in the study. Numbers of eligible individuals and numbers of households (HH) are reduced by non-participation and lack of complete data, either parasitological or questionnaire data.

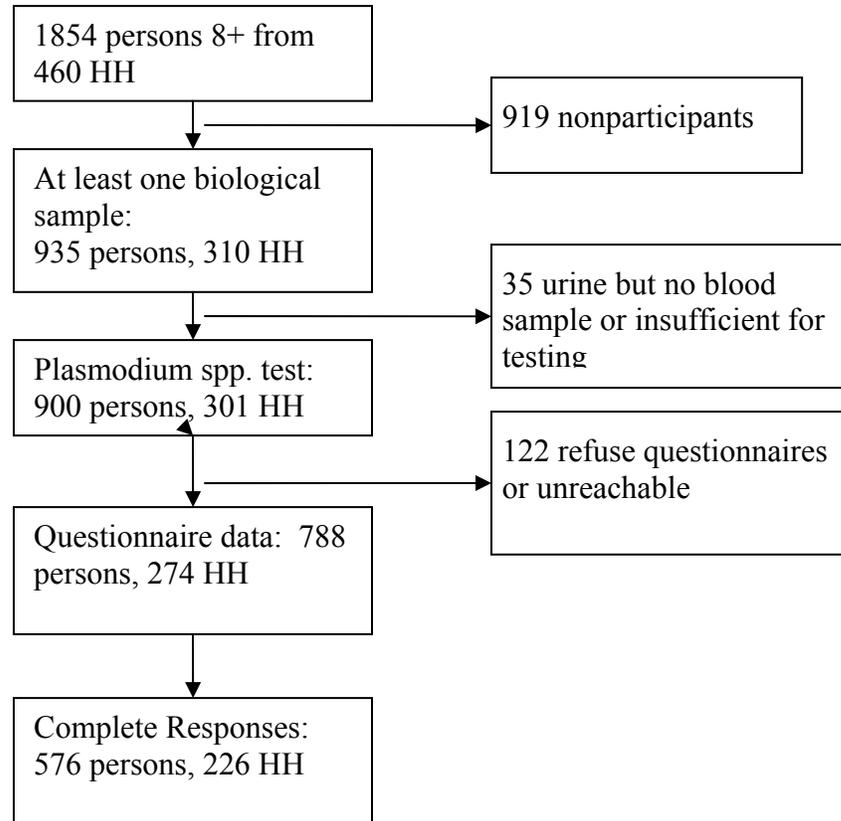


Figure 4.2: Distribution of single and multiple *Plasmodium* spp. infections among positive cases.

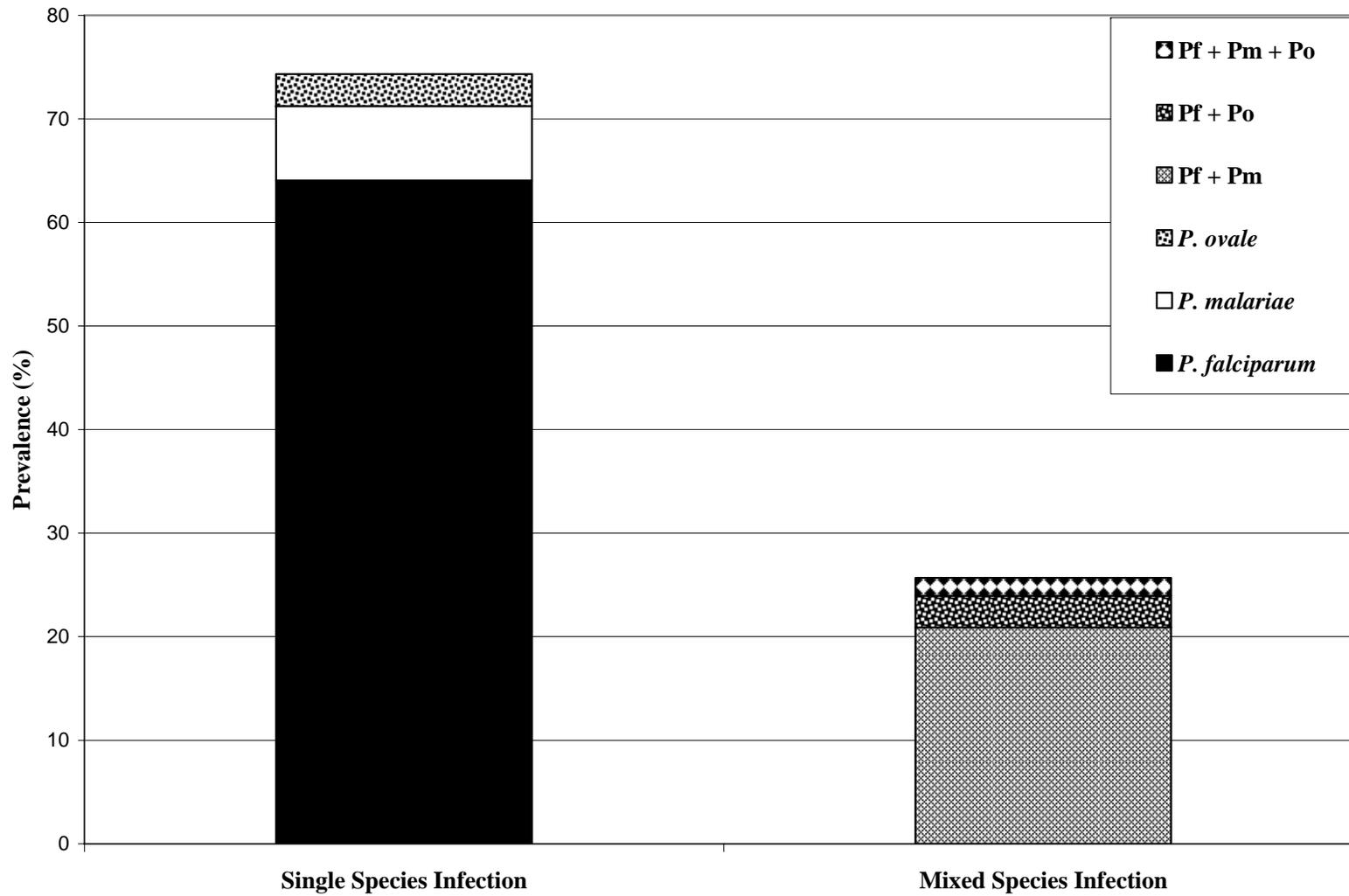
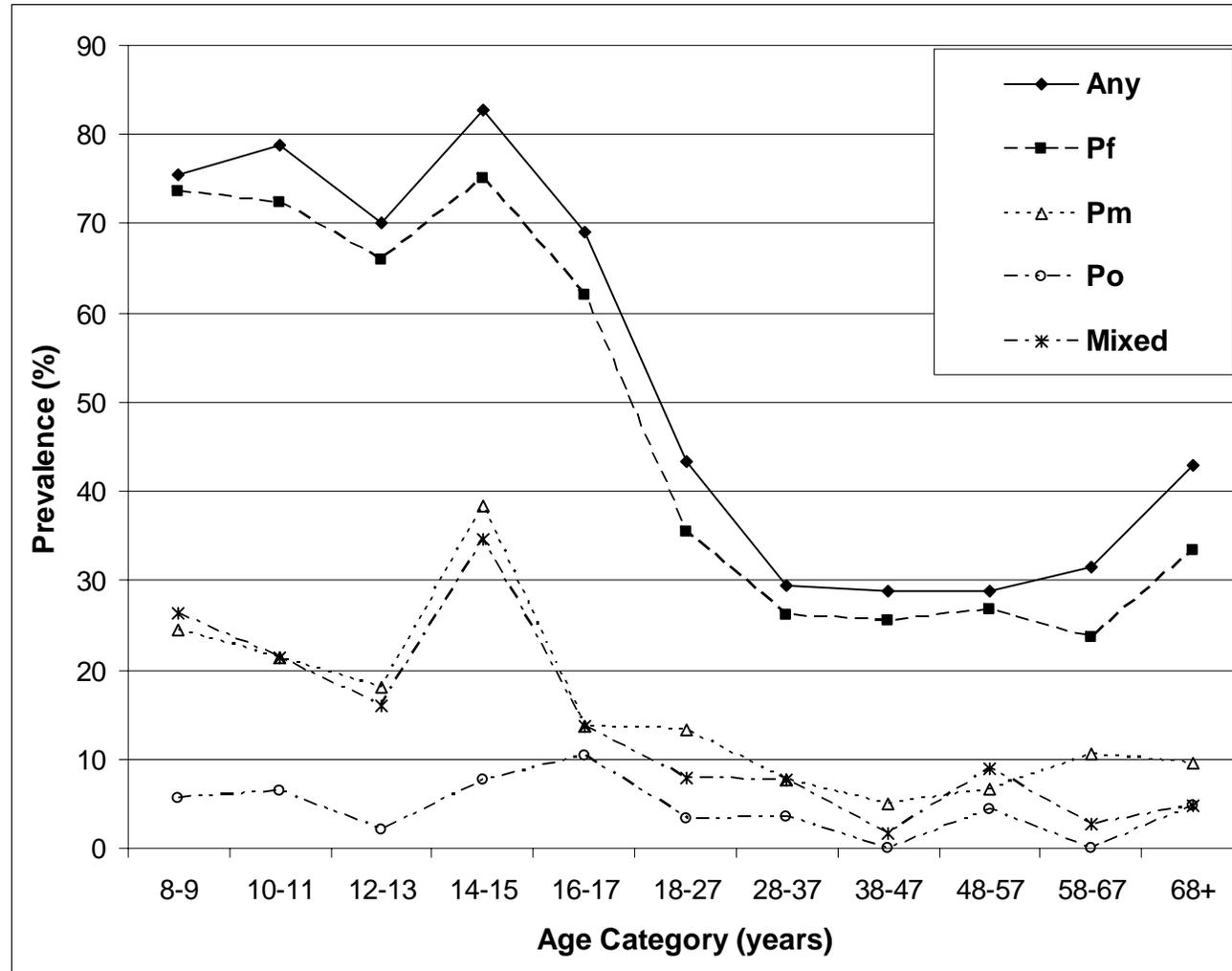


Figure 4.3: Distribution of asymptomatic *Plasmodium* infections by age among study participants.



Note: Pf = *P. falciparum*, Pm = *P. malariae*, Po = *P. ovale*, Mixed = any combination of two or more *Plasmodium* species.

Table 4.1: Demographic, behavioral, and social characteristics of the study population, and *Plasmodium* infection status for children and adults in Kingwede, Kenya.

Characteristic	Children (n=231)	Adults (n=345)	OR (children:adults)	95% CI	p-value
Demographic and Social					
Age (yrs)	12.07 (2.73)	39.76 (15.85)	---	---	---
Male (%)	50.6	32.4	2.23	1.65-3.02	<0.0001
Malaria KAP (0-3)	2.18 (1.01)	2.77 (0.56)	0.56	0.48-0.65	<0.0001
School Level (0-4)	2.24 (0.99)	2.05 (1.80)	1.20	0.95-1.51	0.1193
Regular Income (%)	1.7	84.9	0.01	0.00- 0.01	<0.0001
Bednet use (%)	47.6	59.4	0.61	0.45-0.83	0.0014
Out at night (%)	50.2	80.3	0.24	0.17-0.36	<0.0001
Recent malaria treatment (%)	56.7	62.3	0.79	0.56-1.12	0.1911
Household SEP score	0.18 (1.04)	0.10 (1.03)	1.08	0.93-1.27	0.3215
Household Distance to stream (km)	0.68 (0.37)	0.64 (0.41)	1.04	0.98-1.11	0.2177
Infection status					
<i>S. haematobium</i> (%)	40.7	14.5	4.06	2.78-5.93	<0.0001
Mean eggs (per mL urine)	2.1 (5.9)	1.0 (8.3)	2.89	0.92-9.08	0.0694
<i>S. haematobium</i> intensity (1-4)	0.6 (0.9)	0.2 (0.6)	1.50	1.31-1.72	<0.0001
Heavy <i>S. haematobium</i> (>5eggs/mL) (%)	11.3	2.6	4.67	2.15-10.12	<0.0001
<i>Plasmodium</i> spp (%)	75.8	34.0	6.55	4.40-9.75	<0.0001
<i>P. falciparum</i> (%)	70.1	28.7	6.22	4.24-9.12	<0.0001
<i>P. malariae</i> (%)	24.2	9.0	3.18	1.98-5.10	<0.0001
<i>P. ovale</i> (%)	6.1	2.7	2.72	1.24-5.95	0.0125
Mixed spp.	23.4	6.1	4.64	2.65-8.12	<0.0001
Heavy Plasmodium spp.(in positives)	36.3	13.0	3.79	1.97-7.29	<0.0001
Heavy Pf (in positives)	20.8	3.8	6.66	3.46-12.82	<0.0001
Heavy Pm (in positives)	4.8	1.7	2.80	1.02-7.68	0.0453
Heavy Po (in positives)	2.2	0.6	3.82	0.75-19.5	0.1079
Heavy Mixed spp. (in positives)	9.1	0.9	10.92	3.17-37.57	0.0001

Table 4.2: GEE models estimating odds ratios (95% confidence intervals) of infection with any combination of single- or multiple-species *Plasmodium* parasites among children and adults in Kingwede, Kenya. Bivariate associations are shown in the first column for the entire study population. Full models including all significant bivariate associations and reduced models are shown separately for children and adults.

