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INFECTIONS AND CHILDHOOD CANCER IN MALAWI

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ABSTRACT

The causes of childhood cancers are not well understood. That infections are believed to play an important role in childhood cancer development is of particular interest in sub-Saharan Africa, where infectious diseases are common. The objectives of this thesis were to identify childhood cancers associated with HIV, malaria, EBV and HHV-8, and to investigate child and maternal factors associated with Burkitt lymphoma and Kaposi sarcoma.

In Blantyre, Malawi, 305 children diagnosed with cancer and 212 of their mothers, were recruited. Risk factor data were collected using a brief questionnaire and blood samples tested for infections.

Case-control analyses were conducted to compare 148 Burkitt lymphoma cases and 22 Kaposi sarcoma case with a control group comprising 104 children with cancers other than those known to be associated with HIV.

The prevalence of HIV was 6% among children with Burkitt lymphoma and 2% in controls (OR=12.4, 95% CI 1.3 to 116.2). Tumour risk increased with increasing titres of antibodies against EBV and malaria. In comparison with those who had low titres against both EBV and malaria, the highest risk of Burkitt lymphoma was among those with high titres against both infections (OR=13.2, 95% CI 3.8 to 46.6). Reported use of mosquito nets was protective against Burkitt lymphoma.

The prevalence of HIV was 81% among children with Kaposi sarcoma (OR=762.7, 95% CI 44 to 13376), and risk increased with increasing HHV-8 antibodies. Prevalence of infections was also examined among children with other cancer types and no associations were identified, although the number of cases was small. Few maternal factors were found to be associated with cancer in children.

This research demonstrates that infections play a particularly important role in increasing the risk of Burkitt lymphoma and Kaposi sarcoma in children in sub-Saharan Africa. Prevention or early treatment of these infections may be vital in the control of childhood cancer.

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LIST OF ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
ALL	Acute Lymphoctyic Leukaemia
ARL	AIDS-related Lymphoma
ART	Anti-Retroviral Therapy
ASR	Age Standardised Rate
BL	Burkitt Lymphoma
CCTVM	Centre for Clinical Vaccinology and Tropical Medicine
EBV	Epstein Barr Virus
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked Immuno Sorbent Assay
FNA	Fine Needle Aspirates
GMT	Geometric Mean Titre
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HHV-8	Human Herpes Virus type 8
HIV	Human Immunodeficiency Virus
HL	Hodgkin Lymphoma
HPV	Human Papilloma Virus
IARC	International Agency for Research on Cancer
ICCC	International Classification of Childhood Cancer
ICD-O	International Classification of Diseases for Oncology
IFA	Indirect immunofluorescence
IQR	Interquartile range
KS	Kaposi Sarcoma
KSHV	Kaposi Sarcoma associated Herpes Virus
NHL	Non-Hodgkin Lymphoma
NOS	Not otherwise specified
NPC	Nasopharyngeal Carcinoma

OD	Optical density
ORs	Odds ratios
PfSE	*Plasmodium falciparum* Schizont Extract
PR	Parasite rates
QECH	Queen Elizabeth Central Hospital
RMS	Rhabdomyosarcoma
RPR	Rapid Plasma Reagin
SD	Standard deviation
SEER	Surveillance Epidemiology and End Results
SRBCT	Small round blue cell tumour
TPHA	*Treponema pallidum* haemaglutination assay
UNAIDS	The Joint United Nations Programme on HIV/AIDS
UNICEF	United Nations Children's Emergency Fund
VCA	Viral Capsid Antigen
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION, BACKGROUND AND OBJECTIVES

1.1. Introduction

This chapter gives a brief overview of the epidemiology of cancer focusing in particular on cancer in children. The role of infections in the aetiology of cancer is briefly discussed. The role of the Human Immunodeficiency Virus (HIV) in cancer development is highlighted. Some limitations in studying childhood cancer in Africa are outlined, the objectives of this study are explained and the layout of the thesis described.

1.2. Cancer overview

Globally about 10 million new cancer cases are diagnosed annually, and this is projected to further increase by 50% to 15 million new cases by the year 2020 (Stewart et al.,2003). This estimate is based on cancer incidence and mortality data, current trends in smoking and other lifestyle factors compiled by the World Health Organisation and the International Agency for Research on Cancer. The World Health Organization (WHO) estimates that there were 58 million deaths worldwide in the year 2005 and 13% of these were caused by cancer. This is more than the percentage of deaths caused by HIV/AIDS, tuberculosis, and malaria put together (World Health Organisation.,2005). More than 50% of the world's cancer burden occurs in developing countries, making cancer a major cause of morbidity and mortality (Stewart et al.,2003).

1.3. Childhood cancer

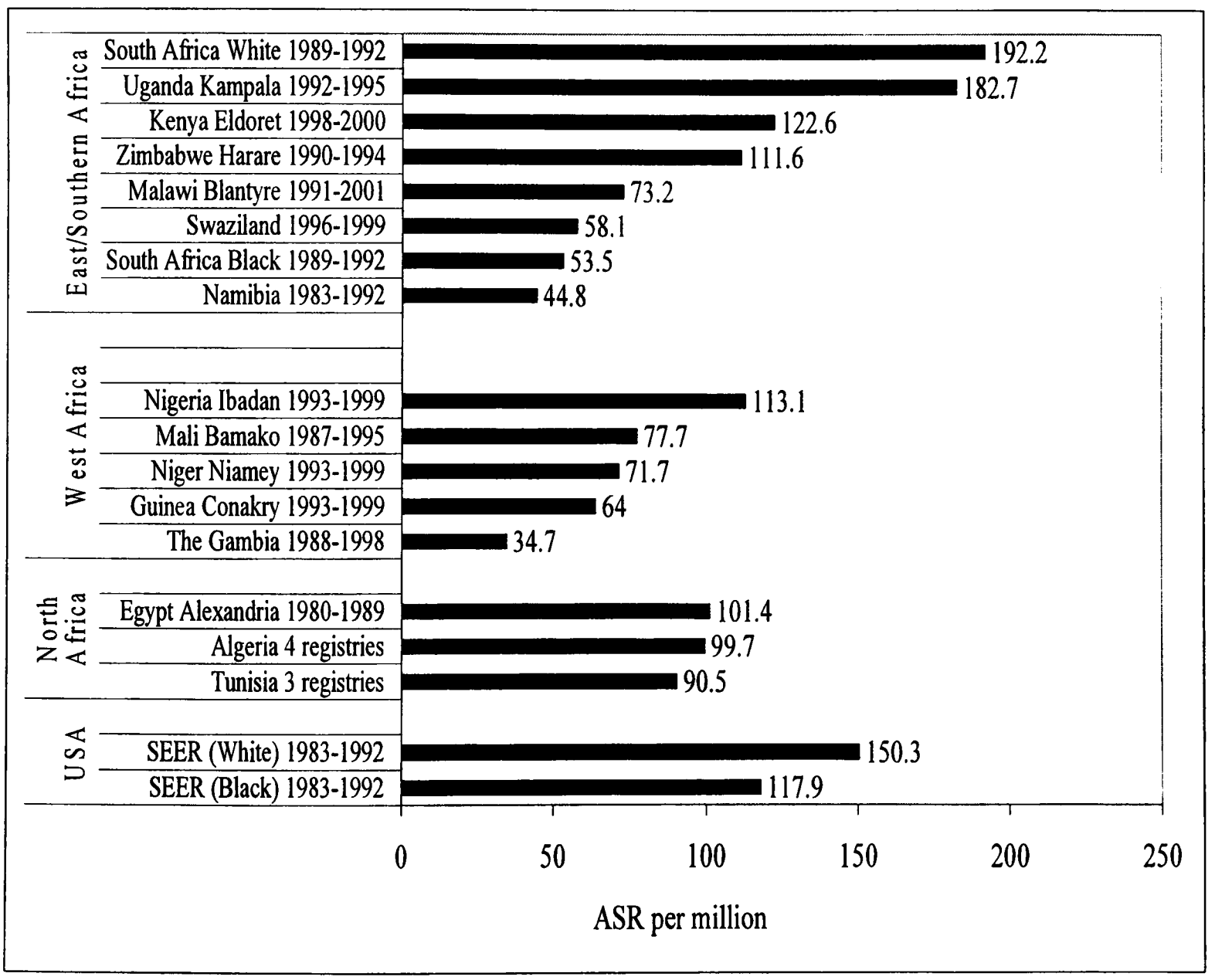
Cancer is relatively rare in children. Registry data on cancer shows that childhood cancer in developed countries occurs typically in the range of 110 to 150 per million children per year (Stiller et al.,2004). Routinely collected data from African countries show that childhood cancer occurrence ranges from 70 to 180 per million children per year (IARC,1998). Developing countries account for only 5% of the world's economic resources and more often than not have inadequate health care programs. Despite this, more than 85% of paediatric cancer cases occur in developing countries (Yaris et al.,2004). Figure 1.1 is a compilation of childhood cancer data available from African countries. The data are derived from the texts 'International Incidence of Childhood Cancer Volume 2' and 'Cancer in Africa: Epidemiology and Prevention' (IARC,1998, 2003). Where overall child mortality is high, the relative importance of cancer is obscured by the high mortality due to infections and complications of pregnancy and delivery, and cancer is likely to be under-reported.

There are around 160,000 global childhood cancer registrations each year, but it is estimated that between 225,000 and 250,000 children get cancer each year. Therefore approximately 60,000 to 90,000 are not registered for various reasons including not presenting to hospital, the correct diagnosis is not made or the cancer is treated but not registered (Eiser et al.,2006). An International Union against Cancer (UICC) report states that childhood cancer rates appear to be lower in sub-Saharan Africa than in developed countries, except in regions where HIV infection is endemic, and a large group of child cancer is due to HIV-associated lymphomas and Kaposi

sarcoma. In reality, there is likely to be significant under-reporting of cases (Pisani et al.,2006).

The cancer burden in Africa appears to be rising. This is attributed to factors that have persistently overwhelmed the continent including infectious diseases, poor food supply, poverty, and an increasing number of people exposed to a more affluent lifestyle, especially tobacco (Morris,2003). These exposures may be important determinants of cancer development in African children (Wild et al.,2003).

Figure 1.1: Age standardised rates of childhood cancer (aged ≤ 15 years) in African countries



1.3.1. Childhood cancer survival

The most striking feature of the epidemiology of childhood cancer over the last two decades is the continuing improvement in survival due to increasing efficacy of treatment. Significant improvements in unselected series of cases have been documented in Europe (Gatta et al., 2002; Ajiki et al., 2004; Steliarova et al., 2004). Despite 80% survival rates in developed countries, most children with cancer in developing countries will die due to lack of adequate medical care (Gemma et al.,2002). Direct measures of population-based survival probabilities of cancer patients in developing countries are sparse and virtually unknown for children. This lack of information reflects the slight attention given to this group of diseases when healthcare resources are limited and other diseases such as malaria and HIV are more serious public health problems. Surviving children remain at increased risk of cancer and other diseases throughout their life and therefore require continued monitoring.

1.3.2. Childhood cancer diagnosis in Africa

In general, data on cancer incidence and mortality are less complete and less accurate in developing than in developed countries. The latter have more resources to invest and maintain population-based cancer registries. Deficits in diagnostic materials and knowledge limit the accurate diagnosis of cancers in developing countries. Not all cancer types are diagnosed histologically, and other methods of diagnosis such as radiology are not widely available, making it difficult to diagnose some childhood cancers such as central nervous system tumours. This may result in under ascertainment of cases. Most of what is known about childhood cancer in African countries is based on pathological and hospital-based case series. The disadvantage of this is that there is over-representation of cancers that are easily biopsied, and the

data collected are difficult to interpret because they are not population-based (IARC,2003). In most circumstances, it is not possible to obtain an accurate estimate of childhood cancer incidence in African countries, and most data presented as incidence figures are actually derived from routine hospital-based data collection.

1.4. Childhood cancer aetiology

Marked improvements in cancer treatment have not been matched by similar insights into cancer aetiology, and the cause or causes of the majority of malignancies in children and young adults remain unknown. There is little evidence to link the majority of childhood cancers with the well known carcinogens implicated in adult-onset cancer. The relatively higher numbers of adults with cancer have enabled the ascertainment of causative factors, such as alcohol and smoking. Whereas the small numbers of children with cancer have made it difficult to evaluate the role of environmental factors in cancer development. Small childhood cancer numbers may only be part of the issue; it may be that some childhood cancers do not have specific environmental causes.

1.4.1. Genetic associations

Recognized genetic syndromes and familial aggregations account for a small proportion of all childhood cancers, probably less than 5% (Anderson et al.,2000). Examples include Down's syndrome and neurofibromatosis, genetic conditions that predispose to a range of tumours (Little,1999). Retinoblastoma has become the paradigm for the role of genetic factors in cancer aetiology. Knudson coined the "two-mutation hypothesis" in which he postulated that all retinoblastomas occur as the result of two mutations in the Rb gene (Knudson,1971). Similarly, there are

several genes thought to be involved in the aetiology of Wilms tumour (Green et al.,1996).

1.4.2. Infections

Specific infectious agents are now recognized as major causes of certain cancers, for example Epstein Barr virus for Burkitt lymphoma, HHV-8 for Kaposi sarcoma, hepatitis B and C for hepatocellular carcinoma (Stewart et al.,2003). Specific infections and their relationship with childhood cancer are discussed later in this and subsequent chapters. The role of specific infections and their relationship with cancer in children is one of the major focuses of this thesis.

1.5. Infections and cancer

The International Agency for Research on Cancer (IARC) has found sufficient evidence to recognize some pathogens as definitely carcinogenic, and others as probably carcinogenic to humans (IARC,1994, 1997). This applies to both adult and child cancers. Recent estimates suggest that up to 23% of human cancers are attributable to infectious agents, and that developing countries have a higher proportion of these cancers (about 23% compared to 10% in developed countries) (Pisani et al.,1997). The implication of these relationships with infections is that vaccinations can potentially be used to prevent infection-associated cancers. The use of vaccinations has reduced the incidence of numerous infectious diseases worldwide, and this tool can be used to reduce or stop the occurrence of infection-associated cancers. For example, the incidence of hepatocellular carcinoma caused by the hepatitis B virus (HBV) declined by 50% after effective vaccination against HBV in children in Taiwan was introduced, demonstrating that vaccination can and will eventually become a major factor in cancer prevention (Lee et al.,2003). The

Gambia Hepatitis Intervention Study (GHIS) was initiated in 1986 (The Gambia Hepatitis Study Group,1987). It aims to evaluate the protection provided by HBV vaccination administered during the first year of life against primary infection, the development of chronic carriage, and primary liver cancer. It is expected that HBV vaccination will result in a reduction in mortality from hepatocellular carcinoma. Immunization against hepatitis B is now recommended by WHO for all countries, but is still unavailable in many places where it is most needed. Other strategies such as screening blood for infections e.g. hepatitis B and C could also help to reduce cancer occurrence.

Cancer is considered a multistage phenomenon and infectious agents are a necessary yet not sufficient factor for the development of certain cancers such as Kaposi sarcoma (IARC,1997). A better understanding of the role of infectious agents in the aetiology of cancer is a public health imperative, because such cancers are theoretically preventable by vaccination or early treatment of infections. Table 1.1 gives an overview of cancer-causing infections, categorised into established associations and suspected or possible associations for both adults and children.

1.6. Infections associated with cancer in African children

A description of infections associated with the most frequent cancers in African children including lymphomas and Kaposi sarcoma is now presented. This description is not limited to childhood cancers because most of the data available about infectious causes of cancer are derived from studies involving adults. The infections are described in order of importance based on the number of childhood cancers they are associated with.

Table 1.1: Carcinogenic human infections

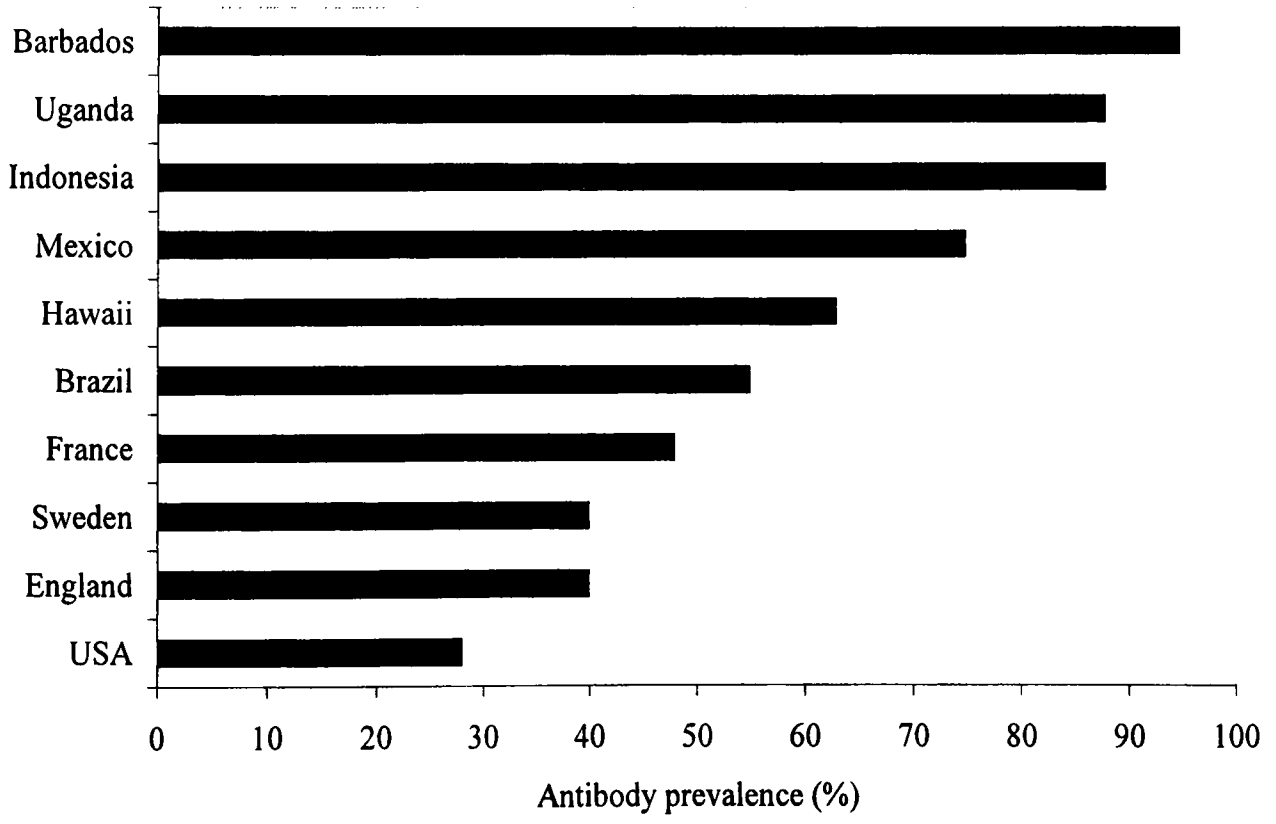
Infections	Cancer with established associations	Cancers with suspected associations
Viruses		
Human papilloma virus (HPV)	Cervical cancer	Skin squamous cell carcinoma Conjunctival carcinoma Other ano-genital cancers
Hepatitis B virus (HBV)	Hepatocellular carcinoma	
Epstein Barr virus (EBV)	NHL Post-Transplant Lymphoproliferative Disease Burkitt lymphoma CNS lymphoma Nasopharyngeal carcinoma Hodgkin lymphoma	Leiomyosarcoma Gastric adenocarcinoma
HHV-8	Kaposi sarcoma Primary effusion lymphoma Castleman's disease	
Human T cell lymphotrophic virus type 1 (HTLV-1)	Adult T cell leukaemia/lymphoma	
Hepatitis C virus (HCV)	Hepatocellular carcinoma	NHL Oral cancer
[a]HIV	NHL Kaposi sarcoma	Conjunctival squamous cell carcinoma Hodgkin lymphoma Leiomyosarcoma Cervical cancer
Bacteria		
Helicobacter pylori	Gastric adenocarcinoma, Gastric NHL	
Helminths		
Schistosoma haematobium *Schistosoma japonicum*	Bladder carcinoma	Colorectal carcinoma Primary liver cancer
Opisthorchis viverrini *Clonorchis sinensis*	Cholangiocarcinoma Cholangiocarcinoma	

[a]Not directly oncogenic
Adapted and modified from (Herrera et al.,2005).

1.6.1. Epstein Barr Virus

EBV was first discovered in 1964 in B lymphocytes cultured from Burkitt lymphoma tissue of an African child (Epstein et al.,1964). EBV is a member of the herpes virus family, and is a group 1 carcinogen (IARC,1997). EBV is a ubiquitous infection worldwide and by the third decade, 80 to 100% of all people are carriers of the virus (IARC,1997). EBV antibody prevalence in the 4 to 6 year old population of different countries from the developed and developing world is shown in Figure 1.2 (IARC,1997). Over 80% of children in developing countries have been infected with EBV by the age of three (Evans et al.,1989).

Figure 1.2: Prevalence of EBV in children aged 4 to 6 years in various populations
Data from (IARC,1997)



Infection of humans with EBV usually occurs by contact with oral secretions (Cohen,2000). In developing countries, infection is acquired in the first few years of life, and associated risk factors for infection include low socio-economic status, large

family size and poor hygienic standards (IARC,1997). EBV has been found in the saliva of African mothers (Gerber et al.,1976; Mbulaiteye et al.,2006). African populations have numerous practices involving the exchange of saliva including use of saliva in traditional medical and feeding practices that may facilitate EBV transmission (Wojocicki,2003). Infants who acquire EBV infection by 15 months of age are more likely to have parents with the least education, the most inadequate housing, greater number of children in the compound and mother sharing a bed with the infant (Biggar et al.,1978). Children from poor socio-economic backgrounds have been found to be infected with EBV at an earlier age than children from higher socio-economic status backgrounds (Biggar et al.,1981). EBV infection in early infancy remains essentially silent, with no obvious symptoms (Biggar et al.,1978). In developed countries, infection is often delayed, and when acquired during the teenage years may cause infectious mononucleosis (IARC,1997). Evidence is now emerging that EBV may also be transmitted sexually (Higgins et al.,2007).

Primary infection of B cells by EBV results in a rapid expansion of infected lymphocytes, and is followed by a marked EBV-specific cytotoxic T cell response (Hsu et al.,2000). EBV establishes a life long latent infection in the B cells, although this may be triggered into a replicative phase (Cohen,2000). EBV is associated with lymphoproliferative disease in patients with congenital or acquired immunodeficiency, including patients with severe combined immunodeficiency, recipients of organ or bone marrow transplants, and patients with AIDS (Crawford,2001). These patients have impaired EBV-specific T cell immunity and are unable to control the proliferation of EBV infected B cells. The oncogenic

potential of EBV is reflected in its ability to infect and transform B lymphocytes into continuously growing cell lines (Crawford,2001).

Cancers associated with EBV infection in adults and children include non-Hodgkin lymphoma (NHL) including Burkitt lymphoma, Hodgkin lymphoma, nasopharyngeal carcinoma, lymphomas associated with immunosuppresion, and immunodeficiency-related leiomyosarcoma (Hsu et al.,2000).

1.6.2. Human herpes virus type 8

Human Herpes Virus type 8 (HHV-8) is also known as Kaposi sarcoma-associated herpes virus (KSHV). This newly discovered herpes virus was first detected in Kaposi sarcoma tissue obtained from patients with AIDS (Chang et al.,1994). It has been detected in every epidemiological form of Kaposi sarcoma, and is now believed to be a necessary cause of this disease (IARC,1997). HHV-8 is not a ubiquitous virus, it is most prevalent in groups or populations at highest risk of developing Kaposi sarcoma, such as HIV infected homosexual men in the USA and in African populations where the tumour has long been endemic (Boshoff,1999). Prevalence of HHV-8 infection in adults may be up to 50 to 70% in some African populations (IARC,1997). A review of HHV-8 prevalence in Africa showed that HHV-8 was common in many African countries, including places where Kaposi sarcoma was almost unknown before HIV and that it is as common in women as in men (Dedicoat et al.,2003).

In the 1990s studies revealed that homosexual and bisexual men in the United States and Australia had a relatively high prevalence of HHV-8, which was associated with

a high number of sexual partners and a history of sexually transmitted diseases (Beral et al.,1992; Grulich et al.,1997). This led to the hypothesis that HHV-8 may be transmitted sexually. Large amounts of HHV-8 DNA were detected in saliva of homosexual adult males, this was thought to suggest that the virus may be transmitted via saliva (Koelle et al.,1997; Pauk et al.,2000).

Transmission of HHV-8 to children is thought to be via saliva exchange between mother and child and between siblings as they interact with each other (Plancoulaine et al.,2000; Mbulataiye et al.,2003). Children may be exposed to saliva during play and sharing of eating utensils, or other practices that involve exchange of saliva. As saliva is the body fluid that most often harbours HHV-8, and at greatest concentrations, it is likely that any behaviour involving the exchange of saliva may facilitate transmission (Pauk et al.,2000; Wojocicki,2003). The low HHV-8 DNA seroprevalence in children younger than five years argues against the idea that transmission to the child from the mother is primarily during pregnancy, delivery, or breastfeeding (Mantina et al.,2001). Some studies have suggested that the prevalence of HHV-8 antibodies increases with age, and infection may be acquired early in life through routes other than sexual transmission (Mayama et al.,1998; Andreoni et al.,1999; Andreoni et al.,2002; Mbulataiye et al.,2003).

A study in Rome further investigated the vertical transmission of HHV-8 (Sarmati et al.,2004). Two hundred and forty-five pregnant women who underwent amniocentesis for genetic screening were evaluated. Amniotic fluid, maternal blood and neonatal cord blood samples from HHV-8 seropositive women were tested for the presence of HHV-8 DNA sequences by PCR. No evidence of HHV-8 DNA was

found in amniotic fluid or in neonatal cord blood for women in whom HHV-8-DNA was detected in blood. The findings of this study strengthened the impression that vertical transmission of HHV-8 infection is rare or even absent, and supported the results of epidemiological studies suggesting that horizontal transmission plays a major role in the spread of HHV-8 infection among children in endemic populations.

HHV-8 is associated with Kaposi sarcoma, primary effusion lymphoma and multicentric Castleman's disease in both adults and children (Schulz,1998).

1.7. HIV infection and cancer

Immunodeficiency, be it congenital, therapeutic or infectious in origin, increases the risk of certain, but not all types of cancer (Newton et al.,2001). A common feature of malignancies that arise because of immunodeficiency is that specific infectious agents appear to be important in their aetiology; not only in immunodeficient subjects but also in the general population (Beral et al.,1999). According to the Joint United Nations Programme on HIV/AIDS (UNAIDS), more than 40 million people are infected with HIV worldwide, the majority being in sub-Saharan Africa, which was home to 2 million children under 15 years living with HIV (UNAIDS et al.,2006). Studying HIV infected people has provided an opportunity to investigate the role of the immune system in the aetiology of cancer as well as possibly identifying new cancers with an infectious cause.

HIV is distinguished from other cancer-causing viruses in that there is little evidence that it has a direct oncogenic role in the development of a specific cancer. It provides an environment that facilitates a variety of opportunistic infections because of its

damaging effect on the immune system (Herrera et al.,2005). HIV appears to facilitate the development of a number of cancers, all of which are either known, or thought, to be caused by other infectious agents. Patients infected with HIV are at a significantly increased risk of developing cancer compared with the general population (Beral et al.,1991; Beral et al.,1998; Mueller,1998, 1999; Biggar et al.,2000). Although many cancers have been reported to be increased in people with HIV infection, for only a few is the evidence sufficiently strong and consistent to conclude there is a definite increase in risk.

HIV is causally associated with Kaposi sarcoma, non-Hodgkin lymphoma and conjunctival carcinoma (Kuper et al.,2000). Recent evidence for Hodgkin lymphoma and childhood leiomyosarcoma suggests a definite increase in risk associated with HIV infection (McClain et al.,1995; Pollock et al.,2003). The relative risks for HIV-seropositive compared with HIV-seronegative people for cancers associated with HIV tend to be very large, generally 10-fold or greater (Beral et al.,1998). As a result of the immune reconstitution afforded by effective combination anti-retroviral therapies, the epidemiological and clinical profile of cancers in the setting of HIV infection has changed. There has been a significant decline in some AIDS defining cancers such as Kaposi sarcoma (Cheung et al.,2005).

The number of children with HIV infection who develop a malignancy is poorly defined. In 1994 the Centres for Disease Control and Prevention (CDC) published a revised classification of paediatric AIDS which lists primary brain lymphomas, small non-cleaved cell (Burkitt) non-Hodgkin lymphoma, immunoblastic or large-cell lymphoma of B cell or unknown immunologic phenotype, as well as Kaposi sarcoma

as AIDS-defining events (Centers for Disease Control and Prevention.,1994). Leiomyosarcomas were also included as a sign of a moderately symptomatic stage.

Relative to adults, there are few published data from analytical studies on the risk of cancer in HIV infected children. This is primarily because both cancer and HIV infection are less common in children than in adults. A study from the United States showed that, subsequent to a diagnosis of AIDS, children were at an increased risk of non-Hodgkin lymphoma (based on 42 cases; including "sporadic" Burkitt lymphoma, immunoblastic lymphoma and primary lymphoma of the brain), Kaposi sarcoma (based on 4 cases), and leiomyosarcoma (based on 3 cases) (Biggar et al.,2000). HIV positive children in Britain had an increased risk of developing Kaposi sarcoma and non-Hodgkin lymphoma (Evans et al.,1997). These children had an estimated prevalence of HIV associated malignancies of 3%. A cohort of HIV positive children in Italy had an estimated prevalence of HIV associated malignancies of 2% (Aricò et al.,1991). The sample size was small, out of 388 HIV infected children, 7 developed tumours including non-Hodgkin lymphoma, acute leukaemia, Kaposi sarcoma and hepatoblastoma.

Despite the fact that HIV infection is more prevalent in parts of sub-Saharan Africa than elsewhere, there is only one published study from Africa that investigated the scale of the excess risk of cancer in HIV infected as compared to uninfected children (Newton et al.,2001). The results showed that HIV infection increased the risk both of Kaposi sarcoma and of the African endemic form of Burkitt lymphoma. Results for acute lymphoblastic leukaemia (ALL) were suggestive of an association with HIV infection, but were not significant.

It is notable that the spectrum of cancers affecting children in the general population is different from that in adults. Furthermore, unlike adults, the great majority of HIV infected children acquire the virus in the first months of life, while the immune system is developing and prior to exposure to many other immunological challenges.

1.7.1. HIV associated malignancies

1.7.1.1. AIDS-related lymphoma

In developed countries, adults with AIDS are 60 times more likely than the HIV negative general population to develop non-Hodgkin lymphoma, and 1000 times more likely to develop Burkitt lymphoma or central nervous system lymphomas (Beral et al.,1991). AIDS-related non-Hodgkin lymphoma often occurs extranodally, in the central nervous system, gut or bone marrow, and usually takes an aggressive clinical course (Mitsuyasu et al.,2004).

More than half of AIDS-related lymphomas (ARL) are associated with EBV and/or HHV-8 infection. (Boshoff et al.,2002). In HIV infected individuals, chronic antigen stimulation may lead to oligoclonal B cell expansion. AIDS-related non-Hodgkin lymphoma is believed to arise as a result of continued stimulation of B cell proliferation because of HIV, EBV, and other infections, all of which occur in the setting of profound T cell immunodeficiency (Grulich et al.,2000). HIV also induces the expression of a number of cytokines that can further increase B cell activation. Non-Hodgkin lymphoma is a late manifestation of HIV infection and the incidence of disease is highest in patients with marked immunosuppresion.

Few data are available on the risk of non-Hodgkin lymphoma in children in Africa. To date, 1 case-control study has compared the risk of non-Hodgkin lymphoma in HIV positive children (Newton et al.,2001). The estimation of risk for Burkitt lymphoma in HIV positive children found an OR of 7.5 (95% CI, 2.8 to 20.1) based on 33 cases. An autopsy study conducted in Cote d'Ivoire in 1991 revealed that none of the 78 autopsied HIV positive children (median age =17 months) had non-Hodgkin lymphoma. The authors inferred that survival of HIV positive children in Africa appears too short to permit the significant development of additional non-Hodgkin lymphoma.

The scale of the excess risk between non-Hodgkin lymphoma and HIV appears weaker in Africa than in developed countries, and different explanations have been put forward. Early acquisition of EBV in childhood in Africa could lead to lower lymphoma risk. A lower susceptibility to non-Hodgkin lymphoma among African populations than among white populations has also been hypothesised, since in the USA the proportion of non-Hodgkin lymphoma as AIDS-defining illness and incidence rates of non-Hodgkin lymphoma in the general population were lower in black than white people (Dal Maso et al.,2003). The likelier explanation of the apparent lack of non-Hodgkin lymphoma in Africa, however, is under-ascertainment of non-Hodgkin lymphoma and competitive mortality from other AIDS-associated illnesses. Patients with severe immunodeficiency in this part of the world are likely to die from infectious diseases before developing non-Hodgkin lymphoma.

