A CASE-CONTROL STUDY OF ENVIRONMENTAL AND BEHAVIOURAL RISK FACTORS ASSOCIATED WITH MULTIPLE PARASITIC INFECTIONS IN WESTERN KENYA

BY

JUMA ELIJAH OMONDI (BSc.)

I56/78193/2009

A thesis submitted in partial fulfillment of the requirements for the award of the degree of Master of Science (Applied Parasitology) of the University of Nairobi.

June, 2012

DECLARATION

I, Elijah Omondi Juma, hereby declare that this is my original work and has not been presented for a degree in any other university.

Sign.....Date.....15/6/2012.....

Elijah Omondi Juma

This thesis has been submitted with our approval as supervisors.

Sign.....Date.....15/6/2012.....

1. David Odongo, PhD
School of Biological Sciences
University of Nairobi

Sign.....Date.....14/6/12.....

2. Eric Fèvre, PhD
International Livestock Research Institute (ILRI), Nairobi
School of Biological Sciences, University of Edinburgh, UK

Sign.....Date.....14/6/12.....

3. Lian Doble, DVM, MSc. MRCVS
International Livestock Research Institute (ILRI), Nairobi
School of Biological Sciences, University of Edinburgh, UK

ACKNOWLEDGEMENTS

I wish to acknowledge the kind contribution of the following people and institutions that helped me with the technical knowledge, skills, equipment, finances and emotional support which enabled me to piece together this work. First and foremost, I wish to acknowledge the contributions made by my project supervisors Dr. Eric Fèvre, Dr. David Odongo and Dr. Lian Doble. Through their supervisory role and technical guidance, I was able to understand and master fundamental steps in carrying out scientific research as well as the critical details of data analysis, reporting and thesis writing. My heartfelt gratitude also goes to ILRI staff working within the PAZ project in Busia, for the technical support they gave me as well as their hospitality while conducting my fieldwork. I also wish to extend my humble gratitude to ILRI as an institution, both in Busia and Nairobi, for according me the chance to use some of their facilities to conduct my research work. I am grateful to the University of Nairobi for granting me a scholarship to pursue a master's degree course at the School of Biological Sciences (SBS) through the Board of Postgraduate Studies (BPS). Last but not least, I wish to thank my family and close friends for providing me with emotional and moral support which was invaluable in helping me achieve this goal.

DEDICATION

I dedicate this thesis to my siblings and my best friend, Becky, for their inspiration, companionship and emotional support during the course of this research work.

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ABBREVIATIONS AND ACRONYMS

AIC	Akaike Information Criteria
aOR	Adjusted Odds Ratio
AP	Activity Points
BCT	Buffy coat Concentration Technique
CLR	Conditional Logistic Regression
CT	Computerised Tomography
DALYs	Disability Adjusted Life Years
EITB	Electro-Immuno-Transfer Blot
ELISA	Enzyme-Linked Immunosorbent Assay
GIS	Geographical Information System
HAT	Human African Trypanosomiasis
HCT	Hematocrit Concentration Technique
Ig	Immunoglobulin
IL	Interleukin
ITNs	Insecticide Treated bed Nets
ILRI	International Livestock Research Institute
LLINs	Long Lasting Impregnated bed Nets
MDGs	Millennium Development Goals
MRI	Magnetic Resonance Imaging
PAZ	People Animals and their Zoonoses
RS	Remote Sensing
SPSS	Statistical Package for the Social Sciences
STH	Soil Transmitted Helminths
T h	T helper
UoN	University of Nairobi
UTM	Universal Transverse Mercator
WGS	World Geodetic System
WHO	World Health Organisation

ABSTRACT

Many rural African villages are characterized by high prevalence of endemic parasitic diseases with cases of multiple parasite infections often too being common. This study was conducted in the Western Province of Kenya within a 45 km radius from Busia town, falling within the Lake Victoria Crescent Zone. The study aimed at determining whether there was a relationship between contact with particular environmental features, or specific human social behaviour and the risk of infection with multiple parasitic diseases. The sample frame for this nested case-control study comprised the 467 individuals randomly selected and sampled from this study site as part of a larger, on-going, cross-sectional study. The participants had been screened for a variety of zoonotic and non-zoonotic diseases and questionnaires administered to obtain information on specific aspects of their social behaviour. From the sample frame, 24 subjects with multiple parasitic infections defined as cases, were randomly selected and matched for age and sex with 24 other individuals who had one or no infection, herein defined as controls. Cases and controls were followed to their daily activity points using a GPS waypoint data of the activity points mapped. The study revealed high prevalence of parasitic infections. The infections co-occurred spatially within the same geographic setting with cases of sampled individuals bearing multiple infections at any one time being common. Cases and controls displayed a relatively homogeneous social behaviour and visited nearly the same activity points. Conditional logistic regression analysis did not reveal any significant difference between cases and controls with respect to any difference in their social behaviour and interaction with potentially risky environments and thus perceived odds of acquiring parasitic infections. These findings are an initial step to further research into the dynamics of human-environment-parasite interaction in an environment where parasitic infections are co-endemic. The findings will also be vital in helping design long-term strategies for control.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.0. General Introduction

Many rural villages in Sub-Saharan Africa are characterised by a high prevalence of endemic parasitic disease among the inhabitants (Ali, 1997). The diseases are associated with intense poverty in low-and middle income countries where access to safe drinking water and sanitation facilities is limited (Mehraj *et al.*, 2008; Ngui *et al.*, 2011). A five year literature review by Harhay *et al.*, 2010, indicate that children of school going age are the principal sources of gastrointestinal polyparasitism infections through environmental contamination and bear the greatest burden of these infections which include, but not limited to: severe weight loss, acute malnutrition and wasting, stunted growth and anaemia. The same is reported in studies done in Cote d Dİvoire (Keiser *et al.*, 2002), Zimbabwe (Sangweme *et al.*, 2010) and Brazil (Parraga *et al.*, 1996), which show that school going children bear greater burden of gastrointestinal polyparasitism than the rest of the population. The application of spatial tools such as geographical information system (GIS) and remote sensing (RS) coupled with better understanding of human demographic and behavioural risk factors associated with multiple parasite co-infection offers the prospect for improved mapping of such infections, their surveillance and effective control (Brooker, 2006; Brooker and Utzinger, 2007).

The environments inhabited by humans play host to the parasites as well as their host vectors (Heller *et al.*, 1998; Matthys *et al.*, 2007). Variations in temperatures and rainfall influence the behaviour and geographical distribution of vectors, due to their effect upon vegetation distribution and in turn vector breeding habitats (Martens *at al.*, 1995; Harhay *et al.*, 2010).

Human social behaviour is also a risk factor in the epidemiology of parasitic diseases. There is therefore an intrinsic relationship between the environment, the parasite, its host vector and the human host (Macpherson, 2005).

The study was conducted in the Western Province of Kenya within a 45km radius from Busia town encapsulating a variety of environmental and demographic areas found within the wider Lake Victoria Crescent zone. The study involved risk profiling, mapping and delineation of high risk environments and human behaviour for acquisition and transmission of the parasitic infections described in section 2.0.

1.1. Literature Review

1.1.1. Multiple parasitic infections

Polyparasitism is defined as a condition in which an individual is infected with multiple parasite species (Gibson *et al.*, 2011). Different species of parasites often overlap within the same geographical settings in the tropics resulting in high prevalence of polyparasitism in parasite endemic areas (van Eijk *et al.*, 2009; Gibson *et al.*, 2011). A study conducted in a geo-helminths and *Schistosoma japonica* endemic area of Leyte, The Philippines, revealed a high prevalence of *Schistosoma* and hookworm as well as hookworm and *Trichuris* infections occurring concomitantly (Enzeamama *et al.*, 2008). A similar study in Unguja, Zanzibar, conducted by Knopp *et al.*, (2010), revealed a high prevalence of concurrent infection between *S. haematobium* and soil transmitted helminths namely: *Strongyloides stercoralis*, hookworm, *Trichuris trichiura* and *Ascaris lumbricoides.* In Kintapo North Municipality, Central Ghana, hookworm was reported to occur concomitantly with malaria (Humphries *et al.*, 2011).

1.1.2. Immune regulation mechanisms in multiple parasitic infections

Multiple parasitic infections may invoke a variety of biological interactions between the host's immune system and the invading parasite. These interactions may be synergistic and therefore increase the severity of infection (LaBeaud *et al.*, 2009; Gibson *et al.*, 2011), or antagonistic in which case the multiple infections will be less severe than single infections (Murray *et al.*, 1978). Definitive studies on the synergistic or antagonistic effects of harbouring multiple parasite infections on an individual's immune system are still limited. According to a review by Brooker *et al.*, (2007), there are varied outcomes to such studies done in different parts of the world. Studies on immune regulation mechanisms in cerebral malaria and STH, *A. lumbricoides* co-infection scenario in Thailand revealed that there was a protective effect for *A. lumbricoides* infection on the risk of cerebral malaria and acute renal failure denoting an antagonistic effect. In Senegal, however, a similar study pointed to a positive association between *A. lumbricoides* infection and occurrence of severe malaria denoting a synergistic effect (Brooker *et al.*, 2007).

Attempts have been made to explain the precise mechanism of immune regulation in malaria-geo-helminths co-infection scenario. In synergistic situations, chronic helminths infections induce Type II hypersensitivity reaction and regulatory response characterised by up-regulation of IL-(4), IL-(5), IL-(3), as well as elevated levels of serum IgE (Hartgers & Yazdanbakhsh, 2006). This kind of immune response may alter the development of an appropriate pro-inflammatory response to initial malaria infection and in effect tilt anti-*Plasmodium* antibody responses towards the production of non-cytophilic immunoglobulins which would not be effective against malaria (IgG1 & IgGM) instead of cytophilic ones necessary for immunity (IgG1 & IgG3). Helminths may also modulate antigen presenting cells such as dendritic cells which are involved in the initiation and maintenance of immune

responses in parasitic infections. Dendritic cells are involved in phagocytosis of *Plasmodium* - infected red blood cells and present the antigens through the MHC class II restricted pathway. (Hartgers & Yazdanbakhsh, 2006). In antagonistic situations, high levels of helminths-induced IL-(10) may act to down-regulate the effects of interferon (IFN-γ) and tumor necrosis factor (TNF)-α, and therefore scale down malaria pathology (Brooker *et al.*, 2007).

1.1.3. Human and environmental risk factors for multiple parasitic infections

Human behaviour is complex and is compounded by the unique array of cultural, religious, ethnic, racial, age and gender variables. Differences in human behaviour have a profound effect on the epidemiology of parasitic diseases. Changes in population dynamics also contribute to behaviour change and ultimately the epidemiology of parasitic diseases. Explosive population growth particularly in the developing world has resulted in accelerated migration of people into new regions for exploitation of virgin territories, conversion of virgin lands into agricultural fields and development of large commercial projects of irrigation and dam construction (Macpherson, 2005). This movement is implicated in dissemination of existing parasitic diseases and the emergence of new ones. Movement of immigrants, refugees and tourists carrying along their mix of cultural practices and customs and behavioural patterns also compound the situation (Wilson, 1995).