Characteristics	Any Infection	Children		Adult	
	Bivariates	Full	Reduced	Full	Reduced
Age*	6.55 (4.40-9.70)	1.01 (0.89-1.14)		0.98 (0.96-1.00)	0.98 (0.97-1.00)
Male	1.59 (1.13-2.24)	0.93 (0.51-1.70)		1.75 (1.01-1.11)	1.60 (0.94-2.72)
Bednet	0.61 (0.43-0.87)	1.06 (0.55-2.04)		0.70 (0.41-1.19)	
MKAP	0.60 (0.49-0.75)	0.95 (0.66-1.37)		0.62 (0.41-0.94)	0.57 (0.37-0.87)
School Level	1.00 (0.91-1.11)				
Outdoor night activity	0.51 (0.25-0.74)	0.60 (0.31-1.15)		1.01 (0.55-1.88)	
Recent malaria treatment	0.68 (0.48-0.96)	1.01 (0.55-1.83)		0.60 (0.37-0.97)	0.57 (0.36-0.92)
Regular Income	0.18 (0.13-0.26)	---		0.72 (0.41-1.29)	
HH SEP†	0.82 (0.69-0.97)	0.82 (0.69-0.97)	0.76 (0.55-1.05)	0.78 (0.61-1.01)	0.73 (0.57-0.92)
HH distance to stream‡	0.76 (0.50-1.15)	0.90 (0.40-2.04)		0.65 (0.33-1.26)	
<i>S. haematobium</i> egg burden‡	1.48 (1.16-1.87)	1.04 (0.76-1.42)		1.00 (0.68-1.46)	

MKAP = Malaria knowledge, attitudes and practice; HH= household; SEP=socio-economic position factor score.

* = Bivariate odds ratios compare infection in children (8-17) to infection in adults (18+). Stratified multivariable odds ratios treat age as a linear variable centered on the mean within each age category.

† Centered on grand mean of the study population.

‡ Ordinal values from 0-4 as described in methods.

Table 4.3: Full and reduced multivariable GEE models for single and mixed *Plasmodium* spp. infections in children aged 8-17. Values represent odds ratios and corresponding 95% confidence intervals.

Characteristics	<i>P. falciparum</i>		<i>P. malariae</i>		<i>P. ovale</i>		Mixed species	
	Full	Reduced	Full	Reduced	Full	Reduced	Full	Reduced
Age†	0.97 (0.86-1.10)		1.01 (0.98-1.03)		1.04 (0.84-1.30)		0.98 (0.85-1.12)	
Sex	1.00 (0.55-1.82)							
Bednet	0.93 (0.48-1.79)							
MKAP	0.94 (0.67-1.33)		0.96 (0.89-1.03)				0.84 (0.58-1.22)	
Outdoor night activity	0.72 (0.39-1.33)		0.98 (0.88-1.09)					
Recent malaria treatment	0.98 (0.56-1.71)		0.93 (0.84-1.03)	0.94 (0.84-1.04)	0.58 (0.19-1.74)		0.56 (0.31-1.01)	0.61 (0.33-1.10)
Household SEP†	0.78 (0.58-1.05)	0.77 (0.58-1.03)	0.98 (0.93-1.02)					
Household Distance to stream†	0.79 (0.37-1.71)							
<i>S. haematobium</i> intensity category‡	1.14 (0.83-1.56)		1.02 (0.97-1.09)		1.46 (0.95-2.26)	1.46 (0.96-2.23)	1.39 (1.00-1.94)	1.39 (0.99-1.95)

† Centered on the grand mean for the study population.

‡ Ordinal values from 0-4 as described in methods.

Table 4.4: Full and reduced multivariable GEE models for single and mixed *Plasmodium* spp. infections in adults aged 18-86. Values represent odds ratios and corresponding 95% confidence intervals.

Characteristics	<i>P. falciparum</i>		<i>P. malariae</i>		<i>P. ovale</i>		Mixed species	
	Full	Reduced	Full	Reduced	Full	Reduced	Full	Reduced
Age†	0.98 (0.96-1.00)		1.00 (1.00-1.00)		0.99 (0.94-1.04)		0.98 (0.94-1.02)	
Sex	1.63 (0.90-2.94)							
Bednet	0.63 (0.36-1.12)							
MKAP	0.65 (0.42-1.04)		1.00 (0.95-1.05)				0.74 (0.32-1.72)	
Outdoor night activity	1.19 (0.60-2.35)		0.98 (0.90-1.05)					
Recent malaria treatment	0.60 (0.37-0.99)	0.57 (0.35-0.91)	0.94 (0.88-1.01)	0.94 (0.88-1.01)	0.30 (0.07-1.33)	0.29 (0.07-1.31)	0.27 (0.11- 0.69)	0.28 (0.11-0.73)
Regular Income	0.62 (0.33-1.16)		1.07 (1.02-1.13)	1.07 (1.01-1.13)	0.61 (0.21-1.81)		2.37 (0.69-8.14)	
Household SEP†	0.77 (0.59-1.00)	0.73 (0.57-0.94)	0.98 (0.96-1.01)					
Household Distance to stream†	0.50 (0.23-1.09)	0.47 (0.22-1.01)						
<i>S. haematobium</i> intensity category‡	1.18 (0.84-1.67)		0.99 (0.93-1.06)		1.23 (0.87-1.76)		1.13 (0.49-2.58)	

† Centered on the grand mean for the study population.

‡ Ordinal values from 0-4 as described in methods.

Table 4.5: Multivariable models of *Plasmodium* infection intensity (any combination of single or multiple species) in infected individuals, measured as an ordinal, categorical variable. Values represent odds ratios and corresponding 95% confidence intervals. Bivariate associations are shown in the first column for the entire study population. Full models including all significant bivariate associations and reduced models are shown separately for children and adults.

Characteristics	Both	Children		Adults	
	Bivariate	Bivariate	Full	Bivariate	Full
Age †	1.48 (1.27-1.74)		0.98 (0.94-1.03)		1.00 (0.99-1.01)
Male	1.08 (0.90-1.30)	1.02 (0.80-1.31)	1.05 (0.85-1.29)	1.05 (0.83-1.32)	1.08 (0.87-1.33)
Bednet	1.05 (0.88-1.25)	1.22 (0.96-1.54)	1.21 (0.99-1.47)	0.91 (0.73-1.13)	0.94 (0.75-1.19)
MKAP	0.96 (0.86-1.07)	1.00 (0.87-1.14)	1.03 (0.91-1.17)	1.11 (0.95-1.30)	1.06 (0.90-1.25)
Outdoor night activity	0.73 (0.61-0.86)	0.84 (0.67-1.06)	0.77 (0.62-0.94)	0.76 (0.55-1.05)	0.74 (0.53-1.02)
Recent antimalarials	0.84 (0.70-1.01)	0.79 (0.62-1.01)	0.86 (0.69-1.08)	0.88 (0.71-1.09)	0.89 (0.72-1.09)
Regular Income	0.97 (0.87-1.08)			1.01 (0.77-1.33)	1.00 (0.76-1.33)
Household SEP†	1.22 (0.82-1.83)	0.96 (0.84-1.09)	0.94 (0.84-1.05)	0.92 (0.80-1.05)	0.91 (0.79-1.05)
<i>S. haematobium</i> burden‡	1.14 (1.04-1.24)	1.08 (0.97-1.20)	1.02 (0.92-1.13)	1.09 (0.94-1.25)	1.05 (0.92-1.20)
Mixed species infection	1.96 (1.57-2.47)	2.02 (1.50-2.74)	1.98 (1.47-2.67)	1.57 (1.24-2.00)	1.51 (1.16-1.97)

† Centered on the grand mean egg concentration for the study population.

‡ Ordinal values from 0-4 as described in methods.

Table 4.6: Age-stratified multivariable models estimating odds of species-specific *Plasmodium* spp. infections among infected individuals. Values represent odds ratios and corresponding 95% confidence intervals.*

	<i>P. falciparum</i>		<i>P. malariae</i>		<i>P. ovale</i>	
	Children	Adults	Children	Adults	Children	Adults
<i>P. falciparum</i>	---	---	0.19 (0.05-0.70)	0.01 (0.00-0.08)	0.04 (0.01-0.24)	0.34 (0.07-1.61)
<i>P. malariae</i>	0.19 (0.06-0.63)	0.01 (0.00-0.14)	---	---	0.35 (0.05-2.38)	---
<i>P. ovale</i>	0.04 (0.01-0.27)	0.02 (0.00-0.52)	0.40 (0.07-2.36)	0.05 (0.00-2.22)	---	---
Age†	0.93 (0.72-1.21)	0.98 (0.94-1.02)	0.98 (0.86-1.11)	0.98 (0.95-1.02)	1.01 (0.78-1.31)	1.01 (0.97-1.05)
Recent treatment	0.54 (0.13-2.31)	---	0.57 (0.30-1.08)	0.42 (0.15-1.15)	0.43 (0.13-1.37)	0.40 (0.12-1.37)
<i>S. haematobium</i> burden‡	1.98 (0.74-5.26)	---	1.19 (0.86-1.67)	1.11 (0.45-2.73)	1.84 (1.12-3.03)	0.98 (0.45-2.16)
Household SEP†	0.75 (0.39-1.44)	---	0.89 (0.66-1.20)	0.84 (0.46-1.51)	0.75 (0.45-1.26)	1.65 (0.75-3.61)

* NB some cells are missing results because statistical power was insufficient.

† Centered on the grand mean for the study population.

‡ Ordinal value from 0-4 as described in methods.

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CHAPTER 5

SYNERGISTIC EFFECTS OF *PLASMODIUM* SPP. AND *SCHISTOSOMA HAEMATOBIIUM* CO-INFECTION ON ANEMIA AND STUNTING IN CHILDREN IN COASTAL KENYA.

INTRODUCTION

Concomitant infection with multiple parasites is common in most human populations inhabiting tropical regions of the world [1-3]. Despite the high prevalence of polyparasitism, and well established morbidities associated with single parasite infections, little is known about the health effects of co-infections [4, 5]. Pathogenic effects of co-infections may be synergistic or antagonistic depending on the host-specific biologic responses to each parasite involving, for example, shared pathways or immune responses (Figure 5.1). Identifying synergistic effects between common parasites should help inform health policies, especially in heavily burdened regions with limited resources. Knowledge of antagonistic relationships between parasitic infections is also essential in cost-benefit analyses of interventions, treatments and vaccines.

Anemia is an important cause of disease globally, contributing to 23% of nutrition-related disability-adjusted life years (DALYs) lost [6]. More than 83 million African children under five years old (yo) are estimated to have anemia (blood hemoglobin concentration <11 g/dL) [7] with prevalence estimates ranging between 49% and 76% in malaria endemic regions [8]. *Plasmodium* infection is known to cause

anemia, especially in children and pregnant women [8-10]. Infection with parasites of the genus *Schistosoma* also is associated with increased risk of anemia [11, 12]. The biological mechanisms by which these parasites deplete iron stores of human hosts differ and the relative importance of each needs further elucidation. It may be that the effects of multiple infections are synergistic due to differing mechanisms of initiating and maintaining anemia. Alternatively, common pathogenic pathways between these parasites might lead to antagonistic relationships, evidenced by less than additive statistical associations. Several recent publications support hypothesized biological interactions among helminth species [13] and between hookworm and *Plasmodium falciparum* [14] with respect to anemia in children; however research on potential interactions between *Plasmodium* and *S. haematobium* is lacking [5].