1.7.1.2. Kaposi sarcoma

It was the appearance of aggressive forms of Kaposi sarcoma in the USA in the early 1980s that heralded the onset of the HIV epidemic in western countries. Although the

incidence of Kaposi sarcoma has increased in populations at high risk of HIV in northern Europe and the USA, it existed at such a low level before the onset of the epidemic that it remains a relatively rare tumour. However, parts of Africa with a high prevalence of HIV and where Kaposi sarcoma was relatively common even before the era of AIDS, have seen an explosion in the incidence of the disease. Cancer registry data shows that there has also been an increase in the reported cases of childhood Kaposi sarcoma in sub-Saharan Africa. Before the AIDS epidemic, the reported Kaposi sarcoma incidence in children between 1960 and 1971 was 2.5 per million children per year in Uganda, and increased to 55.8 per million children per year during 1991 to 1997 (Wabinga et al.,1993). In Africa, there is a bimodal peak in incidence of Kaposi sarcoma, with an early peak in childhood (4 to 10 years) and a second peak in young adulthood (30 to 40 years), which continues to shift to an earlier age of onset in the backdrop of the AIDS epidemic (Orem et al.,2004). In a Ugandan case–control study, HIV infection was associated with an odds ratio of 95 (95% CI, 28.5 to 315.3, based on 36 cases) for Kaposi sarcoma in children (Newton et al.,2001). Kaposi sarcoma is further discussed in Chapter 5.

1.7.1.3. Leiomyosarcoma

Leiomyosarcoma is a very rare tumour, occurring in less than 2 cases per 10 million HIV negative children per year (Lack,1986). A much higher than expected frequency of leiomyosarcoma has been reported in HIV infected children in developed countries. Visceral sites are commonly involved including the lungs, spleen, and gastrointestinal tract (McClain et al.,1995; Granovsky et al.,1998; Biggar et al.,2000; Pollock et al.,2003). Although leiomyosarcoma incidence has been found to be increased in children in developed countries, studies in African children have not examined this association. This is probably because few or no cases are identified.

Leiomyosarcoma has been included in the revised CDC classification of paediatric HIV disease as an AIDS-defining cancer (Centers for Disease Control and Prevention.,1994). Molecular techniques have revealed EBV DNA in leiomyosarcoma tumour tissue of HIV infected children, and in the setting of HIV-induced immunodeficiency, EBV may contribute to the pathogenesis of smooth-muscle tumours in children (McClain et al.,1995; Granovsky et al.,1998).

1.7.1.4. Hodgkin lymphoma

Although not considered an AIDS-defining malignancy, HIV related Hodgkin lymphoma is similar to ARL in that there is an increase in occurrence and a clear relationship between the incidence of disease and progressive immunodeficiency (Cheung et al.,2005). The histologic subtypes common among HIV infected people include mixed cellularity and lymphocyte-depleted variants. Male predominance, a higher prevalence of B symptoms, and more extra nodal disease on presentation are the main characteristics of Hodgkin lymphoma in HIV patients (Mitsuyasu et al.,2004). Virtually all HIV related Hodgkin lymphomas are EBV positive. Most studies demonstrating increased incidence of Hodgkin lymphoma in HIV infected individuals were conducted in developed countries, few data are available from African countries (Goedert et al.,1998; Rabkin,1998; Franceschi et al.,1999; International Collaboration on HIV and Cancer,2000; Grulich et al., 2002).

In adult studies conducted in Africa, no excess of Hodgkin lymphoma was identified among HIV infected patients, although the number of cases studied was small (Newton et al.,1995; Sitas F et al.,2000; Newton et al.,2001; Mbulaiteye et al.,2006). The occurrence of childhood Hodgkin disease in Africa has not been addressed,

probably because of the rarity of the disease which might be as a result of under-diagnosis.

1.7.1.5. Other infection-cancer associations

It has also been postulated that childhood acute lymphoblastic leukaemia (and possibly non-Burkitt lymphoma) can arise as an abnormal response to a common infection. This may occur as a consequence of population mobility and mixing (Kinlen,1993, 1995) or subsequent to delayed infection following a failure of the immune system exposure and modulation mechanism in infancy (Greaves,1997). The latter "hygiene hypothesis" evokes a mechanism involving deficient immunological exposure of infants to specific or multiple common childhood infections leading to an abnormal response to delayed infection, resulting in the development of malignancy.

However, no deficit of infections was found in children later diagnosed with ALL in a recent study (Roman et al.,2007). Children diagnosed with ALL had significantly more clinically diagnosed infectious episodes in infancy than did controls. Cases who had more than one neonatal infectious episode tended to be diagnosed with ALL at a comparatively young age; the mean age at ALL diagnosis was 37.7 months for cases with two or more episodes versus 45.3 months for cases with only one episode or none ($P<0.01$). The authors suggested that dysregulated immune response to infection in the first few months of life promotes transition to overt ALL later in childhood. Although there is a large body of epidemiological data accruing on this subject, no reports have identified any specific infectious agent, common or rare, as a cause or co-determinant of childhood ALL.

1.8. Paediatric HIV

Children may acquire HIV in utero, during labour, at delivery or, after birth through breastfeeding. The vast majority of children, more than 90% acquire the infection from their mother (Peckham et al.,1995; Miotti et al.,1999). In 2001, more than 2.6 million pregnant women had HIV infection and more than half a million transmitted the virus to their infants (UNAIDS,2002). Without anti-retroviral treatment during pregnancy, the rates of mother to child transmission range from 15 to 25% in developed countries and 25 to 48% in developing countries. Breastfeeding is estimated to increase the risk of transmission by 10 to 15% (Dabis et al.,1993; Dabis et al.,2002). HIV infection may be transmitted through blood transfusions and the use of contaminated needles and syringes. Strategies such as the screening of all donated blood and avoidance of the inappropriate use of blood and/or its products have been enforced worldwide to combat transmission via this route (UNAIDS,2002).

UNICEF ranked the effect of HIV/AIDS as one of the top five concerns for children in 2004 (UNICEF,2003). It is estimated that 14 million children have been orphaned by AIDS, and 11 million of those are in sub-Saharan Africa. By 2010, the number of orphaned children is expected to be close to 20 million (UNAIDS et al.,2003). Analyses of mortality causes have shown that HIV/AIDS is an increasingly important cause of under-5 mortality in Africa (Walker et al.,2002). Pooled analyses of HIV child mortality data in Africa showed that by 1 year, a third of infected children are estimated to have died, and more than half of children infected at birth will have died by the age of 2 years (Newell et al.). HIV infection in children represents a chronic condition with a wide spectrum of clinical disease, varying from

no symptoms to AIDS. Children with HIV infection suffer from the same common childhood illnesses as those who are not infected. In advanced HIV disease, opportunistic infections may occur, and the disease progresses rapidly without access to anti-retroviral treatment (Connolly et al.,1998; Chakraborty,2004). Some children with HIV infection have an adult pattern of disease with HIV-related symptoms appearing 10 or more years after initial infection. Accelerated HIV disease progression in African children may occur because of factors, associated with poverty, including limited access to proper nutrition, clean water and health care, as well as exposure to a high prevalence of infectious agents in the community (Chakraborty,2004). Child mortality estimates from community-based cohorts demonstrate that the children of HIV-infected mothers have higher mortality rates than the children of uninfected mothers, and that child mortality is closely linked to maternal health status (Newell et al.,2004).

1.9. Study objectives

The main objective of this study is to identify cancer types among children in Malawi (a sub-Saharan African country) that are associated with HIV and other infections. Factors associated with poverty and infectious disease exposures are also explored. The specific objectives are:

1. To identify infectious agents associated with childhood malignancies diagnosed in Malawi. In particular, to identify cancers that are associated with HIV infection as a means of identifying cancers that may have another underlying infectious cause.

2. To specifically investigate childhood and maternal factors including infections, that are associated with the risk of Burkitt lymphoma, the commonest childhood cancer in Malawi and Kaposi sarcoma.

1.10. Research justification

Few studies in Africa have evaluated the relationship between childhood cancer and HIV infection. Cancer registries in sub-Saharan Africa during the HIV era have shown an increased incidence of Kaposi sarcoma in children, including but not exclusively, in Zambia, Zimbabwe, Malawi and Uganda (Wabinga et al.,1993; Chintu et al.,1995; Chitsike et al.,1998; Sinfield et al.,2007).

There is inadequate information about the role of other infectious agents in the aetiology of childhood cancer in Africa. The study of cancer in immunodeficient populations offers a unique opportunity to investigate the role of the immune system in controlling the development, growth, and dissemination of tumours. Furthermore, with current technology, an infectious cause of cancer might be more easily identified in the immunodeficient than in the immunocompetent because for certain viruses such as HHV-8, viral genomes and gene products are more easily detected in an individual, as immune function declines (Chang et al.,1994; Whitby et al.,1995; Newton et al.,1999). Information gained from the study of HIV associated cancers is likely to be relevant to the HIV uninfected population as well.

1.11. Overview of thesis structure

The study described in this thesis was conducted in Blantyre, Malawi. Chapter 2 provides a description of the study setting, methodology, data management and analyses. The various laboratory tests conducted are also described.

A literature review on Burkitt lymphoma is presented in Chapter 3. This was not described in this chapter because Burkitt lymphoma was the main focus of this study, and therefore required an extensive literature review which was best discussed in a separate chapter. A description of Burkitt lymphoma in African children, aetiology and associated risk factors are presented. Chapter 4 describes the analyses, results and discussion of the Burkitt lymphoma case-control study. Chapter 5 describes the analyses, results and discussion of the Kaposi sarcoma case-control study.

Chapter 6 describes the prevalence of infections in different cancer types including: Wilms tumour, retinoblastoma, rhabdomyosarcoma and other cancers. These 3 cancers were described because together with Burkitt lymphoma, and Kaposi sarcoma, they were the most common child cancers recruited during the study period. Results and discussion of the descriptive analyses of Wilms tumour, retinoblastoma and rhabdomyosarcoma are presented. Detailed descriptions of the associations found between HIV and childhood cancer types in this study are presented in Chapters 4, 5 and 6. Prevalence of infections among different cancer types are not presented before the case-control analyses results despite being the primary objective. This is largely because the case-control analyses of Burkitt lymphoma and Kaposi sarcoma clearly demonstrate the role that infections can

potentially play in cancer development, making it easier to understand infections in other cancer types. Another factor that necessitated this arrangement is that there were very few cases of other cancer types, resulting in any comprehensive analyses lacking adequate power.

Chapter 7 provides a summary of the prevalence of infections across different cancer types, and the case-control analyses of Burkitt lymphoma and Kaposi sarcoma. A concise summary of all the major findings of the study is presented. The thesis conclusions and proposals for future work are offered here.

CHAPTER TWO

STUDY SETTING, DESIGN AND METHODOLOGY

2.1. Introduction

This chapter provides information regarding the setting, design and methodology for this study. The field work was carried out in Malawi and therefore starts with background about the study setting; then moves on to describe the epidemiology of childhood cancer, HIV and malaria in Malawi. The laboratory techniques employed, data management and analyses are presented. A description of the presentation of results is also provided.

2.2. Malawi background

Malawi is a landlocked country located in Southern Africa, surrounded by Zambia, Mozambique and Tanzania, see map in Appendix 2.1 (United.Nations.,2004). Lake Malawi runs down Malawi's eastern border with Mozambique. The Rift Valley runs the entire length of the country, passing through Lake Malawi in the Northern and Central regions, to the Shire Valley in the south. Malawi has a sub-tropical climate, with two distinct seasons, the rainy season from November to May, and the dry season from May to November. The country is divided into three regions that are further divided into 27 districts. The study was conducted in Blantyre, which is the commercial capital and located in the Southern region. Malawi currently ranks amongst the world's least developed countries and approximately 85% of the population live in rural areas (National.Statistical.Office.,2006). The economy is predominately agricultural, with tobacco, tea and sugar being the main exports.

2.3. General: About Malawi

2.3.1. Demographic characteristics

The estimated population in 2005 (based on the 1998 census data), taking into account the effects of increased mortality due to AIDS, lower life expectancy, higher infant mortality and death rates was 12.3 million (National.Statistical.Office.,2000). The life expectancy at birth for males and females is 40 and 44 years respectively. The population is predominantly composed of young people, the overall median age being 16.4 years. Children less than 15 years old constitute 47% of the population. The population of children less than 5 years in 2004 was estimated to be 2.2 million. The birth rate in 2004 was 44 births per 1,000 population. The child (under 5 years) mortality rate was 133 deaths per 1,000 live births. These statistics suggest that about 1 in every 8 children born in Malawi dies before their fifth birthday (National.Statistical.Office.,2004, 2005).

The Malawi welfare report of 2005 reports that the national employment rate was 78%, and 72% of those were subsistence farmers who did not receive a formal income (National.Statistical.Office.,2005). Over 55% of households in Malawi either owned an axe, sickle or radio. About 50% of the population of Malawi were below the poverty line.

2.3.2. HIV/AIDS

Sub-Saharan Africa remains by far the region worst affected by the HIV/AIDS epidemic (UNAIDS et al.,2006). UNAIDS reports that in 2006, there were an estimated 940,000 people living with HIV/AIDS in Malawi, and that 91,000 of these

were children (UNAIDS et al.,2006). The overall countrywide adult prevalence rate is approximately 14% (7 to 21%), but higher in urban areas like Blantyre (UNAIDS et al.,2004; UNAIDS et al.,2006). The occurrence of HIV infection in children less than 15 years old admitted to hospital can be as high as 20% (Rogerson et al.,2004).

Sentinel surveys revealed that from 1985 to 1993, the HIV seroprevalence among women attending antenatal clinics in major urban areas such as Blantyre increased from 2 to 30% (Taha TE et al.,1998; UNAIDS et al.,2004). A peak prevalence of 32% was seen among urban women aged 25 to 29 years in 1996 (Taha TE et al.,1998; National.AIDS.Control.Programme.,2001). In 1998, 26% of antenatal clinic attendees tested positive for HIV (UNAIDS et al.,2004). By 2001, HIV prevalence in this age group of women had fallen to 17%. In 2003, 18% of women aged 15 to 24 attending antenatal clinics tested HIV positive (UNAIDS et al.,2004). With reported transmission rates from mother to child ranging from 25 to 48% in developing countries, this route is an important way in which the virus is spread (Dabis et al.,1993; Dabis et al.,2002).

A Malawi demographic health survey reports that urban residents have a significantly higher risk of HIV infection than rural residents; while 18% of urban women were HIV positive, the corresponding proportion for rural women was 13%. However, since 85% of Malawi's population live in rural areas, the greatest burden of HIV infection is in the rural population (National.Statistical.Office.,2004). Women and girls make up almost 57% of adults living with HIV in sub-Saharan Africa (UNAIDS et al.,2006).

2.3.3. Malaria

Malaria is the leading cause of morbidity and mortality, especially among children under the age of five years and pregnant women (National.Statistical.Office.,2005). Malaria accounts for 40% of all outpatient visits to health facilities. *Plasmodium falciparum* is the most common malaria parasite harboured. Malawi has geographical variations in malaria transmission. Along the lakeshore, and the Shire River valley, transmission is perennial with a peak in the January to April rainy season, while on the plateau, about 1000 meters above sea level, transmission is seasonal from December to April/May. With regard to prevalence of reported clinical malaria (diagnosis not verified by microscopy), 27% of the total population received treatment for malaria in 2002. This figure varies between districts ranging from 14% (196,477/1,423.274) in Lilongwe to 33% (274,271/828,767) in Blantyre (Ministry of Health and Population. et al.,2003).

2.3.4. Malaria preventive measures

Malaria causes huge economic losses, which contribute to keeping the country as one of the world's poorest. A 2004 health survey of 13,664 households showed that 42% of households in Malawi have at least 1 mosquito net, less than 18% have more than 1 net, and the average number of mosquito nets per household is 0.7 (National.Statistical.Office.,2004). Economically 'better off' households were found to be more likely than poorer households to own a mosquito net. Older children were less likely than younger children to sleep under a bed net, and children living in urban areas were much more likely to use a mosquito net than children living in rural areas.

2.3.5. Queen Elizabeth Central Hospital

Queen Elizabeth Central Hospital (QECH) is the major referral centre in the southern region, and is located in Blantyre which is the largest city and a major commercial centre. The hospital catchment population was approximately 3,000,000 in 2003 (Planning Department. et al.,2004). The QECH is a government teaching hospital with 1,100 beds, of which 200 are for children. The paediatric department houses a separate 16 bed paediatric oncology ward to which most children with suspected cancer are admitted. Children with bone malignancies are admitted to the paediatric surgical ward. Children with eye malignancies are treated in a separate adult and child eye unit. Children are referred to the QECH mainly from the southern part of the country and often travel great distances to reach Blantyre.

2.4. Childhood cancer in Malawi

Table 2.1 shows the numbers and percentages of childhood cancers (from 0 to 15 years) diagnosed in Malawi for the period 1967 to 1995. These data are derived from reports that detail the profile of childhood cancer cases diagnosed in the national pathology series at Queen Elizabeth Central Hospital. Lymphomas were the most common cancer types and Burkitt lymphoma the most common subtype. The number of Kaposi sarcoma cases suggests that there has been an increase in occurrence of the tumour over the years. Brain tumours and leukaemias represented very few cases, and in some instances, none at all.

Cancer registry data shows that the four most frequently occurring childhood cancers in Malawi are currently Burkitt lymphoma, Kaposi sarcoma, retinoblastoma and Wilms tumour (Dzamalala et al.,2005). By far the most common cancer is Burkitt

lymphoma, representing about a third of all malignancies diagnosed in children. In a 1991 to 2001 review of cancer in children (age range 0 to 15 years) in Blantyre, the age standardised rate (ASR) for Burkitt lymphoma was 25.9 per million (IARC,2003). That Kaposi sarcoma is the second most frequent paediatric cancer (ASR=11.8 per million) is thought to be a consequence of the increasing number of HIV infected children, as has been seen in other African countries (Chintu et al.,1995; Chokunonga et al.,2000; Banda et al.,2001; Sinfield et al.,2007).

Table 2.1: Childhood (0 to 15 years) cancer case series from Blantyre, Malawi for the period 1967 to 1995. Modified from (IARC,2003).

Cancer type	1967-76 (Molyneux,1979)		1985-93 (Mukiibi et al.,1995)		1991-95 (Banda et al.,1999)	
	No	Percent	No	Percent	No	Percent
Leukaemia	0	0	18	2.3	6	1.1
[a]ALL	0	0	10	1.3	1	0.2
Lymphoma	187	45.9	472	59.7	283	51.7
Burkitt lymphoma	**121**	**29.7**	**368**	**46.5**	**199**	**36.4**
Hodgkin lymphoma	11	2.7	38	4.8	22	4.0
Other lymphomas	55	13.5	66	8.3	62	11.3
Brain and spinal neoplasms	0	0	6	0.8	1	0.2
Neuroblastoma	13	3.2	0	0	0	0
Retinoblastoma	56	13.8	89	11.3	46	8.4
Wilms tumour	27	6.6	50	6.3	27	4.9
Bone tumours	14	3.4	16	2.0	16	2.9
Soft tissue sarcomas	48	11.8	66	8.3	120	21.9
Kaposi sarcoma	**18**	**4.4**	**32**	**4.0**	**88**	**16.1**
Other soft tissue tumours	30	7.4	34	4.3	32	5.9
Other	62	15.2	74	9.4	48	8.8
Total	407	100	791	100	547	100

[a] Acute lymphoblastic leukaemia

A recent descriptive review of cancer in children admitted to QECH over a 6 year period (between 1998 to 2003) revealed a successive increase in the overall number of childhood cancers, from 87 in 1998 to 170 in 2003 (Sinfield et al.,2007). The perceived increase in cancer was attributed to more referrals secondary to improved awareness among parents and clinicians. The authors thought there may be a real increase in all cancer types, although the basis for this is not clear. Brain tumours, leukaemias and leiomyosarcomas were not frequently reported. This was thought to be because brain tumours are difficult to diagnose on purely clinical grounds and may be missed by primary clinicians, or not referred from distant medical centres knowing that often very little treatment can be provided. Leukaemias were also thought to be under diagnosed in this series. The effect of HIV on cancers other than Kaposi sarcoma was uncertain because not all children with cancer had an HIV test done. Histological verification of cancer diagnosis was available for 49% (348/707) of cancers, although this figure varied by cancer site being lowest for retinoblastoma (7%) and highest for hepatic tumours (100%).

2.5. Child cancer classification

The International Classification of Diseases for Oncology (ICD-O) third edition is used to group cancers in adults (Fritz et al.,2000). ICD-O is a dual classification with coding systems for both topography and morphology of tumours. The topography code describes the site of origin of the neoplasms. The morphology code describes the cell type of the tumour and its biologic activity. Cancers in children are highly specific and differ in many ways from cancers found in adults. They are histologically very diverse and some histological types of childhood tumour such as

rhabdomyosarcomas occur in many different sites. As classification of child cancers by site would not be ideal, a special classification was developed.

The Birch and Marsden classification was the first internationally accepted classification of childhood cancers (Birch et al.,1987). This classification was based on the first edition of the ICD-O and diagnostic groups were defined in terms of morphology and not site of presentation. The publication of the second edition of the ICD-O and the 10th revision of the International Classification of Diseases necessitated an update of the childhood classification to allow for the new and expanded coding of cancer which had been introduced (Kramárová et al.,1996). The resulting International Classification of Childhood Cancer became the standard for presentation of international data on childhood cancer incidence and survival. The International Classification of Childhood Cancer (ICCC-2) of 1996 was intended to be a continuation of the 1987 childhood classification.

Improved diagnostic methods prompted a third edition of the ICD-O (ICD-O-3) which introduced numerous new morphology codes particularly for leukaemias and lymphomas (Fritz et al.,2000). To accommodate this new knowledge, a third edition of International Classification of Childhood Cancer (ICCC-3) was also introduced (Steliarova-Foucher et al.,2005). The ICCC-3 classifies tumours coded according to the ICD-O-3 into 12 main groups, which are split further into 47 subgroups. Level 1 includes the 12 main diagnostic groups; level 2 includes 47 diagnostic subgroups. Level 3 is an optional "extended classification" and comprises 2 to 11 divisions of selected diagnostic subgroups (The classification tables are shown in Appendix 2.2). This division of some diagnostic subgroups reflects the availability of detailed

information that permits homogeneous groups of tumours to be distinguished within them, allowing their separate study. These divisions are optional and distinct from the 12 main diagnostic groups because their use may not always be possible due to inadequate diagnostic methods.

2.6. Malawi cancer registration

The Malawi National Cancer Registry was established in 1989 and is located at the QECH complex. It was initially a histopathology-based registry, recording data on cancer cases diagnosed in the pathology laboratory at the QECH, which received specimens from hospitals throughout the country. In 1993, a population-based component was added to the registry activities, and now covers urban and rural Blantyre (with a population of about 1 million), including 10 other hospitals within and outside Blantyre. The registry staff aim to identify all cancer cases in children and adults from the urban and rural Blantyre population. The registry is computerized and employs a combination of active and passive methods of case finding. The registry receives copies of reports on all cancer cases diagnosed in the central pathology laboratory at QECH. Cancer information from all hospitals and major clinics is recorded in the form of cytology, histology and haematology reports obtained from the histopathology and haematology departments of the College of Medicine. The registry staff regularly visit inpatient wards and oncology outpatient clinics of the hospital, and other hospitals in the district.

2.7. Study design

The objectives and outline of the research reported in this thesis are explained in Chapter 1. In short, the primary aim was to estimate the prevalence of HIV and other

infections among children with cancer using a cross-sectional study design. The secondary aim was to investigate other childhood factors in addition to infections and maternal factors (including sexual behaviour) associated with the risk of childhood cancers, using a case-control study design. For this secondary aim, the primary focus was on Burkitt lymphoma and Kaposi sarcoma.

2.7.1. Ethics

The protocol for this study was written by the author, and submitted to the Oxford Tropical Research Ethics Committee which granted approval in August 2004. The College of Medicine Research and Ethics Committee in Malawi reviewed the protocol and granted approval in March 2005.

2.7.2. Justification of case identification

All cancer patients aged 15 years and younger admitted to QECH between July 2005 and August 2006 were considered for inclusion. The study population base was defined as all potential 'hospital users'. It was assumed that all patients will, in general, have gone through similar selective procedures with equal chances of referral to the hospital. Children referred to the hospital were thought to be representative of all the children with cancer in the overall catchment area of the hospital.

2.8. Research methods

2.8.1. Prevalence study

Prevalence studies are sample surveys where the primary aim is estimation of the percentage of the study group exposed to one or more risk factors. Clearly of major

importance in this type of study is obtaining a sample which is representative of the population of interest. It was therefore important that all cancer types were eligible for recruitment. All children admitted to the QECH with cancer or suspected of having cancer were eligible for recruitment.

2.8.2. Case-control study of Burkitt lymphoma

Burkitt lymphoma patients were classified as cases, and children with other cancers and non-malignant conditions as controls.

2.8.3. Case-control study of Kaposi sarcoma

Kaposi sarcoma patients were classified as cases, and children with other cancers and non-malignant conditions as controls.

2.8.4. Use of 'other cancers' as controls.

The crucial aspect about controls in any well designed case-control study is that they should arise from the same population from which the cases came. The intention in hospital-based studies is to select cases and controls from hospital admissions that are likely to reflect the exposure rates to the study factor in the population from which they arose (Stolley et al.,1982). In hospital-based studies, 'other cancers' can be used to act as controls for a specific cancer type.

The use of "other cancers" as controls is not a commonly used study design, but has distinct advantages in this setting. Principally, biases in the form of selection bias, recall bias and interviewer bias are reduced (Smith et al.,1988; Infante-Rivard,2003). Another advantage of using the hospital as a recruitment base is that the chance of overmatching for factors of interest, such as environmental exposures when

'neighbourhood' controls are used, is avoided. In general, hospital controls are likely to have a comparable recall of their exposure to risk factors as cases, because they are facing a similar problem, and are more likely to remember exposures better than non-hospital controls.

In a hospital-based cancer case-control study, an alternative method would be to use non-cancer patients from surgical wards to act as controls. Cancer patients are often referred from diverse regions of the country, but surgical controls often live relatively close to the hospital. The problem this creates is that cases and controls would have come from different background populations and as a result may have had a range of different exposures. Surgical cases for this study would not have made good controls as they are thought to arise from a different population (Prof. E Molyneux Blantyre Malawi, personal communication). The use of 'other cancers' as controls has been used successfully in studies of cancer, both in Western and African populations (Smith et al.,1988; Newton et al.,1995; Sitas F et al.,2000; Newton et al.,2001).

2.8.5. Recruitment

Children and their mothers were recruited when the child was admitted to hospital suspected of having cancer. Between July 2005 and August 2006, 310 children presented on the paediatric oncology ward with a provisional diagnosis of cancer. Five of these children were excluded from the study because they were aged over 15 years. Recruitment was restricted to children 15 years and younger because in Blantyre, older children are not treated by paediatricians, but by adult physicians and are not classified as children. In general, older children were not recruited because

recruitment was based in the paediatric oncology ward, and it was difficult logistically to recruit older children with cancer being treated in other wards. Five children older than 15 years were recruited because they were treated in the paediatric oncology ward, but they were excluded from the analyses. The classification of childhood as 0 to 14 or 15 years has been used in other classifications and reviews (IARC,1998; Little,1999; IARC,2003; Steliarova-Foucher et al.,2006). Children were excluded from the study if consent was not provided by the parents or guardians (consent form shown in Appendix 2.3b).

New cases that appeared after the study started, and cases diagnosed with cancer prior to the study onset were recruited and included in the analyses. Approximately 87% (n=257) of all cancers recruited were incident cases, whose tumour diagnosis was established during the study period (2005 and 2006). About ten percent (n=28) of cancers were diagnosed 1 year before the study started, and 3% (n=10) of cancers diagnosed between 2001 and 2003. The disadvantage of using both incident and prevalent cases is that prevalent cases will include patients who may have had the disease for some time, and may have changed their habits (or "exposures") because of the disease compared to incident (new) cases. The old cases were included in the analyses as they constituted a small proportion of the total sample.

2.8.6. Cancer diagnosis

When possible, tumour tissue samples were collected using fine needle aspiration (FNA) by the oncology ward staff, and cancer diagnoses made at the College of Medicine in Blantyre by a trained pathologist using cytology examination. For 162 cancers FNA and cytological examination of the tumour was done to establish a

diagnosis. For some malignancies including Burkitt lymphoma and other haematological malignancies, a lumber puncture and bone marrow aspirate was done for staging purposes. When a FNA was not possible because the tumour was deep seated, or when the cytology report was not helpful (e.g. reactive cells, insufficient sample) a section of tumour tissue was obtained using a trucut needle and histologically examined for 42 cancers. Radiological investigations included an abdominal ultrasound, chest radiograph and sometimes computed tomography. For 90 cancers, diagnosis was made on clinical grounds only, without the benefit of histology. Table 2.2 shows the mode of diagnosis for all children recruited.

Table 2.2: Mode of diagnosis for all children

Basis of diagnosis	**Number**	**Percent**
Clinical	90	30
Cytology	162	53
Histology	42	13
Missing	11	4
Total	305	100

Cancer diagnoses were classified using the ICCC-3 (see Appendix 2.2). It was not possible to use the subgroups of the 12 main cancer groups in some instances, as adequate diagnostic detail was lacking from the pathology reports, largely because of inadequate diagnostic methods. Appendices 2.4a to 2.4c show all the cancer types and non-malignant conditions recruited and their mode of diagnosis. For this report, cytological and histological methods of diagnosis were grouped and referred to as histological or morphological verification of diagnosis.

2.8.7. Data collection

Details about cancer diagnosis were abstracted using standardised forms from clinical records and the histopathology report if available (forms shown in Appendix 2.5). A standard information sheet, available in English and the local language (Chichewa) was provided; basic translation methods were used (see Appendix 2.3). Informed consent was mandatory before the interview was conducted. Five local nurses were recruited and trained to administer questionnaires to the mother or guardian of the child (shown in Appendices 2.6 and 2.7). The questionnaires were based on questionnaires used for a similar study in Uganda (Carpenter et al.,2007). The questionnaires were modified for the Malawian population, such as proxies of socioeconomic status. They were piloted in Malawi for a week before the recruitment started, and minimal changes made to optimise interview data collection. Most interviews were conducted in the local language. For each child a minimum amount of socio-demographic data was collected from the parent or guardian. Information about the child's vaccination status was abstracted from the health passport (see Appendix 2.5). Height and weight for both mother and child were measured and recorded during the interview. Information about demographic and socio-economic factors, experience at (or around) the child's birth, household characteristics and sexual and reproductive histories were obtained from 212 mothers using a standardised questionnaire. All questionnaires were checked for completeness by the author.

As part of normal clinical work-up, all children seen for the first time in the paediatric oncology ward with suspected cancer were tested for HIV infection with appropriately trained staff providing pre and post-test counselling. Two millilitres of

whole blood stored in a 5ml ethylenediaminetetraacetic acid (EDTA) tube (BD Vacutainer®) were collected opportunistically from children at a time when blood was being drawn for other purposes as part of routine care.

Two samples of maternal blood were collected from 204 mothers, 5mls into a serum separator tube (BD Vacutainer®), and 5mls into an EDTA tube (BD Vacutainer®). Consent was sought for a maternal HIV test and the necessary pre and post-test counselling provided. This result was made known to the mother if she wished by a trained counsellor. If HIV positive, she was referred to the appropriate clinic in the hospital. The guardian was also informed of the child's HIV results by the paediatric oncology ward staff, as part of the child's clinical care. The mother and child blood samples were identified by a unique study identification number.

2.8.8. Study size determination

2.8.8.1. Prevalence study

The primary aim of the prevalence study was to estimate prevalence of HIV and other infections in different cancer types. When planning this study it was important to consider the effect that study size would have on the precision of these estimates. Sample sizes were calculated using the expected prevalence of HIV infection as the main estimate of interest. Other infections such as EBV and HHV-8 were not used to estimate the sample size because of the uncertainty of how large, or small the prevalence estimates were likely to be. The required sample sizes were estimated assuming precision of ± 8% of the expected HIV infection prevalence. The table below shows different sample sizes required for prevalence of HIV ranging from 10% to 80%. As can be seen, to estimate a true prevalence of HIV of 10% within ±

8% precision, the confidence interval of the estimate would be from 2 to 18%, and 54 patients would need to be recruited. Based on data from a case-control study on cancer in Uganda, the prevalence of HIV in Burkitt lymphoma cases was expected to be about 30%, thus with a precision of ± 8% the expected sample size for Burkitt lymphoma cases was 126 (Newton et al.,2001). Kaposi sarcoma was expected to have an HIV prevalence of about 80%; therefore a sample of 96 patients at 8% precision was anticipated.