Human activities around the world lead to alterations in climate resulting in changes in the ecosystem, communities and populations (Harwel *et al.*, 2002; Sutherst, 2004). These changes in turn influence transmission cycles and disease incidences of vector-borne diseases in a number of ways (Portier *et al.*, 2009): Changes in precipitation and temperature patterns directly affect vector-borne diseases through host-vector interaction, and indirectly through changes in the ecosystem such as humidity, soil moisture, water temperature, salinity and

acidity (Portier *et al.*, 2009; Harhay *et al.*, 2010). Subsequently, vector-borne diseases increase in their geographic distribution. Changes in land-use practices, vegetation cover, environmental contamination with human excreta and development of drug resistant pathogens also result in geographical expansion of vector-borne parasitic diseases (Harwell *et al.*, 2002). This phenomenon therefore calls for critical analysis of changes in environmental variables which act as risk to health as a means to preventing and controlling vector-borne parasitic diseases (Don, 1994; Keiser *et al.*, 2005). Different parasitic infections are transmitted under different environmental conditions as discussed below.

1.1.4.1. Malaria

More than two billion people are presently living at the risk of contracting malaria with a global annual incidence of clinical malaria estimated at over 300 million cases. More than one million deaths attributed to malaria alone are reported annually, with children under the age of five years of age living in sub-Saharan Africa being at highest risk (Keiser *et al.*, 2005). Malaria disease burden accounts for the loss of an estimated 46.5 million disability adjusted life years (DALYs) with nearly 90% of the burden being concentrated in sub-Saharan Africa (WHO, 2004). About 90% of incidence of malaria disease burden is related to environmental factors (WHO, 1997). Temperature, rainfall, relative humidity and wind patterns and their changes such as El Niño effect (Coupon-Johnson, 2000) are environmental factors which have an important effect on vector density, the vectors' reproduction habitats, longevity, development and survival of the pathogen (Kovats *et al.*, 2003; Dale *et al.*, 2005).

Malaria distribution pattern reflects a combination of factors involving vector distribution within the environment, human-vector contact and human host factors. Establishment of water resource development projects (dams and irrigation schemes) constitutes a major

environmental risk factor for malaria transmission since such projects alter ecosystems and can substantially change the dynamics of malaria risk close to their location (Brooker *et al.*, 2004; Keiser *et al.*, 2005).

1.1.4.2. Human African trypanosomiasis (HAT)

Human African trypanosomiasis (HAT), often known as sleeping sickness has a focal distribution, being reported in only specific regions in 36 African countries. Sixty million people are estimated to be at risk of infection in some 259 discrete areas where the vector, the tsetse, is found (Cattand *et al.*, 2001; WHO, 2006). At least 300, 000 are believed to be infected with HAT, but only 10-15% of cases are often diagnosed and treated (Cattand *et al.*, 2001).

Land cover and topology has been associated with the risk of contracting HAT (Odiit *et al.*, 2006; Cecchi *et al.*, 2009). Incidence of sleeping sickness is frequently linked to distance to tsetse infested habitats such as long vegetation swamps or riverine areas, mangrove areas and regions of rice cultivation among others (Odiit *et al.*, 2006).

In Kenya, the disease is endemic in the western part of Nyanza province (Lambwe Valley) and Western province, mainly Teso, Busia and Bungoma district (Rutto and Karuga, 2009; Thumbi *et al.*, 2010; Von Wissmann *et al.*, 2011). Over the last two decades, incidences of sleeping sickness have been low and sporadic owing to concerted vector control, treatment of animal reservoirs and changes in land-use practices which have rendered the environment inhabitable for the tsetse vectors (Rutto and Karuga, 2009; Thumbi *et al.*, 2010).

1.1.4.3. Soil-transmitted helminths (STH)

Soil-transmitted helminths (STH) infections are endemic in communities where unsanitary environments and poor personal hygiene are commonplace, as is typical of the majority of

developing countries, especially in the humid tropics (Yodmani *et al.*, 1982; Gryseels *et al.*, 2006). Soil transmitted helminths include: *Ascaris lumbricoides*, *Trichuris trichiura*, and Hookworm (*Ancylostoma duodenale & Necator americanus*). It is estimated that between 120 to 215 million cases of morbidity are attributable to *Ascaris lumbricoides* annually, 90 to 130 million due to hookworm and 60 to 100 million due to *T. trichiura* infections (Chan *et al.*, 1994). More recent estimates of the disease burden due to STH puts the figure at more than one billion people worldwide. The global burden of STH could be as high as 39 million disability adjusted life years (DALYs), rivaling that of malaria (Bethony *et al.*, 2006).

Contamination of soil with eggs of these parasites constitutes the most important risk factor for STH as well as for zoonotic helminths infections (Rai *et al.*, 2000). It is estimated that between 20 to 64% of the soils in the developing world are contaminated with the eggs of STH due to open and indiscriminate defecation, poor or non-existent sanitation, the use of untreated night soil as fertilizer and poverty (Pezzani *et al.*, 1996; Uga *et al.*, 1997). Studies have established that contact with the infested soil on bare feet as well as ingestion of embryonated infective eggs via unwashed hands or on raw or unwashed vegetables lead to accelerated rates of transmission (Rai *et al.*, 2000; Ulukanligil *et al.*, 2001).

1.1.4.4. Schistosomiasis

Disease burden related to schistosomiasis is staggering. An estimated 207 million people are infected with schistosome while at least 500 million people are at risk of acquiring the infection (Steinmann *et al.*, 2006). Schistosomiasis infection often result in severely reduced life expectancy, chronic morbidity and disability, absenteeism for school going children and reduced agricultural activity for those in their active years. In particular, heavy infections of schistosomiasis results in daily blood loss in stool and urine over a period of years, lesions of

the genito-urinary system including calcification of the bladder, gastrointestinal damage, impairment of liver function, and hepatosplenomegaly (Hunter, 1993).

Ecological variables such as density of snail population and infection rates, environmental temperature, determine the size and distribution of parasite populations (WHO, 1993; Kariuki *et al.*, 2004). Common habitats of snail intermediate hosts are usually scattered widely across expansive geographical zones but with high focal distribution. Transmission levels also vary according to physical characteristics of the surface water, ranging from stagnant to flowing water and from small streams to large water bodies such as dams and lakes (WHO, 1993; Sama *et al.*, 1994; Brooker *et al.*, 2006). Contact with cercariae infested surface water either during occupational engagement or as a result of recreational activities in such environments is a major risk factor for contracting schistosomiasis (Ndassa *et al.*, 2007). Activities which result in the longest duration of water contact and thus contributing to the highest risk of infection include fishing, laundry and swimming.

Development of mega water projects such as dams, aquaculture and fisheries, water reservoirs and irrigation schemes has led to increased incidences and spread of schistosomiasis infections as infected populations migrate to occupy the new developed areas thus compounding the epidemiology of schistosomiasis infections (WHO, 1993; Brooker *et al.*, 2006). Rapid upsurge in schistosomiasis transmission rates has been documented in several countries in Africa, Latin America and Asia. Construction of a dam on the river Volta in Ghana, irrigation in the Nile valley in Egypt and Sudan, development of irrigation schemes in northern Cameroon are just some of the few examples of documented water development projects which resulted in phenomenal increase in prevalence of schistosomiasis from ranges of between 5 to 90% in the affected areas (WHO, 1993; Handzel *et al.*, 2003; Brooker *et al.*,

2006). In East Africa, prevalence of schistosomiasis infection along the great lakes is mainly related to distance from Lake Victoria or other small lakes within the region (Handzel *et al.*, 2003; Kabatereine *et al.*, 2004).

1.1.4.5. Human taeniasis

Human taeniasis is a food-borne infection caused by infestation of the gastrointestinal tract by adult tapeworms; *Taenia solium* or *T. saginata* (the beef tapeworm). Cysticercosis is a tissue infection with the larval cysticercus or metacestode stage of pig tapeworm, *T. solium* (Prasad *et al.*, 2007). Taeniasis is a disease of major public health importance in many developing countries of Latin America, Africa and Asia (Nguekam *et al.*, 2003). Averages of 50 million people are infected with *T. solium* with at least 50, 000 deaths being reported worldwide with an additional 20 million cysticerci infections (Bimi *et al.*, 2012).

The presence of individuals with intestinal infection of *T. solium* in the immediate environment increases the risk of transmission of *T. solium* infection to other members of the community. In the developing world, poor sanitary conditions including limited or lack of toilet facilities is commonplace. In such environments, individuals harbouring *T. solium* infections practice open field defeacation in nearby land close to homesteads where food grains and vegetables are grown. The infection is propagated when individuals pick up the infection through contamination of the hands by touching or eating of contaminated vegetables and grains or by contamination of the hands via the peri-anal region. Freely roaming pigs also feed on the faeces and thus become infected with *T. solium* eggs which are subsequently transmitted to humans when such pork is consumed raw or undercooked (Prasad *et al.*, 2007; Bimi *et al.*, 2012).

Eating raw or undercooked pork/beef, handling of pork especially in the slaughter slabs or engaging in pork preparation therefore constitute some of the major risk factors for acquiring taeniasis infection; Nguekam *et al.*, 2003; (Li *et al.*, 2007; Bimi *et al.*, 2012). Migration from endemic to non-endemic areas leads to the spread of the disease to environments where the infected individuals migrate to. This disease is therefore increasingly being reported in affluent countries (Allan *et al.*, 1996; Prasad *et al.*, 2007).

There is generally little information on the epidemiology of human taeniasis with low prevalence being reported in many endemic communities (generally ≤1%). This is due to lack of sensitive and specific diagnostic tools for the collection of reliable epidemiological data (Margono *et al.*, 2001; Nguekam *et al.*, 2003). However, substantial progress has been made to refine the diagnostic procedures so as to improve sensitivity and specificity (Li *et al.*, 2007). A more refined method involving the detection of *Taenia*-specific antigens in faeces (copro-antigens) has been developed and shown to significantly increase the number of positive cases detected in community-wide surveys as opposed to microscopy (Allan *et al.*, 1996).

1.1.4.6. Gastrointestinal protozoan infections

According to WHO (2002), 3.5 billion people are infected with gastrointestinal parasites out of which 450 million suffer illness from the parasites. This situation is aggravated by the fact that over 2.6 billion people worldwide still do not have access to proper sanitation facilities (UNICEF & WHO, 2004). Infections caused by these parasites are essentially cosmopolitan in distribution but are more common in tropical and subtropical regions especially in Haiti, Mexico, Brazil, El Salvador, tropical Africa, the middle East and South Asia (Yazar *et al.*, 2004; Schuster and Ramirez-Avila, 2008).

Gastrointestinal protozoan infections include isosporiasis, balantidiasis, giardiasis, cryptosporidiosis and amoebiasis. Risk factors associated with gastrointestinal parasitic protozoan infections are primarily related to environmental contamination through indiscriminate defecation and the use of night soil as fertilisers (Wani *et al.*, 2007). Infective oocysts are realised into the environment as a consequence of these practices. The oocysts are washed into water reservoirs by rain water run-off thereby contaminating the natural water sources. When such water is consumed, untreated or by simple chlorination alone, the affected individuals pick infection through this route (Flanagan, 1992). Transmission of infection is also accelerated in environmental set-ups where there is overcrowding. In such environments, person-to-person transmission of infection is common because of the close interaction between individuals and the potential breach in personal hygiene. Overcrowding in built environment adds another dimension to the risk of infection in the sense that in such environments, the potential risk of contamination of public water supply is so high; this coupled to potential for post-treatment contamination through cross-connections, damages or during repairs to the mains water supply pipes (Cohen *et al.*, 2008; Flanagan *et al.*, 1992). Proper sanitation through proper disposal of human excreta greatly reduces contamination in the environment of faeces and thus reduces the transmission of faecal-oral infections (Corrales *et al.*, 2006).