Stunting, or low height-for-age, is another important morbidity globally, estimated to affect as much as one third of young children (<5 yo) in developing countries [15]. Height-for-age, weight-for-age and weight-for-height measurements are used as proxy measures for malnutrition depending on the hypothesized etiology. In the case of chronic infections, height-for-age measurements are used to assess deficient growth. Schistosome infections are known to contribute to malnutrition in those children with chronic exposures [16, 17]. Associations between chronic *Plasmodium* infection and stunting are less clear, although some evidence does support a positive relationship [18, 19]. No published studies have explicitly investigated the nutritional impacts of *Plasmodium-S. haematobium* co-infection [5]. This may be due to differences in the age ranges of those at highest infection risk for these two genera, with very young children (<2 yo) generally experiencing the greatest nutritional impacts from malaria [20]. Since

helminth infection prevalence is low in this age range, studies of co-infection have not been high on public health research agendas. However, in endemic zones, asymptomatic *Plasmodium* infection prevalences remain high throughout adolescence (see Chapters 2 and 4 and reference [21]), during this period of rapid growth and development. Possible interactions between these parasites in adolescents merit further consideration [14].

Extant epidemiologic research has found evidence of negative health consequences of polyparasitism, including malnutrition (reviewed in [5]), organ pathology [22, 23], and low birth weight [24], although these trends are inconsistent [3, 25-29]. Coherent summaries of polyparasitism research findings remain elusive due to the multifactorial nature of infection, the range in specific parasite species studied, variations in infection intensities, and the important role of local social and environmental context in determining infection dynamics. In addition, few studies to date have used appropriate statistical methods to formally assess biological interaction which requires analysis on an additive scale instead of the more commonly employed multiplicative scale [13, 30].

This study evaluated associations between *Plasmodium* spp. and *S. haematobium* infections and negative health outcomes such as anemia and stunting in a cross-sectional population of school-aged children in Coastal Kenya. Specifically, we quantified the burden of anemia and stunting in this population and assessed evidence of biological interactions among parasite species on these morbidities.

METHODS

Study design

This cross-sectional study of data collected in May-July 2006 in Kingwede, Kenya was part of a village-wide polyparasitism study focusing on *Plasmodium* spp., *S. haematobium* and *W. bancrofti* infections. Detailed information on the study site and data collection methods was previously presented (Chapter 2). In total, 48.5% of the 1854 individuals aged 8 and older in Kingwede were screened for presence of *Plasmodium* spp. and 49.5% for *S. haematobium* infection. Of individuals contributing both questionnaire data and parasitological samples, over half (52.4%) were infected with *Plasmodium* spp., over one quarter (27.8%) with *S. haematobium*, and 17.5% with both genera of parasites. In this study population, 48.5% (384/791) of participants were between 8 and 17 years old. Children in this age range who provided urine and blood samples, and who provided child assent as well as informed consent from a parent, were eligible to participate in this study. Of the 371 eligible children, 104 (28.0%) lacked information on covariates needed for the analyses including weight and height measurements, and household SEP giving a total sample size of 267 children. Previously reported analyses showed no significant differences between participants and non-participants (Chapter 2). Approval for this study was granted by the University of Michigan Institutional Review Board and the Kenya Medical Research Institute (KEMRI).

Infection Intensity

Infection burden for *S. haematobium* was determined by duplicate examination of two midday urine samples collected on consecutive days. Presence of *S. haematobium*

eggs was determined by microscopic examination of filtered urine. Two 10 ml aliquots of each urine sample were filtered using 12- μ m pore Nucleopore filters (Nucleopore, Pleasanton, CA) and mounted on microscope slides for examination at the study site [31]. The average number of eggs per 10mL urine was used to define infection intensity levels with 100 eggs per 10mL considered moderate to heavy infection [32, 33] and 0-99 light infection.

Infection burden for *Plasmodium* spp. was determined by identifying and quantifying circulating parasites from fingerprick blood (~200 μ L) samples that had been collected in potassium ethylenediaminetetraacetic acid (K-EDTA)-coated Vacutainer tubes, stored at -20C and sent to Case Western Reserve University in Cleveland, OH. *Plasmodium* spp. was detected in these preparations using polymerase chain reaction/ligase detection reaction fluorescent microsphere-based assay (PCR/LDR-FMA) as previously described (Mehlotra, Kasehagen et al. 2002; McNamara, Thomson et al. 2004; McNamara, Kasehagen et al. 2006). Briefly, DNA was extracted, amplified and added to a multiplex, species-specific ligase detection reaction (LDR) where species-specific primers hybridized to target sequences and are subsequently labeled with oligonucleotide probes with fluorescent capacity. A Bio-Plex array reader (Bio-Rad Laboratories, Hercules, CA) was used for detection of fluorescence in a species-specific manner. Samples were considered positive if they had a median fluorescent intensity (MFI) greater than two standard deviations above those of negative controls (MFI above 205, 260, or 220 for *P. falciparum* (Pf), *P. malariae* (Pm) and *P. ovale* (Po), respectively). Standardized methods of quantifying intensity of *Plasmodium* spp. infections using PCR data are lacking, thus empirical distributions were used to define

low and high *Plasmodium* spp. infection intensity based on a standardized MFI score. First, values below the aforementioned cut-off values were set to zero. Subsequently, the MFI were divided by the maximum observed value to give a range from 0 to 1. This method was used for each *Plasmodium* species. A summed value was then created to represent intensity of any *Plasmodium* spp (possible range 0-3, actual range 0-1.29). Based on the distribution of this score, light *Plasmodium* spp. infections were defined as 0-0.65, and moderate to heavy as ≥ 0.65 .

Anemia and Stunting

Anemia was determined from fingerprick blood of participants that was tested for hemoglobin levels on site using a blood hemoglobin photometer (HemoCue, Inc, Lake Forest, CA). Anemia was defined as blood hemoglobin less than 11g/dL (severe anemia < 8g/dL).

Stunting was based on height and weight measurements recorded for participants and used in anthropometry calculations. Anthropometric measures were converted to height-for-age, Z-scores (HAZ) using the National Center for Health Statistics year 2000 reference values in EpiInfo (version 2000, Atlanta, Georgia). Moderate stunting was defined as $-2 \geq \text{HAZ} > -3$ and severe stunting as $-3 \geq \text{HAZ}$ [34].

Confounders

Potential confounders of the relationship between anemia and infection by *Plasmodium* spp. and by *S. haematobium* were identified in peer-reviewed literature [5, 8, 14, 20, 35, 36] and in previous analyses (Chapter 2). A similar assessment was done for the stunting and infection [5, 34, 37]. The risk and intensity of *Plasmodium* infection is known to vary by age, socioeconomic position (SEP) and nutritional status [38-42].

Although evidence of sex differences in malaria prevalence is lacking, pregnancy is known to lower immunity to *Plasmodium* parasites, particularly in first and second pregnancies [24, 35, 43, 44]. The risk and intensity of *S. haematobium* infection also varies by age, SEP, and nutritional status with some evidence of sex effects as well [45-47]. As risk of anemia and stunting is also predicted by these factors, they were measured and controlled for in multivariable analyses.

Data used in determining household socio-economic position (SEP) was collected by questionnaire administered to heads of households asking about asset ownership. Other household-level information was collected by interviewers who observed house quality (e.g. roofing and construction material, presence of window screens, etc) and by demographic data on total household size. An asset index, constructed using principal components factor analysis of questionnaire responses, was used to assess household SEP [48, 49]. Assets measured include electricity, radio, television, bicycle, motor vehicle, land ownership, domestic animals and toilet. The score also included information on crowding, quality of house construction, and numbers of full and part time workers in a household. Missing data necessary for construction of the household SEP score were first imputed using the IVEware (Imputation and variance estimation software) SAS macro [50]. Each individual was assigned an SEP score corresponding to their household and this household SEP score was treated as a continuous variable.

Nutritional status was measured using weight-for-height Z-scores (WAZ) as a proxy. WAZ were calculated using the method described above for HAZ. Moderate malnutrition was defined as $-3 < \text{WAZ} \leq -2$ and severe malnutrition as $\text{WAZ} < -3$ [34].

Infection Interaction

Departures from additivity were assessed in order to identify potential synergistic or antagonistic effects of concomitant heavy-intensity *Plasmodium* spp. and *S. haematobium* infections on prevalence of anemia and stunting, respectively. Four categories of co-infection were necessary for these analyses: i) a reference group defined as children with no infection or with light infection of either species, ii) heavy *S. haematobium* only, defined as children with heavy *S. haematobium* infection and no or light *Plasmodium* infection, iii) heavy *Plasmodium* spp. only, defined as children with heavy *Plasmodium* spp. infection and no or light *S. haematobium* infection, and iv) heavy co-infection, defined as children carrying heavy infections of both parasites. Interactions between heavy infections were examined (instead of presence vs. absence of infection) due the well-documented relationship between intense infection and morbidity [4, 12, 32, 51]. In addition, previous investigations of co-infections between these parasites have found evidence of intensity-dependent associations between infections [4, 13, 25, 52].

Statistical Methods

All data were double entered in Microsoft Access and analyzed using SAS 9.1 (SAS Institute, Inc, Cary, NC). Outcome variables of interest in these analyses were anemia and stunting. Variables of primary interest in these analyses were *S. haematobium* and *Plasmodium* spp. infection intensity. Confounders included age, sex, household SEP, and malnutrition as measured by WAZ.

General estimating equation (GEE) models with exchangeable correlation matrix structures and Poisson distributions were used to estimate associations between the prevalence of outcomes of interest (anemia and stunting) and infections. This analytic

approach accounts for household clustering in the data and estimates fixed effects of variables averaging across households.

Identifying interactions between infections in multiplicative models with prevalence ratios as the estimates of effect is challenging as the formal definition of biologically relevant interaction relies on risk ratios estimated from additive models [30]. The interaction parameter of interest can be indirectly estimated using the interaction contrast ratio (ICR), the synergy index (SI), or the attributable proportion (AP) [53]. The ICR measures the excess risk due to interaction relative to the risk without either exposure (infection). AP measures the proportion of the disease due to interaction in those with joint exposures. When the two exposures do not interact, ICR = 0 and AP=0. Finally, SI is a measure of the excess risk from joint exposure when interaction exists relative to the excess risk from joint exposure when interaction is not present. The null value for SI is 1. These measures must be modified to assess additive interaction for case-control or cross-sectional studies when odds ratios are not good estimates of risk ratios (i.e. when the outcome is not rare in the source population). Formulas used to estimate these measures are provided in Appendix 1 [53].

Finally, percentages of anemia and stunting in the study population attributable to each infection were evaluated for sex and age-specific strata using a weighted sum approach [54, 55]. The weighted population attributable fraction (PAF) is calculated as follows:

$$PAF_w = \sum_j N_{cj} PAF_j / \sum_j N_{cj} \quad (1)$$

where N_{cj} is the frequency of anemia in each stratum. The same method was used to calculate age- and sex-weighted attributable fractions (AF) in each exposure group:

$$AF_w = \sum_j N_{cj} AF_j / \sum_j N_{cj}. \quad (2)$$

RESULTS

One quarter of the study population was anemic, one fifth stunted, and 22% thin for their age (Table 5.1). Over 70% were infected with at least one species of *Plasmodium* parasite, 14% of whom harbored heavy infections. More than 40% had *S. haematobium* infection and 8.5% were heavily infected. Over 30% of children carried both genera of parasites with almost 10% heavily burdened. Approximately half of the study population was 8-12 years of age and half 13-17. Fifty-two percent were male. Sixty-nine percent of the participating children had low household SEP scores (Table 5.1).

Bivariate analyses showed several interesting associations (Table 5.1). First, boys were more likely to be stunted than were girls, and older children were more likely to be stunted than were younger children (OR=1.68 (1.09-2.58)). Next, under-weight children were more likely to be anemic than were those of normal weight (OR=1.62 (1.05-2.51)) and were also more likely to be stunted (OR=8.33 (5.20-13.08)), although this estimate was less precise. Finally, several associations with light infections were significant in this study population. Those with light *Plasmodium* spp. infection were more likely to be stunted than those who were not infected (OR=1.91 (1.09-3.34)), while those with light *S. haematobium* infection were less likely to be anemic than were the uninfected (OR=0.61 (0.38-0.97)).