Table 2.3: Required sample size based on precision

Confidence level	Precision	Expected HIV prevalence	Expected size of confidence interval	Sample size
95%	8%	80%	72% to 88%	96
		30%	22% to 38%	126
		20%	12% to 28%	96
		10%	2% to 18%	54

2.8.8.2. Case-control studies

Burkitt lymphoma patients were classified as cases, children with other cancers and non-malignant conditions were recruited as controls. As Burkitt lymphoma is the most frequent child malignancy in Malawi, obtaining a control group large enough to detect any differences in infections prevalence was a particular challenge. The main assumption for this study was that there is an association between Burkitt lymphoma and HIV, based on the study conducted in Uganda (Newton et al.,2001). The adequacy of different sample sizes was assessed by considering the precision of the odds ratio estimate from the case-control study of Burkitt lymphoma i.e. by examining the expected size of the confidence interval. The likely scenario

expected was a 2:1 case-control ratio, as there are more Burkitt lymphoma cases than controls.

In order to achieve statistically reliable results, the numbers of cases required would depend on the scale of the excess risk. In Uganda, out of 33 Burkitt lymphoma cases, 30% were HIV positive, and a statistically significant odds ratio of 7.5 was found. The non-cancer reference group had an HIV prevalence of 6%, which is comparable to the 10% that is said to exist in Malawian children (Prof Molyneux, Malawi personal communication). Table 2.4 shows that even with a case sample size of 175 detection of an odds ratio of 3 was achievable. A total number of 305 children with cancer were recruited, 148 were Burkitt lymphoma cases, 146 other cancers and 11 were non-malignant conditions.

Table 2.4: Estimates of the sample size for Burkitt lymphoma (Calculated using Epi-Info software (CDC,2006))

Case: Control ratio	Controls with HIV infection	Odds ratio	Precision of odds ratio estimate (95% CI)	BL Cases	Controls	Total
2:1	10%	3	1.4 to 6.5	175	88	263
		4	1.9 to 8.6	104	52	156
		5	2.3 to 10.7	75	38	113
		6	2.8 to 12.8	59	30	89
		7	3.3 to 14.9	49	24	73

2.8.9. Blood processing

The maternal and child blood samples were aliquoted on site at QECH using sterile pipettes into sterile cryovials. For each mother and child sample, 3 or 4 250µl aliquots were created depending on how much blood was available. An aliquot of

maternal blood was tested for syphilis and HIV on site and the remaining maternal and child aliquots were stored at -20°C on site. After data collection, all the blood sample aliquots were transported to the UK while stored in dry ice. The blood aliquotes were analysed for antibodies against malaria in Oxford, EBV and HHV-8 antibodies in London.

2.8.9.1. HIV testing

HIV testing was conducted on 291 child and 201 maternal blood samples onsite in Malawi. Blood was tested using Determine HIV (Abbott Laboratories, Illinois, USA) as a screening test and Uni-Gold™ HIV (Trinity Biotech PLC, Ireland) as a confirmatory test. Child blood samples were analysed by a technician in the department of paediatrics, who was blinded to the child's identity and diagnosis. The maternal blood samples were tested by a technologist in the main hospital laboratory who was also blinded. Uni-Gold™ was done only when the Determine test was positive. Both tests were conducted according to the manufacturer's instructions. Specimens were considered as true negative if they reacted negatively with the screening assay. Specimens reactive with the screening assay were retested using the confirmatory assay. If specimens were concordantly positive using the two assays, they were considered as true positives. Discordantly reactive sera were further tested by a third assay, SD HIV-1/2 ELISA 3.0 (Standard Diagnostics, Inc Kyonggi-do Korea), whose outcome was considered as definitive.

HIV testing in young children up to 18 months old presents unique problems because maternal IgG antibodies to HIV are passed via the placenta (in perinatally acquired infection) to infants born to HIV seropositive mothers, and the presence of the

antibodies in a young child does not definitively indicate HIV infection (Rogers et al.,1994). A positive antibody test in this age group could mean that the mother is infected and/or the infant is infected and additional testing is necessary for definitive diagnosis (Ginsburg et al.,2006). Standard antibody tests for HIV cannot distinguish between maternal and infant antibodies. Polymerase Chain Reaction (PCR) amplifies the HIV DNA in peripheral blood mononuclear cells to increase the probability of detection in children younger than 18 months.

For this study PCR testing was not conducted for children less than 18 months old (n=16) because it is not routinely available at QECH. The HIV status of 4 (3 retinoblastoma and 1 non-malignant) of the 16 children under 18 months old was not known, and the rest were HIV negative. Although antibody tests are not recommended for children less than 18 months old, the fact that none of the children below this age were HIV positive provided a confidence, albeit not verified, that the children may indeed have been HIV negative.

2.8.9.2. Syphilis testing

Syphilis tests were conducted onsite in Blantyre on 203 maternal blood samples. A technologist in the QECH main laboratory performed blinded syphilis testing. Testing was done using the Rapid Plasma Reagin (RPR) test (Abbot Determine, Alloa, Scotland) and if positive, confirmed by the *Treponema pallidum* haemaglutination assay (TPHA) (Biotech TPHA kit, Biotech Laboratories Ltd, Suffolk, UK). Both tests were conducted according to the manufacturer's instructions. Women were defined as having current or prior syphilis infection if they had positive RPR and TPHA tests. If found to have syphilis, the mother was referred

to the hospital sexual infections unit and treated in accordance with Malawi treatment guidelines.

2.8.9.3. Malaria antibodies

The author analysed 204 maternal and 284 child blood samples for antibodies against malaria at the Centre for Clinical Vaccinology and Tropical Medicine (CCTVM) at the Churchill Hospital in Oxford. The blood samples were tested using an Enzyme-linked Immuno Sorbent Assay (ELISA). The principle of the test is that anylates in plasma are measured by capturing them in a microplate coated with a specific antigen. The general details of this method are described elsewhere (Egan et al.,1995). The technique was modified to use a *Plasmodium falciparum* schizont extract (PfSE) as the malaria antigen. The individual optical density (OD) values were corrected by subtracting the mean OD of the lysate antigen-coated wells from that of the corresponding red blood cell antigen-coated wells.

Blood samples were designated positive for malaria antibodies if the OD value was equal to or greater than the mean plus 2 standard deviations of the OD values of European control samples. The child samples were considered to be highly reactive if they were equal to or greater than the median of the Burkitt lymphoma control samples. The maternal samples were considered to be highly reactive if they were equal to or greater than the median of the mothers of the Burkitt lymphoma control samples. There were no negative maternal samples.

Optical density readings were used as a surrogate measure of malaria antibody titres. For the purposes of analyses, children were categorized into two groups:

negative/low (OD < 0.2), medium/high (OD ≥ 0.2) while mothers were categorised as either: low (OD < 1) or medium/high (OD ≥ 1). The difference in category cut-off points between maternal and child samples is expected because the levels of malaria antibodies increase with age, thus the maternal cut-off points were higher (de Souza et al.,2002).

2.8.9.4. EBV and HHV-8 testing

Testing for antibodies against EBV and HHV-8 were conducted at the Wolfson Institute for Biomedical Research based at University College London. Testing for antibodies against EBV was done for 283 child and 204 maternal samples. Testing for antibodies against HHV-8 was done for 284 child and 204 maternal samples. The investigator was blinded to the identity, diagnostic group and other laboratory test results of the sample donors. Human IgG EBV antibodies were detected using indirect immunofluorescence (IFA). Details of the testing procedure for antibodies against EBV antigens are described elsewhere (Akre et al.,1999). The results are expressed as the end-point dilution (titre) that the last positive signal was still observed. Cut off for positivity was set at titre greater than or equal to 1:40.

With respect to EBV antibodies, children were categorized into one of three groups: low (antibodies levels of 1:640 or less), medium (antibodies levels of 1:1280 to 1:2560), and high (antibody levels of 1:5120 to 1:20480). Mothers were categorised as low (antibody levels of 1:640 or less), medium/high (antibody levels of 1:1280 to 1:20480).

HHV-8 antibody testing for both lytic and latent HHV-8 antigens was conducted using an in house HHV-8 MAP ORF K8.1 and 73 ELISA (developed by Dr D Lagos). The details of the assay are reported elsewhere (Nascimento et al.,2007). The cut off of the assay was calculated as the mean +5 times the standard deviation of known HHV-8 seronegative samples.

Optical density readings were used as a surrogate measure of HHV-8 antibody titres. For analyses purposes, children and mothers were categorized into one of two groups: negative (OD < 0.85) or positive (OD >1.25). Five maternal and 3 child samples tested for HHV-8 antibodies were found to be equivocal (OD>0.85 and ≤ 1.25) and were not included in the HHV-8 analyses.

2.8.10. Data management and analyses

All data collected were entered into a password-protected Microsoft Access database designed by the author for this study (Microsoft, 2003). The database was accessible only to the principal investigator. Data were double entered into separate databases and compared to identify and correct data entry errors. Data cleaning was done using range and consistency checks. The data were exported into STATA computer software (version 9.2) for analyses (StataCorp, Release 2007). The names of the participants were excluded from all analyses and were identified by a study number to ensure anonymity.

For prevalence estimates, tables were created for cancers including Burkitt lymphoma, Kaposi sarcoma, Wilms tumour, retinoblastoma and rhabdomyosarcoma. To compare the characteristics of each of the specific cancer case groups 2 x 2 and 2

x n frequency tables were created. Proportions, chi-square for heterogeneity and linear trend and two-tailed *P* values were computed where comparison of prevalence estimates were required. For categorical data where the sample size was small, when the "expected values" in any of the cells of the table was below 5, Fisher's exact test was used to calculate significance, and the two-sided *P* value reported (Kirkwood et al.,2003). The student's t test was used to compare means of normally distributed continuous variables, while the Wilcoxon rank-sum test was used to compare variables that were either not continuous or not normally distributed. The degrees of freedom are presented alongside the t statistic as a subscript for example t_{102}.

Analyses of data for Burkitt lymphoma and Kaposi sarcoma were conducted using a case-control study methodology. Odds ratios were estimated by maximum likelihood using unconditional logistic regression. All ORs relating to data on children were adjusted for child's age (under 5 years, and 5 years and over), sex and residence (rural and urban). Odds ratios relating to data on mothers were adjusted for mother's age (under 30 years, and 30 years and over), and residence (rural and urban). In order to avoid confounding by HIV, analyses that involved variables associated with child health, infections and maternal reproductive and sexual behaviour were restricted to HIV negative children and mothers. In all the final multivariate models, a *P* value less than or equal to 0.05 was considered statistically significant, and of borderline significance if between 0.06 and 0.1.

2.9. Presentation of results

Detailed results tables are shown in the appendix section. Burkitt lymphoma case-control results are presented in Appendices 4.1 to 4.9 and Kaposi sarcoma case-

control results in Appendices 5.1 to 5.6. Some of the results are presented within the text of Chapters 4 and 5. Test of significance are presented in the text and main tables.

The results of the case-control studies of Burkitt lymphoma and Kaposi sarcoma are presented in categories which are comprised of several variables. The categories include: description of the cases and controls, child general characteristics, possible proxies of infection, mother general characteristics, and mother's sexual and reproductive history. Child and maternal measures of infections report the results of laboratory tests done for specific infections. Possible proxies of infection are defined as variables that are likely to give an indication of exposure to infections and include questionnaire data about whether the child parents were alive. Appendices 4.10 to 4.17 provide a description of the case-control analyses of Burkitt lymphoma including markers of poverty in the multivariate analyses.

Chapter 6 contains descriptive analyses of Wilms tumour, retinoblastoma and rhabdomyosarcoma, and the prevalence of infections across different cancer types. The main results tables are presented in Appendices 6.1 to 6.5. These tables also give a description of the characteristics of all the cancers recruited, including the non-malignant conditions, and a column shows the characteristics of other cancers excluding Burkitt lymphoma, Kaposi sarcoma, Wilms tumour, retinoblastoma and rhabdomyosarcoma.

Appendices 7.1 to 7.7 provide a description of the adjusted seroprevalence of HIV, malaria, HHV-8 and EBV (for all children, and restricted to HIV negative only)

across the different cancer types included in the analyses. The seroprevalence rates are adjusted using child age, sex and residence. These data are referred to in the results chapters when appropriate.

2.10. Discussion

The reality of whether a child is referred to hospital is dependant on proximity of child residence to hospital, family socio-economic status, and if the family believes that treatment in hospital would benefit the child. Primary care health workers may not refer a child because the diagnosis of cancer is not made (especially for haematological malignancies like leukaemia). The health worker may also feel that treatment is not available and therefore referring the patient would be pointless. The population recruited is assumed to be representative of patients who would be referred to hospital. The above mentioned biases that could not be measured are important and need to be considered when interpreting the results. It was crucial to have unbiased recruitment of all eligible candidates.

'Other cancers' have been used as controls, the disadvantage with this is that these patients may come from the right catchment population, but may not reflect the exposure prevalence in the population from which the cases came. The primary assumption when using 'other cancers' as controls is that different cancers are caused by different exposures. A disadvantage of using 'other cancers' as controls may be that the exposure under study may actually cause cancers of several sites (Smith et al.,1988). The effect of this problem is to bias the relative risk estimate downwards. The extent of the bias is dependent on the proportion of other cancers in the control

group which can be attributed to the exposure, and on the prevalence of the exposure in the catchment population.

The ideal study design would have been to randomly select cases from a general population-based sample. This is practically expensive and time-consuming, and is often impossible in a developing country where records are not always centralised and communication is problematic. Recruiting all hospitalised cancer patients was both practical and beneficial in this setting. It was practically easier to obtain biologic material from hospital cases as they were having blood tests done as part of routine care.

Hospital based studies are advantageous from a practical perspective and when designed well, can reduce the effect of bias. The patients included in this study formed a representative sample of the population seen at QECH during a specific time period.

2.11. Summary

This chapter described the study site in Malawi and the recruitment process. Between July 2005 and August 2006, 305 children were recruited. HIV testing was done for all child and maternal blood samples. Syphilis testing was done for all maternal blood samples. All blood samples were transported to the UK and tested for malaria, EBV and HHV-8 antibodies. Microsoft Access was used to manage the database, and STATA used to analyse the data.

CHAPTER THREE

BURKITT LYMPHOMA LITERATURE REVIEW

3.1. Introduction

In this chapter some of the clinical and epidemiological features of Burkitt lymphoma are described. The focus is on endemic Burkitt lymphoma, which occurs in children in Africa and Papua New Guinea. A historical description of the pioneering work done by Dr Denis Burkitt in describing this tumour is presented. The epidemiology of Burkitt lymphoma in African children is described, together with an account of possible risk factors.

3.2. Historical background

In 1958 Dr Denis Burkitt first described the tumour that bears his name, as being a tumour affecting the jaws of African children (Burkitt,1958). Burkitt lymphoma is the most frequent childhood cancer in tropical Africa.

3.3. Definition

Burkitt lymphoma is the eponym given to a malignant tumour of the haematopoietic system. It is a monoclonal proliferation of B lymphocytes characterized by small non-cleaved cells that are uniform in appearance and produce a diffuse pattern of tissue involvement (Hanxian,2002). Scattered macrophages with phagocyte cell debris impart a so called "starry sky" appearance.

3.3.1. Classification of Burkitt lymphoma

The World Health Organisation classification reports three epidemiological and clinically distinctive subtypes of tumour i.e. endemic, non-endemic and immunodeficiency-associated (Harris et al.,1999; Bellan et al.,2003). These subtypes share many morphologic and immunophenotypic features, but are different in many other respects (Pelicci et al.,1986). All possess chromosomal rearrangements of the *c-myc* oncogene, the genetic hallmark of Burkitt lymphoma (Magrath et al.,1992).

Non-endemic Burkitt lymphoma accounts for 1 to 2% of all adult lymphomas in Western Europe and the United States (Blum. et al.,2004). It usually presents as lower abdominal masses involving the terminal ileum (Wright et al.,1997). EBV is associated with about 20% of all non-endemic cases (Magrath,1991; Shiramizu et al.,1991; Shapira et al.,1998). HIV infected people are a thousand times more likely to develop Burkitt lymphoma than the general population (Beral et al.,1991). Most AIDS related Burkitt lymphomas in adults in western countries are Epstein Barr Virus negative, but are strongly associated with EBV in Africa (Lazzi S et al.,1998). As the focus of this thesis is the endemic form of the disease, the non-endemic and immunodeficiency-associated tumour types will not be further discussed.

3.3.1.1. Endemic Burkitt lymphoma

Endemic Burkitt lymphoma, the form of tumour described by Denis Burkitt, is the most frequent childhood cancer in tropical Africa and Papua New Guinea (Burkitt,1958; Morrow et al.,1976; Burkitt,1983). The peak age of tumour occurrence is at 7 years, with

a male preponderance (Morrow et al.,1976; Olweny et al.,1977; Williams et al.,1978; Olweny et al.,1980). Jaw tumours usually involving multiple quadrants are age dependent and a characteristic feature; occurring more frequently in younger children (Bigger et al.,1979). At least 90% of endemic Burkitt lymphoma are EBV-associated (Olweny et al.,1977; Gesar et al.,1982; Magrath,1991; Shiramizu et al.,1991).

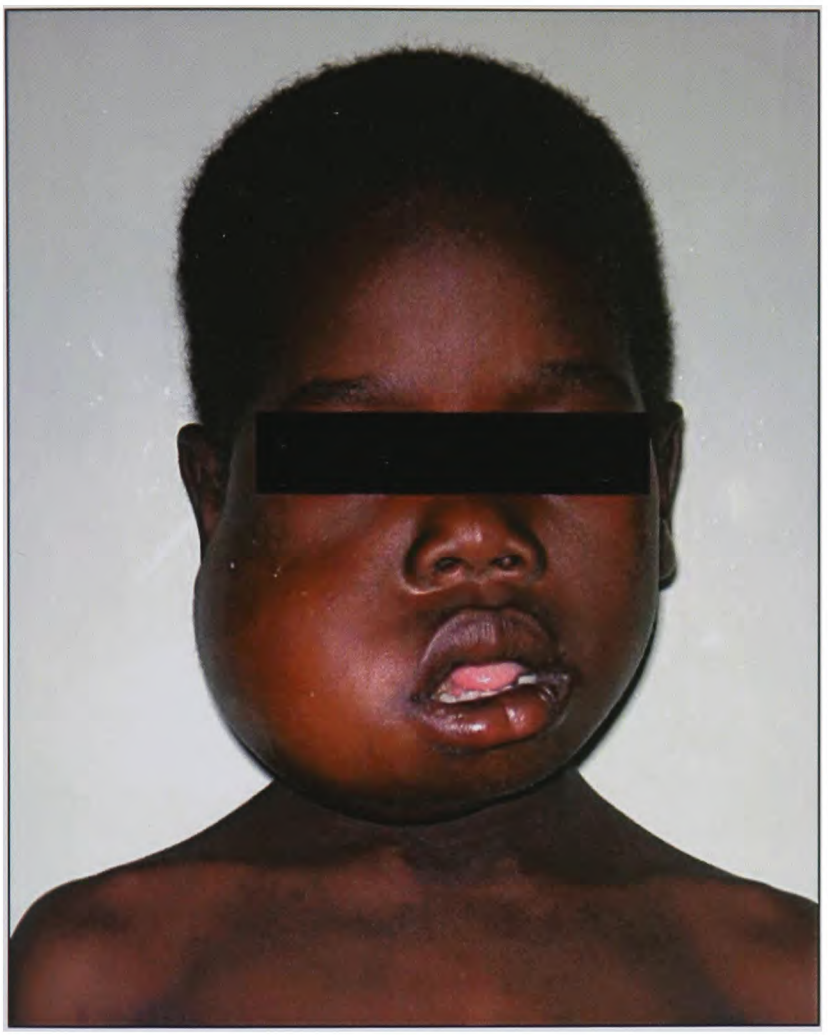
3.4. Clinical features of endemic Burkitt lymphoma

Dr Burkitt observed the characteristics of this subtype of Burkitt lymphoma in 38 patients aged from 2 to 14 years, with the majority of cases occurring between 2 and 7 years (Burkitt,1958). Endemic Burkitt lymphoma commonly starts in the maxilla or mandible and may involve other regions of the body such as the adrenals, kidneys and liver. Following further research, this became recognised as "The Lymphoma Syndrome" (Burkitt,1983).

Endemic Burkitt lymphoma in sub-Saharan Africa has protean clinical manifestations, and is almost always multifocal when the patient presents to hospital. The majority of children present with jaw and/or abdominal tumours (see Figure 3.1). For most patients with jaw tumours, the lesions are first evident in the alveolar process of the jaw, leading to loosening of the teeth, and subsequent displacement and premature shedding (Burkitt,1958; Burkitt et al.,1961). This is typically described as 'dental anarchy' and is shown in Figure 3.2 (Nkrumah et al.,1985). Jaw tumours may involve one or more bones of the jaw and may grossly enlarge and cause facial disfigurement resulting in difficulties closing the mouth, eating and breathing (see Figure 3.3). Maxillary tumours

may expand and spread to the orbit leading to progressive oedema of the eyelid and conjunctiva, protrusion of the eye and ultimately destruction of the involved eye.

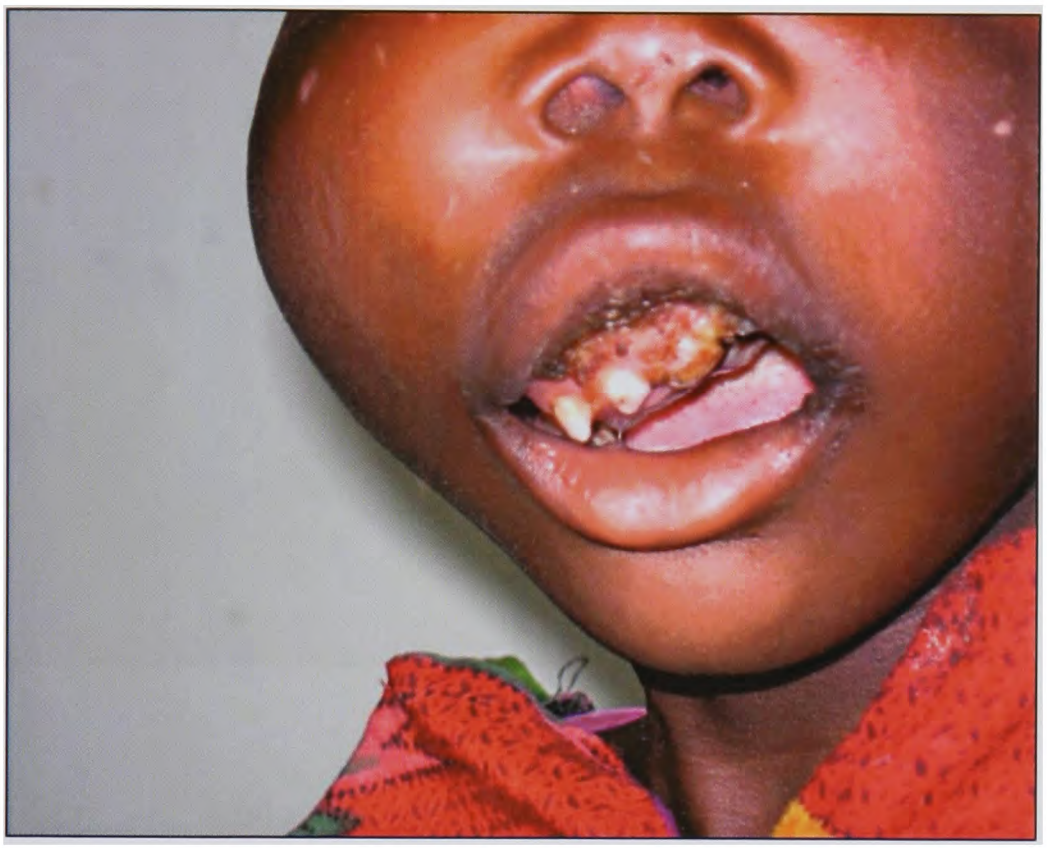
Figure 3.1: Typical Burkitt lymphoma jaw tumour. (Picture taken in Blantyre and reproduced with permission)



Abdominal tumours usually present with abdominal swelling, with or without malignant ascities (see Figure 3.4). Multiple abdominal structures may be riddled with the tumour including the kidneys, ovaries, liver and the retroperitoneal space. Large tumours can occur in the retroperitoneal space and para-aortic lymph nodes may be enlarged. Tumour that spreads to the retroperitoneal or extradural space may result in flaccid paraplegia, either by compromising blood supply to the spinal cord or by the tumour directly

compressing on the spinal cord. The patient will present with gradual weakness and eventual paralysis of the lower limbs.

Figure 3.2: 'Dental anarchy' of Burkitt lymphoma. (Picture taken in Blantyre and reproduced with permission)



The tumour may also present in the long bones (Burkitt et al.,1961). There may be diffuse deposits in the bone marrow or small osteolytic lesions which become confluent, destructive, and may ultimately lead to pathological fractures. Bone tumours may also present as changes in the shape of the bone before a pathological fracture occurs (see Figures 3.5). Frequently involved are the long bones, scapula, ribs and pelvis. There is usually no pain until the disease has progressed considerably. Burkitt lymphoma is an important differential diagnosis that needs to be excluded when a child living in an endemic region presents with an unexplained body swelling.

Figure 3.3: Gross facial disfigurement caused by Burkitt lymphoma. (Picture taken in Blantyre and reproduced with permission)

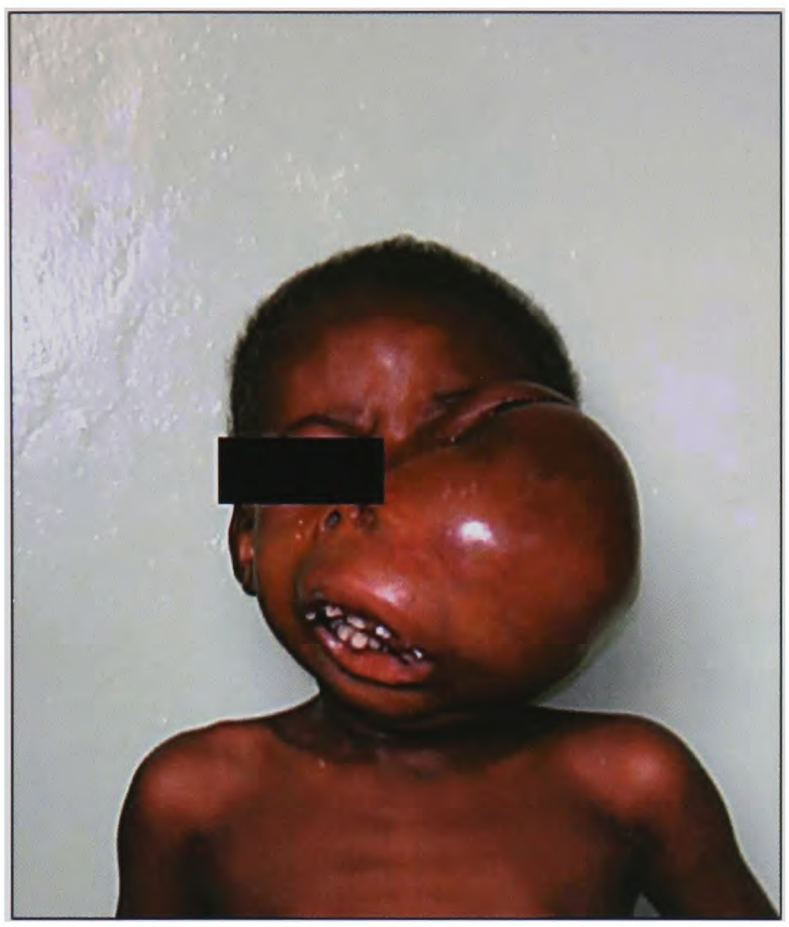


Figure 3.4: Abdominal Burkitt lymphoma. (Picture taken in Blantyre and reproduced with permission)

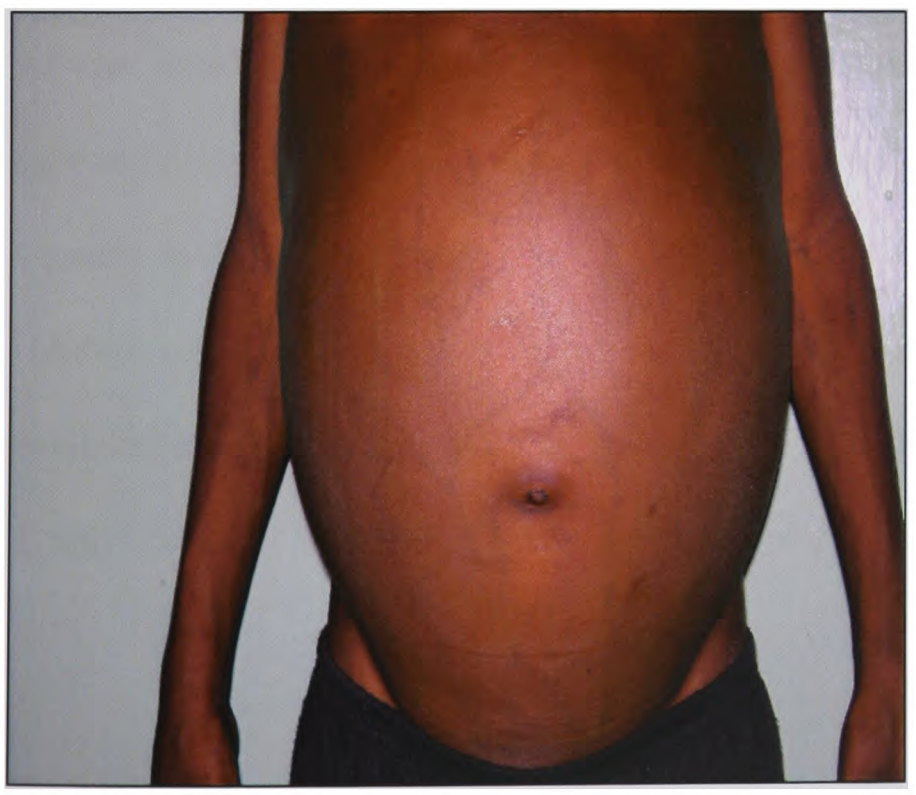
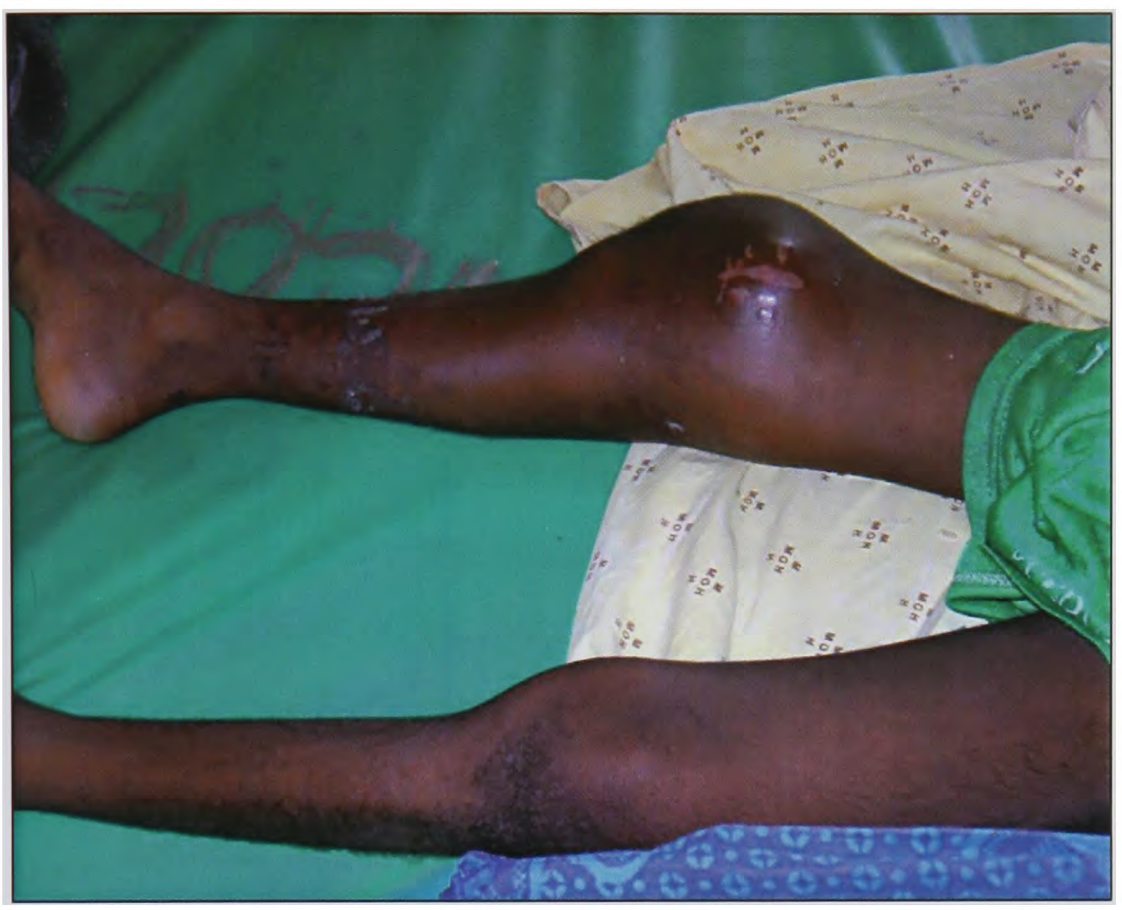