Transmission of intestinal protozoan infections requires no intermediate or reservoir hosts, with the possible exception of *Balantidium coli* which may use pigs as reservoir hosts for human infection (Brook and Melvin, 1964). Common symptoms associated with

gastrointestinal protozoa infections range from intestinal uneasiness, nausea, anorexia, mild fever and chills and diarrhea (Simsek *et al.*, 2004).

1.1.5. Spatial mapping of parasitic infection prevalence

Early detection of disease outbreaks remains a major component of public health disease control strategies (Jiang and Cooper, 2010). To do this, there's need for tools which can reliably provide estimates of geographical distribution of infections and the size of population for which intervention is required (Magalhaes *et al.,* 2011). Several tools have been recently developed for mapping the spatial distribution as well as predicting potential outbreaks of major parasitic infections. Bayesian geostatistical modeling method is one of those tools used to carry out risk mapping enabling the estimation of the relationship between environmental predictors (i.e. land surface temperature, precipitation, normalized difference vegetation index, enhanced vegetation index, distance to permanent water bodies etc.), socioeconomic factors and infection prevalence (Magalhaes *et al.,* 2011; Pullan *et al.,* 2011; Wang *et al.,* 2008). Bayesian models are robust and their outcomes can be used to derive optimal spatial design for prediction of infection prevalence even in un-sampled locations and for the estimation of spatially dependent parameters while giving provision for parameter uncertainty (Magalhaes *et al.,* 2011; Pullan *et al.,* 2011).

Bayesian geo-statistical models are normally used alongside GIS and RS. GIS and RS enhances the delineation and prediction patterns of parasitic infection risks by availing an avenue through which survey data can be integrated on environmental and socioeconomic determinants. During spatial analysis, field survey data with information on either prevalence or intensity of infection and individual level predictor variables such as age, sex and socioeconomic status are integrated onto GIS and geo-located to the remote sensed

environmental data Magalhaes *et al.*, 2011). The combination of geo-statistical models, GIS and RS have been used in mapping of parasitic diseases such as malaria (Gaudart *et al.*, 2009; Gosoniu *et al.*, 2006), schistosomiasis (Wang *et al.*, 2008), HAT (Wadrobe *et al.*, 2010) and Cryptosporidium (Szonnyi *et al.*, 2010) and soil transmitted helminths among others (Pullan *et al.*, 2011).

1.1.5.1. Use of GIS/RS and GPS in mapping parasitic infections

Mapping the geographical distribution of diseases has become a major component of implementation strategy in controlling infectious diseases (Ocaña-Riola, 2010; Suwannatrai, 2011). Recently, significant steps have been made in the use of GIS and RS to understand the ecology and epidemiology of vector-borne diseases, and to develop cost effective ways of identifying targeted populations for treatment (Brooker and Michael, 2000). GIS is used in identification and delimitation of disease endemic areas, and the multiple data obtained overlaid on the map of the endemic area. This is an essential precursor to the planning of disease control and eradication programmes (Brooker *et al.*, 2006).

RS data is used for the identification and mapping of land-cover associated with host and /or organism habitats as indicators of climatic conditions (e.g. precipitation and surface temperature) known to be associated with vector/host population dynamics. RS therefore aids in the detection, surveillance, forecasting and control of disease vectors as well as their associated diseases (Wayant *et al.*, 2010). GIS and RS technologies have been used to map the distribution of vector-borne diseases such as malaria (Dale *et al.*, 1998; Hightower *et al.*, 1998), sleeping sickness (Odiit *et al.*, 2005), schistosomiasis (Dale *et al.*, 2005) and helminths infections among many others (Brooker *et al.*, 2000, 2002).

The Global Positioning System (GPS) is also an important component of the GIS toolkit used for geo-referencing particular details of a disease or infection on the GIS map of the study area (Cattand *et al.*, 2001; Nihei *et al.*, 2005). GPS determines position by measuring the distance from a group of satellites in space, which act as precise reference points. The technology has been applied in various ways in medical research. For instance, in compiling data on snail intermediate host habitat for schistosomiasis control through identification and mapping of the intermediate host breeding habitats such as irrigation ditches or canals (Nihei *et al.*, 2006). In malaria studies, the technology has been used in surveillance and control of malaria and other mosquito-borne diseases by geo-referencing the malaria vector breeding hotspots in the given study areas (Brooker *et al.*, 2004). Recent advances in GPS has eliminated the need for differential correction, allowing such maps to be created cost-effectively with a single inexpensive consumer grade GPS unit (Seto *et al.*, 2001).

1.1.6. Case-control studies

The Case-control study is an experimental design in which cases and non-cases (controls) are selected based on specific criteria, and the frequency of the exposure factors of interest then compared in each group. Cases are subjects who have developed the disease or the outcome of interest at the time they are chosen to participate in the study while controls are those who have not developed the disease at the time of selection (Carneiro *et al.*, 2001; Dohoo *et al.*, 2003). Cases and controls should be representative of the same base experience (Wacholder *et al.*, 1992) and are drawn from a population known as the study base. When cases come from a spatially and temporally well-defined population, the population is called primary study base (Wacholder *et al.*, 1992; Dohoo *et al.*, 2003). In certain circumstances, cases are defined before the base is identified and therefore referred to as secondary base. In this scenario the base is the population from which cases are drawn, and controls are those individuals who

would have been part of the case series if they had contracted the infection. A secondary base is used when it is practically unfeasible to ascertain all cases in a primary study base (Wacholder *et al.*, 1992).

1.1.6.1. Control selection

In selecting participants to be included in the control sample, the researcher has to ensure that the population consisting of the controls is comparable to the one constituting the cases in three major aspects. Firstly, the study base-an individual is eligible to be included in the study base only if s/he would be enrolled as a case if s/he would have been diagnosed with the disease of interest at the time of exposure. This ensures that there is no selection bias which arises when there is an error in selecting individuals or groups to be included in the study (Wacholder *et al.*, 1992). Secondly, non-confounding-confounders which can be determined by measuring (confounders are the disease risk factors which are jointly responsible for the lack of comparability of exposed and unexposed) (McNamee, 2003) can be controlled in the analysis while unknown confounders should result in negligible variability. Non-confounding ensures that there is no major variability between the case and control group which would otherwise result in or prevent the outcome of interest. Thirdly, comparable accuracy-the level of accuracy in measuring the exposure of interest for the cases should be similar to the level of accuracy for the controls, unless the net effect of the inaccuracy can be controlled in the analysis. This eliminates any case of information bias, in which the measurement of information such as exposure to disease differs among the case and control study group (Wacholder *et al.*, 1992).

1.1.6.2. Matching

Matching is a means of improving efficiency in the estimation of the effect of exposure to the outcome variable by preventing a situation in which the distribution of confounding variables is significantly different between cases and controls (Thomas and Greenland, 1983). Certain environmental and socio-economic variables of the subjects are difficult to measure, thus matching in terms of the variables results in reduced confounding (Miettinen, 1985). Matching reduces the possibility of lost efficiency due to significant differences in the distributions of major risk factors between cases and controls. Matching is normally applied to risk factors whose confounding effects need to be controlled for but which are not of scientific interest as independent risk factors in the context of the particular study. In most cases, age, sex and race are used as matching variables because they are often strong confounders and because their effects are usually well known from descriptive epidemiology (Wacholder *et al.*, 1992).

1.2. Justification and significance of the study

A substantially large number of people in the developing world are living at risk of contracting parasitic diseases such as malaria, lymphatic filariasis, STH, schistosomiasis, leishmaniasis, trypanosomiasis, Chagas disease, and onchocerciasis (WHO, 2004; Hotez *et al.*, 2006). Most of these diseases overlap spatially and temporally within the same epidemiologic setting (Raso *et al.*, 2004). Mortality due to infectious and parasitic diseases remains the highest and accounts for one quarter of the entire global disease burden. The greatest burden of these diseases is borne by Africa, Southeast Asia and eastern Mediterranean (Hotez *et al.*, 2004) and these diseases are therefore of major public health significance. Their successful control is critical to attainment of Millennium Development Goals (MDGs) number one, four, five and six which relate eradication of extreme poverty and improved livelihoods through access to better healthcare (WHO, 2006).

Previous research has focused on investigating the risk factors related to individual parasitic infections such as malaria, sleeping sickness or schistosomiasis (Raso *et al.*, 2007). Relatively fewer studies have, however, been conducted to understand the principal risk factors influencing the prevalence and transmission dynamics of polyparasitic infections within individuals despite polyparasitism being common in various epidemiologic settings (Petney *et al.*, 1998; Raso *et al.*, 2006).

The work presented in this thesis addresses some of the spatially explicit risk factors for simultaneous infection with multiple species of parasites, and describes the environmental and socio-demographic risk factors which could offer an explanation to the worrying trend of polyparasitism in rural African villages. This was achieved by gathering spatial and socio-demographic data to risk-profile map to understand a multiple of parasitic diseases and their epidemiological dynamics which characterised their occurrence within the study environment. It is envisaged that the results of this study will assist local communities, policy analysts and health experts to devise appropriate preventive and control measures to the multiple parasitic diseases endemic to the study area. This study approach will also serve as an exploratory and descriptive tool intended to aid in disease monitoring and surveillance as an early control and intervention measure.

1.3. Objectives

1.3.1. General objectives

To define and understand the risk factors associated with multiple parasitic infections in the study population.

1.3.1.1. Specific objectives

1. To define and understand the prevalence of multiple parasite infections in the study area.

2. To define and understand risk factors associated with multiple parasite infections in the study area.

3. To describe the human behavioural risk factors associated with multiple parasite infections.

1.4. Null hypothesis

There is a high prevalence of multiple parasite co-infections in the study area attributable to human behaviour and their contact with contaminated environments.

CHAPTER TWO

MATERIALS AND METHODS

2.0. Study site and study design

The study site is located in the Western Province of Kenya. A 45km radius from Busia town encapsulates the variety of environmental and demographic areas found within the wider Lake Victoria Crescent zone. The location of the study site can be seen in Figure 1.