Sex and age were found to interact in their effects on outcomes of interest and on infection (Figure 5.2). Boys over 12 years old were much more likely to be stunted than younger boys or girls. Older girls were more likely to be anemic than younger girls,

whereas the opposite trend was seen in boys. Heavy *Plasmodium* infection was more prevalent in younger children regardless of sex. Older boys were more likely to carry heavy *S. haematobium* infection whereas the opposite trend was seen in girls. These sex-age interactions necessitated multivariable models to be stratified by sex.

Sex and age-dependent trends in anthropometric variables are presented in Figure 5.3, along with sex and age-specific infection intensity data. In comparison with the US standard, mean height-for-age in these Kenyan boys decreased among those 11 years of age and older. This decline in growth occurred in the same age category in which average *S. haematobium* infection intensity was high. Weight did not appear to change drastically over this age-range in boys. In girls, no clear co-varying trends were evident with respect to infection and height or weight over this age range. It should be noted that for both boys and girls, average height and weight across the age range corresponded to values well below the average of the US standard population. Our results closely resemble those of another study among Kenyan school-children [11, 37].

Effects of infection status on mean blood hemoglobin levels and on mean height-for-age Z-scores stratified by sex are shown in Figure 5.4. Hemoglobin and HAZ were not significantly different in children with heavy single-species infections as compared to the referent group. In contrast, hemoglobin levels were significantly lower in boys carrying heavy co-infection compared to the referent group. A similar, but not statistically significant trend was seen in HAZ scores. The effect of heavy co-infection on these outcomes in girls was not estimable due to small sample sizes.

Sex-stratified multivariable analyses revealed several interesting trends (Table 5.2). Anemia was more prevalent in older girls and in younger boys. Boys and girls with

high weight-for-age scores were less likely to be anemic than were those with low WAZ. Girls, but not boys, from households with higher vs. lower household SEP scores were more likely to be anemic. Prevalence of anemia was higher in girls but not boys carrying heavy *Plasmodium* infection as compared to those with no or low intensity infections. Older boys were more likely to be stunted than were younger boys but no age effect was seen in girls. Unsurprisingly, WAZ and stunting were associated in boys and marginally so in girls. Household SEP appeared to have no effect on prevalence of stunting in this population. *Plasmodium* infections did not influence likelihood of stunting but light *S. haematobium* infections were associated with higher prevalence of stunting in girls but not boys.

Next, the extent to which heavy co-infections with *Plasmodium* spp. and *S. haematobium* additively affected anemia and stunting was assessed in sex-stratified models after adjustment for age, household SEP, and weight-for-age Z-score (for anemia) or mean hemoglobin level (for stunting) (Table 5.3). Results showed very different patterns for males and for females. In boys, neither heavy single nor heavy joint infections were associated with increased prevalence of anemia in multivariable models, however the joint effect of heavy infection with both parasite genera was found to differ from expected values based on an additive model. The synergy index value indicated an antagonistic relationship between the two infections in their effects on anemia ($S = -1.07$). In girls, the small numbers of heavily co-infected participants led to a non-estimable prevalence ratio comparing joint heavy infection to the reference group (no or light infection with both parasite genera). It was still possible to calculate the synergy index since the regression coefficient for heavy co-infection was zero. In this case,

although co-infection was not associated with increased prevalence of anemia, and heavy *Plasmodium* infection was associated with increased prevalence, the synergy index indicated no departure from an additive model (SI=1.09). Similar patterns were seen with stunting as an outcome. Prevalence ratio (PR) estimates for heavy single infections were <1.0 in boys, whereas the PR for heavy co-infection was 3.0. Although these estimates have confidence intervals including the null value, the synergy index revealed departure from additivity (SI =-2.14). For girls, co-infection did not appear to increase prevalence of stunting as compared to the referent group (no or light infections), but only one girl carried heavy co-infection. Similarly, the synergy index for girls provided little support for a departure from additivity in the effect of *Plasmodium* infection and *S. haematobium* infection on stunting (SI=1.10).

Finally, sex- and age-weighted population attributable fractions and exposure-specific attributable fractions were estimated (Table 5.4). Calculations suggest that relatively few (11.4%) cases of anemia in the study population were due to heavy parasitic infection. More specifically, 13% of observed anemia was attributable to acute malnutrition (low WAZ), 14% to heavy *Plasmodium* infection, and <1% to heavy *S. haematobium* infection. In contrast, an estimated 65.0% of stunting in this study population was due to heavy intensity parasitic infection; 67.2% of cases could be attributed to anemia, 61.4% to heavy *Plasmodium* infection and 66.8% to heavy *S. haematobium* infection.

Among those carrying heavy *Plasmodium* infections, 74.7% of anemia cases, but only 7.7% of cases of stunting were attributable to heavy *Plasmodium* infection. Only 8.2% of anemia and 15.8% of stunting in heavily *S. haematobium*-infected children was

due to that infection. Among children with low WAZ scores, 24.1% of anemia cases were attributable to low weight for age. In anemic children, 6.0% of the cases of stunting were attributable to anemia. These analyses were limited by the small sample size in age- and sex-specific strata, and estimates of the excess prevalence of anemia attributable to co-infection were not possible.

DISCUSSION

Concomitant infection with multiple parasites is widespread in many areas of the developing world, especially in tropical climates [1-3]. Despite this reality, detailed estimates of associated morbidities are lacking [5, 14]. Our study attempted to address this gap in knowledge by examining interactions between *Plasmodium* spp. and *S. haematobium* parasites, specifically assessing evidence for synergy or antagonism in effects on anemia and stunting on an additive scale. Results suggest that although important associations exist between heavy single infections and these outcomes, joint infections do not appear to cause significant deviations from additivity. In other words, we found no evidence to support biological interactions in the effects of co-infection on anemia or stunting.

Similar to previously published studies from this region [26, 56-60], *Plasmodium* spp. and *S. haematobium* infections were prevalent in these school-aged children, with 30.7% carrying both parasites concurrently. Furthermore, the proportion of children determined to be anemic (~25%) and stunted (~20%) approximates estimates among school-children in other regions of Kenya [61]. The causes of anemia and stunting are multi-factorial and interrelated (e.g. Figure 5.1). Thus, associations between each individual infection and the outcomes of interest are context-specific; they are likely to

vary based on nutritional status of the population, distributions of other co-endemic parasites, and individual genetic and immunologic characteristics of study participants. Despite limited generalizability of study results, infection-morbidity associations can be examined within the context of the local environment.

Our results show that morbidities varied significantly by age group and by sex (Tables 5.1 and 5.2; Figures 5.2-5.4). Younger boys (8-12 yo) were more likely to be anemic than were older boys (13-17 yo), whereas anemia was more prevalent in older rather than younger girls. These results are consistent with those from other published findings. In a review of several African and Asian countries, school-aged boys were more likely to be anemic than were girls [62]. Another study in coastal Kenya that evaluated the effects of hookworm infection on anemia also found sex differences, with boys having higher prevalence of anemia than girls [55], although that study population was younger. That older girls tended to have more prevalent anemia is likely due to iron loss associated with menstruation [61, 63]. Older boys were much more likely to be stunted than were younger boys, but no significant age effect was seen among girls (Figures 5.2-5.3, Table 5.1). This trend was also observed for weight-for-age z-scores (Figure 5.3 and Table 5.1). Similar results were seen in another study of *S. haematobium* in Kenyan children in which it was suggested that the high intensity *S. haematobium* infections experienced by school-aged boys lead to growth deficits around 10-11 years of age that persist through adolescence [11, 37]. In contrast, a study of malnutrition in adolescent girls from western Kenya found girls aged 12-13 to have a lower prevalence of stunting than older girls, aged 14-18 (20.1% vs. 4.2% [64]).

Next, we examined the extent to which observed sex and age trends in morbidities were mediated by infection profiles. Multivariable analyses showed *Plasmodium* spp. infection to be associated with anemia in girls (Table 5.2). Similar results have been found elsewhere. For example, a study of risk factors for anemia in adolescent girls in Western Kenya found *Plasmodium* infection to be predictive of anemia in younger girls (12-14) but not in older girls (15-18) for whom menstruation was more important [61]. As results of the above-cited study indicate, the relative proportion of anemia cases due to infection, versus malnutrition or other etiologies, likely differs by age. In our study, the strength of association between *Plasmodium* spp infection and anemia in girls varied by infection intensity in a dose-response manner; girls with more intense infections had a higher prevalence of anemia than did those with less intense infections. A similar positive relationship between malaria parasite density and anemia was seen in Cameroon children aged 0-14 [36]. Other dose-response relationships have been noted such as the positive association between anemia and cumulative intensities of helminth infections among school-children in the Philippines [4].

It does not appear that the higher prevalence of malnutrition measured in boys in our study was due to infection. Indeed, the only observed association of stunting with infection occurred among girls with light *S. haematobium* infections, whose prevalence of stunting was higher than those who were uninfected (Table 5.3), while heavy infection had no effect on stunting. This finding supports recent discussions in the literature on the public health significance of light or sub-patent Schistosome infections and the accompanying unrecognized burden of disease [16, 37]. In contrast to our findings, another study in Kenya found evidence of sex differentials in associations between *S.*

haematobium infection and height and weight, with boys experiencing larger and more permanent deficits in height and weight than girls [11, 37]. Although height and weight patterns and *S. haematobium* infection intensities that we found closely resemble those seen in the study by Olds and colleagues [11], our statistical analyses did not support the same conclusions.

The observed age and sex associations with anemia and stunting highlight the complexity of parasite pathogenesis, especially regarding multi-factorial outcomes. The proportion of morbidity attributable to one specific infection is dynamic in space and time, and depends on the growth and development micronutrient needs of individuals. These needs will vary by age and sex, with more dramatic differences observable during adolescent years. Longitudinal studies are needed to identify crucial windows in childhood and adolescent development when parasitic infections may strongly influence risk of developmental morbidities.

Contrary to several recent studies examining biological interactions among parasites in their effects on anemia in school-children, we found no evidence of departures from additivity when examining *Plasmodium* spp. and *S. haematobium* infection. Ezeamama and colleagues studied the joint effects of multiple STH infection in the Philippines [13], and Brooker and colleagues examined additive interactions between *Plasmodium* and hookworm infections in Kenya [14]. Our failure to identify significant deviation from additivity could very well be due to small sample size, especially as very few girls had concomitant heavy infections. Analyses of additive interaction assessed by synergy index values are influenced by the direction of effect of each single species infection. In our study, many of the point estimates of the effect of heavy single-

species infections on anemia and stunting were less than those of the referent group, whereas the opposite was true in the co-infected group. This made synergy index values difficult to interpret.

Although the formal analyses did not find evidence of additive interaction between the effects of heavy infection on anemia or stunting, we did identify a trend in hemoglobin levels in boys that supports infection interaction; hemoglobin levels were significantly lower in boys with heavy co-infections than in those with no or light infections (Figure 5.4). A similar trend was evident in height-for-age Z-scores in boys (Figure 5.4). These results suggest separate biologic pathways for *Plasmodium* and *S. haematobium* infection, leading to more morbidity than would be expected if the etiologies were completely independent. The mechanisms by which *Plasmodium* parasites cause anemia are well understood and include destruction of red blood cells and ‘anemia of inflammation’ which is characterized by disruption of RBC production, iron absorption and metabolism by pro-inflammatory mediators [5, 65, 66]. Chronic infections are more likely to involve dyserythropoiesis rather than hemolysis or cytokine disturbances [66]. Schistosomes cause anemia through chronic blood loss in the urinary tract, but also through destruction of RBCs and dyserythropoiesis [12]. Helminths cause malnutrition and stunting by direct pathophysiology of the gastrointestinal tract, by triggering anorexia, and by intestinal inflammation [67, 68], whereas the mechanisms by which *Plasmodium* infection leads to stunting are not well understood. Some evidence supports the initiation of inflammatory cytokine cascades that trigger anorexia [69, 70]. Thus it is possible that quantitative estimates of anemia and stunting associated with

these two genera of parasites will be greater than additive due to independent biological pathways.

Sex- and age-weighted attributable fraction calculations suggested that relatively few (11.4%) cases of anemia but a majority (65.0%) of stunting cases in the study population were due to heavy parasitic infection. The low percentage of anemia cases attributable to infection may indicate the importance of unmeasured nutritional or genetic variables in assessments of anemia. Another possible explanation is that dichotomization of these infections into heavy versus light/no infection is not biologically appropriate in modeling anemia. For example, Ezeamama and colleagues found evidence that co-infection with multiple, light intensity STH was associated with odds of anemia similar to odds of anemia in those infected with one parasite species at heavy intensity [4].