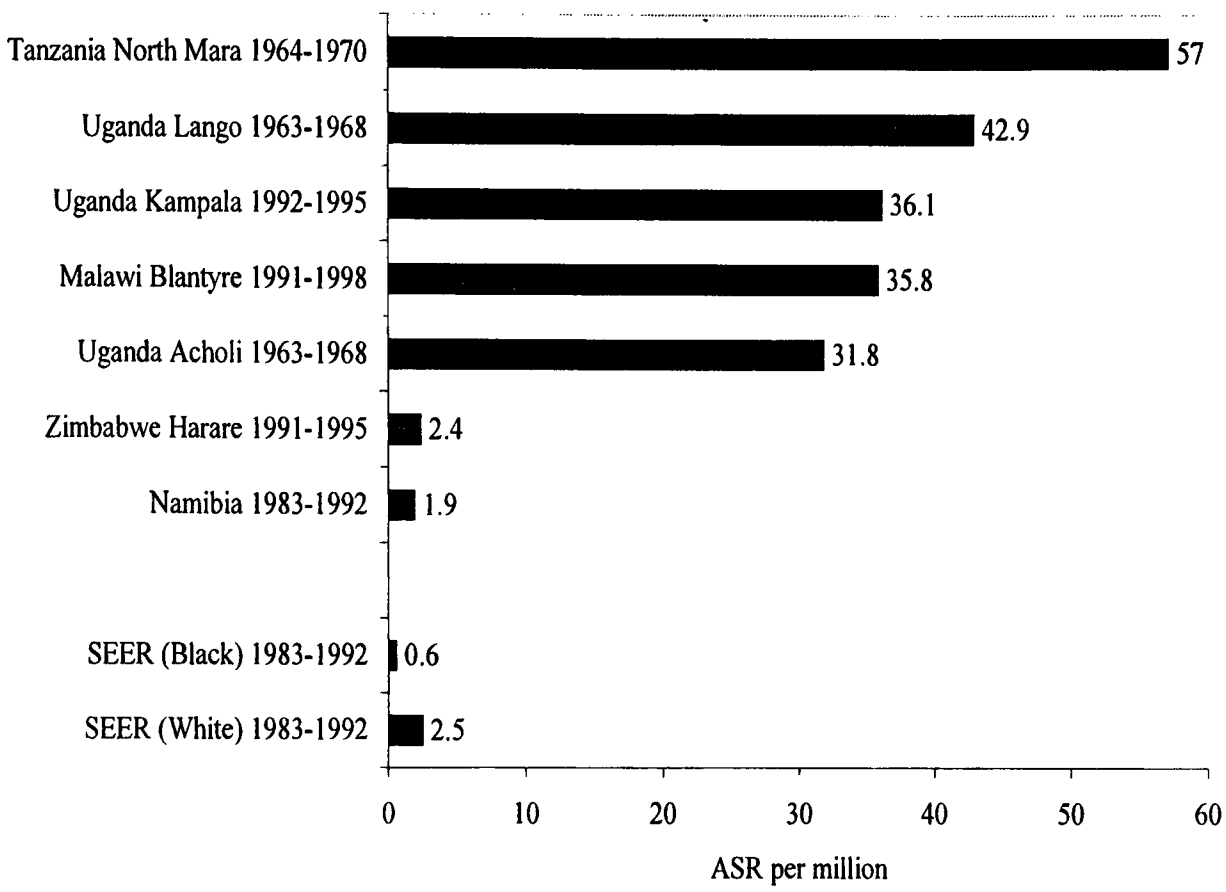
Figure 3.5: Burkitt lymphoma involving the right femur and knee joint. (Picture taken in Blantyre and reproduced with permission)



3.5. Descriptive epidemiology of endemic Burkitt lymphoma

The age standardised incidence rate (based on cancer registry data) varies throughout the African continent, 2.4 per million children per year in Harare, Zimbabwe, 35.8 per million children per year in Blantyre, Malawi and 36.1 per million children per year in Uganda (IARC,1998, 2003). Figure 3.6 shows the incidence of Burkitt lymphoma in children aged 0 to 15 years from countries in Africa where cancer registry data was available and included in the texts International Incidence of Childhood Cancer Volume 2, and Cancer in Africa: Epidemiology and Prevention (IARC,1998, 2003).

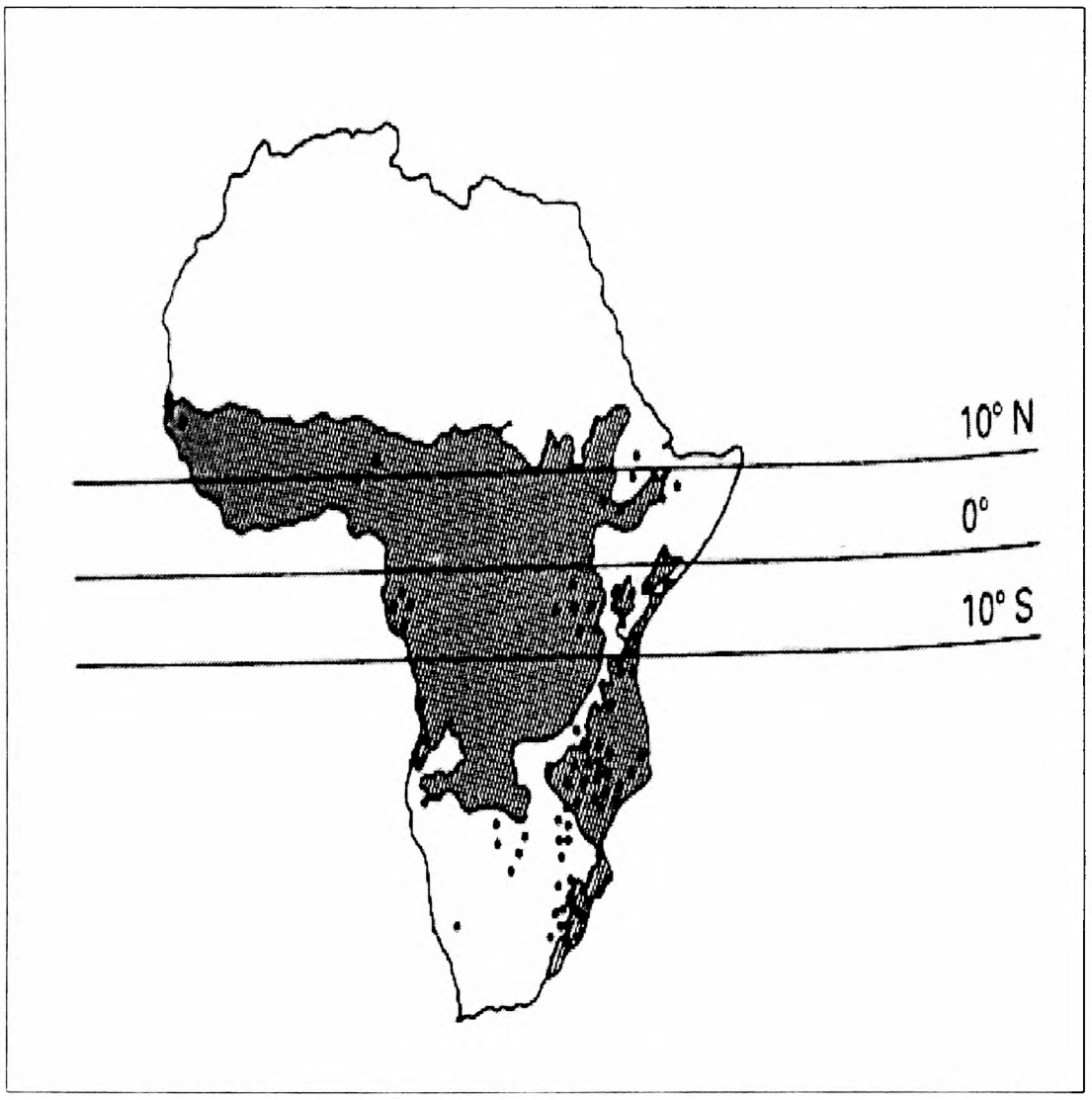
Figure 3.6: Burkitt lymphoma ASR in East and Southern African children 0 to 15 years. Data from (IARC,1998, 2003)



3.5.1. The Lymphoma Belt

Dr Burkitt conducted a postal survey to illustrate the distribution of the tumour in Africa (Burkitt,1962b; Burkitt et al.,1966; Burkitt,1983). Responses revealed that the tumour was frequently seen throughout tropical Africa. A belt lying 10^{o} North to 10^{o} South of the equator with a tail along the east coast was identified, and is now famously known as "The Lymphoma Belt" (see Figure 3.7).

Figure 3.7: 'The Lymphoma belt' in Africa showing the approximate distribution of the tumour. Black dots show cases compiled by D. Burkitt (IARC,1997).



3.5.2. The Burkitt lymphoma tumour safaris.

The edge of the lymphoma belt was defined, and significant findings relating to climatic factors uncovered (Burkitt,1962b). The tumour did not occur in regions where the mean temperature at any time during the year fell below 15°C and the annual rainfall less than 50cm. The high frequency of Burkitt lymphoma in tropical regions and its specific climatic distribution directed attention to an infectious aetiology. Extensive work was conducted in Uganda and Tanzania to explore the theory that environmental co-factors influenced tumour distribution. In Uganda, the tumour was more prevalent in the

northern, eastern and central regions, than the south and west (Burkitt et al.,1966). Patients from the lower incidence areas were older (average age 16.2 years) than those from high incidence areas (average age 8.1 years). There was almost an absence of adult disease in people indigenous to the high incidence regions; but immigrants from tumour free regions showed a susceptibility to the tumour, almost equal to that of the indigenous groups. The authors believed that the immigrants had come in contact with a novel risk factor that made them susceptible to disease. These observed differences were thought to be because of the different levels of malaria endemicity in the various regions.

3.5.3. Age and sex distribution of endemic Burkitt lymphoma.

Few cases are diagnosed before the age of 2 years and most occur between the ages of 5 and 9 years, as illustrated by numerous hospital surveys and case series. Table 3.1 below highlights studies conducted in tropical Africa demonstrating the age and sex distribution of endemic Burkitt lymphoma suggesting that boys are more likely to develop the tumour than girls.

Table 3.1: Age and sex distribution of Burkitt lymphoma in East and Central Africa.

Country, Year	Number of cases	Age range	M:F ratio
Enugu Nigeria 2002 (Oguonu et al.,2002)	44	3 to 14	2.7:1
Uganda 1977 (Olweny et al.,1977)	34	2 to 15	1.8:1
Uganda 1978 (Williams et al.,1978)	202	2 to 16+	1.7:1
Uganda 1980 (Olweny et al.,1980)	291	2 to 20	1.9:1
Kenya 2004 (Mwanda. et al.,2004)	628	-	1.7:1
Lusaka Zambia 1995 (Chintu et al.,1995)	87	6/12 to 14	1.7:1

3.6. EBV and Burkitt lymphoma

3.6.1. EBV association with endemic Burkitt lymphoma: Evidence from serological studies

Sero-epidemiological studies have been fundamental in unravelling the EBV-Burkitt lymphoma link. Two components to the link between EBV and Burkitt lymphoma include the presence of EBV DNA clonally integrated into tumour tissue, and sero-epidemiological associations (IARC,1997). Almost all cases of endemic Burkitt lymphoma are associated with EBV (IARC,1997). Table 3.2 illustrates case series from endemic regions of Africa that explored the association between Burkitt lymphoma and EBV. Some case-series fundamental in establishing the association between EBV and Burkitt lymphoma are described in Table 3.2 below.

Table 3.2: Burkitt lymphoma and EBV association.

Country, Year	Number of cases	EBV association
Kenya 1973 (Nonoyama et al.,1973)	23	96% had 4 to 113 EBV genome equivalents per cell
Uganda 1977 (Olweny et al.,1977)	27	79% EBV-positive 93% of the 15 tumours tested were EBV DNA positive
Uganda, 1983 (Gesar et al.,1983)	70	96% had 38 EBV genome equivalents per tumour cell, 91% had ≥ 160 EBV/VCA titres
Cameroon, 1992 (Prevot et al.,1992)	10	EBV DNA in all 10 cases

3.6.1.1. Case-control studies

One of the first case-control studies exploring EBV antibody titres in Burkitt lymphoma patients was reported in 1969 (Henle et al.,1969). EBV antibody titres in sera from 94 Burkitt lymphoma patients (cases) and different selected multiple control groups were compared. Endemic Burkitt lymphoma patients had higher levels of EBV antibodies than controls. The geometric mean titre (GMT) was 1:326 for Burkitt lymphoma cases, and 1:37 for controls.

A similar project conducted in Ghana in the 1970s evaluated the relationship between EBV antibody titres and Burkitt lymphoma (Nkrumah et al.,1976). Sera from 141 Burkitt lymphoma patients (cases) were examined, and for 75 of these, the EBV antibody titres were compared with those of 54 siblings and 50 age and sex matched

neighbourhood controls. Cases had significantly greater EBV antibody titre levels than all controls. Neither study was able to evaluate the temporal relationship between EBV infection and tumour development.

3.6.1.2. Cohort study and nested case-control study

A classical cohort study evaluating the relationship between EBV and Burkitt lymphoma was conducted between 1972 and 1979 in the West Nile district of Uganda (de Thé et al.,1978). The aim was to investigate the aetiological role of EBV in the development of Burkitt lymphoma. The *a priori* "null" hypothesis stated that there was no causal relationship between EBV and Burkitt lymphoma, suggesting that there was no difference in the antibody status of Burkitt lymphoma cases and non-Burkitt lymphoma controls. The West Nile district, now known as the Nile province, had an average annual Burkitt lymphoma incidence of 2.85 per 100,000 population between 1961 and 1975. Between 1972 and 1974, 42,000 sera were collected from 0 to 8 year old children living in five selected counties of the district. This cohort of children was followed up for eight years, and 16 children (0.04%) developed histologically proven Burkitt lymphoma. The interval between serum collection and the onset of Burkitt lymphoma ranged from 7 to 54 months.

An initial nested case-control analysis was conducted in 1978 from this cohort, and 14 Burkitt lymphoma cases were included (de Thé et al.,1978). For each Burkitt lymphoma case, five controls from the cohort, matched for age, sex, and locality to the index case were chosen. Burkitt lymphoma patients had higher pre-diagnostic EBV antibody titres

than controls (425.5 and 125.8 respectively, $P= 0.01$). This difference was noted as early as four years before the tumour developed.

A second report from the same study in 1982 included 2 more histologically verified Burkitt lymphoma cases (Gesar et al.,1982). The EBV antibody results were added to the previous 14, and the recalculated t test increased the significance of the difference in EBV antibody titres ($P<0.001$). The relative risk of developing Burkitt lymphoma increased multiplicatively by a factor of 5 for each two-fold dilution in anti-EBV titre for all cases of Burkitt lymphoma. A child with EBV antibody titres two dilutions or more above the average for the general child population of the same age and sex was found to have a 30-fold higher risk of developing Burkitt lymphoma. When the analyses were restricted to cases in whom EBV DNA had been demonstrated in tumour tissue, the relative risk for developing Burkitt lymphoma increased multiplicatively by a factor of 9 for each dilution. This study was monumental in establishing the possible timeline of tumour development, and also consolidating that EBV is a central component in the causation of this tumour.

3.7. Malaria and Burkitt lymphoma

Malaria is considered a co-factor in the development of Burkitt lymphoma (IARC,1997). The classification of the endemicity of malaria is based on parasite rates (PR), the percentage of children between 2 and 9 years old with a positive malaria blood slide (Metselaar et al.,1959). Hypoendemic malaria is PR less than 10%, mesoendemic

malaria is PR between 11% and 50%, hyperendemic malaria is PR constantly greater than 50%, and holoendemic is PR in infants (up to 1 year) constantly greater than 75%.

3.7.1. Ecological data

Ecological studies have associated Burkitt lymphoma with holoendemic and hyperendemic malaria (Dalldorf et al.,1964; Burkitt,1969; Kafuko et al.,1970). A relationship has been established using ecological data between malaria parasitemia and the incidence of Burkitt lymphoma (Morrow,1982, 1985). There is a close geographic concurrence between malaria and endemic Burkitt lymphoma within countries and internationally. The 'Lymphoma Belt' demonstrates an overlap between regions that have holoendemic and hyperendemic malaria and Burkitt lymphoma incidence (Haddow,1970; Kafuko et al.,1970).

The transmission dynamics of *P. falciparum* affect both the frequency of infection and the severity of malaria. Infants and children are the most vulnerable to malaria infection, and the maximal effects occur in the first eighteen months (O'Conor,1970). By 3 years of age considerable immunity has been developed. The peak prevalence of malaria parasitemia is at 3 years of age in holoendemic areas.

There is a close parallel between the maximum levels of malaria antibodies and age incidence of Burkitt lymphoma. Figure 3.8 illustrates the average serum malaria gamma globulin (total immunoglobulin) levels at different ages in children living in The Gambia and New Guinea (O'Conor,1970). It shows a difference between the unprotected population and the group given prophylactic anti-malarial treatment. Without the drugs,

the antibody levels rose above 2 grams per 100 ml by the age of two years; the maximum antibody levels were observed at about 3 years of age and remained elevated throughout life. In protected children there was a gradual increase, but the level did not go above 2 grams. In Figure 3.9, the prevalence of parasitemia was found to approach 100% at about 3 years, when there was maximum antibody response to malaria. The authors suggested that the shift to the right of the age distribution curve in Burkitt lymphoma may represent a relationship between the primary effect of malaria and the action of an immediate carcinogen. The number of Burkitt lymphoma cases was high when the number of children with a high level of parasitemia was also high.

Figure 3.8: Comparison of serum gamma globulin levels in unprotected subjects and those receiving malarial suppressants (O'Conor,1970).

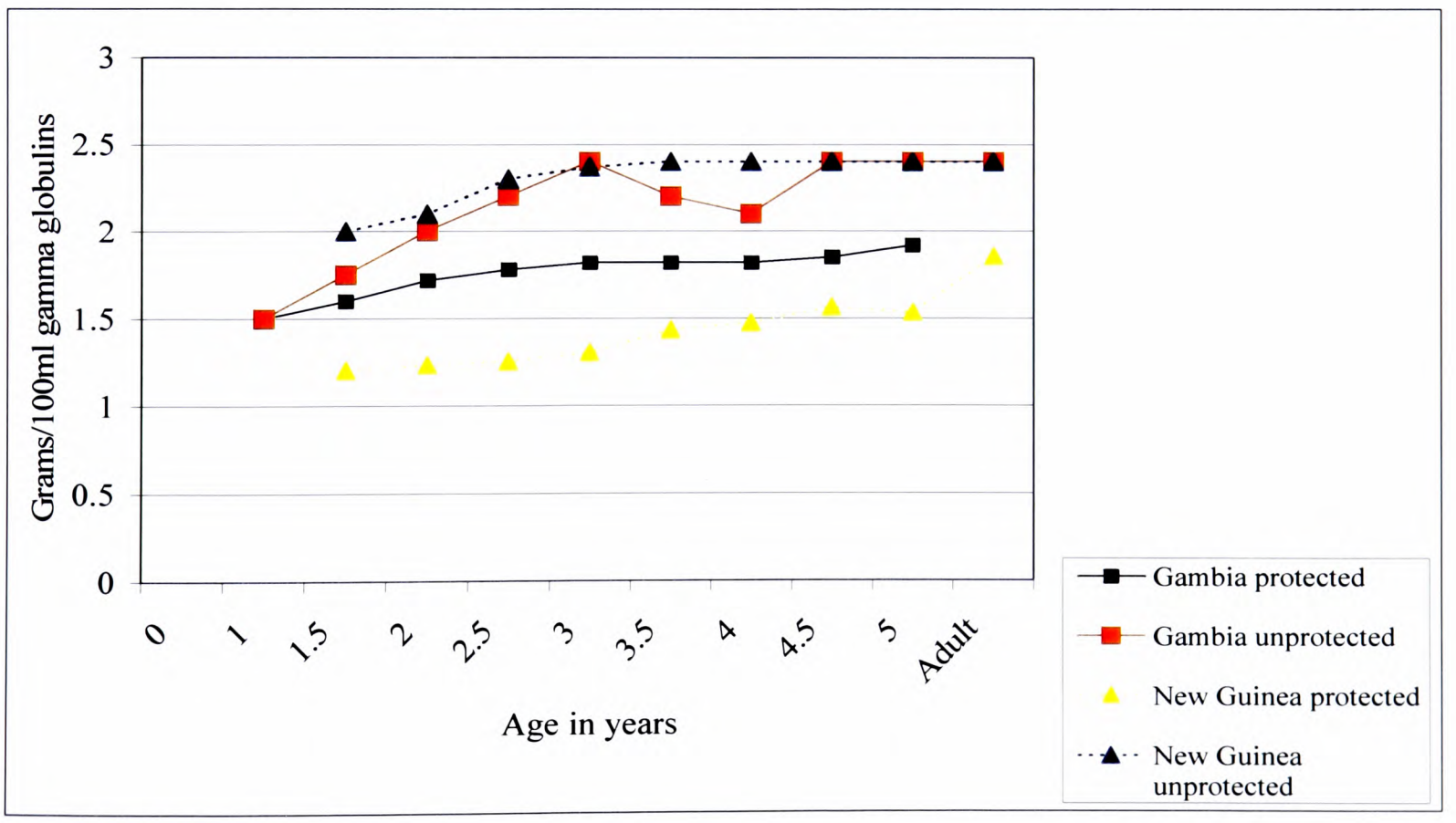
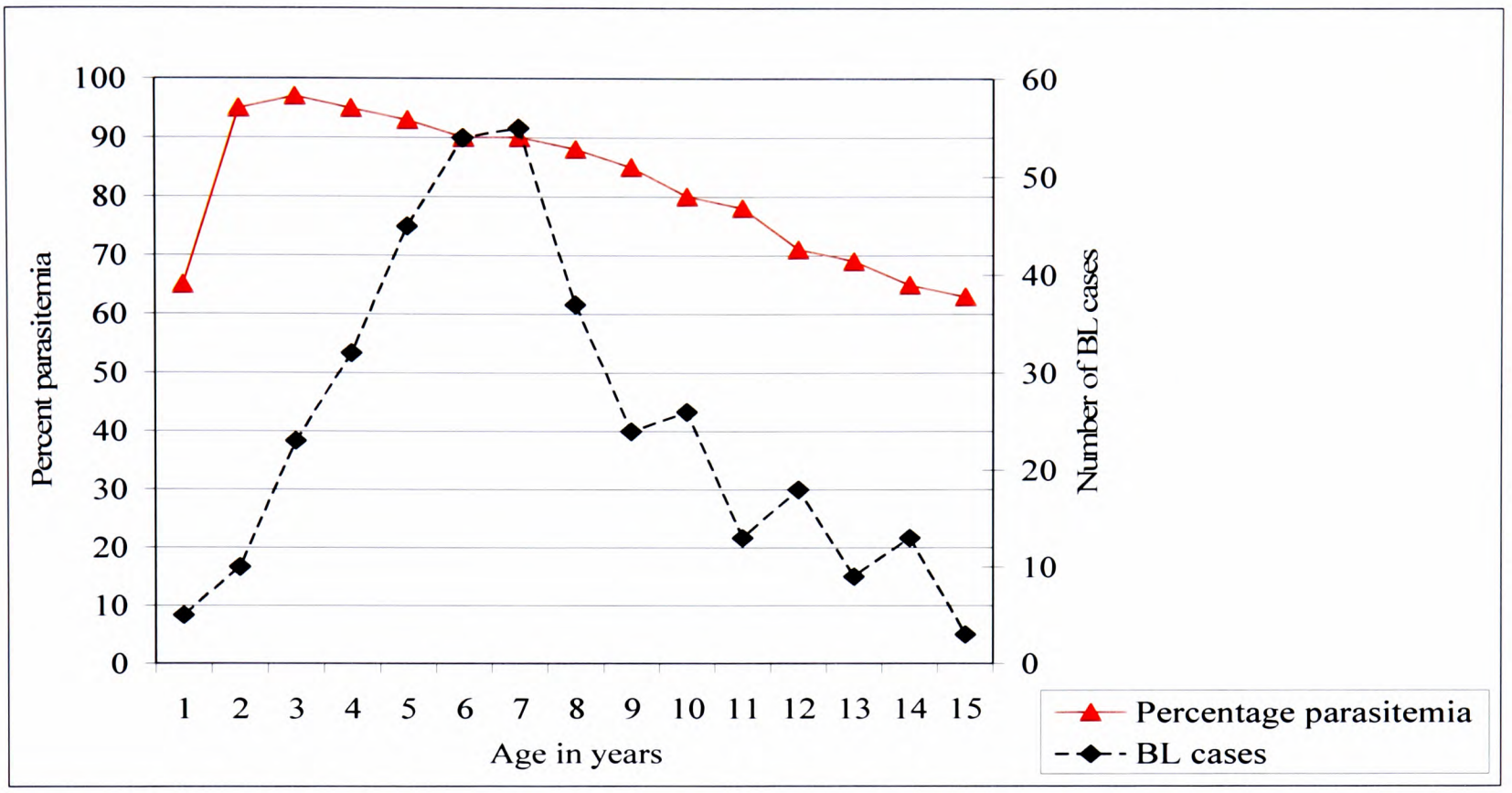


Figure 3.9: Age distribution of 361 cases of Burkitt lymphoma and percentage with malaria parasitemia in Gambian children (O'Conor,1970).



Holoendemic malaria is characterized by recurrent exposure and repeated malaria infections throughout the year (Rochford et al.,2005). In holoendemic areas, malaria has been found to provide a severe, universal, persistent and relentless stimulus to the reticulo-endothelial system and to the immunologic capacity of the host.

In urban areas where malaria transmission is lower, it has been observed that there is a lower incidence of Burkitt lymphoma compared to rural areas, where malaria transmission is higher (Bigger et al.,1979; Biggar et al.,1981; Oguonu et al.,2002). For example, a cross-sectional serological survey conducted in Ghana examined the relationship between malaria and EBV in the aetiology of Burkitt lymphoma (Biggar et al.,1981). Household cluster surveys of rural and urban areas were carried out. Malaria

was more prevalent in the rural areas. The incidence of Burkitt lymphoma was greater in rural areas, and EBV antibodies were similar in both urban and rural areas.

An interventional study conducted in the North Mara region of Tanzania with endemic levels of Burkitt lymphoma initially suggested that the incidence of Burkitt lymphoma declines when the incidence of malaria is reduced (Gesar et al.,1989). Before the trial (1964 to 1976), all 85 cases of Burkitt lymphoma in North Mara occurred in the lowlands (near Lake Victoria), with a high malarial parasitemia rate (28 to 48%), and none occurred in plateau, which had a low malarial parasitemia rate (5 to 24%). During this period, the annual incidence of Burkitt lymphoma was stable at an average of 4.3 per 100,000 population. From 1977 to 1982, the anti-malarial drug Chloroquine was administered to all children below 10 years (approximately 100,000 children) in the North Mara lowland region. During the intervention, the prevalence of malaria parasitemia fell to 11% in 1977 and 13% in 1978. After the trial, the incidence of Burkitt lymphoma fell to a minimum of 0.5 per 100,000 in 1980 and 1981. After 1982, the Burkitt lymphoma incidence levels started to rise, reaching 7.1 per 100,000 in 1984. After the trial, the level of malaria parasitemia started increasing, reaching 61% in 1983. This increase in parasite levels despite the intervention programme was attributed to inefficiency of the drug distribution, and not drug resistance, as Chloroquine resistance is reported to not have developed in this area until 1982. It has been argued that during the time period the study was conducted, a similar trend of decreasing Burkitt lymphoma incidence was observed elsewhere in Africa (Gesar et al.,1985). The decline of Burkitt lymphoma incidence began about 2 years before the malaria suppression (data not

shown); therefore, malaria suppression may not have been the primary cause of tumour incidence decline. The results of this study are still questionable.

3.8. EBV and malaria

The highest malaria parasite loads are found among children aged 6 to 18 months, which is also the age at which primary EBV infection usually occurs (Rochford et al.,2005). Unlike many infectious diseases, *P. falciparum* malaria only elicits protective immunity in children after several years of cumulative exposure.

The effect of malaria infection on EBV reactivation and its contribution to the genesis of endemic Burkitt lymphoma is not entirely understood. To examine this question and explore the nature of EBV infection in malaria endemic areas, the presence of EBV DNA in plasma from 73 children with and without acute malaria in Ghana was evaluated (Rasti et al.,2005). Viral DNA was detected in 40% of plasma samples (47% in the malaria infected and 34% in the non-malaria group) and was absent in plasma from Ghanaian adults and healthy Italian children. This suggested that viral reactivation may be common among children living in malaria endemic areas, and may contribute to the increased risk for endemic Burkitt lymphoma. The authors suggested that children living in malaria endemic regions are often exposed to many other infectious/parasitic diseases and have a relatively poor nutritional status that could indirectly contribute to impaired control of EBV.

A study in Uganda compared EBV DNA loads in plasma and saliva samples from Ugandan children with acute malaria at the time of diagnosis and 14 days after malaria treatment, in children without malaria, and in children with Burkitt lymphoma (Donati et al.,2006). EBV DNA was detected in 31% (13/42) of the plasma and in 79% (34/43) of the saliva samples from children with acute malaria. EBV DNA was detected in only 5% (2/38) of the plasma and in 93% (38/41) of the saliva in the after malaria treatment samples. In children without malaria and with Burkitt lymphoma, EBV DNA was found in 18% (7/40) and 89% (23/26) of the plasma samples respectively. In children with malaria, anti-malarial treatment led to clearance of plasma viral load in 85% of the cases but did not affect the EBV levels in saliva. The clearance of circulating plasma EBV after treatment may suggest a relationship between active malaria infection and EBV reactivation.

A 2002 study in Kenya compared the EBV loads in children living in areas with different malaria endemicity, holoendemic and sporadic (Moormann et al.,2005). This study evaluated the effect of persistent malaria exposure on EBV load. EBV load was evaluated in groups of children with different exposures to malaria, adults, and children with Burkitt lymphoma. The plasma EBV levels in children aged 1 to 4 years living in the malaria holoendemic areas were significantly higher compared to the same age children living in areas where malaria transmission was sporadic ($P<0.01$). This is the same age group of children in whom malaria morbidity and mortality is the most severe, and precedes the mean age (5 to 7 years) at which Burkitt lymphoma cases occur. The proposed explanation for these findings was that malaria facilitates polyclonal B cell

activation, and the higher EBV load in the children 1 to 4 years of age in malaria holoendemic regions may reflect an indirect expansion of EBV infected B cells. Children with Burkitt lymphoma had significantly higher EBV loads than controls. It is possible that the elevated EBV loads in these children resulted from the presence of tumour cells rather than latently infected B cells, as Burkitt lymphoma is a systemic disease. An alternative explanation is that EBV specific immunity is suppressed. Whittle et al. demonstrated that, during an episode of acute malaria in children, spontaneous outgrowth of EBV transformed B cells occurred at a greater frequency, which they thought suggested impaired EBV-specific immunity (Whittle et al.,1990). It has been proposed that *P. falciparum* malaria infection increases the risk of Burkitt lymphoma by increasing the number of EBV infected B cells during an episode of malaria (Lam et al.,1991). The consequence of repeated malaria infections and early EBV infection may be an increase in the number of EBV infected B cells.

A summary of the evidence of the role malaria and EBV play in the aetiology of Burkitt lymphoma based on the above discussed studies is now presented.

- The plasma EBV levels in children younger than 4 years living in the malaria holoendemic areas appear to be higher than EBV levels in same aged children living in an area where malaria transmission is sporadic.
- Malaria causes polyclonal B cell activation. The higher EBV load in children in holoendemic malaria regions could reflect an indirect expansion of EBV infected B cells.