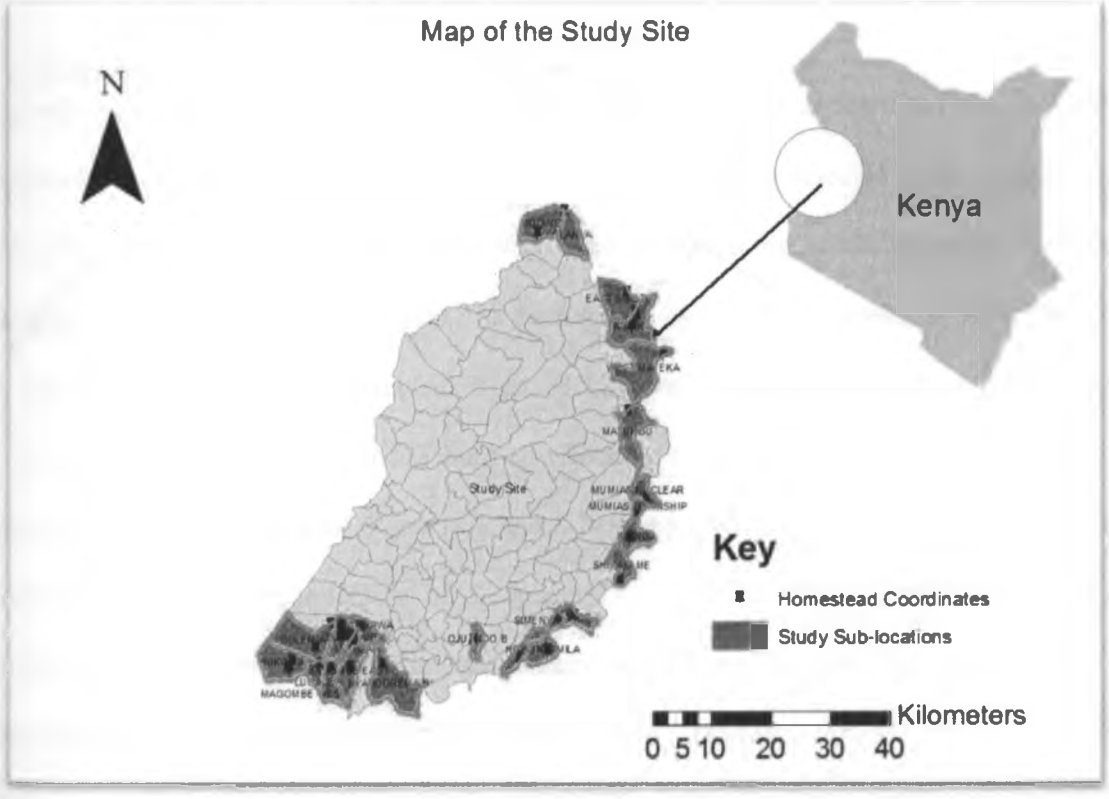

Figure 1: Map of the study site showing the sub-locations sampled and the homestead locations

This ecosystem has a high human and livestock density with a hot and wet climate. Agriculture is the primary industry, with small-holders growing both crops and keeping livestock. Most families in this region follow a sedentary lifestyle.

This research study was conducted within the framework of a Wellcome Trust funded 'People, Animals and their Zoonoses' (PAZ) project, a collaboration between the University of Edinburgh, the Kenya Medical Research Institute (KEMRI) and the International Livestock Research Institute (ILRI). The main activity of the PAZ project was a cross-sectional study in which humans and animals were sampled and diagnostic tests carried out for a wide range of zoonotic and non-zoonotic diseases. The unit of sampling was the homestead, with the number of homesteads sampled within each sub-location being proportional to the cattle population within that sub-location. Each homestead had a unique PAZ homestead number and GPS coordinates taken at the time of sampling.

2.1. Diagnostic methods in parasitic infections

Trained and experienced personnel from the PAZ laboratory used standardised parasitological diagnostic techniques to screen for parasitic infections in the selected study participants. Formalin-ether concentration procedure was used to screen for infective stages of faecal parasites namely: *G. lamblia* cysts, *E. histolytica* cysts, *B.coli* cysts *I. belli* oocysts, *C. parvuum* oocysts, *A. lumbricoides* eggs, Hookworm (*N. americanus* & *A. duodenale*), *T. trichiura, Schistosoma* sp. and *Taenia* sp (Becker *et al.,* 2011). Ziehl-Neelsen technique was also used for detection of *C. parvum* and *I. belli* oocysts (Garcia *et al.,* 1983). Kato-Katz technique was used for detection of *S. mansoni, A. lumbricoides, T. trichiura* and other helminths ova. Thick and thin blood smears were used to identify the presence of haemoparasites (trypanosomes, microfilariae and malaria) (Katz *et al.,* 1973). Microheamatocrit centrifugation technique (mHCT) and quantitative buffy coat technique (QBC) were also used to detect the presence of trypanosome parasites and microfilariae (Chappuis *et al.,* 2005). In addition, questionnaire data on a variety of homestead, herd and

individual risk factors for zoonotic and non-zoonotic diseases were collected from every participant in the study.

2.2. Case-control definition and sample selection

The individuals who were selected to take part in the PAZ cross-sectional survey constituted the sample frame for this study. This sample frame had a total of 467 individuals with 150 individuals having no parasitic infection at all; 138 bearing single infections while 179 individuals had multiple parasitic infections. Criteria for categorizing individuals from the sample frame into cases and controls were stipulated as laid out below:

Cases were defined as individuals in the sampling frame who had multiple parasitic infections of two or more parasites mentioned in section 2.1. Controls were defined as individuals who had only a single parasitic infection at the time of screening or did not have any infection at all. Thirty individuals who met this case definition criterion were randomly selected from the sample frame. An additional 30 individuals meeting the control definition criterion and matched for age and sex with their case-counterparts were randomly selected from the sample frame. An additional back-up sample of 10 individuals (5 cases and 5 controls) was set aside from the sample frame as a back-up sample. This group of individuals was to be engaged in the study in case there was decline from some participants or incase the participants had relocated from the study area. Questionnaires data corresponding to the selected case-control study subjects were extracted from the PAZ database to analyse specific aspects of their social behaviour which could be potential risk factors for parasitic infections. The study received ethical approval from the Kenya Medical Research Institute Ethical Review Committee (file number SSC 1701).

2.3. Follow-up protocol

Each individual was traced back to their homestead in order to initiate the follow-up process. Upon locating the homesteads, participants were informed about this next phase of study to the extent they understood and consented to participate. During the follow-up process, the follow-up researcher was blinded with regard to the case or control status of the study participants. Information on the participant's daily activities and routes followed was taken and recorded in a spreadsheet such as shown in Table 1 to signal the start of the follow-up exercise.

Table 1: Participant follow-up data sheet

Participant ID+ Homestead ID	Homestead coordinates (Northings and Eastings)	Activity point (AP)	Activity type	Time spent on AP	Number of visits per day to AP	Comment
e.g. 00006548+85	00.131563 034.08942	Borehole	Drawing water for domestic use	30 minutes	*2	-A protected borehole, cemented and sealed.

The 'trace on' feature on the hand-held GPS unit was checked to start marking the track followed. The follow-up researcher then followed each study participant to their regular APs in the order in which they were recorded in the spreadsheet.

During the tracking process, waypoints of each AP was recorded in the hand-held GPS unit as participant ID +AP1, 2, 3, etc., depending on the number of APs. At the end of each follow-up, the feature 'trace off' was checked on the GPS unit to end the tracking process. The track and waypoints data stored in the GPS unit were downloaded and saved into a computer as shapefiles unprojected using DNR Garmin which is a software application available on line at (http://www.dnr.state.mn.us/mis/gis/tools/arcview/extensions/DNRGarmin/DNRGarmin.html)

and allows the management, manipulation, and saving of data from a Garmin GPS receiver for use in GIS programs such as ArcMap. The data were first projected to World Geodetic System (WGS) 1984 Universal Transverse Mercator (UTM) zone 36N to conform to the coordinate system of the study area. The data were then plotted on ArcGIS 9.1 (ESRI, Redlands, CA)as shown in Figure 2.

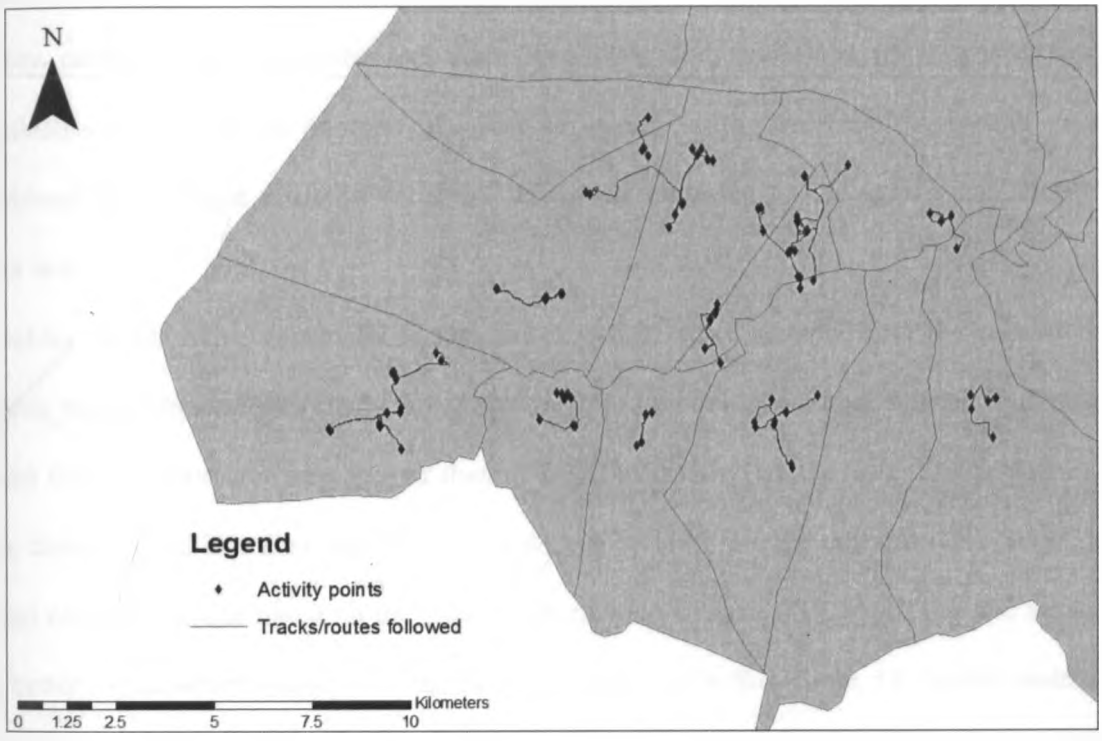

Figure 2: A section of ArcGIS map of the study site showing activity points and routes followed.

2.4. Data Analysis

There were two sets of data: questionnaire data and geographical data. Questionnaire data were obtained from questionnaires which were administered to the respondents to verify aspects of their behaviour which could be quantified as risk factors for acquisition of parasitic diseases. Geographical data were obtained from follow-ups to the respondents. Questionnaire data were analysed using STATA v10 (StataCorp, College Station, TX) while Geographical

data were analysed using ArcGIS 9.1(ESRI, Redlands, CA) and R version 2.14.0 (R Development Core Team, 2012). To study the association between status and the covariates, conditional logistic regression model was fitted, but first, a univariate analysis was performed to help screen covariates to be included in the model. This was done based on either standard Chi square or Fisher's exact test. In a situation where the number of observation per variable was below five, Fisher's exact test was used so as to give accurate measurements (Woodward, 2005; Bower, 2003), yet in the case where the number of observation per variable was more than five (Camilli and Hopkins, 1978), Pearson Chi square was used. Variables with zero cells in any category were excluded and some variables were combined, if it was deemed practicable to increase the number of counts i.e. contact with 'river' and 'riverbank' were combined into a single response variable-'river'- and subjected to Chi square and/ Fisher's exact test.