The relative importance of each infection in predicting anemia and stunting varies; among those carrying heavy *Plasmodium* infections, 74.7% of anemia cases were attributable to heavy *Plasmodium* infection, while only 8.2% of anemia in heavily *S. haematobium*-infected children was due to that infection. For stunting, infection-specific attributable fractions were similar (61.4%, 66.8%, respectively). This suggests that treating heavy *Plasmodium* infections may reduce more cases of anemia than treating heavy *S. haematobium* infection with little differential effect on stunting. However, the total proportion of cases of anemia attributable to either infection was relatively low in this population, indicating a minimal role for public health intervention via infection control.

The cross-sectional design of this study prohibits unequivocal assessment of temporality; anemia could predispose children to parasitic infections, or to more intense

infections [71]. In addition, the potential for unmeasured confounders must be considered. This study did not control for other infections, known to be risk factors for anemia and/or stunting that could affect susceptibility to, or intensity of *Plasmodium* spp. or *S. haematobium*, such as soil-transmitted helminthes (STH) or HIV. Although STH, such as hookworm, are probably widespread, *S. mansoni* is not endemic to this region and HIV rates are fairly low, especially in children (7% prevalence in rural Kenya and 7.9% prevalence in Coast province overall, [72]). Such infections also could have effects on immune responses thereby influencing the *S. haematobium-Plasmodium* spp. associations. Similarly, this study lacks data on pregnancy status of girls. Pregnant girls and women have elevated risk of anemia, and malaria-associated morbidity in primigravidae is particularly high [24, 43, 44]. Nutritional status of participants is also lacking although analyses were adjusted for weight-for-age which is a proxy measure of acute malnutrition [34]. Our reliance on blood hemoglobin levels to define anemia precludes differentiation between iron-deficiency anemia (IDA) and non-iron deficiency anemia (NIDA), which have different etiologies and vary by age, at least in populations of school-aged girls [61]. Finally, alpha + Thalassemia status of our study population was not assessed and has been estimated to affect nearly half of people in the study area [73]. Alpha+ Thalassemia (heterozygous or homozygous) has been shown to protect against anemia in Kenyan children with asymptomatic *Plasmodium* infection, and may protect against anemia resulting from other inflammation-mediated etiologies as well [74]. Thus, although generalizability of our study results may be limited, missing data on alpha+Thalassemia status is likely to lead to conservative estimates of the association between *Plasmodium* infection and the outcomes studied here.

Despite these limitations, our study improves upon previous reports of *Plasmodium-S. haematobium* co-infection [25, 27, 75] by investigating infection intensity and specific morbidities, as well as by explicitly examining biologically relevant infection interactions through formal assessments of deviations from additive models. To our knowledge, the only other study that has assessed biological interactions between infections on anemia using these analytic techniques focused on STH [13]. In addition, we have provided sensitive measures of infection burden in the population through the use of PCR to diagnose *Plasmodium* infections. Our findings suggest that hemoglobin levels and growth in school-aged children may be adversely affected by heavy co-infection with these two parasites.

Figure 5.1: Causal diagram of hypothesized relationships among parasitic infections and associated morbidities [16].

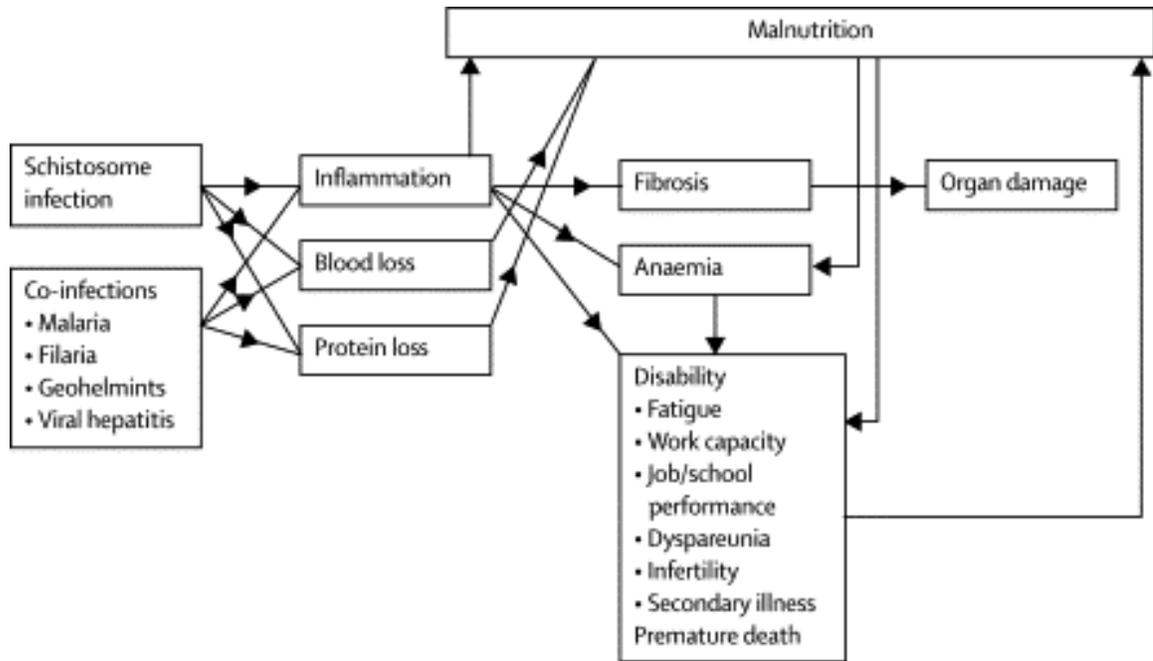
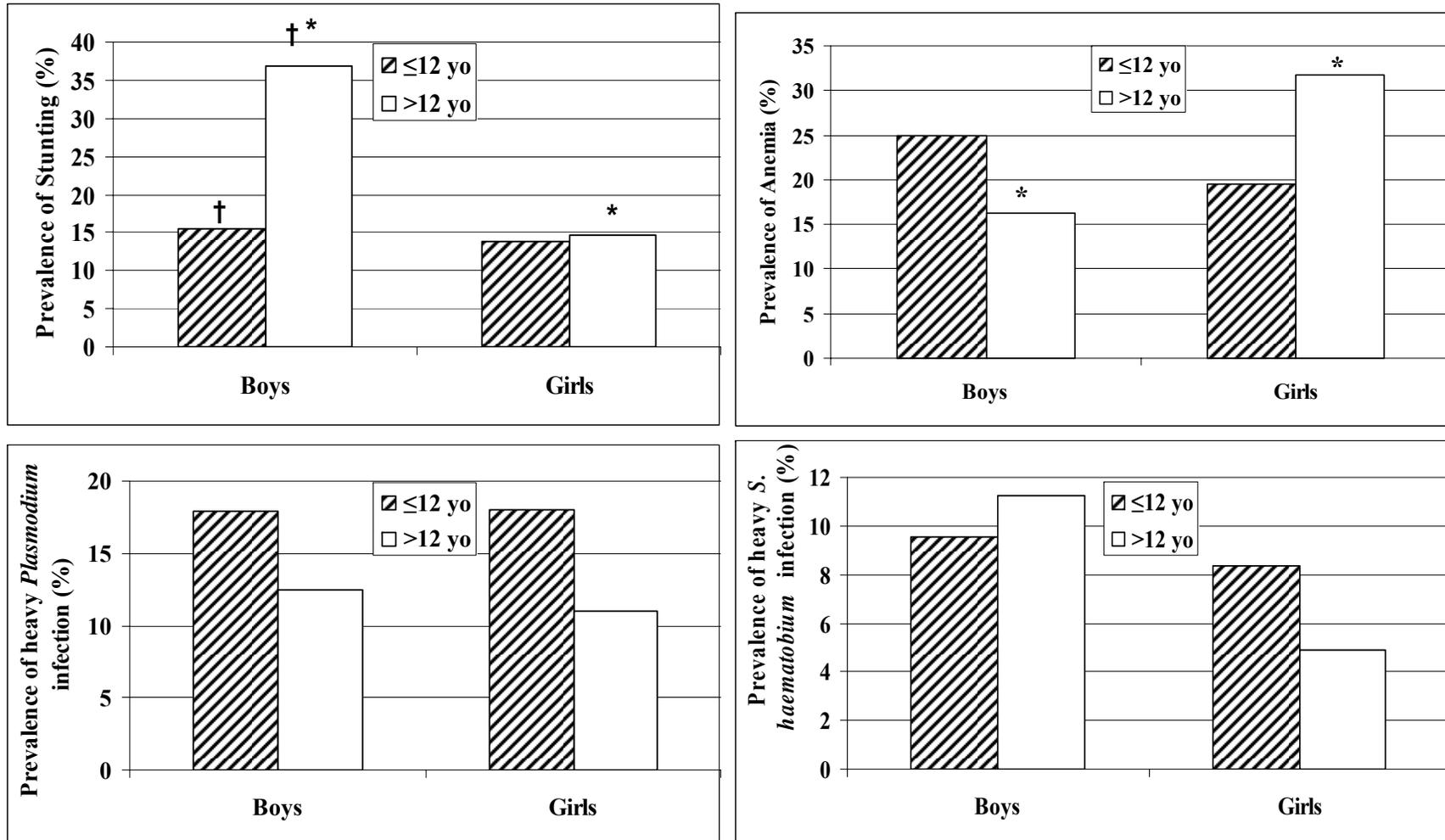


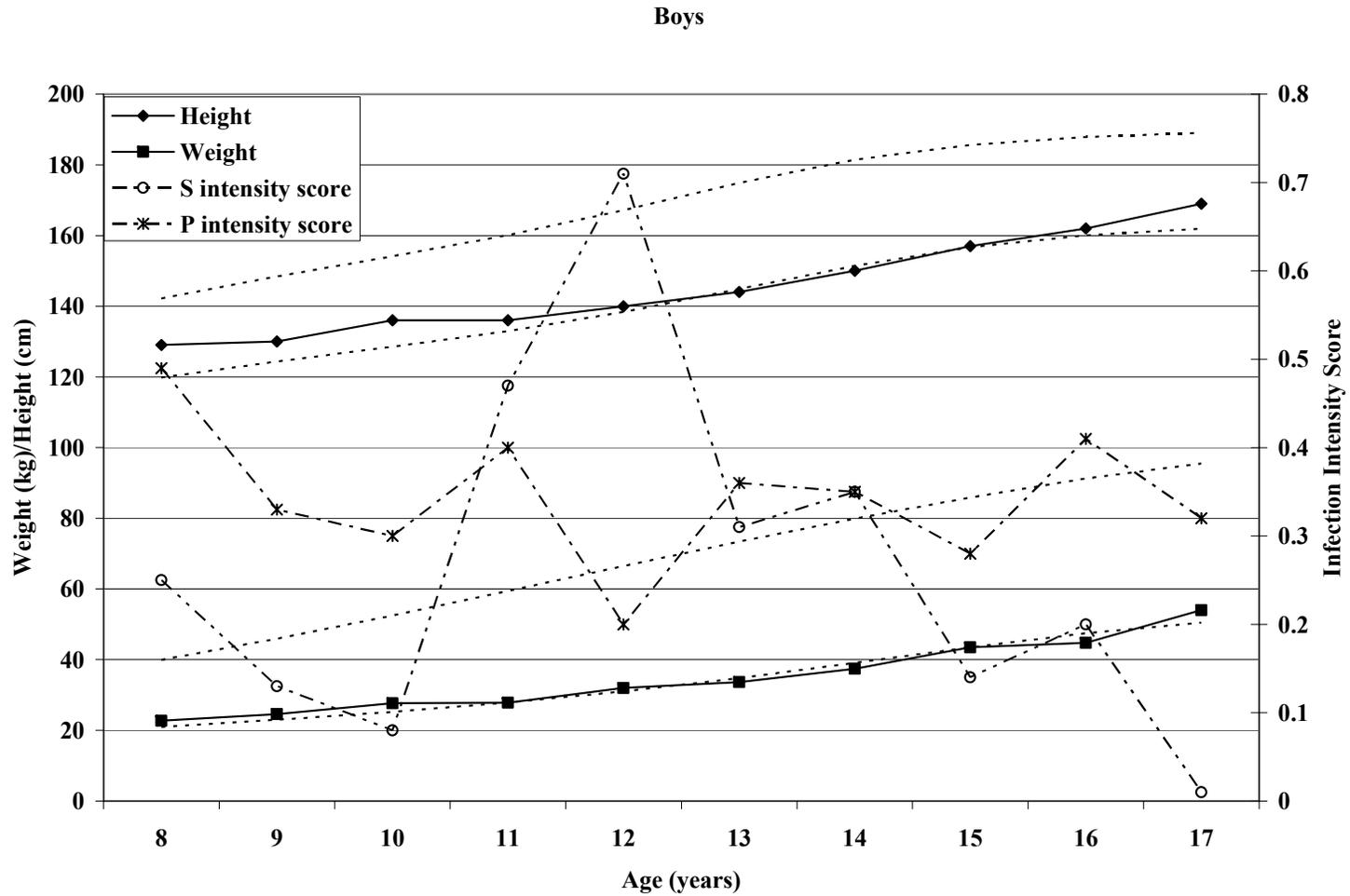
Figure 5.2: Prevalence of anemia, stunting, heavy *Plasmodium* and heavy *S. haematobium* infection by age and sex.