- *P. falciparum* malaria infection may increase the risk of Burkitt lymphoma by increasing the number of EBV infected B cells during an episode of malaria.
- In patients with acute malaria and circulating EBV DNA, the plasma EBV load appears to be cleared after anti-malarial treatment, suggesting a relationship between active malaria infection and viral reactivation.
- Patients with Burkitt lymphoma apparently have higher EBV plasma loads compared to non-Burkitt lymphoma controls.
- In urban areas where malaria transmission is lower, there is apparently a lower incidence of Burkitt lymphoma compared to rural areas.
- The incidence of Burkitt lymphoma may decline when the incidence of malaria is reduced.

3.8.1. Three stage model

Morrow modified a three stage model of pathogenesis that was initially suggested by Klein in 1982 (Klein,1979; Morrow,1982). This model addresses the pathogenesis of Burkitt lymphoma, incorporating the roles of malaria and EBV. Malaria increases the number and turnover rate of EBV infected B lymphocytes, and thus increases the likelihood of the emergence of malignant cytogenic changes. The three stage model, hypothesises that some kind of third event would be required beyond EBV and malaria that facilitates tumour formation. The three stages are:

1. Initiation phase

Primary EBV infection is regarded a necessary factor. Most children who develop the tumour are infected with a high dose of EBV, or after primary infection high EBV

multiplication, leading to the immortalization of a large number of B lymphocytes (de Thé et al.,1978; Gesar et al.,1982).

2. Promotion phase

This is a critical period in which there is increased B cell stimulation, and a decreased control of B cell proliferation (Evans,1985). Malaria has a mitogenic and immunosuppressive effect on the immune system. Infection with malaria leads to an increased intensity of polyclonal expansion during which a malignant cell may develop. Chronic severe malaria leads to an intense host response with proliferation of the lymphoreticular system. This proliferation provides a much higher statistical opportunity for abnormal cells to evolve. This phase is determined by both epidemiological and immunological factors shown in Table 3.3.

Table 3.3: African Burkitt lymphoma: Factors that may be associated with the promotion phase (Evans,1985).

Factor
Polyclonal expansion of EBV infected B lymphocytes
High EBV viral capsid antigen antibody titres
Holoendemic malarial infection stimulating further B cell proliferation
Occurrence of a chromosomal translocation
Other mitogenic factors enhancing proliferation or depressing the suppressor T cell response
Nutritional state
Genetic susceptibility
Age and sex

3. Clinical illness promotion factor

Cells no longer controlled by normal growth inhibiting mechanisms have developed, and lead to tumour formation. Genetic factors and effective immune surveillance may be important in preventing tumour formation (Evans,1985).

3.9. HIV and Burkitt lymphoma in children

Case series have provided equivocal results suggesting that there is no association between HIV and endemic Burkitt lymphoma (Lazzi S et al.,1998; Sinfield et al.,2007). However, they have not been able to adjust for important confounding factors for HIV infection including residence and age.

One case-control study has demonstrated that HIV infection may be significantly associated with Burkitt lymphoma in children under 15 years (OR 7.5, 95% CI 2.8-20.1, based on 33 cases) (Newton et al.,2001). Burkitt lymphoma occurs at an increased frequency in HIV-seropositive individuals, but is less frequent among immunosuppressed transplant recipients (Gong et al.,2003). Whether this is because immunosuppresion alone is insufficient to facilitate its development, or that there is something specific about the immune dysfunction caused by the HIV that is important, is not clear. In the West HIV-associated Burkitt lymphoma resembles the sporadic form of the disease found in the West: sequences of EBV DNA can be found in about half the cases and most have a characteristic c-myc/immunoglobulin gene translocation. The increased B cell activation accompanying HIV infection may increase the likelihood of such mutational events. However, c-myc/immunoglobulin gene translocations can be

found in circulating B cells in the peripheral blood of otherwise healthy HIV-seropositive individuals and their presence does not appear to correlate with the subsequent development of a lymphoma. This suggests that further oncogenic steps are required for the development of Burkitt lymphoma.

3.10. Socio-economic factors and Burkitt lymphoma

Socio-economic factors appear to play a role in the development of Burkitt lymphoma. Burkitt lymphoma patients appear more likely to live in rural areas (Biggar et al.,1979; Bigger et al.,1979; Biggar et al.,1981; Oguonu et al.,2002). This is thought to be because rural areas are more likely to have a higher incidence of malaria than urban areas. An assessment of family and environmental circumstances among Burkitt lymphoma patients in Uganda revealed that patients had more siblings than their age and sex-matched nearest neighbour controls (Morrow et al.,1974). Burkitt lymphoma patients had a higher percentage of sibling deaths than controls, and they had more people sharing the rooms in which they slept than controls. There was no difference in distributions by birth order between Burkitt lymphoma patients and controls. The families of Burkitt lymphoma patients lived in poorer houses than controls, and the families of Burkitt lymphoma patients moved their residences more frequently than controls. The above findings may reflect differences in transmission of EBV between family members, and the lack of prevention of malaria in rural families.

3.11. Plant products and Burkitt lymphoma

Extracts from the Euphorbiaceae family, of the *Euphorbia* genus are thought to be co-factors in the development of Burkitt lymphoma (van den Bosch et al.,1993; MacNeil et al.,2003). It is postulated that the plant's geographic distribution could potentially explain the geographical distribution of the tumour, and the lower tumour incidence in urban areas. *Euphorbia tirucalli* (Figure 3.10) commonly called the milk bush or pencil tree originates from Africa and the rain forests of the Amazon. It is a relatively common garden plant in Africa, and is used as a barrier fence due to its thick mass and rapid growth. The plant is used as an insect repellent, for treatment of snakebites and the latex is used for skin tumours and syphilitic ulcers (Tropicalplantdatabase). The latex is a rich source of terpenes including phorbol esters. An important phorbol ester, *4-deoxyphorbol ester* has been implicated in the development of Burkitt lymphoma in some studies (Ito et al.,1983; Lin et al.,1983; Mizuno et al.,1983; Benjamin et al.,1984; Aya et al.,1991).

3.11.1. Plant distribution.

E. tirucalli has been observed near the homes of Burkitt lymphoma cases (Mizuno et al.,1983; van den Bosch et al.,1993). The area of the 'Lymphoma Belt' overlaps with the areas where the plant grows. In Malawi, the possible role of EBV promoting plants in the aetiology of Burkitt lymphoma was investigated by visiting the homes of patients with and without the tumour (van den Bosch et al.,1993). Seventy-six Burkitt lymphoma cases were identified, and 3 age and sex matched controls were recruited for each case. One control was a non-cancer patient admitted to hospital at the same time as the index case, and the other 2 were neighbourhood controls. The results showed a significant

association between a case of Burkitt lymphoma in a house and the presence of *E. tirucalli* in or around the yard (OR 7.96, *P*= 0.012). However, this result was based on only 5 cases.

Figure 3.10: *Euphorbia tirucalli* (Hull,2003).

Interaction between plant derived terpenes and human cells (which may lead to the formation of Burkitt lymphoma), was suggested when soil samples collected from the ground under *E. tirucalli* plants were evaluated (Ito et al.,1983). Ether extracts of the soil at a specific concentration induced EBV early antigen, in a cell line that grows in suspension derived from a patient with Burkitt lymphoma. Methanol extracts of the stalks, leaves and roots of *E. tirucalli* enhanced EBV mediated cell transformation of human lymphocytes (Mizuno et al.,1983).

Human B lymphocytes exposed to both EBV and purified 4-deoxyphorbol ester derived from the plant *E. tirucalli* in vitro develop a high frequency of chromosomal rearrangements. The most commonly affected chromosome is chromosome 8 which shows abnormalities in Burkitt lymphoma cells (Aya et al.,1991). Mucosal B cells latently infected with EBV may be induced to reactivate the viral lytic cycle. Dilute concentrations of *E. tirucalli* latex were effective at inducing viral reactivation in EBV positive cells (MacNeil et al.,2003). Children are thought to be easily exposed to the plant latex and convey it to their mucous membranes using their hands as it is used as a play item.

3.12. Dietary associations and Burkitt lymphoma

Carcinogens, particularly volatile *N*-nitrosoamines from smoked salted fish, and mutagens in other preserved food samples, have been invoked as co-factors in Asian nasopharyngeal carcinoma (IARC,1997). The fish caught in Lake Malawi is treated in various ways including sun-drying, salting or smoking, and is an important dietary component, especially along the lakeside. Data linking some forms of salted fish with nasopharyngeal carcinoma in Asia which is also EBV associated makes it reasonable to suggest that dietary factors may have a role to play in the aetiology of Burkitt lymphoma (Griffin,2000). Variations in cooking and preserving methods could be assessed for their effect on the concentration of *N*-nitroso compounds found in the diets of children in Burkitt lymphoma endemic regions. Evidence remains scant.

3.13. Sickle cell trait and Burkitt lymphoma

Sickle-cell trait has been shown to protect substantially against severe *P. falciparum* malaria (Allison,1954). Sickle-cell trait provides a survival advantage over people with normal haemoglobin in regions where malaria is endemic. For that reason, attempts have been made to determine if children with the sickle-cell trait are less likely to develop Burkitt lymphoma. It was hoped that an inverse association of Burkitt lymphoma with sickle-cell trait might provide strong evidence for the role of intense falciparum malaria (Morrow,1985).

Studies conducted in malaria endemic African countries that have a large population of children with the sickle-cell trait suggest that children with the sickle-cell trait may have a lower risk of Burkitt lymphoma (Williams,1966). Other studies have shown inconsistent results (Pike,1970; Nkrumah et al.,1976). A 1997 IARC review concluded that the issue of sickle-cell trait being protective against Burkitt lymphoma is still unresolved, mainly because few large studies have been done (IARC,1997).

3.14. Burkitt lymphoma seasonal variation

A review of Burkitt lymphoma occurrence in the West Nile region of Uganda revealed an almost twofold excess of Burkitt lymphoma in the second half of the year, the season of greatest rainfall over the period 1966 to 1973 (Williams et al.,1974). This review compared the incidence of other diseases over the same time period and revealed no evidence of a seasonal effect as seen in Burkitt lymphoma. The seasonal effect of Burkitt lymphoma was stratified by age and sex, and it was evident in all the subgroups. The

authors believed the seasonal effect to be a real finding for the following reasons: Burkitt lymphoma occurrence increased in the rainy season, this is a period when access to medical facilities would be limited, thus not confounded by ability to get to hospital. There was no seasonal effect noted for any other disease, and the total number of outpatients did not increase during this period, but decreased as they would have expected. The authors suggested that a plausible interpretation would be that during a certain period of the year, between 3 and 4 months, there is an initiating event that is more likely than during the rest of the year. Suggested triggering events were increased insect density during the rainy season, increased malaria transmission and contamination of drinking water, spells of drought followed by severe malnutrition may also trigger tumour development by impairing the immune system. A similar type of review in Ghana which also has seasonal rainfall did not show an obvious pattern of seasonality (Biggar et al.,1979).

Investigation of seasonal variation in diagnosis and onset of Burkitt lymphoma is limited by the small numbers of cases, difficulties in defining date of onset, and variations in access to diagnostic facilities. The studies are further limited by the lack of a definition of an appropriate comparison population that would take account of seasonal variation of births, deaths and population movement. It is also possible that seasonal clustering may be attributable to seasonal variation in access to care, itself constrained by transportation to medical facilities.

3.15. Space-time clustering

A cluster is an aggregation of a relatively uncommon disease in space and/or time in amounts that are believed or perceived to be greater than could be expected by chance alone (Last,2001). Space-time clustering is a statistically significant excess of cases of a disease occurring within a limited space-time continuum. Space and/or time clusters of Burkitt lymphoma cases were apparently identified in Africa as early as 1967 and have been considered an important epidemiological feature of the disease. Pike and colleagues first reported space-time clustering of Burkitt lymphoma in the West Nile district of Uganda in 1967 (Pike et al.,1967). Cases diagnosed between 1961 and 1965 were recruited. Thirty-six cases with known addresses and accurate diagnosis were included in the analyses. Patients whose dates of onset were close tended to live close together, suggesting an 'epidemic' character. A subsequent IARC review concluded, however that that the significance of the clusters is "uninterpretable statistically" (Day et al.,1985).

Space-time clustering has not been reported significantly in other regions, except in the Bwamba region of Uganda (Morrow et al.,1971). Studies conducted in Ghana did not show evidence of clustering (Biggar et al.,1979). Investigations of clusters may be complicated by more active case seeking in areas of interest than in other areas used for comparison, thus making the significance of the reported cluster difficult to assess.

3.16. Summary

This chapter has described the epidemiology of Burkitt lymphoma in African children. Tumour associated risk factors including infection with EBV, malaria and socio-economic status of cases have been discussed. Risk factors for tumour formation that are still being investigated including plant factors, dietary associations, sickle-cell trait, time-space clustering and seasonality have also been briefly discussed.

The relationship between EBV, malaria and the human host is an intricate process. Because this interaction is medically benign in the vast majority of persons, the development of malignancy is likely to require an unusual confluence of circumstances that leads to a persistent and/or substantial disruption of the normal balance between maintenance of the virus and its elimination by the immune system (Hsu et al.,2000).

CHAPTER FOUR

BURKITT LYMPHOMA CASE-CONTROL STUDY

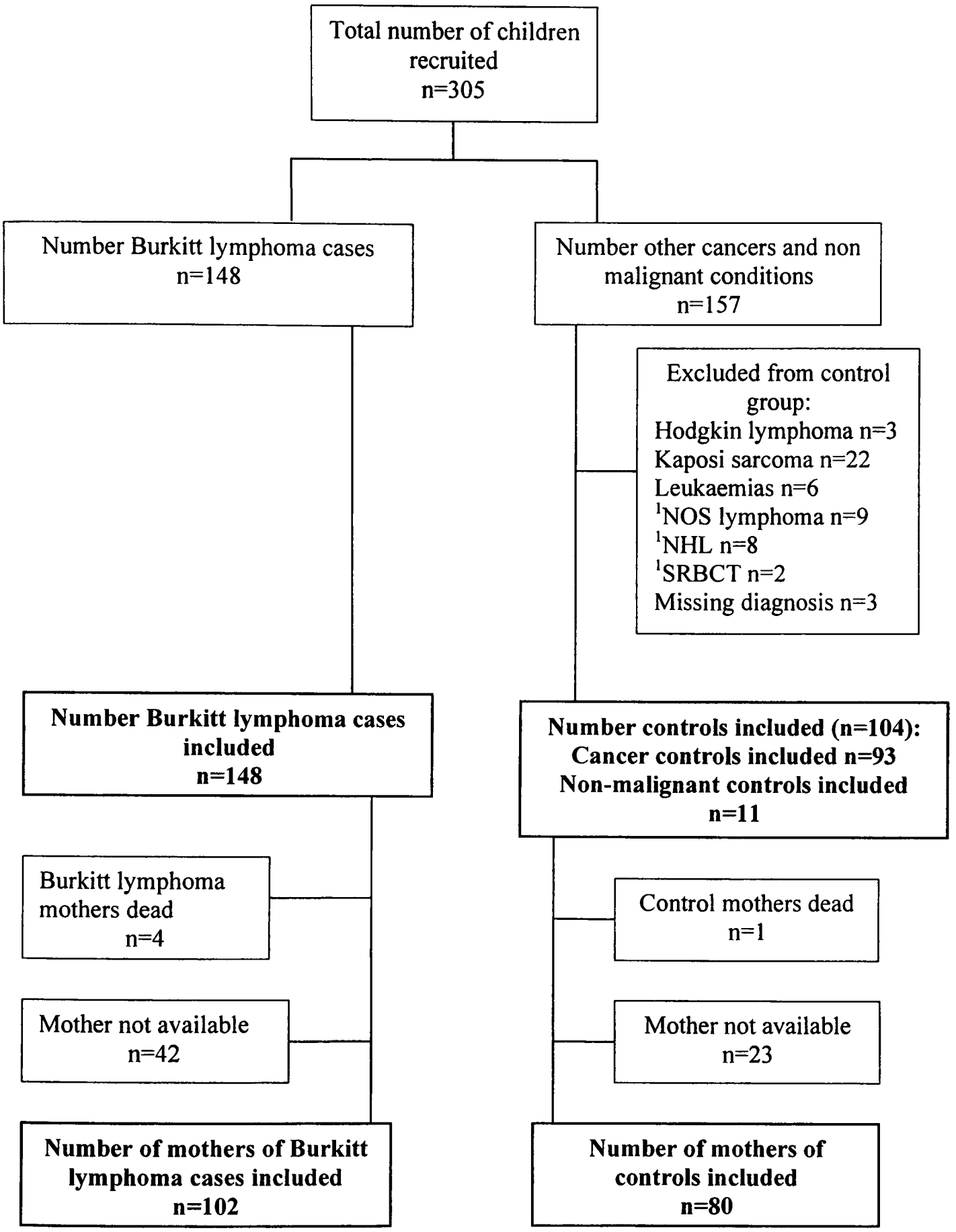
4.1. Introduction

In this chapter, both childhood and maternal risk factors associated with African endemic Burkitt lymphoma in children are investigated. The first objective was to study the role played by specific infections, particularly HIV, EBV and malaria. The second objective was to examine associations with family socio-economic status, child health, and health-related behaviours. An *a priori* hypothesis evaluated was that Burkitt lymphoma is more likely to develop in children who have had more exposure to malaria; children who do not use mosquito nets or have a history of frequent episodes of malaria. Finally maternal risk factors such as reproductive and sexual behaviour were also explored.

4.2. Case-control study of Burkitt lymphoma

Of the total 305 children recruited, 148 were diagnosed with Burkitt lymphoma and included in the analyses. Controls were the 104 children diagnosed with other cancer sites or types. The flowchart in Figure 4.1 illustrates the process used to identify cases, controls and mothers recruited and included in the analyses. All haematological cancers including leukaemias, all non-Burkitt lymphomas and small round blue cell tumours were excluded from the control group because of possible diagnostic overlap and known associations with HIV.

Figure 4.1: Burkitt lymphoma cases and controls recruitment flow chart



[1]See list of abbreviations

4.2.1. Methods

Data were analysed as described in Chapter 2, using a case-control study methodology. All ORs relating to data on children were adjusted for child's age (under 5 years, and 5 years and over), sex and residence (rural and urban). Odds ratios relating to data on mothers were adjusted for mother's age (under 30 years, and 30 years and over), and residence (rural and urban). Analyses for variables that are likely to be influenced by HIV status such as child health, and maternal sexual/reproductive characteristics were restricted to HIV negative children and mothers for both cases and controls. This restriction was necessary because any effect that may be found associated with other exposures might in fact be associated with HIV infection.

Two mother-child pairs had discrepant HIV test results (mother negative and child positive; 1 child with Burkitt lymphoma and 1 child with Wilms tumour). These results suggest laboratory error, and they were excluded from the analyses on that basis.

The Malawi population lives mainly in rural areas and a great majority are subsistence farmers and casual labourers who do not have a steady monetary income. To establish an index of poverty, different markers were created by combining data. Questionnaire data were available for 8 variables used as markers of poverty including, ownership of a phone, television, fridge, radio, chickens, and availability of piped water, land for growing vegetables and electricity. Two groups were created, those 'better off', and 'worse off'. The 'better off' group was defined as

having either a television, fridge, phone, electricity or piped water, in addition to the other attributes listed above. The 'worse off' group did not posses either a television, fridge, phone, electricity or access to piped water, but may have had chickens, radio or land for growing vegetables. Using the composite measure of poverty, there was little difference between cases and controls, with 62% of cases (n=63) and 51% (n=41) of controls categorised as 'worse off'. Adjustment for markers of poverty made no material difference to the results in part because it was correlated with region of residence (see Appendices 4.10 to 4.17).

4.2.2. Laboratory tests

Tumour diagnosis for all tumours was verified at the College of Medicine in Blantyre, Malawi. A proportion of diagnoses were made on clinical grounds only, without the benefit of cytology/histology. A description of child and maternal blood sample collection and testing for antibodies against HIV, malaria, HHV-8 and EBV is described in Chapter 2.

4.2.3. Description of controls

A list of other cancers and non-malignant conditions used as controls for Burkitt lymphoma cases is provided in Table 4.1 and Appendix 2.4b.

4.3. Results

Tables showing the results of the full analyses of the Burkitt lymphoma case-control study are shown in Appendices 4.1 to 4.9. All ORs presented in the text are adjusted as described in Section 4.2.1.

Table 4.1: Control diagnostic groups

Diagnosis	Number	Percent
Wilms tumour	30	28.0
Retinoblastoma	18	17.3
Rhabdomyosarcoma	18	17.3
Non-malignant	11	10.6
Neuroblastoma	6	5.8
Hepatocellular carcinoma	4	3.9
Malignant Teratoma	4	3.9
Bone tumours	4	3.9
Ovarian tumours	3	2.9
Abdominal tumours	1	1.0
Brain tumours	1	1.0
Hepatoblastoma	1	1.0
Soft tissue tumours	1	1.0
Squamous cell carcinoma	1	1.0
Yolk sac tumours	1	1.0
Total	104	100

4.3.1. General characteristics of case children

Seventy-five percent (n=109) of cases and 74% (n=73) of controls had histologically verified diagnoses. Table 4.2 shows the anatomical distribution of tumour primary site for Burkitt lymphoma cases: just over half (56%) presented with jaw lesions, although abdominal lesions were also relatively frequent (39%). However, 84% had metastatic spread of tumour at diagnosis (results shown in Appendix 4.1).

Table 4.2: Primary site of tumour for Burkitt lymphoma cases

Primary site	**Number**	**Percent**
Jaw	83.0	56.1
Abdomen	57.0	38.5
Extremities	2.0	1.4
Head and neck	2.0	1.4
Kidneys	0	0
Orbit	2.0	1.4
Groin	0	0
Bone marrow	0	0
Missing	1.0	1.4
Total	148	100

Burkitt lymphoma cases were on average 2 years older than controls (mean age 7.1 years and 5.1 years respectively) (Table 4.3). Sixty percent (n=89) of cases and 55% (n=57) of controls were boys. Approximately 80% (n=111) of cases and 70% (n=69) of controls resided in rural areas. Children from rural areas appeared more likely to be diagnosed with Burkitt lymphoma, although this was not statistically significant (OR=1.5, 95% CI 0.8 to 2.9, *P*=0.2).

Table 4.3: Age distributions of Burkitt lymphoma cases and controls

Child age (in years)	**Cases**	**Controls**	**Test statistic** (rank sum)
0 to 4	19 (13%)	57 (55%)	
5 to15	126 (87%)	46 (45%)	
Missing	*3*	*1*	
Number	145	103	
Median (IQR)	7 (5 to 9)	4 (2 to 8)	
Mean (SD)	7.1 (2.6)	5.1 (3.9)	z =-5.5 *P*<0.0001
95% CI of mean	6.7 to 7.5	4.4 to 5.9	

Fifty percent (n=49) of cases and 43% (n=30) of controls had a history of having consumed sun-dried fish (OR=1.4, 95% CI 0.6 to 3.4, *P*=0.09). Nineteen percent (n=19) of cases and 30% (n=21) of controls reported having eaten smoked or salted fish (OR=0.4, 95% CI 0.2 to 1.2, *P*=0.09).

4.3.2. Possible markers of infectious exposure

All of the controls, and 98% of the Burkitt lymphoma cases were reported to have shared their bed with at least 1 other person. There was no difference between cases and controls in the mean number of people who shared a bed with the child (shown in Table 4.4).

Three percent (n=4) of mothers of children with Burkitt lymphoma and 1% (n=1) of mothers of the controls died prior to recruitment of the child. Nine percent (n=13) of fathers of children with Burkitt lymphoma and 5% (n=5) of fathers of the controls had died prior to recruitment. Detailed results of the analyses of possible markers of infection are presented in Appendix 4.3.

Table 4.4: Number of people sharing bed with Burkitt lymphoma cases and controls

Number share bed	**Cases**	**Controls**	**Test statistic** (rank sum)
0 to 1	45 (46%)	28 (37%)	
2 to 7	53 (54%)	48 (63%)	
Missing	*4*	*4*	
Number	98	76	
Median (IQR)	2 (1 to 2)	2 (1 to 2)	
Mean (SD)	1.7 (0.9)	1.9 (0.9)	z=1.1 *P*=0.3
95% CI of mean	1.5 to 1.9	1.7 to 2.1	

4.3.3. Child health for HIV negative children

All analyses of child health factors were restricted to HIV negative children whose mothers had answered questions about the child's health. Sixty-six percent (n=97) of Burkitt lymphoma cases and 68% (n=71) of controls were included in the analyses. Children whose HIV status was not known were excluded (1 case and 10 controls).

A large percentage of cases and controls had not been previously admitted to hospital (83% and 80% respectively). Somewhat more cases (82%) than controls (75%) had reported previous episodes of malaria (OR=1.4, 95% CI 0.7 to 3.1, *P*=0.3). There was no difference in the number of self-reported lifetime malaria episodes between cases and controls (OR=1.0, 95% CI 0.8 to 2.3, *P*=0.9) (see Table 4.5). Detailed results are shown in Appendix 4.4.

Table 4.5: Frequency of self-reported malaria for HIV negative Burkitt lymphoma cases and controls

Malaria frequency	**Cases**	**Controls**	**Test statistic** (rank sum)
0 to 3	55 (63%)	46 (72%)	
4 to 10	33 (37%)	18 (28%)	
Missing	*9*	*10*	
Number	88	64	
Median (IQR)	3 (1 to 4)	3 (1 to 4)	
Mean (SD)	3.0 (2.4)	2.6 (2.2)	z =-0.99 *P*=0.32
95% CI of mean	2.5 to 3.5	2.0 to 3.1	

In light of findings from an as yet unpublished study from Uganda (Carpenter et al.,2007), new questions regarding use of mosquito nets were added to the questionnaire 8 months into the study. Data were only available for 29 (20%) cases

and 25 (24%) controls who were HIV negative. Further results are shown in Appendix 4.5

Eighty percent (n=19) of control households and 60% (n=17) of Burkitt lymphoma households reportedly owned mosquito nets (OR=0.2, 95% CI 0.03 to 1.2, *P*=0.08). Thirty-seven percent (n=9) of control households and 16% (n=4) of Burkitt lymphoma households reported having more than 2 mosquito nets in the home (OR=0.3, 95% CI 0.1 to 1.4, *P*=0.1). The difference in mean number of mosquito nets between case and control households was of borderline statistical significance (see Table 4.6 below).

Table 4.6: Number of mosquito nets reported for HIV negative Burkitt lymphoma cases and controls

Number mosquito nets	**Cases**	**Controls**	**Test statistic** (rank sum)
0 to 2	21 (84%)	15 (63%)	
3 to 9	4 (16%)	9 (37%)	
Missing	*4*	*0*	
Number	25	24	
Median (IQR)	1 (0 to 2)	2 (1 to 3)	
Mean (SD)	1.2 (1.1)	2.0 (1.9)	z =1.8 *P*=0.07
95% CI of mean	0.7 to 1.6	1.2 to 2.9	

Seventy-nine percent (n=19) of controls and 57% (n=16) of cases reported having used mosquito nets (OR=0.2, 95% CI 0.03 to 0.9, *P*=0.04).

4.3.4. Maternal characteristics

Questionnaire data was available from 102 of mothers of children with Burkitt lymphoma and 80 of mothers of controls. Only 72% (n=73) of mothers of children

with Burkitt lymphoma and 86% (n=69) of mothers of controls knew their age, but little difference in age was evident between the two groups (see Table 4.7 below). Detailed results are shown in Appendix 4.6.

Table 4.7: Age distribution of mothers of Burkitt lymphoma cases and controls

Mother age (in years)	**Cases**	**Controls**	**Test statistic** (rank sum)
18 to 29	36 (49%)	42 (61%)	
30 to 55	37 (51%)	27 (39%)	
Missing	*29*	*11*	
Number	73	69	
Median (IQR)	30 (27 to 34)	28 (24 to 33)	
Mean (SD)	31 (6.9)	29.6 (7.7)	z=-1.6 *P*=0.1
95% CI of mean	29.4 to 32.6	27.7to 31.4	

Seventy-one percent (n=70) of mothers of children with Burkitt lymphoma and 70% (n=55) of mothers of controls had primary school education or higher (OR=1.1, 95% CI 0.5 to 2.5, *P*=0.8).

4.3.5. Maternal sexual and reproductive history for HIV negative mothers

Analyses of maternal sexual and reproductive characteristics were restricted to HIV negative mothers: 80% (n=82) of mothers of children with Burkitt lymphoma and 73% (n=58) of mothers of controls. The HIV status of 8 mothers was not known, and they were excluded from the analyses (4 Burkitt lymphoma, and 4 controls; 1 retinoblastoma, 1 rhabdomyosarcoma, 1 hepatocellular carcinoma and 1 non-malignant).

Ninety-one percent (n=72) of mothers of case and 91% (n=51) of mothers of controls reported having had a regular partner at the time of recruitment (OR=1.7, 95% CI 0.4 to 7.3, *P*=0.4). Mothers of cases had on average more pregnancies than mothers of controls (OR=3.9, 95% CI 1.2 to 12.2, *P*=0.05) and more live births than mothers of controls (OR=5.2, 95% CI, 1.6 to 16.6, *P*=0.01) (see Tables 4.8 and 4.9). Detailed results are presented for these ORs in Appendix 4.7.

Table 4.8: Number of maternal pregnancies, for HIV negative mothers

Number pregnancies	**Cases**	**Controls**	**Test statistic** (rank sum)
1 to 4	33 (41%)	36 (64%)	
5 to 13	47 (59%)	20 (36%)	
Missing	*2*	*2*	
Number	80	56	
Median (IQR)	5 (4 to 6)	4 (2 to 5)	
Mean (SD)	5.4 (2.3)	4.1 (2.5)	z =-3.3 *P*=0.001
95% CI of mean	4.8 to 5.9	3.4 to 4.7	

Table 4.9: Number of children born to HIV negative case and control mothers

Number children born	**Cases**	**Controls**	**Test statistic** (rank sum)
1 to 2	7 (9%)	18 (32%)	
3 to 13	73 (91%)	39 (68%)	
Missing	*2*	*1*	
Number	80	57	
Median (IQR)	5 (4 to 6)	4 (2 to 5)	
Mean (SD)	4.9 (2.1)	4.0 (2.4)	z=-2.8 *P* =0.005
95% CI of mean	4.5 to 5.5	3.4 to 4.7	

Sixty-seven percent (n=54) of mothers of cases and 46% (n=26) of mothers of controls had more than 3 children alive. The odds of Burkitt lymphoma were almost 5 fold higher for children who had more than 3 siblings alive (OR=4.5, 95% CI 1.4

to 14.1, *P*=0.01) as compared to children with up to 3 siblings alive. Nineteen percent (n=15) of mothers of cases and 7% (n=4) of mothers of controls reported having children with different biological fathers (OR=2.5, 95% CI 0.8 to 8.5, *P*= 0.1).

Table 4.10: Number of living children of HIV negative case and control mothers

Number children alive	**Cases**	**Controls**	**Test statistic** (rank sum)
1 to 3	26 (33%)	30 (54%)	
4 to 10	54 (67%)	26 (46%)	
Missing	*2*	*2*	
Number	80	56	
Median (IQR)	4 (3 to 5)	3 (2 to 5)	
Mean (SD)	4.2 (1.4)	3.5 (2.0)	z =-2.5 *P*=0.01
95% CI of mean	3.8 to 4.5	2.9 to 4.0	

Fifty-one percent (n=38) of mothers of children with Burkitt lymphoma reported having more than 1 lifetime sexual partner as compared to 28% (n=15) of mothers of controls (OR=3.0, 95% CI 1.2 to 7.6, *P*=0.02).

Table 4.11: Number of self-reported maternal lifetime sexual partners for HIV negative mothers

Number sexual partners	**Cases**	**Controls**	**Test statistic** (rank sum)
1	37 (49%)	39 (72%)	
2 to 4	38 (51%)	15 (28%)	
Missing	*7*	*4*	
Number	75	54	
Median (IQR)	2 (1 to 2)	1 (1 to 2)	
Mean (SD)	1.7 (0.8)	1.5 (0.9)	z=-2.4 *P*=0.02
95% CI of mean	1.6 to 1.9	1.3 to 1.7	

There was no difference in the self-reported prevalence of sexually transmitted infections between mothers of cases and controls (5% and 4% respectively, OR=1.5, 95% CI 0.3 to 9.1, *P*=0.65).

4.3.6. Associations with child serological measures of infections

The results for all the laboratory tests among children are presented in Appendix 4.8.

4.3.6.1. Child HIV status

HIV test results were known for 99% (n=147) of the Burkitt lymphoma cases, and for 90% (n=94) of the controls. The number of HIV positive children for both cases and controls was small at 9 (6%) and 2 (2%) respectively. Unadjusted analysis showed that HIV positive children were almost 3 times more likely to have Burkitt lymphoma. After adjustment for child age, sex and residence, children who were HIV positive were 12 times more likely to develop Burkitt lymphoma (OR=12.4, 95% CI 1.3 to 116.2, *P*=0.03).