Variables which were statistically significant at $p<0.25$ were included into the conditional logistic regression model to create a maximal model (a model is maximal if there is no other model that is component-wise greater than or equal to it (Kavvadias *et al.*, 2000). Variables were then dropped in a step-wise process at $p\geq0.05$ level on likelihood ratio (LR) tests. The model comparison was based on the Akaike Information Criteria (AIC) such that the smaller the better. This study intended to estimate association of within-strata, i.e. within matched pairs and exposure, in this case distance covered in daily movement and exposure to specific environmental features. The results are presented in Section 3.4 and Tables 5 and 6 in the appendix.

CHAPTER THREE

RESULTS

3.0. Response rate

Of the 30 cases and 30 controls who were chosen to take part on the study, five individuals from the case sample declined to take part in the study accounting for decline rate of 16.7% of the total case sample. In addition, four of the case samples had already relocated to other localities away from where they were initially sampled and therefore could not be followed. This constituted 13.3% of the case samples. The back-up sample was then used to replace those who declined to take part in the study including those who had already relocated. From the back-up sample, one individual declined to participate in the study bringing the actual number of cases who were included in the study to 24. This number was then followed-up in their everyday activity points together with their matched controls.

3.1. Parasitological data

3.1.1. Multiple parasite infection prevalence and co-endemicity

A computation was done to ascertain the prevalence of multiple parasite infection in the sample frame as well as in the final 24 case-control sample engaged in the study as well as to get a visual display of parasite co-endemicity in the study area. The results of the survey indicate that out of the 467 individual screened for various parasitic infections and included in the sample frame, 150 did not have any parasitic infection at all, 138 individuals had single infections from one parasite species, 116 individuals were infected with two concurrent infection with two different parasite species, 45 individuals had three multiple parasite infection from three different parasite species, 17 individuals were simultaneously infected with four parasites of different species while only one individual was infected with upto 5 different parasitic species. These results indicate that on average most individuals are infected

with between one and two different parasite species. The number of individuals harbouring between three and five different parasitic infections simultaneously is significantly fewer as compared to those harbouring between one and two concomitant parasitic infections. The results are presented in Figure 3 below.

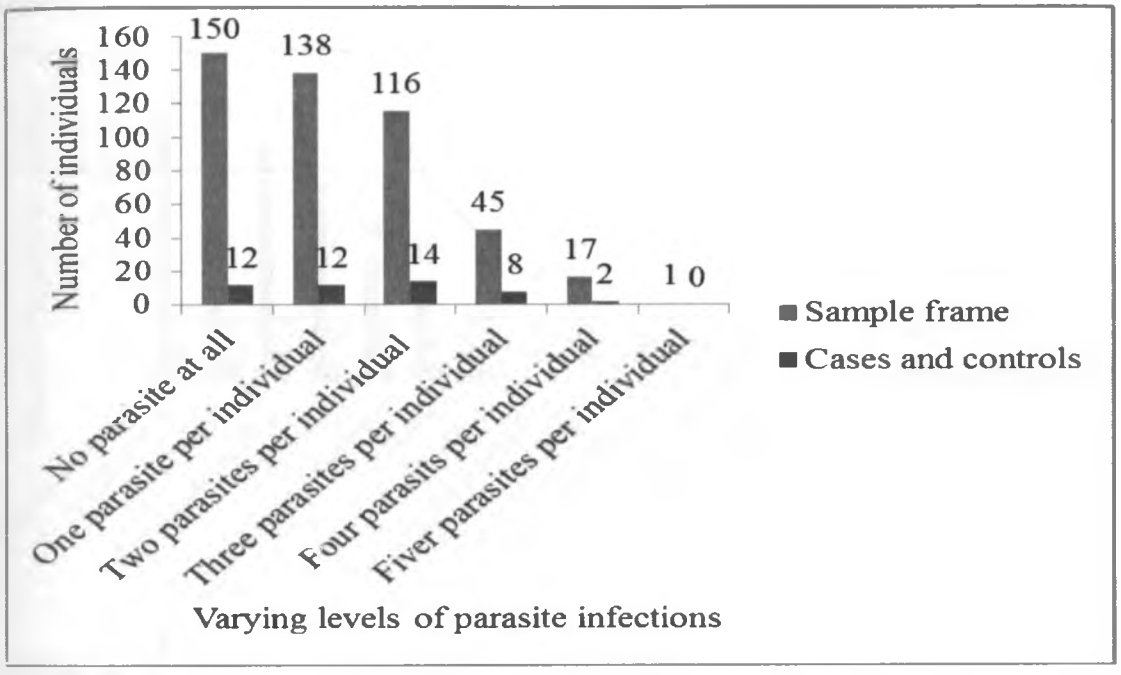

Figure 3: The level of multiple parasite infection in the sample frame as compared to the case- control study sample

Further analysis was done to ascertain the prevalence of the different parasite species in the PAZ sample frame and the 24 case-control pairs drawn out of the sample frame. The results are shown in figure 4. The objective of this comparison was to find out if the randomly selected cases and controls were representative of the PAZ sample frame. The most prevalent parasitic infections were amoebiasis, malaria, hookworm disease, trichuriasis, at an average of over 20% in the sample frame. Schistosomiasis and ascariasis were also prevalent with of over 15% of the sample frame infected. About 5% of those screened had *Giardia* infections. Only one individual was reported with *Cryptosporidium* infection, while none of the subjects had

trypanosome, microfilariae, *Balantidium* or *Taenia* infections. These results indicate that parasitic infection caused by species of *P. falciparum*, *Entamoeba*, *Giardia, Trichuris*, *Cryptosporidium*, *Ascaris*, *Schistosoma* and Hookworm infections are co-endemic and therefore overlap spatially within the geographical set-up of the study area. The results are shown in Figure 4 below.

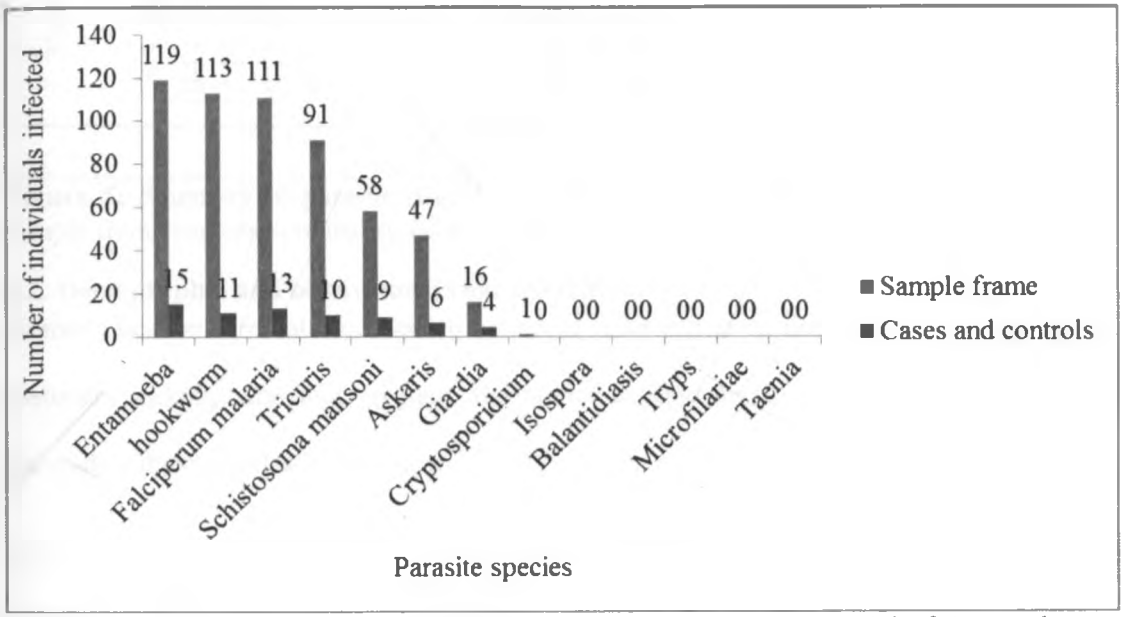

Figure 4: A comparison of the composition of parasites between sample frame and case-control study subjects

Further analysis was done to ascertain the level of parasite prevalence across the different age groups in the PAZ sample frame and the 24 case-control pairs as shown in Figure 5. The highest prevalence of parasitic infections was reported in the lower age brackets of between 5 and 24 years and this decreased progressively with advancing age. Parasite prevalence was comparable between the sample frame and the case control subjects drawn out of it.

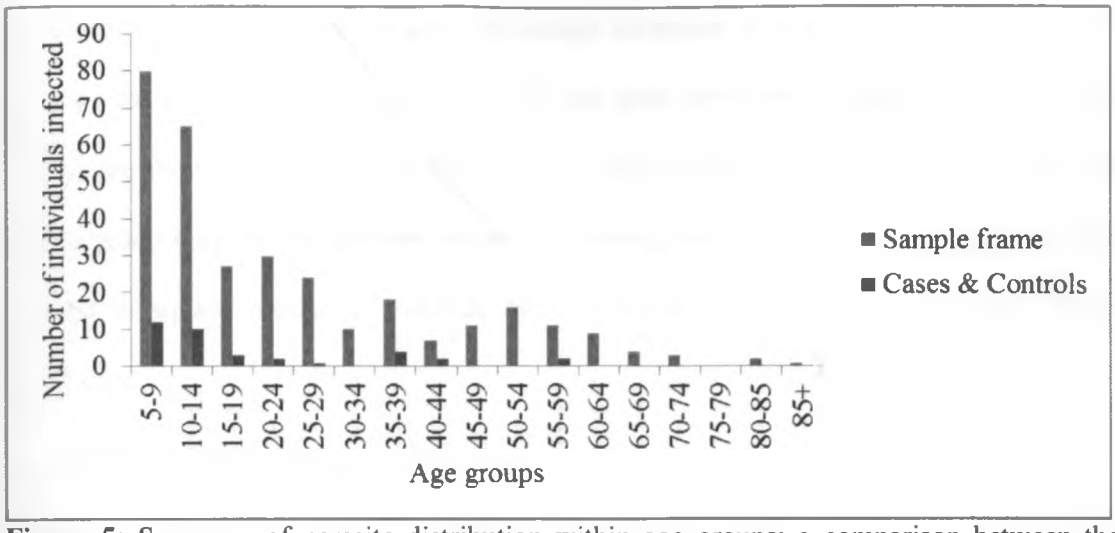

Figure 5: Summary of parasite distribution within age groups; a comparison between the sample frame and case-control subjects.

3.2. Demographic and behavioural characteristics

Human questionnaire data focused on obtaining information on demographic characteristics, treatment seeking behaviour, hygiene and sanitation characteristics and culinary practices of the study subjects as described below.