* Significant difference between boys and girls >12 yo according to results of GEE models (anemia $p = 0.02$; stunting $p = 0.0049$)

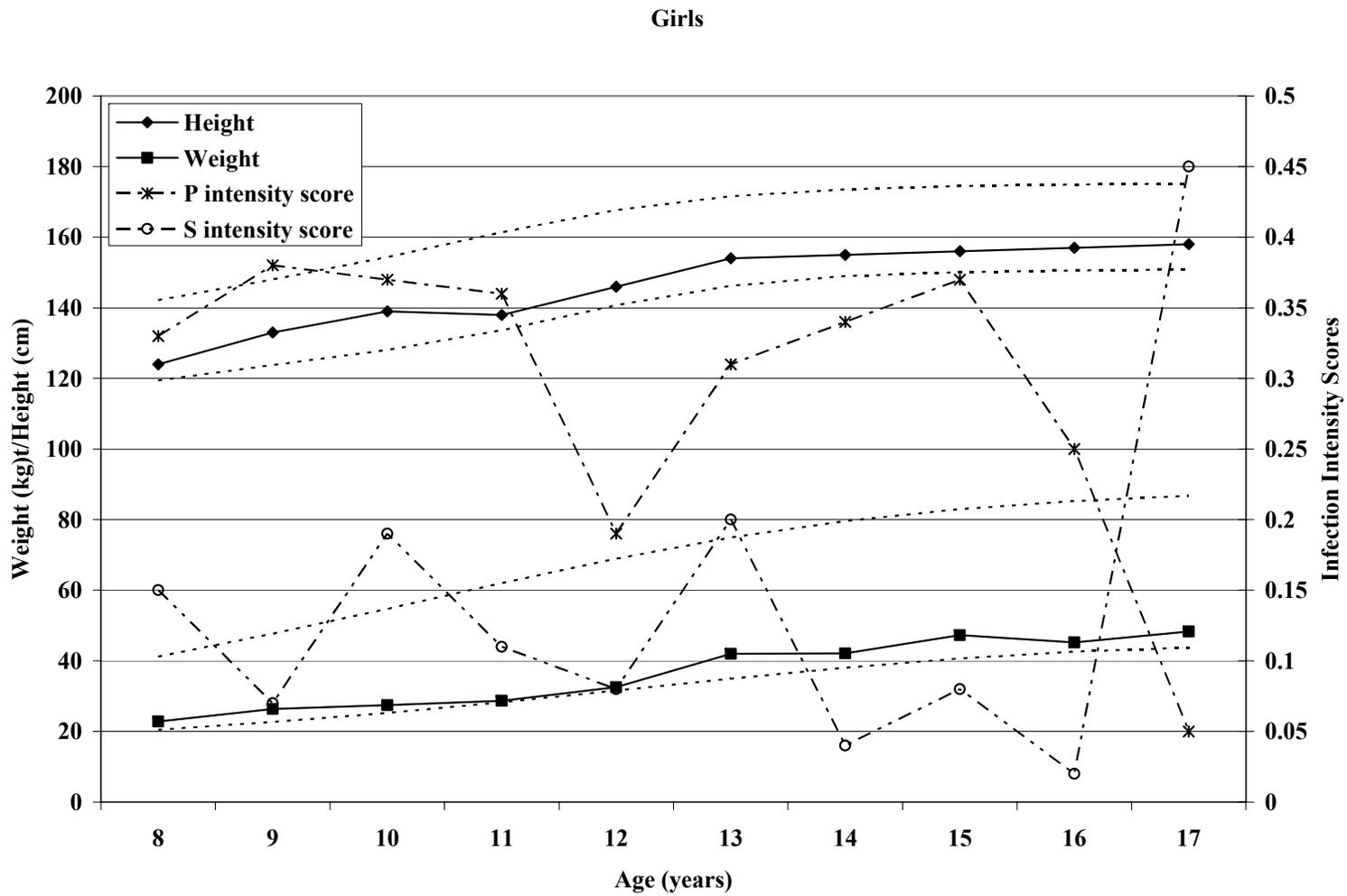
† Significant difference between boys < 12 yo and boys > 12 yo according to results of GEE models (stunting $p = 0.0028$)

Figure 5.3: Average height and weight by age of study participants compared to US reference population (97th and 3rd percentiles, dotted line) for boys (a) and girls (b). Average infection intensity scores for *Plasmodium* and *S. haematobium* are also presented.
(a)



(b)

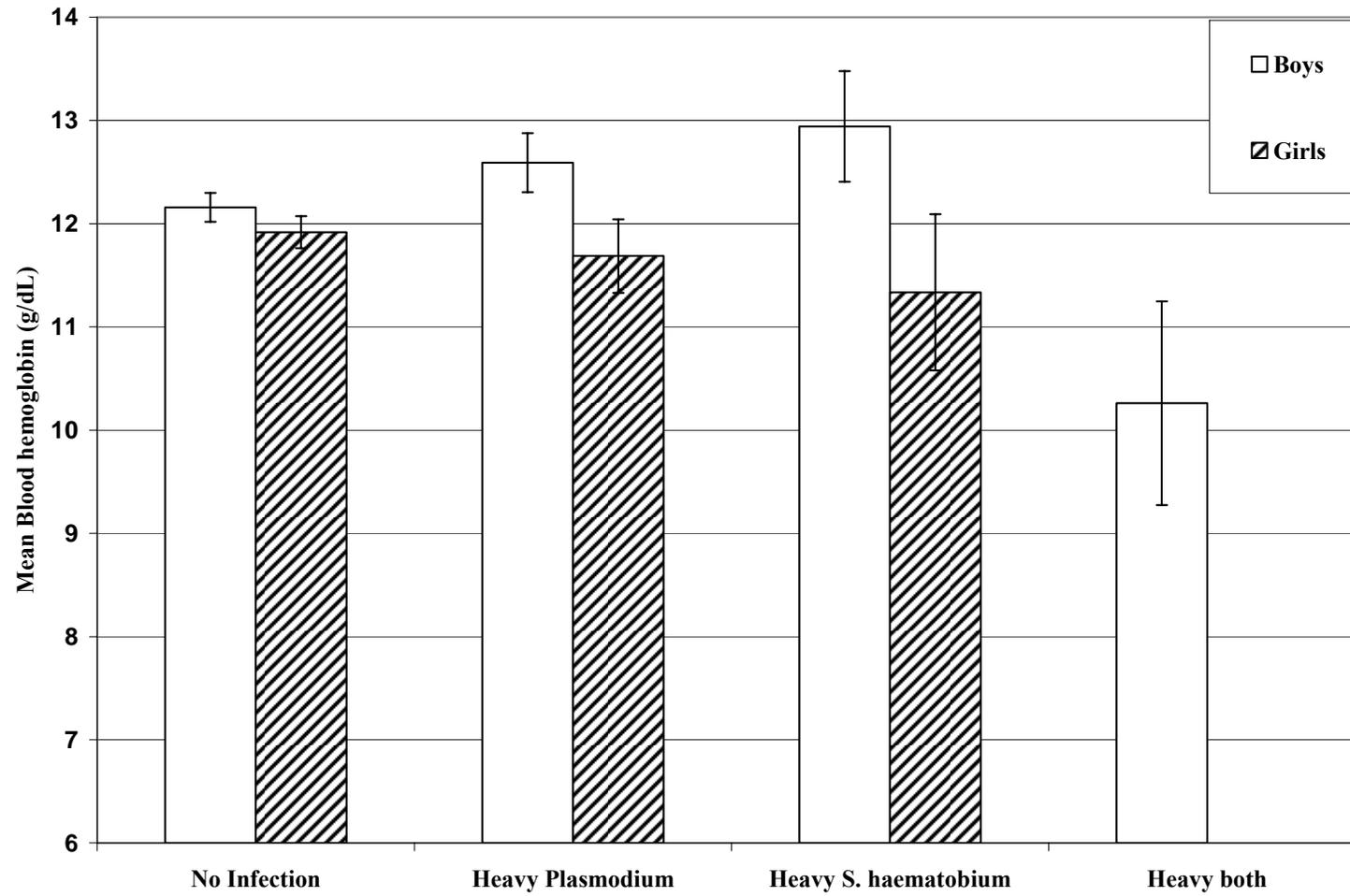
170



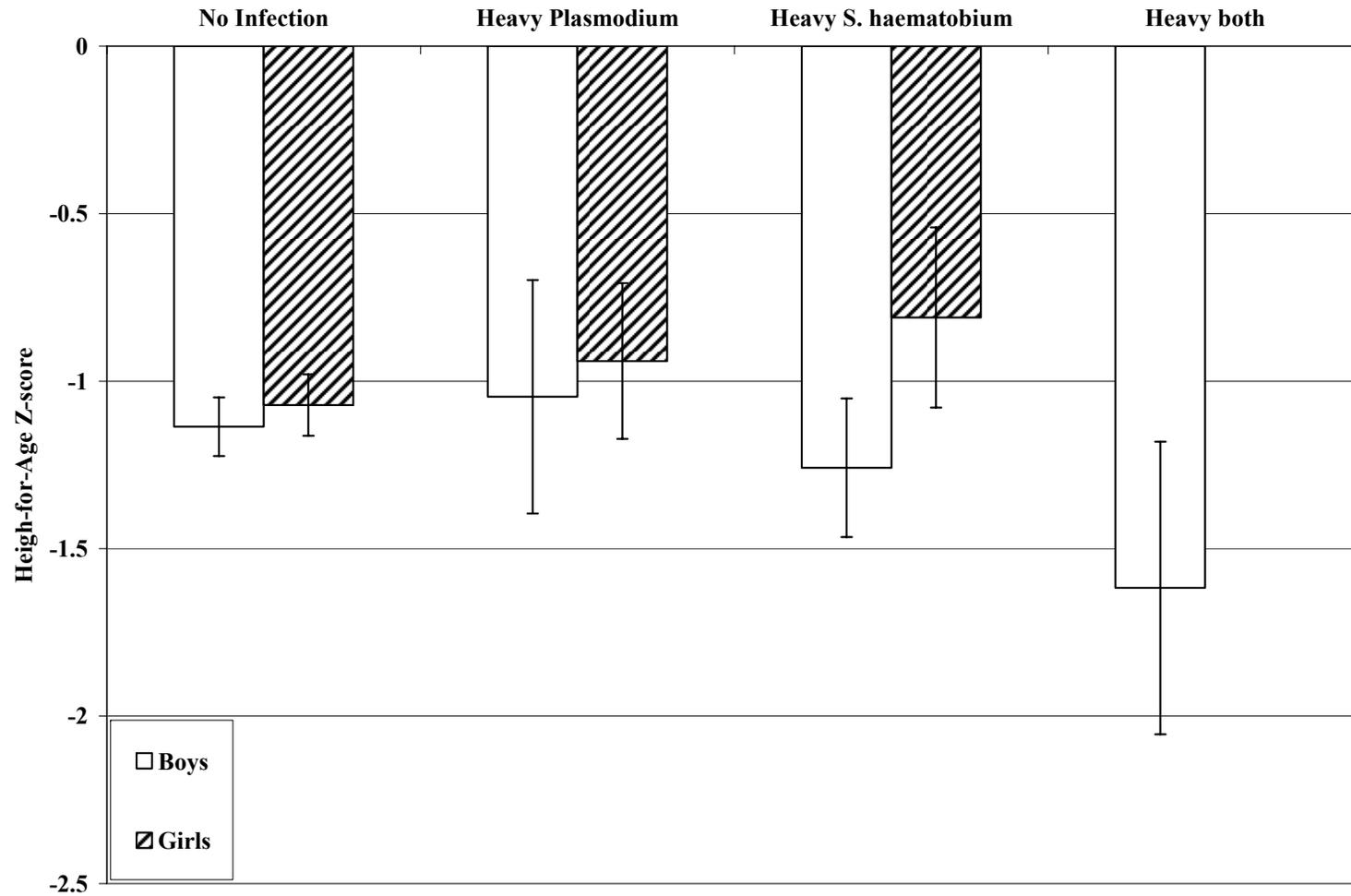
Note: P = *Plasmodium*, S = *S. haematobium*. *S. haematobium* scores were multiplied by 10 for scaling purposes.