Table 4.12: Child HIV status, Burkitt lymphoma cases and controls

Child HIV status	**Cases**	**Controls**	**Adjusted OR (95% CI)**
Positive	9 (6%)	2 (2%)	12.4 (1.3 to 116.2)
Negative	137 (94%)	91 (98%)	1.0
Missing	*1*	*10*	χ^2 (1df)=7.2 *P*=0.03
Restricted to histologically verified cases and controls			
Positive	6 (6%)	2 (3%)	9.7 (1 to 95.7)
Negative	103 (94%)	63 (97%)	1.0
Missing	*0*	*7*	χ^2 (1df)=5.2 *P*=0.02

When the above analysis was restricted to cases and controls that were histologically verified, children who were HIV positive were almost 10 times more likely to develop Burkitt lymphoma (data shown in Table 4.12 above).

The analyses for malaria, EBV and HHV-8 antibodies were restricted to HIV negative children. Children with unknown HIV status were excluded.

4.3.6.2. Child malaria antibodies (for HIV negative children)

The malaria data was log transformed to assume normality. Seventy-one percent (n=91) of cases had high levels of malaria antibodies compared to 47% (n=42) of controls (see Table 4.13).

Table 4.13: Malaria antibodies for HIV negative cases and controls

[1]Malaria antibodies	Cases	Controls	Adjusted OR (95% CI)
Medium/High	91 (71%)	42 (47%)	2.4 (1.2 to 4.4)
Negative/Low	38 (29%)	48 (53%)	1.0
Missing	*9*	*8*	χ^2(1df)=6.9 P=0.01

4.3.6.3. Child EBV antibodies (for HIV negative children)

Sixty percent (n=77) of cases and 21% (n=19) of controls had high EBV antibody levels. The odds of developing Burkitt lymphoma were strongly associated with increasing titres of antibodies against EBV antigens (P_{trend}<0.001, see Table 4.14 below).

[1] Malaria antibody categories: Negative/low (OD < 0.2), Medium/high (OD ≥ 0.2)

Table 4.14: EBV antibody titres for HIV negative cases and controls

[2]EBV antibodies	Cases	Controls	Adjusted OR (95% CI)
Low	12 (9%)	38 (43%)	1.0
Medium	39 (31%)	32 (36%)	4.1 (1.6 to 10.1)
High	77 (60%)	19 (21%)	14.8 (5.8 to 38.5)
Missing	*10*	*9*	χ^2 (1df)=37.8 P<0.001
Number	128	89	
Median (IQR)	10240 (2560 to 10240)	1280 (640 to 2560)	
Mean titres (SD)	7109 (5058)	2992 (4064)	z=-6.93 P<0.001
95% CI of EBV	6224 to 7994	2136 to 3848	(rank sum)

The joint effects of EBV and malaria antibodies on the odds of Burkitt lymphoma were examined by estimating adjusted (for child age, sex and residence) odds ratios for children with raised levels of EBV antibodies only, raised levels of malaria antibodies only, and raised levels of both EBV and malaria antibodies, compared with children with low levels of both. Children with high levels of both EBV and malaria antibodies were estimated to be at 13 times the odds of being diagnosed with Burkitt lymphoma (OR=13.2, 95% CI 3.8 to 46.6, P<0.001). These data are shown in Table 4.15 below.

[2] EBV antibody categories: Low (≤ 1:640), Medium (1:1280 to 1:2560), High (1:5120 to 1:20480)

Table 4.15: Odds ratio estimates assessing joint effects of serological test results for EBV and malaria in case and control children. (Excluding HIV positive and missing cases and controls)

[3]EBV antibodies	[4]Malaria antibodies	Cases/Controls (126/89)	Adjusted OR 95% CI
Low	Negative/Low	5/22	1.0
	Medium/High	7/16	1.4 (0.3 to 6.3)
Medium/High	Negative/Low	32/25	5.7 (1.6 to 20.7)
	Medium/High	82/26	13.2 (3.8 to 46.6) χ^2 (1df)= 31 P<0.001

4.3.6.4. Child HHV-8 status (for HIV negative children)

HHV-8 antibodies were detected in 40% (n=51) of cases and 36% (n= 32) of controls.

4.3.7. Associations with maternal serological measures of infections

The results for all the laboratory tests for mothers are presented in Appendix 4.9. Sixteen percent (n=16) of mothers of children with Burkitt lymphoma and 24% (n=18) of mothers of controls were HIV positive (OR=1.3, 95% CI 0.6 to 2.9, P=0.5). The HIV status of 4 case and 4 control mothers was unknown.

Further analyses involving maternal EBV, malaria and HHV-8 were restricted to HIV negative mothers. Mothers whose HIV status was unknown were excluded. Sixty-one percent (n=50) of mothers of children with Burkitt lymphoma had high malaria antibody levels compared to 51% (n=29) of mothers of controls (OR=1.2, 95% CI 0.5 to 2.7, P=0.6). Seventy-nine percent (n=65) of case mothers and 70%

[3] EBV antibody categories: Low (≤ 1:640), Medium/High (1:1280 to 1:20480)
[4] Malaria antibody categories: Negative/low (OD < 0.2), Medium/high (OD ≥ 0.2)

(n=40) of control mothers had high EBV antibody titres (OR= 2.1, 95% CI 0.8 to 5.7, *P*=0.1). The levels of EBV antibody titres across number of sexual partner groups for case mothers were explored in Table 4.16. There was no statistically significant correlation between number of sexual partners and EBV antibody titres (*r*=-0.02).

Table 4.16: Comparison of maternal EBV antibody titres and number of self-reported lifetime sexual partners

Maternal EBV titres	**1 partner**	**2 to 4 partners**	**Test statistic**
Number	37	38	
Median (IQR	2560 (1280 to 5120)	2560 (1280 to 5120)	
Mean (SD)	4065 (3819)	4783 (6031)	z =-0.2 *P*=0.9
95% CI of mean	2792 to 5338	2801 to 6765	(rank sum)

HHV-8 antibodies were detected in 69% (n=53) of mothers of children with Burkitt lymphoma and 62% (n=35) of mothers of controls. Eighty-seven percent (n=71) of mothers of children with Burkitt lymphoma and 90% (n=52) of mothers of controls did not have current or previous syphilis infection (OR=1.2, 95% CI 0.4 to 3.8, *P*= 0.75).

4.3.8. Mother-child pairs measures of infections

Sixty-six percent (n=97) of Burkitt lymphoma cases and 69% (n=72) of control mother-child pairs were available for analyses.

4.3.8.1. Mother-child HIV status

Four of the 9 HIV positive Burkitt lymphoma cases had mothers available. Three of the case mother-child pairs and 1 of the control mother-child pairs had concordant HIV positive child and mother results. Thirteen cases and 17 controls who were HIV

negative had HIV positive mothers (Table 4.17). There was a higher proportion of HIV positive concordance between Burkitt lymphoma cases and their mothers than between controls and their mothers.

Table 4.17: Mother-child pairs HIV status, Burkitt lymphoma cases and controls

Child HIV status	**Mother HIV status**			
	Cases (n=96)		**Controls** (n=70)	
	Positive	Negative	Positive	Negative
Positive	3	0	1	0
Negative	13	80	17	52
Percentage concordantly positive	3%		1.4%	
	exact=0.64 *P*=0.05			

4.3.8.2. Mother-child malaria antibody status

For malaria, percentage concordantly high was defined as high child malaria antibody levels, and medium/high mother antibody levels. There was a higher proportion of high malaria antibody concordance between Burkitt lymphoma cases and their mothers than between controls and their mothers (Tables 4.18).

Table 4.18: Mother-child pairs malaria antibody status, for HIV negative Burkitt lymphoma cases and controls

Child malaria antibodies	**Mother malaria antibodies**			
	Cases (n=92)		**Controls** (n=70)	
	Low	Medium/High	Low	Medium/High
Negative/Low	14	14	24	14
High	26	38	13	19
Percentage concordantly high	41%		27%	
	χ^2 (1df) =3.5 *P*=0.06			

4.3.8.3. Mother-child EBV status

There was no correlation between child and maternal EBV antibody titres for both cases and controls. Table 4.19 shows the median and interquartile range of maternal EBV antibodies across child EBV categories. The median maternal EBV antibody titres were the same for almost all categories of child EBV titres.

Table 4.19: Mother-child pairs EBV antibody status, Burkitt lymphoma cases and controls

Child EBV antibodies	**Mother EBV antibodies (Median (IQR))**	
	Cases (n=93)	**Controls** (n=67)
Low	2560 (1280 to 5120)	2560 (1280 to 2560)
Medium	2560 (1280 to 10240)	5120 (2560 to 10240)
High	2560 (1280 to 5120)	2560 (640 to 10240)
	r=-0.09 P=0.36	r=0.01 P=0.99

4.3.8.4. Mother-child HHV-8 status

For HHV-8, percent concordantly positive was defined as positive HHV-8 status for both mother and child. There was no difference in the proportion of HHV-8 positive concordance between Burkitt lymphoma cases and their mothers and between controls and their mothers (Table 4.20).

Table 4.20: Mother-child pairs HHV-8 antibody status, HIV negative Burkitt lymphoma cases and controls

Child HHV-8 antibodies	**Mother HHV-8 antibodies**			
	Cases (n=92)		**Controls** (n=69)	
	Positive	Negative	Positive	Negative
Positive	28	8	21	5
Negative	31	25	25	18
Percentage concordantly positive	30%		30%	
	χ^2 (1df) =0 P=1.0			

4.4. Discussion

This case-control study of Burkitt lymphoma reports on both childhood and maternal risk factors for this cancer among children in Malawi. Specific attention has been focused on the potential role played by infectious diseases in children and their mothers.

Seventy-five percent of cases and 74% of controls had a histological verification of diagnosis, which is relatively high for an African series (Chokunonga et al.,2000; Sinfield et al.,2007). Burkitt lymphoma cases in most instances presented with metastatic spread of tumour at diagnosis. This may simply be a manifestation of the disease process since Burkitt lymphoma is a fast growing tumour with a very short doubling time of about 1 to 2 days, in combination with late tumour presentation (Ferry,2006). The jaws were the most common sites for primary tumour presentation, as has also been shown in other studies on Burkitt lymphoma in African children (Burkitt,1958; Burkitt et al.,1961).

Most case children were older than 5 years, the mean age at diagnosis was 7.1 years, which is similar to other studies (Morrow et al.,1976; Olweny et al.,1977; Williams et al.,1978; Olweny et al.,1980). In accordance with results from previous epidemiological studies from sub-Saharan Africa, Burkitt lymphoma is more likely to occur in boys (Morrow et al.,1976; Olweny et al.,1977; Williams et al.,1978; Olweny et al.,1980). The reasons for this are unclear, although it is true of most paediatric cancers. In some developing countries there is evidence that the sex

distribution is skewed because affected girls are less likely to be treated, but there is no evidence to support this in Malawi (Pearce et al.,2001).

In accordance with results from previous epidemiological studies from sub-Saharan Africa, this study has shown clear evidence that the odds of Burkitt lymphoma increased with increasing titres of EBV antibodies in the child (de Thé et al.,1978; Gesar et al.,1982). An association with high EBV antibody titres in the mother was also suggested, although there was no correlation between child and maternal EBV antibody titres. This is plausible because EBV may be acquired from other family members and not the mother, therefore a direct relationship between maternal and child EBV antibody titre was not expected (IARC,1997). Because Burkitt lymphoma is a systemic disease, it is possible that the raised antibody titres of EBV were as a result of the tumour rather than the cause of the disease (reverse causality), although the results of this case-control study are compatible with a prospective investigation (de Thé et al.,1978).

This study has also demonstrated a relationship between presence of high malaria antibodies in the child and increased risk of Burkitt lymphoma. The odds of Burkitt lymphoma increased with increasing malarial antibody titres in the child. Additional analyses demonstrated that Burkitt lymphoma mother-child pairs, compared to control mother-child pairs had more high malaria antibody concordant pairs. This probably illustrates that both mother and child, and possibly other family members are at high risk of malaria, and not necessarily that maternal malaria antibody levels have an impact on tumour formation in the child. This finding may suggest that malaria prevention may have the potential to decrease the risk of Burkitt lymphoma

in African children. Additional analyses would have included comparing malaria antibody levels against use of mosquito nets among controls. The expected result may have been that controls that used nets had lower malaria antibody levels than those who did not. This was not done because of the small sample resulting from the late introduction of relevant questions in the study.

Of particular interest is the evidence suggesting that EBV and malaria antibodies may act jointly in the development of Burkitt lymphoma. Compared with those who had low levels of both EBV and malaria antibodies, children with high levels of both antibodies had a 13 fold excess risk of developing Burkitt lymphoma. Although based on small numbers, this finding suggests that EBV and malaria may act in combination to increase the risk of Burkitt lymphoma in children. If real, the exact nature of the interaction between EBV and malaria is unclear. EBV viral load has been found to be highest in children in high malaria endemicity areas (Moormann et al.,2005). The two hypotheses that have been put forward to explain this are: malaria leads to reactivation and proliferation of the B cells that are latently infected with EBV, and malaria may lead to suppression of EBV-specific T cell immunity, which allows EBV to proliferate (Rochford et al.,2005). This dual action may be needed to facilitate tumour formation.

While cases and controls had the same self-reported prevalence of previous malaria episodes, these subjective measures are unlikely to give specific information about the child's history of malaria infection. The case-control analyses of Burkitt lymphoma demonstrated that the questionnaire data on exposure to malaria and serological malaria exposure data were discrepant, calling into to question the

reliability of the questionnaire data. Previous evidence for an association between Burkitt lymphoma and malaria has rested on ecological data describing an overlap in malaria endemic areas and occurrence of Burkitt lymphoma as described in Chapter 3 (Morrow,1982, 1985). Data suggesting a reduction in the occurrence of Burkitt lymphoma after malaria suppression programmes has also been important in strengthening the evidence for association between malaria and Burkitt lymphoma (Gesar et al.,1989). In this study, questionnaire data showed that households that did not have mosquito nets were more likely to have a child at increased risk of Burkitt lymphoma. While data were limited, children who developed Burkitt lymphoma had not previously used mosquito nets. This study has shown a relationship between the lack of use of mosquito nets by children and increased risk of Burkitt lymphoma. Although the sample size was small, the result was statistically significant. This relationship may suggest that use of mosquito nets could prevent not only malaria but also Burkitt lymphoma for which malaria is a risk factor. These data provide substantial evidence that malaria may be an important component in the aetiology of Burkitt lymphoma particularly when taken in conjunction with serological findings.

The evidence suggesting that HIV may act as a co-factor in the development of Burkitt lymphoma is interesting. The odds of Burkitt lymphoma were found to be increased in HIV positive children. This association with HIV has been demonstrated in only one other study conducted in Uganda (Newton et al.,2001). Most other reports on Burkitt lymphoma in African children have been cases series or reports on routinely collected data and have not been able to adjust for child age, sex and residence which are important confounding factors when addressing the issue of HIV. HIV may be acting as an indirect co-factor in the aetiology of Burkitt

lymphoma as it does in the aetiology of other HIV-associated lymphomas. HIV infection eventually reduces the effectiveness of T cell based immune functions (Douek,2003). It may do this by reducing the EBV-specific T cell function of the immune system; thereby leading to proliferation of EBV infected B cells and eventual tumour formation (Cohen,2000). Reducing the occurrence of HIV or treatment of HIV may lead to a reduction in the incidence of Burkitt lymphoma in Malawian children.

The majority of cases originated from rural districts which appears consistent with the lower occurrence of Burkitt lymphoma in urban areas as illustrated in previous studies (Morrow et al.,1974; Biggar et al.,1979). An explanation may be that children who live in rural areas may be exposed to a factor that predisposes them to developing the tumour, such as malaria, which has a higher prevalence in rural areas. It has been suggested in other studies that children of low socio-economic status might be at increased risk of Burkitt lymphoma (Morrow et al.,1974; Biggar et al.,1981; Oguonu et al.,2002). People who live in rural areas are of a lower socio-economic status and are usually not able to prevent episodes of malaria by using mosquito nets or other chemicals, which they may not afford to buy. This study shows that children living in households that were 'worse off' were at greater risk of Burkitt lymphoma. Markers of poverty may be a surrogate for the higher prevalence of malaria in poor rural areas and possible inability of the household to buy insecticides, mosquito nets or get treatment for malaria.

Dietary components as co-factors in the aetiology of Burkitt lymphoma have not been widely researched. As discussed in Chapter 3, some reports have suggested that

children who ate smoked or salted fish would be predisposed to Burkitt lymphoma, but this study has not found any evidence to support this view. It is important to add that the dietary data collected are self-reported, and are likely to be unreliable.

The odds of Burkitt lymphoma increased with increasing family size, as shown by the higher number of Burkitt lymphoma case siblings. This may be linked to exposure of the index child to EBV infection and re-infection from other family members as described in Chapter 2.

The odds of Burkitt lymphoma were also increased significantly with increasing number of maternal lifetime sexual partners. This finding is interpreted cautiously because number of sexual partners is self-reported, and the data provided may be inaccurate. An association with high EBV antibody titres in the mother was suggested. These findings although based on small numbers, coupled with the finding of increased sexual partners may suggest that mothers of Burkitt lymphoma cases may be exposed to EBV (could also be multiple strains of EBV) through sexual contact (Ranjit Thomas,2006; Higgins et al.,2007). The mother may then pass on EBV to their child, which predisposes them to tumour development. However, further analysis did not show any correlation between number of self-reported lifetime sexual partners and levels of EBV titres. Additional analyses also did not show any correlation between high maternal and child EBV titres. Mothers of children with Burkitt lymphoma were more likely to have a partner. There also appeared to be an increased risk of Burkitt lymphoma with history of self-reported sexually transmitted infections in the mother. However, when mothers were tested

for syphilis which is more reliable than self-reported data, there was no association. There was no increased risk of tumour associated with maternal HIV status.

4.5. Summary

The case-control study of Burkitt lymphoma in Malawian children strengthens the evidence associating Burkitt lymphoma with EBV and malaria. The evidence of an association between Burkitt lymphoma and HIV in children has also been enhanced. Associations found with reported use of mosquito nets in the home may suggest that malaria prevention has a role to play in decreasing the risk of Burkitt lymphoma in Africa. The role of maternal sexual history in the aetiology of Burkitt lymphoma remains uncertain.

CHAPTER FIVE

KAPOSI SARCOMA CASE-CONTROL STUDY

5.1. Introduction

Kaposi sarcoma was first described in 1872 by Hungarian dermatologist, Moritz Kaposi who reported five men with aggressive "idiopathic multiple pigmented sarcomas of the skin" (Antman et al.,2000). The tumour has a variable clinical course ranging from minimal mucocutaneous disease to extensive organ involvement.

This chapter starts by describing the clinical variants and features of Kaposi sarcoma. The epidemiology of this cancer among African children, about which little is known is summarised. Particular consideration is given to the aetiological role played by infections, especially HHV-8 and HIV. Data collection methods and data management of the case-control study have been described in Chapter 2. Results of the case-control analyses of Kaposi sarcoma are reported. This chapter concludes with a discussion of the key findings from these analyses.

5.2. Classification

Kaposi sarcoma is a spindle-cell tumour and is thought to be of endothelial cell origin (Antman et al.,2000). The tumour is usually classified into four clinical variants: classic, endemic, post transplant (or iatrogenic) and AIDS-associated (or epidemic) Kaposi sarcoma. Histologically, the tumours are identical, although behaviour and clinical features vary.

Classic Kaposi sarcoma is the name given to cases which occur mainly in elderly HIV uninfected people primarily from Southern European and Mediterranean countries. The median age at diagnosis is 65 years and it is much more common in men than in women, with a ratio as high as 15 to 1 (Antman et al.,2000). Typically, the disease involves the skin; often on the lower extremities. Endemic Kaposi sarcoma occurs in equatorial countries of Africa. This form of the disease often involves the skin and lymph nodes, and is seen in both children and adults. The median age at diagnosis of endemic Kaposi sarcoma is 40 years (IARC,1997).

Post transplant Kaposi sarcoma is observed in immunosuppressed organ-transplant recipients and patients receiving immunosuppressive treatment and tends to behave more aggressively (IARC,1997). In some instances, the disease regresses following cessation of immunosuppressive treatment.

AIDS-associated (or epidemic) Kaposi sarcoma develops in people who are infected with HIV. AIDS-associated Kaposi sarcoma carries a variable clinical course ranging from minimal mucocutaneous disease to widespread organ involvement. The lesions may involve the skin, oral mucosa, lymph nodes, lungs, and visceral organs.

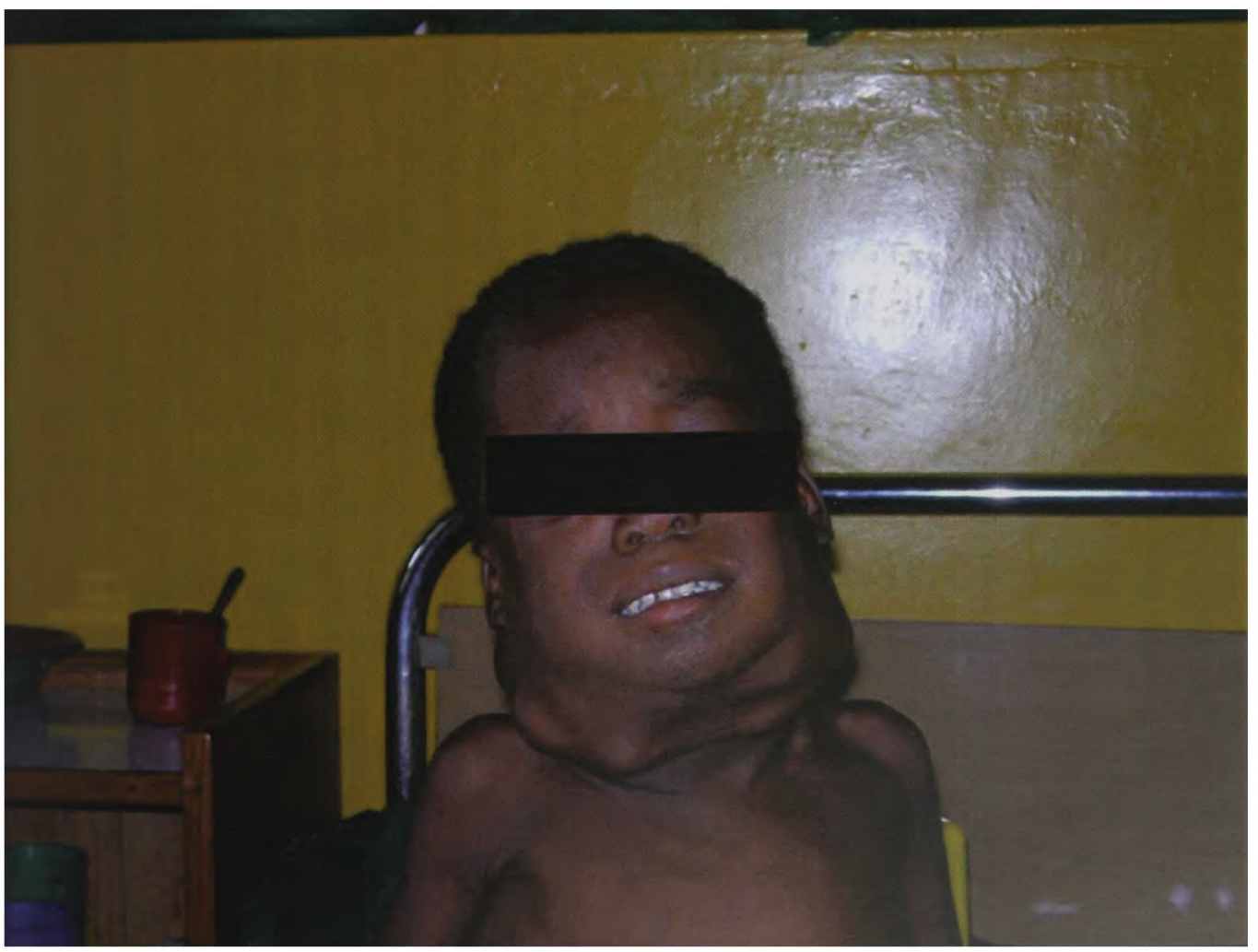
5.3. Clinical presentation of Kaposi sarcoma in African children

The presentation of Kaposi sarcoma in African children has a distinctive clinical pattern. It often involves multiple lymph nodes with either absent or sparsely sited skin lesions. Three distinct clinical patterns were identified in a study of 100 children (HIV negative and positive) with Kaposi sarcoma in Uganda in 1996 (Ziegler et

al.,1996). The first was dominated by lymphadenopathic Kaposi sarcoma lesions in the head and neck region. This is illustrated in Figure 5.1 which shows a 4 year old HIV positive child with head and neck lymphadenopathy. The second pattern was characterised by lesions in the inguinal region, including inguinal lymphadenopathy, with skin and ano-genital involvement. The third pattern involved solitary Kaposi sarcoma tumours on the extremities and in the abdomen.

HIV positive children with Kaposi sarcoma tend to be older than the HIV negative cases, and are more likely to present with generalised lymphadenopathy, with or without oedema (Athale UH et al.,1995; Kasolo et al.,1997; Sinfield et al.,2007). HIV positive cases are less likely to present with cutaneous or oral Kaposi sarcoma lesions.

Figure 5.1: A 4 year old HIV positive child with Kaposi sarcoma. (Picture taken in Blantyre and reproduced with permission)



Kaposi sarcoma that is associated with HIV/AIDS tends to have a more aggressive clinical course than in HIV uninfected children, although it may even regress in patients taking anti-retroviral therapy. The most common causes of morbidity include cosmetically disfiguring cutaneous lesions, lymphoedema or gastrointestinal and pulmonary involvement. Kaposi sarcoma in children is invariably fatal in Africa with respiratory failure secondary to pulmonary involvement being the most common cause of mortality.

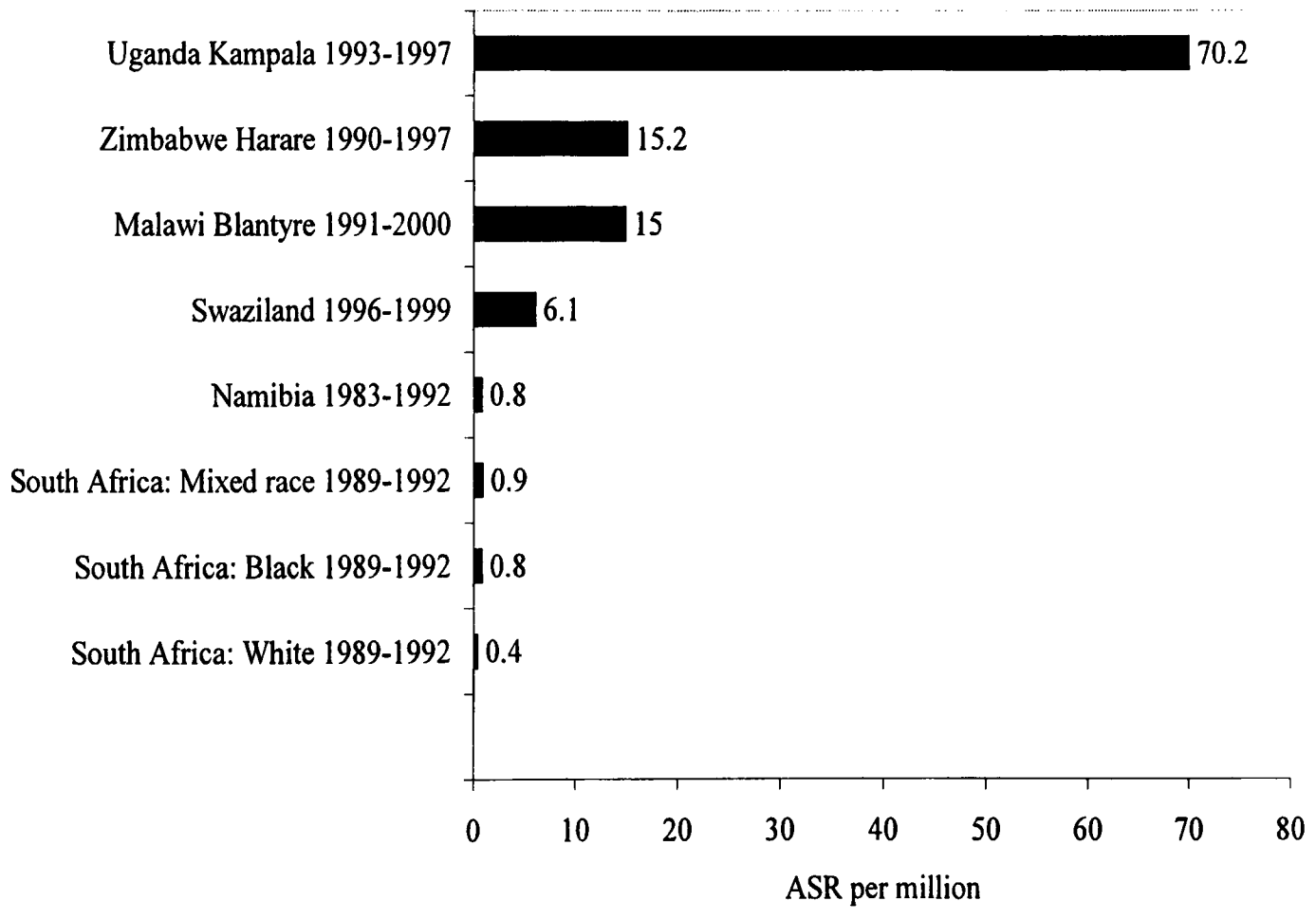
5.4. Epidemiology of Kaposi sarcoma in Africa

Kaposi sarcoma has been reported in Africa since the beginning of the 20th century. It presented as an indolent disease of the skin and lower limbs and was predominantly observed in elderly men and infrequently among women. The tumour was rarely seen in children before the AIDS pandemic (IARC,2003). Before the HIV epidemic in sub-Saharan Africa, Kaposi sarcoma showed great geographic variation in occurrence, and was thought to be as common in parts of sub-Saharan Africa, such as Uganda and eastern former Zaire, as colon cancer is in Europe and the United States (Cook-Mozaffari et al.,1998). In adults, there has been a progressive rise in incidence both in men and in women, in the era of HIV, such that in some countries it is now more common than all other cancers together (Wabinga et al.,1993; Ziegler et al.,1996; Chokunonga et al.,1999).

Most cancer registry data reports on disease in adults and very few childhood cancer cases are documented. As a result not much is known about childhood Kaposi sarcoma in Africa. Incidence rates in children have been reported to range from 1 per million children per year in Namibia to 70 per million children per year in

Uganda (IARC,1998). Figure 5.2 shows routinely collected cancer registry data for Kaposi sarcoma in children across Africa and is derived from two IARC reviews on childhood cancer (IARC,1998, 2003). The registration rates of childhood Kaposi sarcoma appears to be rising, and this may largely be due to the rising incidence of paediatric HIV infection. In some African countries such as Uganda, Malawi, Zimbabwe and Zambia, it is now one of the most common cancers in children (Wabinga et al.,1993; Athale UH et al.,1995; Chintu et al.,1995; Chokunonga et al.,2000; Sinfield et al.,2007). The occurrence of childhood Kaposi sarcoma appears to be substantially higher in Africa than anywhere else in the world, probably reflecting the background prevalence both of HIV and of the underlying cause, HHV-8 (IARC,1998).