The minimum age bracket of the subjects in the case-control study was 5-9 years while the maximum age bracket was between 55-59 years. The ratio of male to females in the case-control study was1:1.5 (i.e. 19 males to 29 females). There were 10 male cases matched with 10 male controls and 14 female cases matched with 14 female controls. Only 21% of the sampled cases and controls were married, majority were children of school going age and therefore single. The average level of education in both cases and controls was primary school. Less than 15% of the sampled cases had secondary education while there was no control who had secondary education. Respondents not in the school going age were either farmers or traders. Over 75% of those sampled had lived in the study area for at least five years or more. A summary of the demographic characteristics is given in Table 3.

3.2.1. Treatment seeking behaviour

Respondents were asked to state where they sought treatment for common ailments. Results showed that over 80% of the respondents sought treatment in hospitals while less than 5% either visited chemists, private clinics or did not seek treatment at all. The results of these analyses are shown in Table 2. A statistical test of significance was done to veryfy if there was an association between the different treatment seeking behaviours and case or control status. Peason Chi square results (x^2 =4.005, df=3, p>0.05) results revealed that there was no significance.

3.2.2. Hygiene and sanitation

Hygiene and sanitation practices tested include latrine use, sources of water and water treatment. Over half the respondents were found to use latrines and treat water. Fisher's exact test results were not significant for use of latrine (p-value 0.33) and water treatment (p-value 0.28). Pearson Chi square results revealed no significant association between source of water and its effect on a case or a control status (x^2=4.0, df=4, p>0.05).

Table 2: A summary of the treatment seeking behaviour as compared between cases and controls

Where treatment is sought	Treatment seeking behaviour			
	Case	Proportion	Control	Proportion
Hospital	20	0.87	21	0.88
Chemist	2	0.09	0	0.00
Private clinic	0	0.00	2	0.08
Don't seek treatment	1	0.04	1	0.04
	23	**1.0**	**24**	**1.0**

Table 3: Demographic characteristics of the case-control study subjects

Characteristic		Case (n)	%	Control (n)	%
sex					
	Male	10	41.7	10	41.7
	Female	14	58.3	14	58.3
Marital status					
	Married	6	25.0	4	16.7
	Widowed	1	4.2	1	4.2
	Single	17	70.8	19	79.2
Tribe					
	Luo	4	16.7	10	41.7
	Luhya	18	75.0	11	45.8
	Teso	2	8.3	3	12.5
Religion					
	Roman catholic	11	45.8	6	25.0
	Protestant	1	4.2	3	12.5
	Pentecostal	10	41.7	8	33.3
	Other religions	2	8.3	7	29.2
Level of education					
	No formal education	2	9.1	1	4.2
	Pre-school	4	18.2	5	20.8
	Primary	13	59.1	17	70.8
	Secondary	3	13.6	0	0.0
	tertiary	0	0.0	1	4.2
Occupation					
	Trader	1	5.0	1	5.9
	Farmer	4	20.0	3	17.6
	Student	11	55.0	11	64.7
	Others	4	20.0	2	11.8
Duration lived in the study site					
	< 1 year	1	4.3	3	12.5
	1-5 years	2	8.7	3	12.5
	>5 years	20	87.0	18	75.0

3.2.3. Culinary habits and food sources.

Respondents were questioned on their preference for beef and pork, where they obtained their meat and frequency of eating meat outside their homes to find out if there was a significant difference in this practice between cases and controls. About 80% of the respondents were

found to eat both meat and pork. Fisher's exact test results for eating meat (p=0.36) and pork (p=0.31) revealed no significant association in this practice between cases and controls. There was no significant association between meat source (p=0.5) as shown in Fisher's exact test results. Fisher's exact test results revealed a significant association between eating meat outside the home to being a case or control (p=0.014). Table 4 shows the summarised results of the Chi square analysis for each variable measured.

Table 4: A summary of the Chi square and Fisher's exact test of significance in which status was the response variable

Variable	x^2	*df*	*p*	Fishers 2-tailed	Fisher's 1-tailed
Duration lived in the study site	1.285	2	0.526	-	-
Education	3.447	4	0.486	-	-
Occupation	4.110	3	0.250	-	-
Days per week spent outside home	9.364	6	0.154	1.00	-
Fishing	0.000	1	1	3.59	0.696
Grazing animals	1.50	1	0.221	0.724	0.179
Eating meat	0.505	1	0.447	1.00	0.362
Meat source	0.976	1	0.323	0.537	0.512
Eating pork	0.660	1	0.416	0.022	0.309
Eating meat outside the home	6.241	1	0.012	-	0.014
Water source (wet season)	4.00	4	0.406	-	-
Water source (dry season)	2.432	3	0.488	0.245	-
Involvement in collecting water	2.400	1	0.0.121	0.564	0.122
Treat water	0.751	1	0.386	0.666	0.282
Use latrine	0.762	1	0.383	1.000	0.333
Recent illness	0.167	1	0.683	-	0.500
Where treatment sought	4.005	3	0.261	0.766	-
Recently on medication	0.356	1	0.551	0.489	0.383
Currently on medication	2.087	1	0.149	0.18	0.245
Recently visited hospital				0.188	0.094

3.3. Attendance at activity points

Activity points visited included market, water sources, farms, swamp, rice paddy, lake, irrigation canal, playing grounds and open fields. Cases and controls visited similar activity

points (Figure 6) and thus were exposed to ralatively similar risk factors.

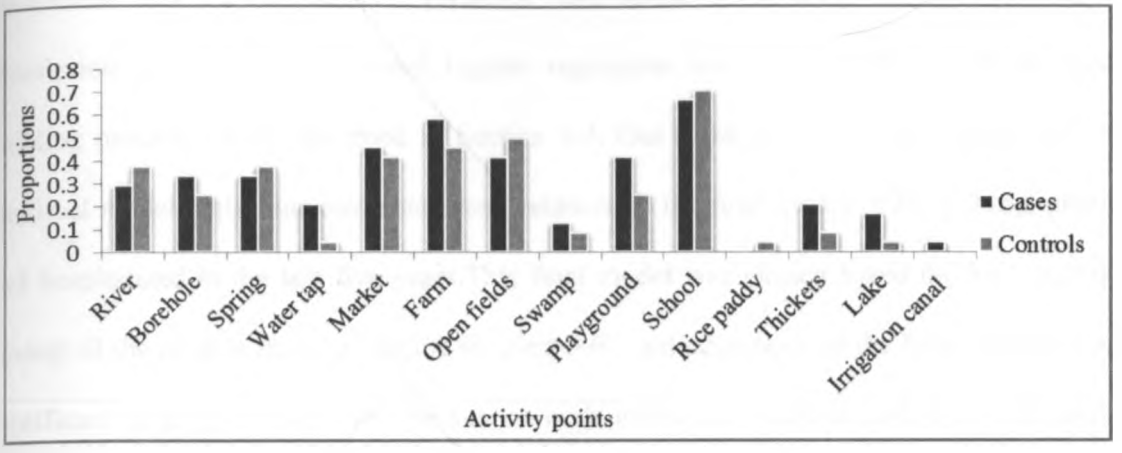

Figure 6: Proportion of cases and controls visiting various activity points

More cases engaged in fishing than controls. There were no case respondents engaged in working in the rice paddies while only a few controls engaged in working in the rice paddies. Univariate analysis done indicated that grazing, feeding livestock, visiting water sources such as pump and tap, not treating water and past incidences of fever and malaria were assoicated with being a case. However, confidence intervals for these predictor variables spanned 1.0 meaning that the association between visiting these activity points and being a case could have been due to chance alone and that the association is not statistically significant. The results are shown in Table 5. Odds ratios for visiting the river, spring, open fields, school and rice paddy were less than 1 meaning that these predictor variables were protective of the outcome. Visiting the borehole, market or swamp did not have any effect on the outcome variable as revealed by the odds ratio of 1. P-values for all the predictor variables were not statistically significant.

3.4. Conditional logistic regression model

3.4.1. Human socio-behavioural risk factors data

A conditional logistic regression model was built to determine whether there was an association between the predictor variables and status of the study subjects. An explicit description of how the conditional logistic regression was done together with the model building process was is described in Section 2.4. Out of all the covariates entered into the maximal model, only four covariates were retained in the final model: tribe, fishing, grazing and hospitalized in the last five years.This final model was chosen based on AIC such that among all the models fitted, it had the smallest AIC- although none of the four covariates was significant at $p \geq 0.05$ level. The results of both univariate analysis and the multivariable conditional logistic regression and presented in Table 5 in the appendix. Overall, there was no signicant effect of tribe (LR test p=0.2096), Luos and Tesos were less likely to be cases as compared to Luhyas , after adjusting for fishing, grazing and hospitalized in the last five years (Luo: OR=0.13, 95%CI0.015 -1.254 ; Teso OR=0.18, 95% CI0.013 - 2.526). For fishing, those who had fished in the last 12 months are 8 times highly likely to be a case than those who didn't (OR=8.07, 95% CI 0.226 - 289.129). Those who graze animals are also 5 times likely to be a case than those who don't. The results also indicate that individuals who must have been hospitalized in the last 5 years were less lilekly to be cases (OR=0.80, 95% CI 0.109-5.882). These results of the model indicate that there was no significant association between tribe, fishing, grazing animals or being hospitalised in the last five years prior to the study and being a case. This is revealed by the confidence intervals spanning 1 and thus denoting no statistical significance.

3.4.2. Environmental risk factors data

A conditional logistic regression model was also fitted to the environmental data. As explained in Section 2.4, a univariate analysis was perfomed to screen the covariates to include in the maximal conditional logistic regression model. Only three covariates were entered in the the maximal modle: visiting water tap, playground and lake. Although none of the three factors was significant at $p \geq 0.05$ level, the model was not reduced because of two reasons: (i) any reduction did not improve the fit statistics; and (ii) the model was already simple. The results are presented in Table 6 in the appendix. The adjusted odds ratios (aORs) indicate that after adjusting for water tap and lake, those visiting the play ground are 1.81 times highly likely to be a case than those who don't- although not significantly so. The results also show that those visiting the lake have 2.84 times higher chance of bieng a case than those who don't visit the lake, after adjusting for water tap and play ground. Those who visit are also at a higher risk of being a case than those who do not visit the water taps (OR= 3.53, 95% CI 0.383 -32.520). However, none of the results were statistically significant given the confidence intervals spanning 1; none of the predictor variables tested were significantly associated with being a case

CHAPTER FOUR

DISCUSSION

4.0. Parasite prevalence and co-endemicity

The most commonly diagnosed parasites were malaria, *Entamoeba*, hookworm, *Trichuris, Schistosoma* and *Ascaris* spp with a 15-20% prevalence rate in the sampled population. Western Kenya is malaria endemic and thus the high prevalence of malaria reported in these results are not surprising (Zhou *et al*., 2011). Possibly the large number of water bodies in the study area and local agricultural practices could be contributing to increase in mosquito vector habitats and consequently an upsurge in the mosquito vectors. A malaria epidemiological survey by Zhou *et al*., 2011 revealed that there has been a resurgence of malaria prevalence in Western Kenya since 2007. Reasons given for this trend include low bed net coverage of less than the expected 80%, rebounce of vector abundance and reducing efficacy of Insecticide Treated bed Nets (ITN) and Long-lasting Insecticide Impregnated bed nets (LLINs).