Figure 5.4: Adjusted mean blood hemoglobin (a) and mean height for age z-score (b) by sex and infection status.*

(a)



(b)



* (a) adjusted for grand-mean centered age, household SEP score, and WAZ score, (b) adjusted for grand-mean centered age, household SEP score, and hgb.

Table 5.1: Study population characteristics and bivariate associations with outcomes of interest (anemia and stunting). Associations are prevalence ratios estimated by GEE models with Poisson distribution.

	N (%)	PR of Anemia*	PR of Stunting†
Anemia (n=352)	24.72%		1.30 (0.81-2.10)
None	75.28%		1.0
Moderate	22.73%		1.26 (0.77-2.05)
Severe	1.99%		1.81 (0.54-5.99)
Stunting (n=346)	20.23%	1.25 (0.80-1.95)	
None	79.77%	1.0	
Moderate	14.16%	1.29 (0.81-2.05)	
Severe	6.07%	1.13 (0.45-2.8)	
Age (years)			
8-12	48.96%	1.0	1.0
13-17	51.04%	0.94 (0.67-1.31)	1.68 (1.09-2.58)
Sex			
Female	47.92%	1.0	1.0
Male	52.08%	0.71 (0.49-1.01)	1.85 (1.16-2.95)
HH SEP (n=322)			
Low	69.01%	1.0	1.0
High	30.99%	1.24 (0.85-1.81)	0.68 (0.41-1.13)
WAZ (n=346)	21.97%		
Normal	78.03%	1.0	1.0
Under-weight	18.79%	1.62 (1.05-2.51)	8.33 (5.20-13.08)
Severe Under-weight	3.18%	0.53 (0.11-2.48)	12.36 (8.00-19.10)
Plasmodium (n=377)	72.68%	1.17 (0.73-1.85)	1.79 (1.03-3.12)
None	27.32%	1.0	1.0
Light Infection	58.36%	1.16 (0.72-1.88)	1.91 (1.09-3.34)
Heavy Infection	14.32%	1.17 (0.63-2.19)	1.35 (0.63-2.92)
S. haematobium (n=378)	41.80%	0.67 (0.45-1.01)	1.29 (0.88-1.89)
None	58.20%	1.0	1.0
Light Infection	33.33%	0.61 (0.38-0.97)	1.30 (0.85-1.99)
Heavy Infection	8.47%	0.93 (0.50-1.72)	1.23 (0.62-2.44)
Co-Infected (n=371)	30.73%	0.71 (0.45-1.11)	1.36 (0.95-1.97)
Heavy Co-infection	1.35%	1.85 (0.50-6.84)	3.03 (0.64-14.36)

* Anemia defined as blood hemoglobin <11 g/dL.

† Stunting defined as height-for-age Z score < 2 standard deviations from mean of standard population.

Table 5.2: Sex-stratified multivariable GEE models with Poisson distribution, adjusted for covariates. Values presented are prevalence ratios and associated 95% confidence intervals.

	Anemia		Stunting	
	Boys (n=140) †	Girls (n=127) †	Boys (n=140) ‡	Girls (n=127) ‡
<i>Plasmodium</i>				
None	1.0	1.0	1.0	1.0
Light	0.87 (0.40-1.87)	2.06 (0.94-4.51)	1.67 (0.81-3.42)	2.07 (0.50-8.51)
Heavy	0.76 (0.28-2.09)	3.17 (1.42-7.10)	1.29 (0.52-3.20)	1.88 (0.34-10.47)
<i>S. haematobium</i>				
None	1.0	1.0	1.0	1.0
Light	0.80 (0.36-1.77)	0.69 (0.36-1.30)	0.96 (0.57-1.60)	2.46 (1.02-5.94)
Heavy	1.18 (0.46-3.07)	1.21 (0.46-3.18)	1.19 (0.58-2.43)	0.87 (0.08-9.89)
Age (years)	0.87 (0.76-0.99)	1.21 (1.07-1.37)	1.21 (1.11-1.32)	1.00 (0.86-1.15)
Household SEP	0.88 (0.60-1.27)	1.31 (1.05-1.64)	0.93 (0.61-1.12)	0.67 (0.41-1.08)
WAZ	0.58 (0.41-0.84)	0.68 (0.51-0.91)		
Blood hgb (g/dL)			0.83 (0.74-0.93)	0.80 (0.62-1.04)

† Controlling for grand-mean centered age, WAZ, and household SEP score.

‡ Controlling for grand-mean centered age, blood hemoglobin level, and household SEP score.

Table 5.3: Multivariate GEE models with Poisson distributions estimating the effects of heavy parasitic infections on prevalence of anemia and stunting on school-aged children with a focus on assessing additive infection interactions.

	Males				
	n	PR of Anemia†	Measures of interaction†	PR of Stunting‡	Measures of interaction ‡
Reference	106	1.0		1.0	
Heavy <i>Plasmodium</i>	19	0.54 (0.15-2.01)		0.66 (0.25-1.75)	
Heavy <i>S. haematobium</i>	11	0.82 (0.19-3.44)		0.99 (0.44-2.24)	
Heavy <i>Plasmodium</i> & Heavy <i>S. haematobium</i>	4	3.98 (0.52-30.31)	ICR= 1.2152 AP= 0.7461 SI=-1.0718	3.03 (0.55-16.83)	ICR=0.9405 AP=0.5733 SI=-2.1352

	Females				
	n	PR of Anemia†	Measures of interaction†	PR of Stunting‡	Measures of interaction ‡
Reference	98	1.0		1.0	
Heavy <i>Plasmodium</i>	20	1.71 (0.93-3.15)		1.10 (0.34-3.59)	
Heavy <i>S. haematobium</i>	9	1.19 (0.48-2.97)		0.52 (0.04-6.12)	
Heavy <i>Plasmodium</i> & Heavy <i>S. haematobium</i>	0	Non estimable, $\beta = 0.0$	ICR= 0.0580 AP= 0.0340 SI=1.0898	Non estimable, $\beta = 0.0$	ICR=-0.0371 AP=-0.0620 SI=1.1016

n= sample size; PR = prevalence ratio; ICR = Interaction Contrast Ratio; AP = Attributable Proportion due to interaction; SI = Synergy Index

† These analyses adjust for grand-mean centered age, weight-for-age Z-scores and household SEP scores.

‡ These analyses adjust for grand-mean centered age, blood hemoglobin level (g/dL) and household SEP scores.

Table 5.4: Age- and sex-weighted attributable fractions (AF) and population attributable fractions (PAF) of heavy infections as predictors of anemia and stunting.

	Anemia AF	Anemia PAF	Stunting AF	Stunting PAF
Heavy <i>Plasmodium</i>†	74.7%	14.3%	7.7%	61.4%
Heavy <i>S. haematobium</i>‡	8.2%	0.9%	15.8%	66.8%
Low WAZ*	24.1%	13.1%	--	--
Anemia**	--	--	6.0%	67.2%

† Estimated using GEE Poisson models with mean *S. haematobium* infection intensity, mean household SEP, and mean WAZ (for anemia) or mean blood hemoglobin (for stunting).

‡ Estimated using models with mean *Plasmodium* infection intensity, mean household SEP, and mean WAZ (for anemia) or mean blood hemoglobin (for stunting).

* Estimated using models with mean *Plasmodium* and mean *S. haematobium* infection intensities, mean household SEP, and mean WAZ (for anemia) or mean blood hemoglobin (for stunting).

** Estimated using models with mean *Plasmodium* and mean *S. haematobium* infection intensities, mean household SEP, and mean WAZ (for anemia) or mean blood hemoglobin (for stunting).

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APPENDIX 1

Formulae for calculating modified ICR, AP and SI estimates based on Odds Ratios as found in [53].

$$ICR = \frac{[OR_{11} (1 + O_{00})/(1+O_{11})] - [OR_{10}(1+O_{00})/(1+O_{10})] - [(OR_{01}(1+ O_{00})/(1+O_{01})) + 1]}{(1+ O_{00})([OR_{11}/(1+ O_{11})] - [OR_{10}/(1+ O_{10})] - [OR_{01}/(1+ O_{01})] + [1/(1+ O_{00})])}$$

$$AP = ICR*(1+ O_{11})/ OR_{11} (1+ O_{00}) = [(1+ O_{11})/ OR_{11}]*([OR_{11}/(1+ O_{11})]-[OR_{10}/(1+ O_{10})]-[OR_{01}/(1- O_{01})]+[1/(1+ O_{00})])$$

$$S = ([OR_{11} (1+ O_{00})/(1+ O_{11})]-1)/([[OR_{10} (1+ O_{00})/(1+ O_{10})]-1) + ([OR_{01} (1+ O_{00})/(1+ O_{01})]-1)]$$

Where O_{00} = Odds of outcome in children with no heavy infection

O_{01} = Odds of outcome in children with single-species heavy infection

O_{10} = Odds of outcome in children with the other single-species heavy infection

O_{11} = Odds of outcome in children with heavy infections with both parasites

OR_{01} = Odds Ratio of outcome comparing O_{01} to O_{00}

OR_{10} = Odds Ratio of outcome comparing O_{10} to O_{00}

OR_{11} = Odds Ratio of outcome comparing O_{11} to O_{00}

CHAPTER 6

CONCLUSIONS

“It is in the power of man to make parasitic maladies disappear from the face of the globe, if the doctrine of spontaneous generation is wrong, as I am sure it is.”
- Louis Pasteur[1]

Polyparasitism is not a new phenomenon. Concomitant infection with multiple parasites has been recognized since the beginning of recorded history [2, 3]. Despite recognition of the ubiquitous nature of multiple infection and calls for integrated control efforts in the 1970s and 1980s [4-8], little progress in parasitic disease reduction has occurred. Only recently have a resurgence of interest in contextual predictors of disease and an increased recognition of the global burden of disease attributable to “neglected tropical diseases” (NTDs) revitalized polyparasitism research [9]. While ours is one of many recent studies focusing on concomitant parasitic infections, it is among very few that incorporates data on contextual variables in the social and environmental domain. Our findings support the importance of context in parasitic infection transmission and highlight the essential role of multifactorial models in exploring infection, co-infection and morbidity.

SUMMARY OF MAJOR FINDINGS AND RESEARCH IMPLICATIONS

Risk Factors for polyparasitism

Single-species infections of globally important diseases such as malaria and urinary schistosomiasis have been the subject of epidemiologic, clinical and

pharmaceutical research for decades, with context-specific risk factors for these diseases having been well described. Despite a geographic overlap in disease distribution and identification of common risk factors of infection, few studies have explicitly focused on identifying predictors of co-infection with these parasites. Those few have focused on different combinations of infections than what was studied in this dissertation, for example concomitant infection with multiple species of soil-transmitted helminthes (STH) [10-14] or *Plasmodium* spp. and STH co-infection [9, 15-32]. Since multiple infections have the potential to interact synergistically in their effects on morbidity, identifying variables that predict co-infection could lead to more effective, targeted health interventions.

Our results identified several important risk factors for *Plasmodium* spp.-*S. haematobium* co-infection that merit review. First, single-infections and co-infections were found to be more prevalent and more intense in children as compared to adults. This pattern, well established in the literature for single species infections, is therefore unremarkable. However, we observed a much higher prevalence of *Plasmodium* infection among adults than is commonly recognized using standard diagnostic methods, thereby highlighting the possibility that the role of adults in maintaining transmission is underestimated [33]. This finding suggests that control efforts should not ignore adults, especially when malaria elimination is the goal. Nonetheless, the higher prevalence of co-infection in children points toward this group as the logical target of interventions under resource-limited conditions.