Figure 5.2: Kaposi sarcoma ASR in Eastern and Southern African children aged ≤ 15 years. Data from (IARC,1998, 2003)



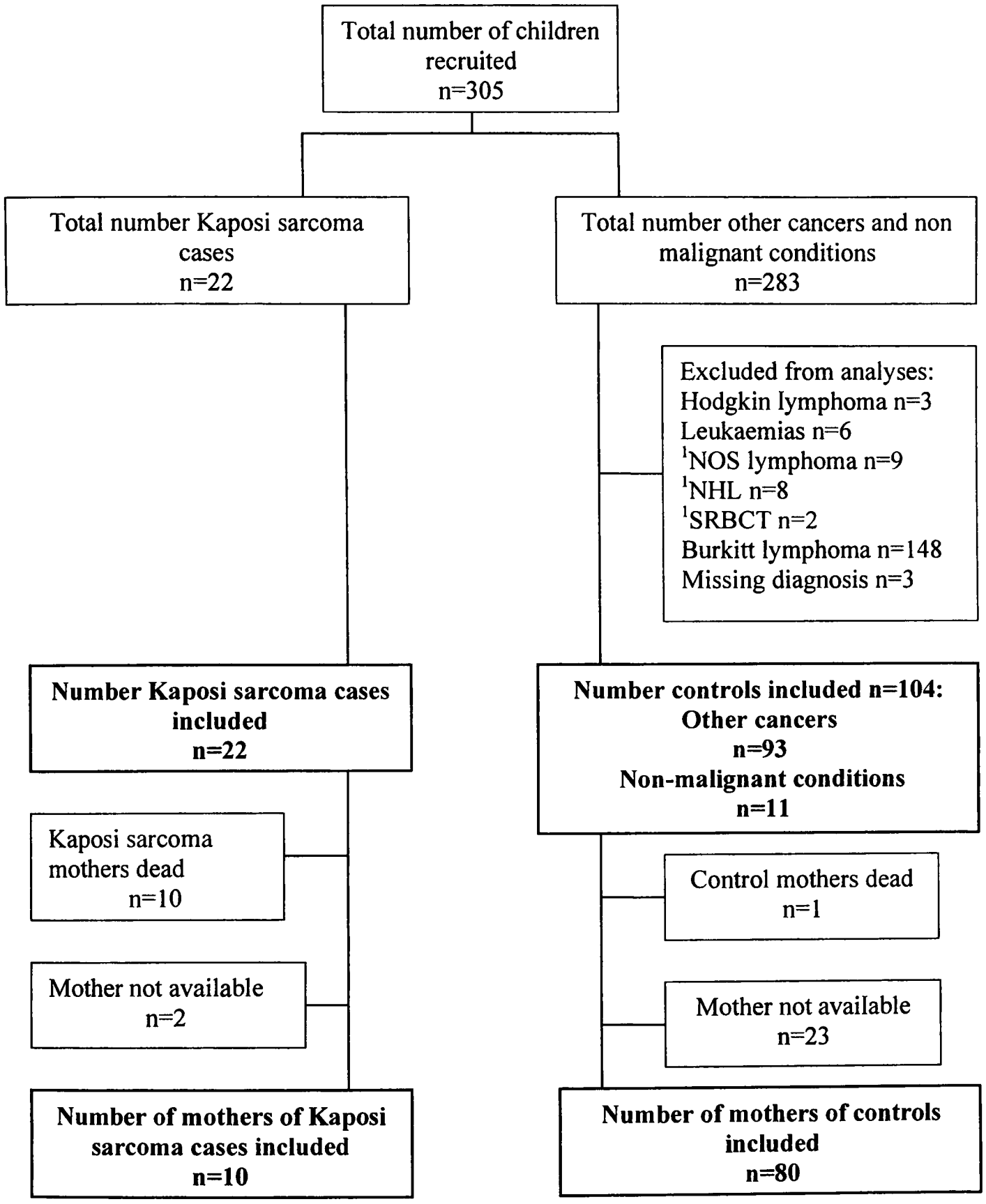
5.5. Kaposi sarcoma: Infectious disease aetiology

HHV-8 has been identified as the underlying causal agent of Kaposi sarcoma, but is not the only factor necessary for tumour formation (IARC,1997). Immunodeficiency caused by HIV or other factors is also known to facilitate development of the tumour. Similar to adults, studies conducted in Europe and Africa have demonstrated that children with HIV infection are at a greater risk of developing Kaposi sarcoma (Aricò et al.,1991; Chintu et al.,1995; Ziegler et al.,1996; Evans et al.,1997; Parkin et al.,1999; Chokunonga et al.,2000; Newton et al.,2001). A case-control study conducted in Uganda described the relationship between Kaposi sarcoma and HIV in children (Newton et al.,2001). Out of 36 Kaposi sarcoma cases recruited, 81% were HIV positive, and when compared to 190 controls, the children who were HIV positive were approximately 100 times more likely to develop Kaposi sarcoma ($P<0.0001$). In a 2006 hospital audit of childhood cancer in Malawi, of the 61 childhood Kaposi sarcoma cases identified, 56 had an HIV test done, and of those, 93% (n=52) were HIV positive (Sinfield et al.,2007).

5.6. Case-control study of Kaposi sarcoma

Of the 310 children recruited, 22 Kaposi sarcoma cases (both clinically and histologically diagnosed) and 104 controls were included in the analyses. Figure 5.3 shows the number of children and mothers recruited and included in the analyses. All haematological malignancies (n=26) and children older than 15 years (n=4) were excluded from the control group. The control group comprised 93 children with cancer and 11 with non-malignant conditions. A description of the controls is presented in Table 4.1 of Chapter 4.

Figure 5.3: Kaposi sarcoma cases and controls recruitment flow chart



[1]See list of abbreviations

5.6.1. Definition of cases and controls

Recruitment criteria for all children and their mothers were described in Chapter 2. Cases were defined as children aged 15 years or younger with a diagnosis (clinical or histologically verified) of Kaposi sarcoma. Controls were defined as children aged 15 years or younger with 'other cancers' or non-malignant conditions. Children with haematological malignancies were excluded from the control group because of the known association with HIV.

5.6.2. Methods

Data were analysed as described in Chapter 2. Analyses of data were conducted using a case-control study methodology. All ORs relating to data on children were adjusted for child's age (under 5 years, and 5 years and over), sex and residence (rural and urban). Odds ratios relating to data on mothers were adjusted for mother's age (under 30 years, and 30 years and over), and residence (rural and urban). In contrast to the Burkitt lymphoma case-control study, analyses that involved variables associated with child health, infections and maternal reproductive/sexual behaviour were not restricted to HIV negative children and mothers because of the small sample size.

5.7. Results

Detailed tables of results are presented in Appendices 5.1 to 5.7. All ORs presented in the text are adjusted as described in Section 5.6.2.

5.7.1. Sites of presentation of Kaposi sarcoma cases

Almost half of all Kaposi sarcoma patients presented with disease in the head and neck region (Table 5.1). This included lesions in the mouth and involvement of head and neck lymph nodes. Primary site of presentation was not known for 4 cases.

5.7.2. Basis of diagnosis

Only 23% (n=5) of Kaposi sarcoma cases had histologically verification of diagnosis as compared to 77% (n=17) of controls.

Table 5.1: Kaposi sarcoma cases sites of clinical presentation

Site	Number	Percent
Head and neck	10	45.5
Extremities	3	13.6
Other lymph nodes	2	9.1
Inguinal	1	4.5
Abdomen	1	4.5
Skin	1	4.5
Missing	*4*	*18.2*
Total	22	100

5.7.3. General characteristics of case children

The results of the analyses of variables included in this category are presented in Appendix 5.1.

Seventy-three percent (n=16) of Kaposi sarcoma cases were older than 4 years at recruitment and were on average 3 years older than controls (Table 5.2). Sixty-four percent (n=14) of cases and 55% (n=57) of controls were boys. Fifty-seven percent

(n=12) of Kaposi sarcoma cases and 30% (n=30) of controls lived in urban areas (OR 2.8, 95% CI 1.0 to 7.5, *P*=0.05).

Table 5.2: Child age distribution of Kaposi sarcoma cases and controls

Child age (in years)	**Cases**	**Controls**	**Test statistic** (rank sum)
0 to 4	6 (27%)	57 (55%)	
5 to 15	16 (73%)	46 (45%)	
Missing	*0*	*1*	
Number	22	103	
Median (IQR)	9 (4 to 12)	4 (2 to 8)	
Mean age (SD)	8.1 (4.0)	5.1 (3.9)	z =-3.0 *P*=0.003
95% CI of mean age	6.3 to 9.9	4.4 to 5.9	

5.7.4. Possible markers of infectious exposure

The variables described below were used as proxies to determine exposure of the index child to infections. The results of the analyses are presented in Appendix 5.2.

Ten mothers of children with Kaposi sarcoma compared to 1 mother of a control child were reported to have died, likely reflecting the high prevalence of HIV in this group (OR= 77.7, 95% CI 8.3 to 723.9, *P*<0.0001). Eight fathers of children with Kaposi sarcoma and 5 fathers of controls were reported to have died (OR= 9.1, 95% CI 2.4 to 34.9, *P*=0.001).

5.7.5. Characteristics of mothers

Only 10 mothers of children with Kaposi sarcoma were available for recruitment. Because of this small sample size, the case-control analyses of maternal characteristics were limited. Four of 8 mothers of children with Kaposi sarcoma and

27 of 69 mothers of controls were older than 29 years. Six mothers of Kaposi sarcoma cases and 65 of 78 mothers of controls had regular partners at the time of recruitment. Of the Kaposi sarcoma mothers that did not have partners, 2 were divorced, and 2 widowed. Detailed results are presented in Appendix 5.3 and 5.4.

5.7.6. Mother's sexual and reproductive history

Questionnaire data were available for 10 (45%) of the Kaposi sarcoma mothers and 80 (77%) of the control mothers. Of the remaining case mothers, 2 (10%) were not available to be interviewed, and 10 (45%) had died. For the control mothers, 23 (22%) were not available and 1(1%) had died as shown in Figure 5.3. Detailed results are in Appendix 5.4.

Results for self-reported number of lifetime sexual partners are shown in Table 5.3. Half of mothers of cases (50%) compared to one-third (34%) of mothers of controls reported 2 or more lifetime sexual partners.

Seven of the 8 mothers of children with Kaposi sarcoma and 68 of 77 of mothers of controls had a vaginal delivery. Thirty percent (n=3) of mothers of Kaposi sarcoma cases compared to 5% (n=4) of mothers of controls had episiotomies conducted (OR=9.2, 95% CI 1.4 to 58.5, P=0.02).

Table 5.3: Number of self-reported maternal lifetime sexual partners, Kaposi sarcoma cases and controls

Number of lifetime sexual partners	**Cases**	**Controls**	**Test statistic** (rank sum)
1	5 (50%)	49 (66%)	
2 or more	5 (50%)	25 (34%)	
Missing	*0*	*6*	
Number	10	74	
Median (IQR)	1.5 (1 to 2)	1 (1 to 2)	
Mean (SD)	1.7 (0.9)	1.5 (0.9)	z =-0.8 *P*=0.41
95% CI of mean	1.0 to 2.4	1.3 to 1.7	

5.7.7. Child serological measures of infections

Detailed results are presented in Appendix 5.5.

5.7.7.1. Child HIV status

HIV test results were available for 21 (95%) Kaposi sarcoma cases, and 94 (90%) controls. Over 80% (n=17) of Kaposi sarcoma cases and only 2% (n=2) of controls were HIV positive. The OR for HIV status was 763, suggesting that HIV positive children were more likely to be diagnosed with Kaposi sarcoma than HIV negative children (762.7, 95% CI 44 to 13376, $P< 0.001$). HIV negative Kaposi sarcoma cases were on average 4 years younger than the HIV positive cases ($P=0.001$) as shown in Table 5.4.

Table 5.4: Child age and HIV status of Kaposi sarcoma cases

Child HIV status			**Test statistic** (rank sum)
Age (years)	**Negative**	**Positive**	
Number	4	17	
Median (IQR)	4 (2 to 7)	9 (5.5 to 12)	
Mean (SD)	4.5 (3.4)	8.8 (3.8)	z =-3.3 *P*=0.001
95% CI of mean	-0.9 to 9.9	6.8 to 10.7	

5.7.7.2. Child HHV-8 status

Seventy-seven percent (n=17) of all Kaposi sarcoma patients had HHV-8 antibodies detected compared to 38% (n=36) of controls (OR=4.8, 95% CI 1.4 to 16.4, *P*=0.01). Eighty-eight percent (n=15) of the 17 Kaposi sarcoma cases with HHV-8 antibodies detected were HIV positive. Restricting analyses to HHV-8 positive cases and controls showed that Kaposi sarcoma cases had higher HHV-8 antibody levels than controls (Table 5.5).

Table 5.5: Comparison of HHV-8 antibody levels between Kaposi sarcoma cases and controls

HHV-8 OD	**Cases**	**Controls**	**Test statistic** (rank sum)
Number	17	36	
Median (IQR)	16.8 (2.4 to 22.1)	0.7 (0.4 to 2.6)	
Mean OD (SD)	14.1 (9.1)	3.18 (4.9)	z = -4.5 *P*<0.001
95% CI of mean	10.0 to 18.1	2.2 to 4.2	

5.7.7.3. Child EBV antibodies

Because of the small sample size, the median EBV titre of the control group was used as the cut-off to form 2 groups: low (≤ 1:1280) and medium/High (≥ 1:2560) instead of the 3 groups used for the Burkitt lymphoma case-control analyses. Ninety-

one percent (n=20) of cases and 47% (n=44) of controls had medium/high levels of EBV antibodies detected. The odds of Kaposi sarcoma was increased in children with high EBV antibody levels (OR=10.9, 95% CI 2.3 to 52.2, $P<0.001$). Although based on small numbers, HIV positive Kaposi sarcoma cases also appeared to have higher EBV antibody titres than HIV negative cases (Table 5.6). This excess was not statistically significant.

Table 5.6: EBV antibody titres in Kaposi sarcoma cases and controls

EBV antibodies	**Cases**	**Controls**	**Test statistic**
Number	22	95	
Median (IQR)	10240 (2560 to 10240)	1280 (640 to 2560)	
Mean (SD)	6778 (4021)	2968 (4031)	z =-4.0 $P<0.001$
95% CI of mean	4995 to 8561	2147 to 3790	(rank sum)
Restricted to HIV positive and negative Kaposi sarcoma cases			
EBV antibodies	**HIV positive**	**HIV negative**	**Test statistic**
Number	17	4	
Median (IQR)	10240 (5120 to 10240)	2560 (1280 to 6400)	
Mean (SD)	7717 (4007)	3840 (4434)	z =-1.8 $P=0.08$
95% CI of mean	5840 to 9595	-3216 to 10895	(rank sum)

5.7.7.4. Child malaria antibodies

Thirty-two percent (n=7) of Kaposi sarcoma cases and 48% (n=46) of the controls had medium/high malaria antibody levels detected.

5.7.8. Maternal serological measures of infections

Of the 10 mothers of children with Kaposi sarcoma who were available to be interviewed, 9 provided blood samples for laboratory analyses. Of the 104 control

mothers, 80 were interviewed and 76 provided blood samples or analysis. The results of the analyses are presented in Appendix 5.6.

Seven out of 9 mothers of children with Kaposi sarcoma, and 18 out of 75 mothers of controls tested were found to be HIV positive. HIV status for 1 case mother and 4 controls mothers was not known. Six of the 9 mothers of Kaposi sarcoma cases and 50 of the 76 control mothers had HHV-8 antibodies detected. Eight of the mothers of children with Kaposi sarcoma and 60 of the 77 mothers of controls had medium/high EBV antibody levels detected. One out of 9 mothers of Kaposi sarcoma cases and 35 out of 77 mothers of controls had high malaria antibodies detected. This difference was statistically significant, although the numbers studied were very small. One out of 9 mothers of cases and 11 out of 77 mothers of controls had current or past syphilis infection.

5.8. Discussion

Kaposi sarcoma has become an increasingly important childhood cancer in the era of HIV/AIDS. This case-control study reports on the risk factors for Kaposi sarcoma in Malawian children. The results presented are from a small group of cases who presented at the oncology ward in Blantyre. The results of the case-control analyses are interpreted cautiously because of the small sample size. The representativeness of these cases of all children with Kaposi sarcoma may be questioned. There is likely to be referral bias against Kaposi sarcoma patients, as primary health care providers associate the tumour with HIV disease, and consequently tend not to refer children for treatment. With the rolling out of paediatric anti-retroviral therapy in Malawi, children with Kaposi sarcoma lesions without other co-morbidities are usually only

seen in the HIV clinic, and not admitted to the oncology ward, and thus were not recruited into the study. It was not possible to determine the number of Kaposi sarcoma cases seen at the hospital but not recruited because the referral or admission diagnosis is often not accurate, and data not centrally stored.

In accordance with results from previous epidemiological studies from sub-Saharan Africa on adult Kaposi sarcoma, there was clear evidence that the odds of Kaposi sarcoma increased with the presence of antibodies against HHV-8 antigens in the child (Sitas et al.,2000; Newton et al.,2003; Ziegler et al.,2003). There was no association found between risk of Kaposi sarcoma in the child and presence of HHV-8 antibodies in the mother, although the numbers were small.

As expected, this study has demonstrated that Kaposi sarcoma is associated with HIV infection in children. The size of the odds ratio is in agreement with what is known about the association between HIV and Kaposi sarcoma in both children and adults (Newton et al.,2001; Mbulataiye et al.,2003). The confidence interval was wide because of the small sample size. Maternal HIV infection was also associated with increased risk of Kaposi sarcoma in the child. Kaposi sarcoma cases are likely to have acquired HIV infection through mother to child transmission either before delivery, during delivery or via breastfeeding (Peckham et al.,1995). A comparison of the characteristics of HIV positive and HIV negative Kaposi sarcoma cases was not possible because of the small sample size. Stratifying age of diagnosis according to HIV status in other studies has shown that HIV negative Kaposi sarcoma cases are generally younger than HIV positive cases, and this is confirmed here (Athale UH et al.,1995; Kasolo et al.,1997; Sinfield et al.,2007). The reason for this is not clear, but

may be associated with differences in the mode of transmission of HHV-8 and the mechanism involved in tumour formation between HIV seronegative and seropositive children.

An unexpected and interesting finding was that compared to controls, antibodies against EBV were elevated in children with Kaposi sarcoma. Kaposi sarcoma cases had on average statistically significant higher EBV antibody levels than controls. A review of the literature revealed a case-control study of Western adult males, nested in the District of Columbia Gay cohort study with similar results (Engels et al.,2003). Multiple markers of HHV-8 and EBV infections as predictors of AIDS-associated Kaposi sarcoma were examined. Levels of circulating EBV were elevated in persons who subsequently developed Kaposi sarcoma as compared to controls. Another study by the same author showed that people with AIDS-associated Kaposi sarcoma had an increased risk for EBV-associated immunoblastic lymphoma (Engels et al.,2001). The authors suggested that poor immune-mediated control of EBV in Kaposi sarcoma might partly explain the heightened risk for immunoblastic lymphoma.

Regarding EBV in the present case-control study of children, the number of Kaposi sarcoma cases was very small, but the odds ratio was large and statistically significant. The analysis was restricted to HIV negative cases and controls to try and evaluate the effect of HIV on the EBV antibody levels. Table 5.6 shows that HIV positive Kaposi sarcoma cases had higher EBV antibody titres than HIV negative cases. A possible explanation for the finding could be that the Kaposi sarcoma cases are HIV infected and thus probably immune suppressed. HIV establishes a progressive infection of the human immune system that leads to fatal

immunodeficiency that reduces the effectiveness of T cell based immune functions (Douek,2003). Regulation of the proliferation of EBV infected B cells is undertaken by EBV-specific T cells, and T cells from patients with AIDS suppress EBV-infected B cells less effectively than do cells from normal controls, resulting in proliferation of EBV-infected cells (Cohen,2000). An implication of this may be that immunodeficient Kaposi sarcoma children may be at risk of developing EBV-associated tumours, and subsequently present with multiple tumours. Another possible explanation for this finding is that children with Kaposi sarcoma may be presenting to hospital with 2 diseases, Kaposi sarcoma and a lymphoma that has not been diagnosed. As most children present with lymphadenopathic disease, it is possible that the lymphadenopathy may be as a result of Kaposi sarcoma and an undetected lymphoma. Histological examination of the lymph nodes is not done in most cases. Therefore, if a lymphoma was present it would be undetected. In this scenario, the raised EBV antibodies might be attributed to the lymphoma. Another aspect that would need to be considered in understanding the high levels of EBV in Kaposi sarcoma cases is possible cross-reactivity between the antigens of EBV and HHV-8 which are both gamma herpes viruses. The details of cross-reactivity are beyond the scope of this thesis.

Of particular interest is the result suggesting that mothers of children with Kaposi sarcoma were more likely to have had an episiotomy at the birth of the index child, a finding not previously reported. The sample size was very small (n=10), but the odds ratio was relatively large and statistically significant. There is no available literature describing a relationship between Kaposi sarcoma and mode of delivery. A possible explanation may be that pregnancy causes reactivation of HHV-8 leading to an

HHV-8 viraemia in the mother. Conducting an episiotomy may expose the child to maternal blood during delivery which might facilitate transmission of both HIV and HHV-8 to the child. Studies have demonstrated that pregnancy may lead to reactivation of other herpes viruses including herpes simplex viruses, HHV-6 and 7, although the role of pregnancy in HHV-8 reactivation is not well known (Dahl et al.,1999; Masahiro Ohashi,2002).

Data from Italy in 2006 suggested that pregnancy may induce HHV-8 replication in HIV infected women (Lisco et al.,2006). To investigate the impact of pregnancy on HHV-8 reactivation in HIV infected women, HHV-8 DNA presence and load were analyzed in peripheral white blood cells and cervico-vaginal secretions from 15 women co-infected with HIV and HHV-8. HHV-8 was detected in maternal white cells in 5 out of the 15 patients from the second trimester and in cervico-vaginal secretions in 5 women mainly from the third trimester. The HHV-8 load significantly increased late in pregnancy in maternal blood and cervico-vaginal secretions and was associated with a significant increase in HHV-8 shedding in the genital tract. The authors suggested that pregnancy may induce HHV-8 replication in HIV infected women, and an augmented HHV-8 load may, in turn, facilitate mother-to-child transmission. Thus the infant may present at birth with high HHV-8 viraemia indicating primary infection from the mother. It is plausible that an HIV and HHV-8 infected mother may have a higher level of HHV-8 viraemia during pregnancy, and the child may acquire the infection during pregnancy or labour. The role of episiotomies in the transmission of HHV-8 to children and how this impacts on Kaposi sarcoma development is not known and requires confirmation in larger studies.

Generally, Kaposi sarcoma is easily recognised clinically. This explains why almost 80% of cases did not have the tumour histologically verified. In accordance with work from Uganda, the majority of cases reported here presented with lesions in the head and neck (Ziegler et al.,1996). The majority of Kaposi sarcoma cases originated from urban areas, which appears consistent with the higher incidence of HIV in urban areas in Malawi (National Statistical Office,2004, 2005). An explanation could be that parents of children who eventually develop Kaposi sarcoma live in urban areas and engage in high risk behaviour that facilitates HIV infection. HIV may subsequently be transmitted to the child who later develops Kaposi sarcoma. Another explanation for the residential area difference may be that children from rural areas with Kaposi sarcoma are not being referred to hospital for treatment because health workers associate the tumour with HIV and may believe that appropriate and adequate treatment is not available.

The parents of Kaposi sarcoma patients were more likely to have died and in most cases the exact cause of death was not known or could not be verified. All Kaposi sarcoma cases whose parents had died were known to be HIV positive from serological tests conducted. It is probable that parents died as a result of HIV-related disease.

The Kaposi sarcoma case-control analyses study had several limitations. With only 22 Kaposi sarcoma cases, the statistical power was modest, and not all Kaposi sarcoma cases were histologically confirmed. These considerations might have lessened the ability to find associations with Kaposi sarcoma.

5.9. Summary

This case-control study of Kaposi sarcoma reconfirms the evidence that this tumour is associated with HIV and HHV-8 infection in children. EBV antibody titres were also found to be raised in Kaposi sarcoma cases. This might have implications for co-morbidities in children with Kaposi sarcoma. The role of maternal factors including HIV, HHV-8 status, and mode of delivery are important in determining development of Kaposi sarcoma in a child. Associations found with reported use of episiotomies during delivery of the child who subsequently develops Kaposi sarcoma may reflect a potential route of transmission for HHV-8.

CHAPTER SIX

PREVALENCE OF INFECTIONS AMONG CHILDHOOD CANCER CASES IN MALAWI

6.1. Introduction

In contrast with Burkitt lymphoma and Kaposi sarcoma, there has been very little epidemiological research conducted regarding Wilms tumour, retinoblastoma, rhabdomyosarcoma and other cancers in African children. In this chapter, the clinical features, epidemiology and characteristics are presented for each of these cancers, and for all the cancers combined. In contrast with the analytical approach applied to Burkitt lymphoma and Kaposi sarcoma, only descriptive statistics are presented for each of these cancers. The aim is to assess child and maternal factors that predispose to tumour formation in the child. To examine each cancer, detailed results are provided in Appendices 6.1 to 6.5. For comparative purposes, data for Burkitt lymphoma and Kaposi sarcoma are also shown. Interpretation of the results is limited by the small number of cases available but these are discussed in the final part of this chapter. As already stated in Section1.11, despite the prevalence of infections across different cancer types being the main objective, the results are presented here and not earlier because the case-control analyses of Burkitt lymphoma and Kaposi sarcoma clearly demonstrated the role that infections can potentially play in cancer development, making it easier to understand infections in other cancer types, and the small sample size other cancer types, resulting in any comprehensive analyses lacking adequate power.

6.2. Wilms tumour

Wilms tumour is an embryonal tumour, accounting for approximately 90% of all kidney cancers diagnosed in children younger than 15 years of age (IARC,2003).

6.2.1. Wilms tumour clinical features

An abdominal mass is the most common feature at presentation. Abdominal pain occurs in 30 to 40% of cases. Other signs and symptoms include hypertension, fever from tumour necrosis, haematuria, and anaemia. Wilms tumour may arise in one of 3 forms; sporadic, familial and those associated with various genetic syndromes (Green et al.,1996). The exact cellular mechanisms involved in the aetiology of the tumour are still being investigated.

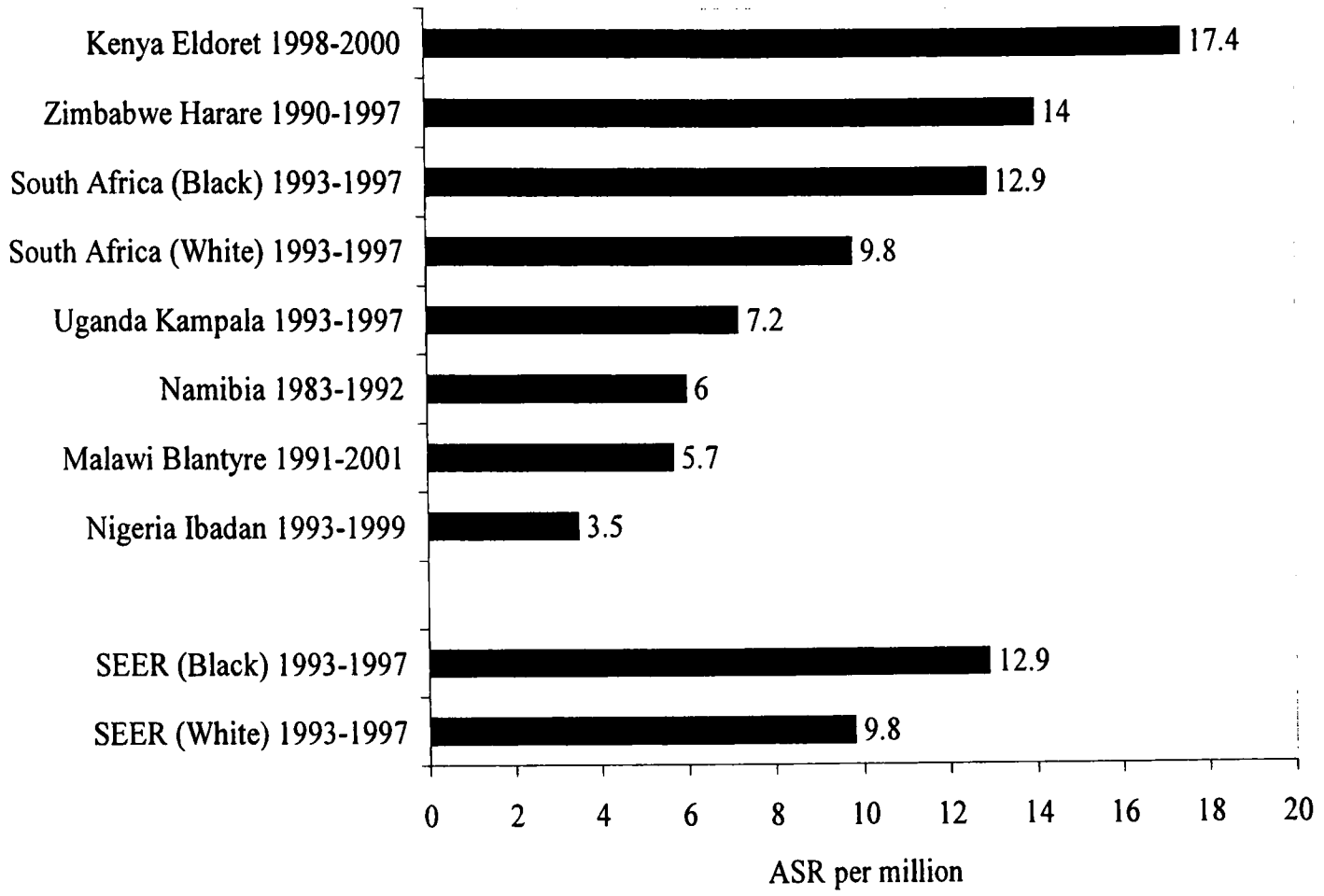
Wilms tumour may have associated anomalies, including aniridia, hemihypertrophy, cryptorchidism, and hypospadias (Green et al.,1996). The constellation of Wilms tumour, aniridia, genitourinary malformations, and mental retardation (WAGR syndrome) occurs in association with a deletion of varying length on chromosome 11. Children with pseudohermaphroditism and/or renal disease (glomerulonephritis or nephrotic syndrome) who develop Wilms tumour may have the Denys-Drash syndrome, which is associated with mutations within the same chromosomal segment as the WAGR syndrome. Hemihypertrophy may occur as an isolated abnormality or as a component of the Beckwith-Wiedemann syndrome, which includes macroglossia, omphalocele, and visceromegaly (Green et al.,1996).

6.2.2. Wilms tumour epidemiology

The reported incidence rate in Africa (derived from cancer registry data) ranges from 3.5 per million children per year in Nigeria to 17.4 per million children per year in Kenya. Figure 6.1 shows cancer registry data on the occurrence of Wilms tumour from Eastern and Southern African countries and SEER data in the 1990s (data from an IARC review) (IARC,2003). The highest incidence of Wilms tumour occurs in the second year of life, and the mean age at diagnosis is 3.5 years (IARC,2003).

In the past 3 decades, the multidisciplinary approach to this tumour by organisations such as the International Society of Paediatric Oncology (SIOP) and the National Wilms Tumour Study Group (NWTSG) has become an example for successful cancer treatment approaches. Survival rates of children with Wilms tumour in developed countries are approximately 80 to 90%. There are also signs that survival is improving in developing countries (Gemma et al.,2002; Davidson et al.,2006; Madani et al.,2006).

Figure 6.1: Registration rates of Wilms tumour (ASR per million) in East and Southern African children aged ≤ 15 years (Data from (IARC,2003))



6.2.3. Wilms tumour aetiology

In 1971, Knudson proposed a model to explain the age of onset and laterality in children with a familial or genetic history of retinoblastoma: the "two-mutation hypothesis" (Knudson,1971). A similar model for Wilms tumour was proposed which predicts that tumour formation depends on two genetic events (Knudson et al.,1972). Here, children with genetic susceptibility are thought to have a constitutional lesion, either inherited from a parent or resulting from a spontaneous mutation. Only one new genetic event is needed, and this greatly increases the likelihood of tumour formation. Paternal employment and exposure to pesticides or metals has been associated with risk of Wilms tumour in some studies although there

is no consistent association for some of these, particularly that between Wilms tumour and paternal employment in agriculture (Little,1999).

6.3. Retinoblastoma

6.3.1. Retinoblastoma clinical features

Retinoblastoma is a paediatric malignant tumour of the developing retina (the nervous tissue of the eye), occurring usually before 5 years of age. The tumour may arise in one or both eyes. Children with retinoblastoma present in a variety of ways. Most children will initially present with leukocoria (also called a white pupillary reflex) instead of a normal healthy black pupil or red reflex, as illustrated in Figure 6.2 (Aventura et al.,2005). A squint is the second most common presentation. The child's eye may turn out (toward the ear) or turn in (toward the nose). Patients may also present with a red painful eye, poor vision and an enlarged or dilated pupil. Proptosis is a more common presenting symptom in most underdeveloped countries because the patients present to hospital when the tumour has already reached an advanced stage. Total destruction of the affected eye, as illustrated in Figure 6.3 is not uncommon in Africa (Chantada et al.,1999).