Malaria prevalence in Western Kenya is mainly associated with environmental factors related to availability of vector breeding habitats; human activities around the vector breeding habitats and proximity of human dwellings to vector breeding habitats, both of which result in human-vector contact. In these settings, malaria transmission is relatively stable throughout the year (Atieli *et al.*, 2011). The topographical layout of the study area is heterogeneous with gentle undulating slopes, hilly and forested areas as well as plains in some parts. All of these environments are punctuated with surface water in the form of rivers, swamps, boreholes and flood plains which are excellent breeding habitats for the mosquito vectors. As such, the high prevalence of malaria reported in the study population could be attributed to the factors described here.

Poor personal hygiene, limited access to safe drinking water and indiscriminate defeacation in the open environment are some of the factors which have been linked to high prevalence of parasitic infections in the developing world (Kvalsvig, 2003). This study revealed a parallel trend in of high prevalence of enteric parasites which could also be attributed to the similar risk factors. Further evidence suggests that growth and development of the eggs of enteric parasites is enhanced by the moist environments and the prevailing tropical climates (Pezzani *et al.*, 1996; Rai *et al.*, 2000). The high prevalence of enteric parasites was therefore similar to others which have been conducted elsewhere in the developing world (Steinman *et al.*, 2006; Gryseels *et al.*, 2006).

There was no case of sleeping sickness reported in the sampled population. Information from the literature indicates that PCR is a more sensitive method of detecting positive cases of HAT as compared to microscopy which may fail to detect all positive cases (Von Wissmann *et al.*, 2011). However, studies done in the last two decades in the study area also reported low and sporadic cases of sleeping sickness. This is attributed to active vector control and changes in land-use practices rendering the habitats uninhabitable by tsetse vectors and thus a reduction in incidence of the disease (Rutto and Karuga, 2009; Von Wissmann *et al.*, 2011).

No case of taeniasis was reported. Past studies point to a low prevalence of taeniasis in endemic areas (Morgano *et al.*, 2001; Nguekam *et al.*, 2003). It is also reported that it can take several years after ingestion of tapeworm eggs before clinical symptoms of the disease begin to manifest. Another explanation given to the low prevalence is that tapeworms inhabit the human host for short periods of time and are subsequently expelled after treatment making their recovery a challenge (Flisser *et al.*, 2006). It is possible that the subjects could have

received treatment prior to the screening thus eliminating the chance of detecting a positive case of taeniasis. A study conducted in two rural Mexican communities on the efficacy of self-detection after treatment with salycilamide resulted in more cases of taeniasis reported by respondents thereby indicating that time after treatment when tapeworms were extracted from faeces was a critical variable in the recovery of tapeworms in faeces (Flisser, 2006). In the case of this present study, since no intervention drug was given to stimulate recovery of positive taeniasis cases and the respondents were not trained on self-detection methods, it is possible that positive cases of taeniasis went undetected since only coproparasitoscopic method was used in diagnosis yet this method is less sensitive compared to coproantigen ELISA (Allan *et al.*, 1996; Flisser *et al.*, 2006). Detection of taenia antigens in faecal matter (coproantigens) has been shown to be a more sensitive method increasing the chance of detecting positive cases in a sample (Allan *et al.*, 1996). Additionally, information in the literature also lends weight to the findings of low prevalence of taeniasis in Western Kenya and indeed the rest of country which puts the prevalence rates at 4-10% for Western Kenya and 2% for the entire country (Phiri *et al.*, 2003; Wohlgemut *et al.*, 2010). The small sample size in this present study could also explain the inability to isolate any positive human taeniasis case.

Although pigs kept under free-range conditions were observed in the study area, no case of balantidiasis was reported. Probably the pigs have low infection rates and subsequently resulting in low transmission rates. There was also no incidence of cryptosporidiosis or isosporiasis. Cryptosporidium has a low prevalence rate in population with some studies putting it at 4% (Gatei *et al.*, 2006) and this could explain its absence in the sampled population given the sample size.

The highest prevalence of parasites was recorded in children with prevalence decreasing progressively with advancing age. It has been reported that in most cases children, more than adults, suffer from poor nutrition, weak immunity and they also come into contact with contaminated environment when they are playing and thus explaining their susceptibility to parasitic infections (Raso *et al.*, 2004).

4.1. Human socio-behavioural risk factors

Several aspects of the respondents' social behaviour were studied; with regard to treatment seeking behaviour, results showed that hospitals and chemists were the primary sources of medical care in case of illness. In the recent past, there has been an up scaling of health education both by government and non-governmental organisations in the rural areas with the ultimate goal of reducing child mortality and improving maternal health. This knowledge may have contributed significantly to respondents' choices of places for seeking treatment. These results mirror previous studies which have also shown that a majority seek treatment in medical facilities as opposed to self-medication (Mbagaya *et al.*, 2005; Sumba *et al.*, 2008).

Majority of the case-control study subjects stated that they used latrines. However, observations from field visits showed that the practice of relieving oneself in the open environment was still common. With many of the children and adults walking on bare feet, this poses a high risk for soil-transmitted helminths and may explain the high prevalence of the soil transmitted helminths within the case-control study subjects. Most respondents treated their drinking water, yet according to the results, water-borne diseases were highly prevalent. It is possible that respondents do not treat their water regularly and this, coupled with contamination during handling leads to high rates of infection. The major water sources are

boreholes, springs and rivers which could be contaminated by surface run-offs especially during rainy seasons and could transmit infective parasites if consumed before treatment.

4.2. Environmental risk factors

The environmental risk factors covariates described in Section 3.4.2. were used to fit a conditional logistic regression model in order to ascertain if there was any association between visiting activity points considered to be risk factors for acquisition of parasitic infections and status of the study subjects. The four covariates namely; fishing, grazing, hospitalization in the last five years and tribe, used to fit the final were not significantly associated with being a case. This could be the effect of a small sample size which has an effect on the statistical power of the data and thus could have obscured clinically important effect in the data (Moher *et al.*, 1994).

A precise definition for cases and controls would have possibly produced different results. If controls were defined as individuals without any infection at all and cases as individuals with one or more infection, it would have provided the chance to compare the lifestyles of individuals in the study sample without any infection against those with at least one infection. In this way it would be relatively easier to see any marked difference in behaviour and activity points visited by those with at least a single infection compared to those without any infection at all. However, the study design was limited by the case and control definition which required that those with multiple parasitic infections are compared against those without any infection or just one infection at most.

The results obtained could also be explained by the fact that different parasites have different epidemiological manifestations under the similar environmental conditions. As such, fishing or working for long hours in a rice paddy may be a risk factor for schistosomiasis, hookworm

or malaria but not so for HAT. Therefore in order to effectively investigate risk factors associated with multiple parasitic infections, epidemiological dynamics attributable to each parasitic infection must be investigated individually. Further evaluation can then be done to find out if epidemiological dynamics associated with individual diseases interact to compound the situation of multiple parasite infections.

Response to socio-behavioural questions could have also been influenced by several factors and biases. Normally infected individuals (cases) are more likely to recall the circumstances leading to the disease, for example, particular sites visited, as compared to non-infected (controls). This is called anamnestic bias (Woodward, 2005). In this case therefore, when the case and control definition is more specific, with cases being diseased individuals while controls being non-diseased individuals, responses by the different groups would be more distinct.

Since the controls in this study also included those who are diseased (individuals with a single infection), it would be expected that the responses given by those having multiple infections is similar to those having just one infection. Any variation in responses by those controls having no infection at all would be obscured by those of controls having a single infection thus resulting in no statistically significant variation in response between cases and controls. It is also possibly that this scenario was compounded by the small sample size for the study.

4.3. CONCLUSION

The study area had a high prevalence of parasitic diseases commonly associated with inadequate sanitary facilities, poor personal and environmental hygiene and a conducive tropical climate. Majority of the study population were also found to have at least two

parasitic diseases occurring simultaneously confirming that multiple parasite infection is a common phenomena among the residents of the study area. The primary sources of healthcare for the respondents are the medical facilities which dot the study area. Thus respondents are more informed of the need to seek medical attention in certified medical facilities as opposed to informal set ups.

The study area has inadequate hygiene and sanitation facilities. The major water sources are boreholes, springs and rivers which may not be safe for consumption. Most respondents therefore treat their water before consumption. The residents of the study area have not completely embraced the culture of using latrines all the time and therefore environmental contamination with human waste is still common. However, it is important to note that a majority of the study population now use latrines on regular basis. Neither eating meat nor sources of the meat were a risk factor for taeniasis infection in according to this study.

Visits to particular activity points considered risk factors for acquiring multiple parasitic infections were also not associated with infection with multiple parasitic infections. The study concludes that there is no difference in socio-behavioural practices and attendance at activity points and the risk of being a case subject. Possibly other factors other than the ones described this study could be linked to the risk of infection with multiple parasitic infections. The results obtained could also be due to the manner in which the cases and the controls were defined. Since it has been established from this study that cases and controls assumed a more homogeneous lifestyle, it would be appropriate to have cases as strictly those who are diseased and controls as those without any infection at all. This would assist in evaluating any

differences in social behaviour and attendance at activity points between diseased and non-diseased.

4.4. RECOMMENDATIONS

There is need for increased access to sanitary facilities and provision of adequate and safe drinking water in order to curb the spread of waterborne diseases. Health education with accurate health messages on the need to maintain proper hygiene and sanitation needs to be up-scaled to reach the remote villages of the study area. The design of this study can be improved by having a more specific definition for cases and controls for differential analysis of the relative risk of acquiring multiple parasitic infections. An initiative to investigate the interaction mechanisms in the risk factors for several parasitic infections occurring concomitantly would help in delineating risk factors for infection with multiple parasitic infections. The study design can also be expanded to include evaluation of other factors such as an individual's immunological state, seasonal changes in vector density and abundance and so on.