Household SEP also was found to have important associations with frequency and intensity of co-infection, but only among adults. The lack of association among children

in our study may be due to the relative strength of other risk factors that we measured, or to a lack of sufficient population heterogeneity in household SEP. Significant associations with bednet use, outdoor nighttime activity, water contact, and household distance to stream were seen in children but not in adults. Alternatively, differences in household SEP may only be relevant in determining likelihood of infection in children in areas of less food security, that is to say where household SEP influences food availability and thus malnutrition. Or, it may be that acquired immunity is ultimately the most important predictor of infection. Thus, other risk factors, including SEP, may play a minimal role in predicting co-infection among children who have more variation in immune-mediated protection than do adults.

Our findings suggest that integrated intervention efforts should focus on school-aged children in order to reach the most heavily burdened subpopulation. Interventions might target behavioral aspects of disease prevention such as increasing uptake and proper use of bednets. This is merited due to the protective association seen between bednet use and co-infection and also due to the suggestion that infections are more common among individuals reporting indoor nighttime activities, indicating that local *Plasmodium* vectors are highly endophilic. Efforts to improve local hygiene, sanitation practices, and education regarding water contact behaviors could also be beneficial. Given adequate resources, interventions should not ignore adults, especially if elimination of these parasites is the goal. Effective interventions for adults may require identification of poor households. Linking poverty alleviation with disease control could be beneficial to this end [34].

Finally, our results suggest the need for multifaceted infection control. We found that children living close to the stream had higher odds of co-infection than those living 1km away, a finding which reinforces the importance of local environmental conditions to infection transmission. Chemotherapeutic and educational interventions should be paired with vector control in order to maximize reductions in infection and associated morbidity.

Associations among infections

Evidence of significant associations among parasites within human hosts is varied and likely to be species and context specific. Observed associations may be due to shared biological mechanisms of pathogenesis, or simply due to shared risk factors or susceptibility to infection. In order to identify potential biological associations between parasite species, our analyses adjusted for contextual factors and other confounders. Results presented in Chapter 2 showed clustering of infection intensities in children, thereby supporting the hypothesis that *Plasmodium* and *S. haematobium* parasites were biologically associated, at least in children.

As has been eloquently argued by Molyneux, treating *S. haematobium* and other helminth infections is inexpensive and has the potential to markedly reduce the burden of disease associated with malaria [35, 36]. Our data provide further justification for integrated control of malaria with NTDs. Targeted control efforts would be most cost-effective if identification of children with high intensity infections is possible. Since intense infections are often associated with recognizable morbidity [15, 22, 37], multi-infection treatment and educational interventions could be provided in health care settings. Targeting highly infected individuals may have the added effect of significantly

reducing transmission [38, 39]. Nonetheless, the current high prevalence of co-infection renders eradication an unrealistic goal and it will remain so if prevention and control efforts are limited to highly infected individuals [39]. Successful disease reduction will require chemotherapeutic intervention, education on prevention, and integrated environmental control efforts.

Results from Chapter 2 have important implications for pharmaceutical and vaccine development as well. Mechanisms underlying the observed associations in infection intensities are likely immunological. Arguments in the literature suggest differential immune responses to *Plasmodium* infection in helminth-infected and uninfected individuals [24, 40]. Chronic helminth infection has been shown to produce Th2 or non-cytophilic immune responses whereas acute helminth and *Plasmodium* infections usually induce Th1 or cytophilic responses [24, 40-43]. Findings of reduced immunity to *Plasmodium* infection (evidenced by reduced malaria incidence) in helminth-infected children could be explained by this Th2/Th1 imbalance. In contrast, the Th1 response to *Plasmodium* infection in helminth-free children may decrease incident cases of malaria but increase risk of severe disease [24, 40, 42, 44]. As vaccines rely on specific immune responses to confer protection from disease, the potential for *S. haematobium* or other parasites to modify or even annul this protective effect merits further investigation [24].

Spatial distribution of infection: the household context

Recent interest in disease mapping has led to coarse-scale studies describing disease distributions in sub-Saharan Africa, particularly for malaria [14]. While these studies provide valuable information on geographic patterns and potential environmental

and climatic risk factors that help predict disease trends, practical application of resulting predictive models may be limited with smaller spatial scales [22]. A better understanding of small-scale spatial distributions of co-infections could help identify locally relevant “hotspots” that merit targeted control efforts. Micro-level heterogeneities in distributions have been found for *S. haematobium* [45-47], *W. bancrofti* [48-51] and malaria [46, 52-54]. Spatially clustered infections may simply be due to the spatial distribution of individuals within communities, but they may also be partially explained by characteristics of local environments and of the individuals that reside therein. For example, health behaviors and genetic factors are likely to be shared among family members [55]. Similarly, household resources can influence risks of infection for household members by altering exposure to parasites (e.g. quality and location of housing, availability of infection control resources such as bednets, latrines, potable water supply) or susceptibility to infection (e.g. food insecurity leading to malnutrition) [6, 56, 57].

Chapter 3 specifically examined the relative role of contextual variables versus individual-level variables in determining clustering of infections within households. Although we identified household-level variables as being important predictors of infection in Chapter 2, and although infections were shown to group within households, these variables did not explain much household variation in infection. Thus, it appears that spatial trends in infection are dependent on the distribution of individual-level characteristics and not on contextual features of household environments. These findings reinforce the suitability of targeted control efforts focusing on school-aged children over community-based interventions.

***Plasmodium* spp. co-infection**

Recently, attention has been paid to mixed species *Plasmodium* infections, particularly Pf-Pv co-infections and possible synergistic or antagonistic relationships between the two species [58-62]. A recent review suggests a pattern of *Plasmodium* spp infection such that an excess of Pf-Pm-Po co-infections occur in areas endemic for these species, yet a deficit of Pf-Pv-Pm co-infections elsewhere [63]. Researchers have used these findings to conclude that hypotheses of heterologous immunity between *Plasmodium* species are unfounded [64-68]. The actual details of species-specific relationships are likely more complex than these generalizations imply. For example, early malariotherapy studies and more recent immunological research show intricate, density-dependent relationships between Pf and Pv, with no evidence of heterologous immunity [69, 70]. Untangling these associations is essential for informed decisions regarding vaccine development and disease control strategies [71].

Results from Chapter 4 cannot be directly compared to those summarized above due to the use of different analytic methodologies. We restricted our analyses to infected individuals instead of using data comprised of both infected and uninfected individuals from the entire study population. In doing so, we were able to report unbiased estimates of association between species by reducing the effect of heterogeneities in individuals' exposures to infectious vectors. In contrast, results from the literature indicate that co-infection frequencies were much less common than expected when analyses were restricted to infected individuals. This finding supports the hypothesis of heterologous immunity between Pf-Pm and Pf-Po. We believe that our analytic methodology and study results provide stronger evidence for inference than those of previously published

cross-sectional studies. In fact, a recent publication calls for such reevaluation of co-infection trends using these kinds of analyses [72]. Our results also revealed higher intensity infections among individuals harboring multiple species of *Plasmodium* parasites than among those with single species infections, a trend that held for both children and adults. Since intensity of infection is often correlated with morbidity, this finding highlights the potential of reducing mixed infections to mitigate burden of disease. Finally, recent use of antimalarial medication was found to significantly decrease the odds of mixed infections in both adults and children, and high intensity co-infection with *S. haematobium* increased odds of mixed infections in children. Encouraging appropriate use of antimalarial medication and treating comorbidities may be an effective means of reducing mixed species infections and associated morbidities.

These results have important implications for vaccine development as well. Malaria vaccine development remains a major field of research. The Bill and Melinda Gates Foundation has declared a lofty, long-term goal of malaria eradication [73, 74]. Meeting this goal will require not only effective vaccines, but massive integrated prevention and control efforts as well. Existence of heterologous immune responses to multiple *Plasmodium* parasites could be of great import to vaccine development, and to the cost-effectiveness and eventual success of these eradication efforts.

Morbidity of co-infection

Perhaps the most important aspect of this research involves inferences provided by analyses of health outcomes associated with single and multiple infections. Epidemiologic studies have identified various ill-health consequences of polyparasitism, including malnutrition (reviewed in [75]), organ pathology [46, 76], low birth weight

[23], and self-reported morbidity [13], although these trends are inconsistent [9, 17, 27, 28, 77-79]. Coherent summaries of polyparasitism research findings remain elusive due to the multifactorial nature of infection, the range in specific parasite species studied, variations in infection intensities, and the important role of local social and environmental context in determining infection dynamics. In addition, formal analyses of the effects on morbidity due to biological interactions between parasite species are few [12, 21].

In line with expectations, we found positive associations between *Plasmodium* spp.-*S. haematobium* co-infection and morbidities, but only when infection intensities were considered. These observed associations were age and sex specific. Despite the significance of these associations, we were unable to identify strong evidence implicating interaction effects of *Plasmodium* spp. and *S. haematobium* infections on anemia or stunting. However, trends in hemoglobin levels and height for age measured as continuous variables provided some qualitative evidence of compounding effects of infection with the two parasites, at least in boys.

Inferences from these analyses were limited by small sample size and low frequency of heavy co-infections; nonetheless, these results reinforce the great potential for integrated control strategies to mitigate disease in heavily parasitized populations. Given synergistic associations between infections, targeting co-infected youth, especially those with intense infections, for inexpensive, anti-helminth treatment could drastically reduce anemia, especially when given in concert with anti-malarial medication [12, 22, 36, 73].

SUGGESTIONS FOR FUTURE RESEARCH

Although the research encompassing this dissertation represents a thorough investigation into *Plasmodium* spp. and *S. haematobium* infection distributions, predictors, associations and morbidities, the scope and scale of the data were limited. Further research on this topic is clearly warranted due to the importance of polyparasitism to public health, evidenced both by the high prevalence and the large burden of disease. Several suggestions are offered to guide future studies.

Parasite species

We focused on only two genera of a wide-array of endemic parasites. Future studies would benefit from inclusion of more species, especially STH and HIV. This is necessary both for identification of species-specific associations that may be mediated by immune responses (such as non-specific inflammation), and for estimation of species-specific effects on morbidity that could be confounded by these other infections.

Spatial scale

Small scale studies, like ours, are needed due to the focal nature of pathogen transmission and the complex interaction of risk factors that determine transmission patterns. Future studies could apply more formal spatial analyses that would help identify hotspots of infection and foci of transmission. In addition, spatial regression analyses would provide a more thorough assessment of how spatial distributions of risk factors predict patterns of infection.

Study design and methodology

Well-designed longitudinal studies are needed in order to avoid temporal ambiguity and account for seasonal variation in infection transmission. Replication of

these studies over a range of infection endemicities also would address questions of generalizability of study results. Malariaology as a science would greatly benefit from sensitive, standardized diagnostic tools for quantification of species-specific *Plasmodium* parasites. Although work of other researchers has shown good correlation of median fluorescent intensity (MFI) values with parasitemia, the relationship between the two is not linear toward the upper and lower limits [80-82]. Validation of our use of MFI as a quantitative measure is needed. Additionally, repetition of our analytical methods in studies of *Plasmodium* co-infections could help to clarify discrepancies in results. Finally, the strong effect of age on all of the outcomes studied indicates the limited generalizability of age-restricted study results. Population-based research, in which associations between parasites is studied across all ages is needed.

Immunological and intervention studies

In order to fully understand the implications of observed associations between parasitic species in human hosts, a better understanding is needed of relevant immunological mechanisms [9, 40, 71]. Care should be taken to design studies with enough power to investigate immune responses to species-specific infection combinations within specific age and infection intensity strata [9]. Due to the ethical limitations of randomized control trials in which effective treatment for pathogenic parasitic diseases is withheld, these immunological questions may be best addressed using intervention studies [75]. Indeed, the need for improved scientific knowledge about the biology, and epidemiology of polyparasitism should not take precedent over what is already known; parasites cause significant morbidity and more accessible, effective treatment and prevention is urgently required.

Despite effective treatment, however, malaria and urinary schistosomiasis continue to cause significant morbidity and mortality in endemic regions throughout the world. Interventions to reduce this burden of disease require enhanced understanding of the basic epidemiology of single- and multiple-species infections. Limited resources and limited global attention to “NTDs” among funders and researchers alike call for integrated control and prevention efforts that have the potential to significantly reduce disease burdens. Addressing the disproportionate disease burden caused by parasitic infections in the developing world is a matter of social justice that requires urgent attention.

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