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APPENDIX

Table 5: Conditional logistic regression model: socio-behavioural risk factors covariates

Risk factor	# subjects	%cases	OR	95% CI	aOR	95% CI
Tribe						
Luhya	29	62.07	1.00	–		
Luo	14	28.57	0.24	(0.061 - 0.963)	0.13	(0.015 -1.254)
Teso	5	40.00	0.41	(0.059 - 2.835)	0.18	(0.013 - 2.526)
Religion						
Roman catholic	17	64.71				
Protestant	4	25.00	0.18	(0.015 - 2.154)		
Pentacostal	18	55.56	0.68	(0.175 -2.660)		
Other Christian	9	22.22	0.16	(0.024 -1.001)		
Marital status						
Single	24	50.00				
Married	10	60.00	1.50	(0.336 - 6.702)		
Widowed	2	50.00	1.00	(0.055- 17.903)		
Residence time						
< 1 yr	4	25.00				
1-5 yrs	5	40.00	2.00	(0.112 -35.807)		
>5 yrs	38	52.63	3.33	(0.318 -34.989)		
Education level						
None	3	66.67				
Pre-school	9	44.44	0.40	(0.026 - 6.176)		
Primary	30	43.33	0.38	(0.031 - 4.689)		
Secondary	4	100.00				
Tertiary	1	0.00				
Occupation						
Student	22	50.00				
Farmer	7	57.14	1.33	(0.240 -7.405)		
Trader	2	50.00	1.00	(0.055 -18.085)		
Other	6	66.67	2.00	(0.302 - 13.265)		
Days out	48	50.00	1.031	(0.778 -1.368)		
Fishing						
No	44	47.73				
Yes	4	75.00	3.29	(0.317 - 34.083)	8.07	(0.226 - 289.129)
Grazing						
No	32	43.75				
Yes	16	62.50	2.14	(0.627 -7.329)	1.50	(0.158 - 14.281)
Feeding livestock						
No	32	43.75				
Yes	16	62.50	2.142	(0.627 -7.329)		
Eat meat						
No	10	50.00				
Yes	38	50.00	1	(0.248 - 4.028)		
Water source						
Borehole	13	46.15				

Pump	6	66.67	2.33	(0.310 -17.545)		
River	9	44.44	0.93	(0.169 - 5.151)		
Spring	5	60.00	1.75	(0.215 -14.224)		
Tap	6	83.33	5.83	(0.525 -64.816)		
Other	9	22.22	0.33	(0.049 -2.257)		
Eat meat outside home						
No	14	21.43				
Yes	29	58.67	5.19	(1.188 - 22.706)		
Collect water						
No	8	62.50				
Yes	40	47.50	0.54	(0.114 -2.584)		
Treat water						
No	23	43.48				
Yes	25	56.00	1.65	(0.528 -5.182)		
Use latrine						
No	10	50.00				
Yes	38	50.00	1	(0.248 - 4.028)		
Illness last 12 months						
No	7	42.86				
Yes	41	51.22	1.4	(0.2778 -7.056)		
Where treated						
Hospital	42	54.76	_	_		
Chemist	2	0.00	_	_		
Private clinic	1	0.00	_	_		
Don't seek treatment	2	1.00	0.83	(0.048 -14.106)		
Had fever						
No	15	46.67				
Yes	33	51.52	1.21	(0.356 - 4.124)		
Had malaria						
No	17	41.18				
Yes	30	53.33	1.63	(0.490 - 5.437)		
Hospitalised last 5yrs						
No	6	66.67				
Yes	42	47.62	0.45	(0.075 -2.756)	0.80	(0.109-5.882)

Table 6: Conditional logistic regression model: environmental risk factors covariates

Risk factor	# subjects	%cases	OR	95% CI	aOR	95% CI
River						
No	32	53.13				
Yes	16	43.75	0.67	(0.205 -2.295)		
Farm						
No	24	45.83				
Yes	24	54.17	1.39	(0.449 - 4.348)		
Borehole						
No	27	51.85				
Yes	21	47.62	0.84	(0.269 -2.644)		
Market						
No	27	48.15				
Yes	21	52.38	1.18	(0.378 -3.710)		
Open field	30	50				
	18	50	1	(0.311 -3.218)		
Spring						
No	38	47.37				
Yes	10	60	1.67	(0.404 -6.870)		
Swamp						
No	43	48.84				
Yes	5	60	1.57	(0.238 -10.365)		
Water tap						
No	42	45.24				
Yes	6	83.33	6.05	(0.650 -56.359)	3.53	(0.383 -32.520)
Play ground						
No	32	43.75				
Yes	16	62.5	2.14	(0.627 -7.329)	1.81	(0.384 -8.556)
School						
No	15	53.33				
Yes	33	48.48	0.82	(0.242 -2.797)		
Thickets						
No	34	50				
Yes	14	50	1	(0.288 -3.472)		
Lake						
No	43	46.51				
Yes	5	80	4.6	(0.474 -44.603)	2.84	(0.299 -26.871)
Irrigation canal						
No	47	48.94				
Yes	1	100	-	-		

Human Individual questionnaire

Individual questionnaire

NR = No Response NA = Not applicable

Individual questionnaire

Date of data entry – auto record
Time of data entry – auto record
Recorder id (drop down list)

Homestead unique ID (barcode scan from book)

Interviewee number (barcode scan and stick in book)

Informed consent?

1. Language of questionnaire administration
Teso□ Luhya□ Luo□ Swahili□ English□
Other□.....

2. Age
<1□ 1-4□ 5-9□ 10-14□ 15-19□ 20-24□ 25-29□ 30-34□ 35-39□ 40-44□ 45-49□ 50-54□ 55-59□ 60-64□ 65-69□ 70-74□ 75-79□ 80-84□ 85+□ NR□

3. Sex Female□ Male□

4. What is your tribal origin? Teso□ Luhya□ Luo□ Kikuyu □ other□.....
NR□

5. What is your religion?
Roman Catholic□ Protestant/other Christian□ Muslim□ tribal religion□
None□ Other□ NR□

6. What is your current Marital status?
Single□ married□ divorced□ widowed□ NR□

Residential history

7. How long have you lived in this village? <1yr□ 1-5yrs□ >5yrs□

Education & Occupation

8. What level of education have you reached?

No formal education□ Pre-school□ Primary□ Secondary□ Tertiary□ College□ University□ vocational or technical school□ other□..... NR□

9. What is your MAJOR Occupation?
Farmer□ Trader□ Shop keeper□ Full time mother□ Student□ Other□..... NR□

10. How many days do you leave your village each week?
(on average or should we ask this week and assume representative?)
1□2□3□4□5□6□7□ days per week less than once a week□ Never □

11. On average, how many hours would you spend outside the village on each trip?
.....hrs

Animal Contact

12. In the last 12 months have you been fishing?
Daily□ at least once a week□ At least once a month□ at least once in the year□ Used to but do not any more□ Never□ NR□

13. In the last 12 months have you been involved with taking animals (your own or someone else's) for grazing?
Daily□ at least once a week□ At least once a month□ at least once in the year□ Used to but do not any more□ Never□ NR□

14. In the last 12 months have you been involved with feeding livestock within or outside the home?
Daily□ at least once a week□ At least once a month□ at least once in the year□ Used to but do not any more□ Never□ NR□

Food Preferences and Acquisition

15. How often do you eat beef?
Daily□ at least once a week□ At least once a month□ at least once a year□ on special occasions□ never□ NR□

16. How to you like your beef cooked?
Boiling□ Barbeque□ Fried□dried (roasted on small fire)□ smoked□ Other□.....

After choosing method →

17. To what extent do you like your beef cooked?
Still red□ slightly pink□ brown on outside□ brown all the way through□ other□..... NR□

18. How often do you eat pork?
Daily□ at least once a week□ At least once a month□ at least once a year□ on special occasions□ never□ NR□

19 How do you like your pork cooked?
Boiling□ Barbeque□ Fried□dried (roasted on small fire)□ smoked□
Other□.....

20. To what extent do you like your pork cooked?

Juicy□ Dry□ white □ with blood□ fully roasted□
Still red□ slightly pink□ brown on outside□ brown all the way through□
other□..... NR□

21. In the last 12 months where do you obtain meat?
Butchery shop□ market□ own herd□ neighbour□ other□ NR□

22. In the last 12 months have you eaten meat outside the home?
Y□ N□ NR□

If Y → Q 49
If N/NR → Q50

23. In the last 12 months where have you eaten meat outside the homestead?
At a neighbours □ By the roadside □ At a hotel □ at School□ Other□

Water & Hygiene

24. Where did you obtain your water from in the last wet season?
Borehole□ River□ Pump□Tap□ Well□ Spring□
Other□..... NR□

25. Where do you obtain your water from in the last dry season?
Borehole□ River□ Pump□Tap□ Well□ Spring□
Other□..... NR□

26. Are you involved in collecting water? Y□ N □ NR□

27. This month, have you treated water before drinking it?
No □ Boil □ add chlorine□ add iodine □ filter □ other □ NR□

28. In the last month how often have you used the latrine when you need to defecate?
Always□ Frequently□ Sometimes□ Never□ NR□

Health status

29. Usually when you feel ill, where to you seek treatment?
Did not seek treatment□ community health workers□ traditional healer□ chemist□ private clinic□ hospital□ self-treatment□ neighbour□ church healer□ other□……..NR□

30. Have you ever had worms in your faeces? Y□ N□ NR□

31. Have you ever had blood in your urine? Y□ N□ NR□

32. Have you ever had fever? Y□ N□ NR□

33. Have you had *diagnosed* malaria? Y□ N□ NR□

34. Have you taken any medicines in the last 1 month Y□ N□ NR□
If Y → 91 If N/NR → 92

35. Are you currently taking any medicines Y□ N□ NR□
If Y → 93 If N/NR → 94.

36. Have you attended hospital/clinic in the last 5 years Y□ N□ NR□
If Y → 96 If N/NR → 97

Informed Consent Document

Study title: Epidemiology of zoonotic infections amongst livestock and their keepers in Western Kenya

Informed Consent Document

Instructions

- **Enumerator to distribute read and explain to participant. Use English, Swahili or local language, as appropriate.**
- **One signed copy for hardcopy file, one signed copy for participant.**

We are visiting you to invite you to participate in a research project which aims to understand the importance of zoonotic diseases in your community. Zoonotic diseases are diseases that you or your family may get from direct or indirect contact with animals. Our objective is ultimately to learn to control these diseases better, and in particular, understand how controlling such diseases in animals may prevent them from infecting people. This is a research project jointly run by the Kenya Medical Research Institute (KEMRI), the International Livestock Research Institute (ILRI) in Nairobi and the University of Edinburgh (UK). It is funded by the Wellcome Trust in the UK.

To carry out this research, we would like to ask you some questions about the animals you keep, the way in which you farm and live, your health and health problems, and also collect some samples for further detailed analysis. We are visiting your home with two teams, one which, with your permission, will collect samples from you, and one which will collect samples from your livestock. The outcome of this research will be a better understanding of zoonotic diseases. Findings from this investigation will help us advise both human and animal health authorities in your region and the rest of Kenya and beyond about improving health.

What is involved

Your participation will take approximately 30 minutes of your time. You have been selected randomly for this project (meaning that everyone in this region had an equal chance of being invited to participate), but you are free to decline participation if you would prefer not to take part. Taking part in this will involve

1) answering some general questions about your health and your home;
2) allowing us to take some measurements like your height and weight;
3) providing us with a sample of your faeces to look for parasites like worms;
4) allowing our qualified technician/nurse to take a 25ml sample of blood from your arm – equivalent of 2 tablespoons, so that we can take these samples to the laboratory (in Busia) and examine the blood for infections that you may have or have had in the past and that can be detected in your blood.

Measurements and samples will be taken by a qualified clinician or technician. There will be some discomfort associated with sampling blood, which will use a needle to collect blood from your arm. This discomfort is transient.

Benefits to participants

We will offer you a general health check as part of this study - by taking measurements like your height and weight and conducting an examination, we can advise you if you appear in good or bad health and suggest whether we think you should attend a clinic for further tests. We will advise you of the most appropriate facility for further consultation if required. If you would like us to, we can also prepare a report which we will send to you to inform you of what parasites we find in your faecal sample and blood sample – eg worms, malaria. This health check and parasitology report that we are offering is free of charge to you, and if you choose not to participate in the sampling for the project, we will none-the-less carry out the health check if you wish: participation is thus entirely voluntary and there is no consequence to you for not participating should you choose not to.

Anonymity/secondary use of material

Beyond the health check and parasitology tests, your participation will be totally anonymous. We will conduct further tests for a range of diseases on your sample, but it will no longer be