Figure 6.2: Retinoblastoma early presentation: White reflex. (Picture taken in Blantyre and reproduced with permission)

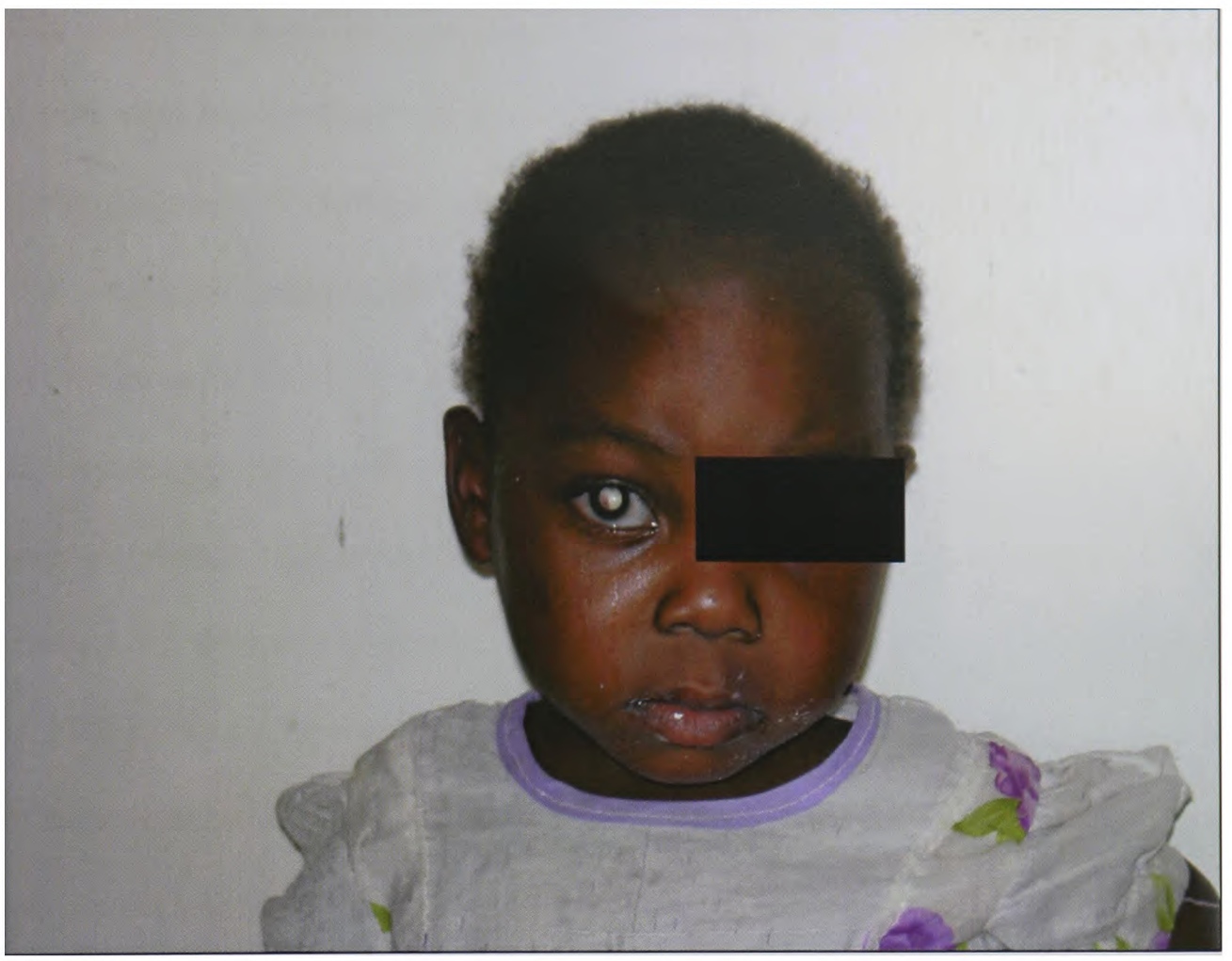
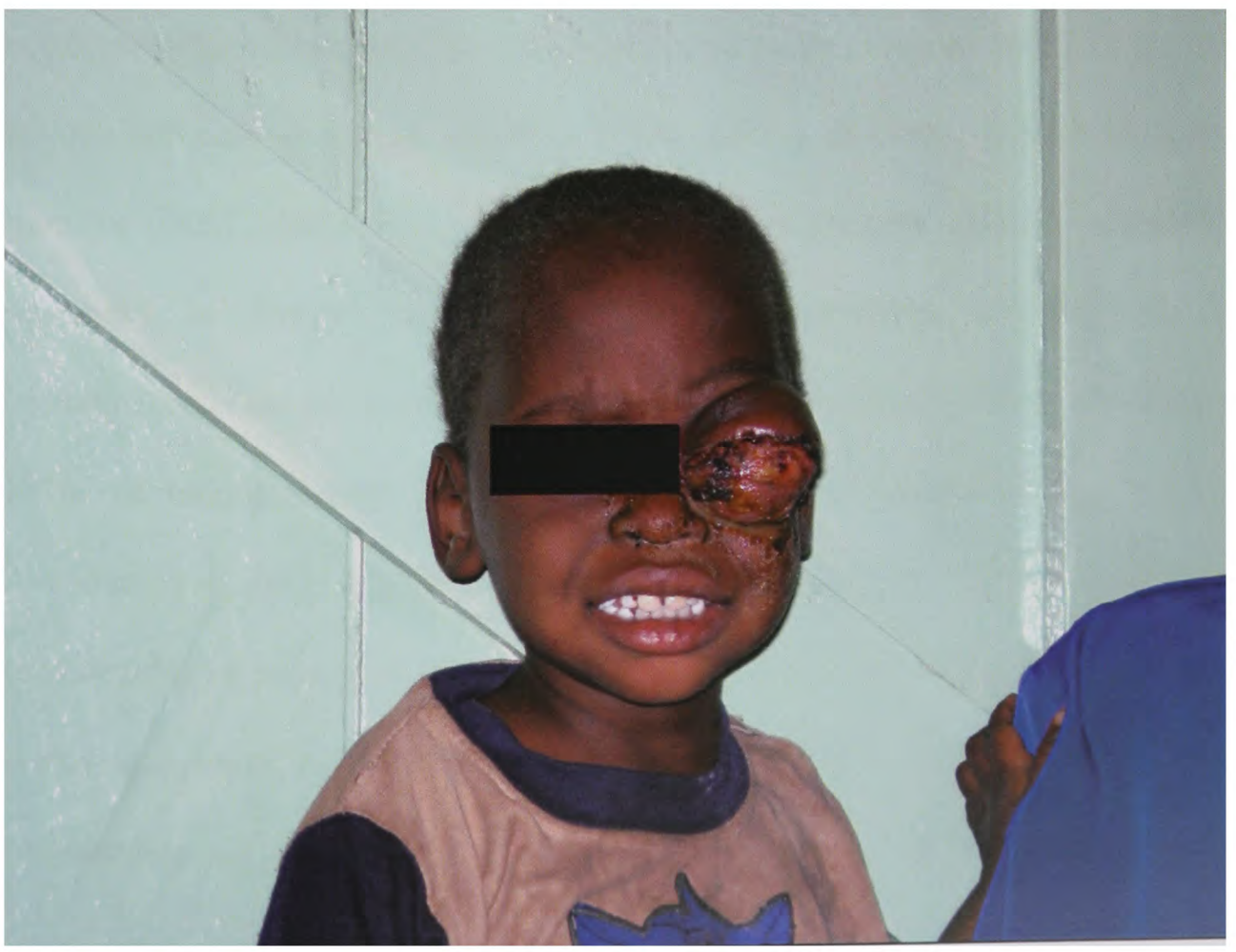


Figure 6.3: Retinoblastoma late presentation; Total destruction of the eye. (Picture taken in Blantyre and reproduced with permission)



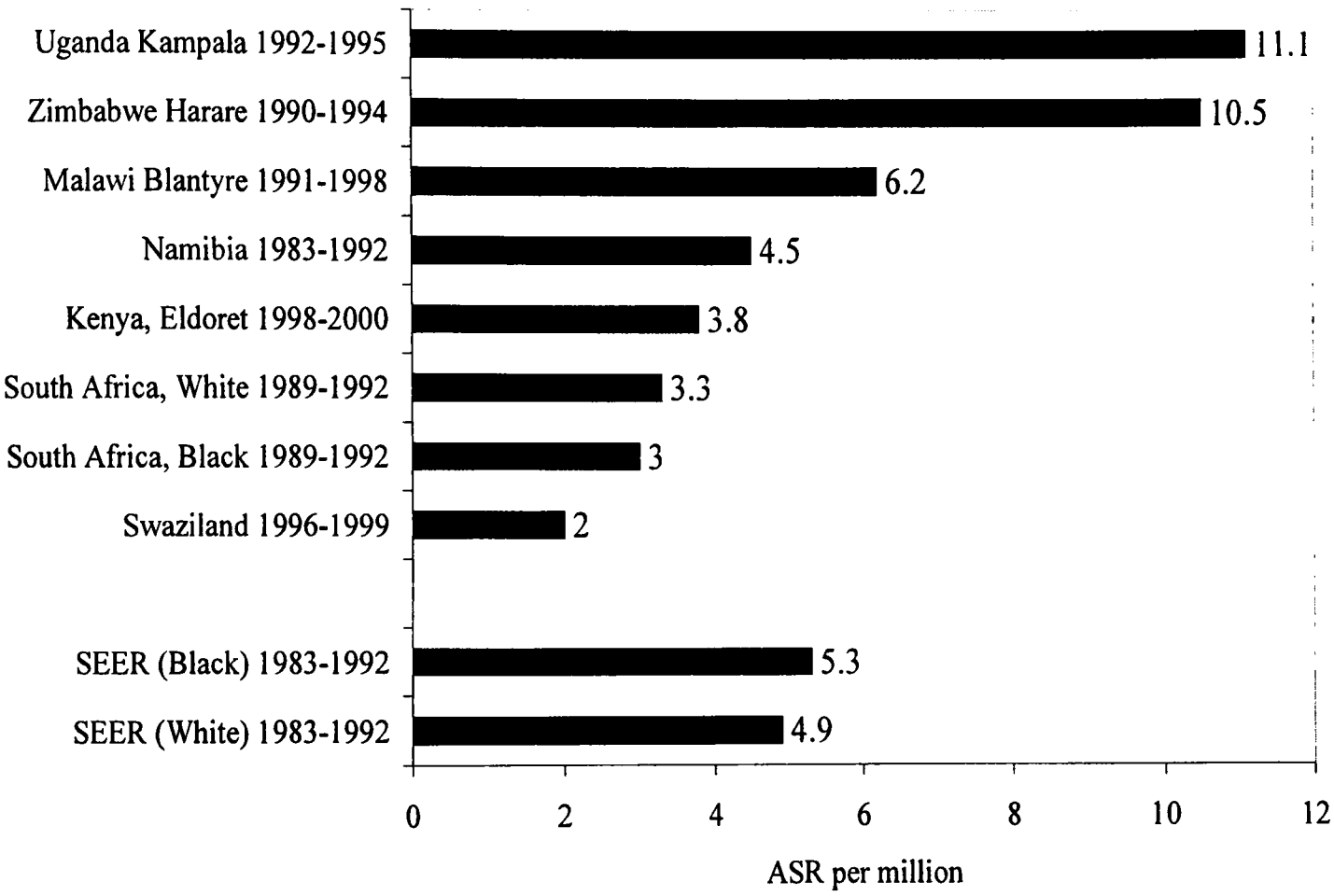
Most data about retinoblastoma in Africa have been from small case series. Very little epidemiological work has been done in Africa, and occurrence of tumour in one or both eyes has not been actively investigated (IARC,2003). Generally, the average age at diagnosis of children with bilateral eye tumours is 13 months, while patients with unilateral retinoblastoma are diagnosed at an average age of 24 months (Aventura et al.,2005). Bilateral disease occurs more frequently in the inherited form of disease. Children with unilateral retinoblastoma are thought to come from low socio-economic backgrounds, and usually present with more advanced disease, because of delayed diagnosis (Chintu et al.,1995; Sahu et al.,1998; Kaimbo et al.,2002). The distribution of tumour laterality in Africa is not known as the number of tumour cases included in case series analyses has been very small and laterality has not been actively sought (IARC,2003).

6.3.2. Retinoblastoma epidemiology

Retinoblastoma is a relatively common childhood malignancy in developing countries. Registration rates have been reported to be between 10 to 24 per million children per year in Africa, and 3 to 5 per million children per year in developed countries (IARC,2003). Figure 6.4 presents cancer registry data on retinoblastoma occurrence in Eastern and Southern African countries (IARC,1998, 2003). Retinoblastoma has the lowest median age at diagnosis of all childhood malignancies (15 to 18 months), with almost all cases diagnosed before the age of 5 years (Aventura et al.,2005). The incidence of retinoblastoma is thought to be increasing in some developing countries, although it is not known if this is as a result of increased tumour awareness in the community or increase in actual tumour numbers, or completeness of registration data (Chintu et al.,1995; Bekibele et al.,2003;

Khandekar et al.,2004). Prognosis in Africa and other developing countries is poor compared to that in developed countries, primarily because of delays in diagnosis (Chantada et al.,1999; Chang et al.,2006).

Figure 6.4: Registration rates of retinoblastoma (ASR per million) in East and Southern African children aged ≤ 15 years



6.3.3. Retinoblastoma aetiology

Like Wilms tumour, retinoblastoma is thought to be caused by two mutational events (Knudson,1971). Familial retinoblastoma arises in children who carry the retinoblastoma gene, which is a mutation in the long arm of chromosome 13 inherited from one or both parents; sporadic retinoblastoma develops as the result of a new mutation. Little is known about the role of non-genetic factors in the aetiology of retinoblastoma.

It has been proposed that an infectious agent may play a role in the aetiology of retinoblastoma, and that this may be passed on from the mother to the child at some stage during pregnancy, at delivery or later during infancy (Bunin,2004). No infection has yet been conclusively identified.

A matched case-control study of retinoblastoma (involving cases from the US and Canada conducted between 1982 and 1985) investigated the hypothesis that post conception exposures may affect the risk of unilateral disease (Bunin et al.,1989). Children whose mothers had low education levels were five times more likely to develop retinoblastoma. Maternal exposure to gestational X-rays was associated with a two-fold risk of disease. Use of peri-conceptional barrier contraception was also found to be a protective factor, and reduced the risk of a child developing retinoblastoma. The use of barrier methods of contraception was associated with a reduced incidence of both retinoblastoma and HPV16/18 infection. The authors suggested a role for HPV in the aetiology of retinoblastoma.

A hospital-based prevalence epidemiological study nested within a larger case-control study conducted in Mexico between 1994 and 1995 further addressed the relationship between HPV infection and retinoblastoma occurrence (Orjuela et al.,2000). Forty children with retinoblastoma and no family history of the tumour were recruited, and 39 tumour tissues were analysed for HPV16/18 DNA. HPV16/18 DNA was detected in 36% (14/39) of the tumour tissues analysed. A case-series conducted in Brazil, also detected HPV16/35 DNA in retinoblastoma tissues. The authors also suggested that HPV may play a role in the aetiology of the tumour (Palazzi et al., 2003). However, a recent American descriptive study did not show

any relationship between HPV and retinoblastoma (Gillison,2007). Of the 40 retinoblastoma tumour tissues analyzed using PCR all of the 18 oncogenic HPV types previously associated with cervical cancer, all were negative for high-risk HPV genomic sequences, and for 19 low risk HPV types. On the basis of evidence accumulated to date, it is therefore unclear if HPV is associated with retinoblastoma development, and if it is, what the biological mechanism might be.

6.4. Rhabdomyosarcoma

6.4.1. Rhabdomyosarcoma clinical features

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children and may arise at any anatomic site (Arndt et al.,1999). Signs, symptoms and prognosis are site-dependent. The most common sites are the head and neck, extremities and genitourinary tract. Other frequent sites include the trunk, orbit and retroperitoneum. Tumours arising in the orbit may lead to proptosis; tumours of the limbs are generally manifested as a painless mass. Most tumours arising in the orbit are embryonal, and are localized at presentation. In contrast, almost half of tumours of the limbs are alveolar on histologic analysis, and in about half of cases there is regional lymphatic spread at diagnosis; the prognosis is therefore much less favourable (Arndt et al.,1999).

Rhabdomyosarcomas have been reported to have associations with familial cancer syndromes characterized by a high risk for early breast cancer, adrenal cortical cancers, gliomas, haematopoietic cancers, and other bone and soft tissue sarcomas (Little,1999). Although the tumour is believed to arise from primitive muscle cells, tumours can occur anywhere in the body except bone (Parham et al.,2006).

6.4.2. Rhabdomyosarcoma epidemiology

Rhabdomyosarcoma is the most frequent soft-tissue sarcoma of childhood in most regions of the world, although in Africa, it is less frequent than Kaposi sarcoma. Estimated registration rates are 4 to 6 per million children per year in Europe, the Americas, and Oceania (Stiller,2004). In Africa, the incidence of rhabdomyosarcoma is thought to be very low, which may be a consequence of under-ascertainment resulting from under-diagnosis.

6.5. Methods

The prevalence of different exposures among children with different cancer sites or types are presented. For these prevalence estimates, tables were created for cancers including Wilms tumour, retinoblastoma, rhabdomyosarcoma and other cancers and non-malignant conditions. As already mentioned, data for Burkitt lymphoma and Kaposi sarcoma are also shown for comparative purposes. The detailed results are shown in Appendices 6.1 to 6.5.

6.6. Results

Descriptive statistics for the 3 cancer types discussed here, and all other cancer types will be presented side-by-side.

Seventy percent (n=196) of all tumours and 73% (n=8) of non-malignant conditions were diagnosed by morphological verification. Almost all Wilms tumour cases were diagnosed by morphological verification (96%). In contrast, Kaposi sarcoma and retinoblastoma cases had the smallest proportion diagnosed histologically (23% and

44% respectively). Fifty-nine percent (n=10) of Rhabdomyosarcoma cases were histologically diagnosed.

6.6.1. Child demographic characteristics

The age range for all cancers and non-malignant conditions was 0 to 15 years. All retinoblastoma cases were aged 4 years and below, and had the youngest mean age compared to all the cancers combined (2.8 years and 6.8 years respectively). Kaposi sarcoma cases were the oldest children recruited, followed by Burkitt lymphoma cases (mean age 8.1 years and 7.1 years respectively). There were more boys than girls for all cancer types (59% and 41% respectively); while there were more girls than boys for the non-malignant conditions (55% and 45% respectively). A greater number of all cancer types lived in rural areas (ranging from 62% to 78%). Only 43% (n=9) of Kaposi sarcoma cases lived in rural areas. Detailed results are presented in Appendix 6.1.

6.6.2. Possible markers of infectious exposure

Details of possible proxies of infection analysed are presented in Appendix 6.2. All mothers of children with Wilms tumour, retinoblastoma and non-malignant conditions were alive at the time of recruitment. In contrast, 45% (n=10) of mothers and 36% (n=8) of fathers of children with Kaposi sarcoma had died. Between 10 and 18% of the fathers of children with other cancers and non-malignant conditions had died.

6.6.3. Mother's reproductive and sexual history

Questionnaire data about maternal reproductive and sexual history was not available for all the mothers because they were not available to be interviewed, either because

they were not at the hospital or they had died. Details of variables described below are presented in Appendix 6.3.

Approximately over 80% of mothers of children with Wilms tumour, retinoblastoma and rhabdomyosarcoma had a regular partner, similar to the mothers of children with non-malignant conditions and other cancers. In contrast, only 60% (n=6) of Kaposi sarcoma mothers had a partner at the time of recruitment.

The number of maternal lifetime sexual partners was self-reported, and the reliability of the data may be questionable. Seventy-one percent (n=15) of mothers of children with Wilms tumour reported having only 1 sexual partner, while 50% of mothers of children with Kaposi sarcoma (n=5) and retinoblastoma (n=7) reported having only 1 sexual partner.

Normal vaginal deliveries were recorded for over 80% of Wilms tumour, retinoblastoma, rhabdomyosarcoma, all cancers and non-malignant conditions. In contrast, only 70% (n=7) of mothers of Kaposi sarcoma cases were recorded as having had a normal vaginal delivery, whilst 30% (n=3) had an episiotomy performed.

6.6.4. Child serological measures of infections

Details of child laboratory test results are presented in Appendix 6.4.

6.6.4.1. Child HIV status

The overall HIV seroprevalence for all cancers excluding known HIV-associated cancers (Kaposi sarcoma, non-Hodgkin lymphoma, Hodgkin lymphoma and not

otherwise specified lymphomas) was 4% (10/237). The HIV seroprevalence for Burkitt lymphoma cases was 6% (9/146), and Kaposi sarcoma had the highest HIV seroprevalence of 81% (17/21). Of the Wilms tumour, retinoblastoma and rhabdomyosarcoma cases tested for HIV, none were HIV positive. It is important to note that 6 out of the 18 retinoblastoma cases did not have an HIV test done. One Wilms tumour mother-child pair had discrepant HIV test results; the child was HIV positive, and the mother HIV negative. Repeat testing was not done for this pair, and they were not included in the analyses. The tables and graph in Appendix 7.1 shows the seroprevalence of HIV across different cancer types adjusted for child age, sex and residence. The graph shows that there were significant differences of HIV seroprevalence across the cancer types.

6.6.4.2. Child malaria antibodies

Medium/high malaria antibodies were detected in 59% of all cancer types. Children with Burkitt lymphoma had the highest medium/high malaria antibody levels (68%). The table and graph in Appendix 7.2 shows the seroprevalence of malaria antibodies across the different cancer types. After adjusting for child age, sex and residence, there was minimal change in antibody seroprevalence across the cancer types, with Burkitt lymphoma cases tending to have higher malaria antibody levels. This was also true when the analyses were restricted to HIV negative children (Appendix 7.3).

6.6.4.3. Child HHV-8 status

Antibodies against HHV-8 were detected in 45% (n=121) of all cancer cases. Fifty-seven percent (n=8) of retinoblastoma cases and only 29% (n=5) and 24% (n=7) of rhabdomyosarcoma and Wilms tumour cases respectively, had HHV-8 antibodies detected. The adjusted seroprevalence graph in Appendix 7.4 shows that there was

significant heterogeneity of HHV-8 seroprevalence across the different cancer types, and after adjustment, the seroprevalence in the retinoblastoma cases increased from 57% to 80%, which was similar to seroprevalence in the Kaposi sarcoma cases. This increase was attributed to the effect of age. This was also true when the analyses were restricted to HIV negative children (see Appendix 7.5).

HHV-8 occurrence in retinoblastoma cases was further analysed. The ages of retinoblastoma cases with HHV-8 antibodies detected were explored and shown in Table 6.1. There were no retinoblastoma cases aged less than 12 months. The data suggests that there was no difference in age between HHV-8 positive and negative retinoblastoma cases.

Table 6.1: Retinoblastoma child HHV-8 status and age in months

Child age in months	**Child HHV-8 antibodies**		
	Positive	**Negative**	**Test statistic**
Number	8	6	
Range	21 to 50	27 to 53	
Median age (IQR)	38 (28 to 43.5)	35.5 (28 to 50)	
Mean age (SD)	36.3 (10.3)	38.2 (11.5)	z=0.5 *P*=0.6
95% CI of mean age	27.6 to 44.9	26.1 to 50.2	(rank sum)

Table 6.2 below describes retinoblastoma mother-child pairs and HHV-8 occurrence. Fifty-seven percent (n=4) of the retinoblastoma mother-child pairs concordantly had HHV-8 antibodies detected. HHV-8 antibodies were also detected in one case whose mother did not have antibodies detected.

Table 6.2: HHV-8 antibody levels in retinoblastoma mother-child pairs

Child HHV-8 antibody levels	Mother HHV-8 antibody levels	
	Positive	Negative
Positive	4 (57%)	1 (33%)
Negative	3 (43%)	2 (67%)
Total	7 (100%)	3 (100%)

6.6.4.4. Child EBV antibodies

Burkitt lymphoma cases had the highest seroprevalence of medium/high EBV antibodies detected. Approximately one third of children with Wilms tumour, rhabdomyosarcoma and other cancers had high antibodies against EBV detected. However, only 7% (n=1) of retinoblastoma cases had high antibody levels detected. The seroprevalence table and graph in Appendix 7.6 shows that there was significant heterogeneity of EBV (medium/high) seroprevalence across the cancer types, with the highest seroprevalence in the Burkitt lymphoma cases. After adjustment, there was minimal change in the seroprevalence in all the diagnostic categories. The adjusted seroprevalence did not change when the analyses were restricted to HIV negative children (Appendix 7.7).

6.6.5. Maternal serological measures of infections

Details of maternal laboratory test results are presented in Appendix 6.5. The maternal HIV seroprevalence for all cancers was 21% (39/188). About three quarters of mothers of children with Kaposi sarcoma were HIV positive. Half of the Rhabdomyosarcoma mothers were HIV positive compared to approximately 20% of mothers of children with retinoblastoma and Wilms tumour.

The seroprevalence of malaria antibodies was approximately 50% for mothers of children with all cancer types except Kaposi sarcoma (11%). The seroprevalence of maternal HHV-8 antibodies for mothers of children with all cancers ranged from 60 to 70%. Mother whose children had non-malignant conditions had a higher seroprevalence of 80%. Between 65% and 92% of mothers had detectable EBV antibodies and syphilis was uncommon for mothers of children of all the cancer groups.

6.7. Discussion

The descriptive analyses of Wilms tumour, retinoblastoma and rhabdomyosarcoma have been presented in this chapter. There were no *a priori* hypotheses formulated because it was expected that the sample size of the specific cancer types would be too small to conduct detailed case-control analyses.

The difference in the age at presentation of the 3 cancers was expected and consistent with previous reports. All retinoblastoma cases were less than 5 years old, and rhabdomyosarcoma occurred in slightly older children. There were more boys presenting with all 3 tumours, and most cases lived in rural areas.

There appeared to be no association between the 3 cancer types and HIV infection, although it is important to note that not all retinoblastoma cases were tested. Of particular note is the unexpected finding that after adjustment for child age, sex and residence, retinoblastoma cases appeared to have a higher HHV-8 seroprevalence than other cancers apart from Kaposi sarcoma. This finding has not been previously reported. Over 50% of retinoblastoma cases whose mothers had HHV-8 detected

were also HHV-8 positive. A proportion had HHV-8 seronegative mothers and may have been infected from another sources (Plancoulaine et al.,2000; Dedicoat et al.,2004).

The supposed association between HHV-8 and retinoblastoma needs to be interpreted with great caution because of the small sample size, and the fact that antibodies detected in some of the cases may have been passively acquired from the mother. The sample size was too small to effectively evaluate the effect of age on HHV-8 antibody presence in the child versus antibody presence in the mother. Other studies have found that HHV-8 antibodies can be passively transmitted from the mother to the child, and that might be the explanation for this finding, especially because the cases were very young (Mantina et al.,2001). In addition, the epidemiology of retinoblastoma does not match that of HHV-8 so this could be a chance finding.

The prevalence of maternal HIV infection for Wilms tumour and retinoblastoma was similar (23% and 21% respectively), but was higher for rhabdomyosarcoma (50%). This might indicate that rhabdomyosarcoma may be associated with maternal HIV infection, although this does not seem likely as all the rhabdomyosarcoma cases were HIV negative. This result also needs to be treated cautiously as only 59% of rhabdomyosarcoma cases had histologically verified diagnosis. Misclassification for this tumour type was a possibility. The other maternal infections tested; HHV-8, EBV, malaria and syphilis did not show a substantial difference in prevalence between the 3 cancer types.

Retinoblastoma cases were less likely to have a morphologically verified diagnosis made compared to all the other cancers; slightly over half of retinoblastoma cases were clinical diagnosed (56%). This is mainly a function of the administrative co-ordination between the eye ward and the pathology department. Retinoblastoma tumour samples are sent for pathological examination after the eye has been removed as part of treatment. The child's family is expected to bear the cost of the histological examination, but as a large proportion of families can not afford to pay, this is not done. Wilms tumour had the most accurate mode of diagnosis done, this may be because in all cases, the child under went surgery where the affected kidney was removed, and a thorough pathological examination conducted post-operatively. Almost half of all rhabdomyosarcoma cases did not have morphological diagnosis made. The reason for this is not known.

Questionnaire data about self reported maternal reproductive and sexual history did not reveal any differences between the Wilms tumour, retinoblastoma, rhabdomyosarcoma and all cancers types for the variables examined.

6.8. Summary

Descriptions of the characteristics of Wilms tumour, retinoblastoma and rhabdomyosarcoma have been presented. Case-control analyses were not done because of the small sample size of the cancers. No obvious differences in the questionnaire variables examined were noted between the 3 cancers. There were no associations between the three tumour types and the infections tested.

CHAPTER SEVEN

SUMMARY AND CONCLUSIONS

7.1. Introduction

This chapter starts by summarising the results obtained from epidemiological research into childhood cancer conducted in Malawi between 2005 and 2006, focusing particularly on the association of specific cancers with four infections studied: HIV, malaria, HHV-8 and EBV. This starts by considering the prevalence of these infections in children diagnosed with cancer. Only cancer types with more than 10 cases were examined: Burkitt lymphoma, Kaposi sarcoma, Wilms tumour, retinoblastoma and rhabdomyosarcoma. The findings of the case-control analyses of Burkitt lymphoma and of Kaposi sarcoma are then summarised. An evaluation of the strengths and limitations of the research undertaken in Malawi are addressed. The chapter concludes with proposed recommendations and conclusions from this epidemiological study.

7.2. Summary of study findings

7.2.1. Prevalence of infectious diseases in children with cancer

This research has provided data on the prevalence of HIV and other infections for different cancer types (see Appendix 7.1 to 7.7, for prevalence estimates and 95% confidence intervals). As expected, prevalence of HIV was highest in children diagnosed with Kaposi sarcoma, with over 80% being HIV positive (Appendix 7.1a, 7.1b). Six percent of Burkitt lymphoma cases were HIV positive compared with 2%

of controls (listed in Table 4.1). The association between HIV and Burkitt lymphoma has been reported in only one other study (Newton et al.,2001). In contrast, Wilms tumour, retinoblastoma and rhabdomyosarcoma were not found to be associated with HIV. Other cancers that are known to be associated with HIV such as other non-Hodgkin lymphomas could not be evaluated in this study because of the small sample size.

Burkitt lymphoma cases had the highest malaria antibody prevalence, with over 60% having high malaria antibodies detected, even after adjusting for child age, sex and residence (Appendices 7.2 and 7.3). The prevalence of HHV-8 antibodies was highest in Kaposi sarcoma cases, with over 80% being HHV-8 positive. Here, the unexpected finding was that retinoblastoma cases had an HHV-8 seroprevalence similar to that of Kaposi sarcoma, although numbers were small (Appendices 7.4 and 7.5). The highest seroprevalence of EBV antibodies was among Burkitt lymphoma cases, with over 90% of cases having antibodies detected. Kaposi sarcoma cases had the second highest EBV seroprevalence of 75%. Other cancers had an EBV seroprevalence ranging from about 50 to 70% (Appendix 7.6).

7.2.2. Findings from the case-control study of Burkitt lymphoma

The case-control study findings clearly demonstrate that infections play a key role in determining the risk of Burkitt lymphoma, particularly malaria and EBV. Burkitt lymphoma cases studied had higher levels of malaria antibodies and higher EBV antibody titres than controls, with the odds of Burkitt lymphoma increasing with increasing titres of EBV antibodies. There was also evidence that malaria and EBV act together to increase the risk of Burkitt lymphoma. That households of children

with Burkitt lymphoma were less likely to report having used mosquito nets also provides strong support to associations with malaria. In addition, this latter evidence is important for emphasising the potential benefit of this anti-malarial intervention for preventing this childhood cancer. These findings also provide important support for previous evidence linking Burkitt lymphoma with malaria, which in contrast, rested largely on ecological data (Kafuko et al.,1970; Morrow,1985).

An unexpected finding was that mothers of children with Burkitt lymphoma reported more lifetime sexual partners than mothers of controls. In contrast with recent suggestions, however, there was no evidence of an association with reported eating of smoked or salted fish (Griffin,2000).

7.2.3. Findings from the case-control study of Kaposi sarcoma

As noted in Section 7.2.1, the infections studied were found to have the strongest associations with Kaposi sarcoma in children. As well as HIV, HHV-8 antibodies were five times more likely to be detected in children diagnosed with this cancer. Compared with control children, Kaposi sarcoma cases had higher HHV-8 antibody levels, and also had higher titres of EBV antibodies detected. In contrast with Burkitt lymphoma, Kaposi sarcoma was not associated with malaria.

Of the 9 mothers of Kaposi sarcoma cases, 7 were HIV positive. Mothers of Kaposi sarcoma cases had a substantially higher percentage of episiotomies conducted compared to mothers of controls (30% and 5% respectively).

7.3. Discussion points

During the study period, it was intended that all children admitted with cancer to the QECH in Blantyre, Malawi, were recruited into this research programme. As a result, the children studied can be expected to form a representative sample of childhood cancer cases attending hospital. Because the hospital records are not accurately and centrally stored, it was not possible to determine if all eligible children were recruited. All parents and guardians who were asked to have their child participate in the study provided informed written consent, as part of ethical requirements.

Almost three quarters of all the cancers recruited had histological verification of tumour diagnosis. The high diagnostic rate was achieved by a thorough attempt to follow up all cytological and histological specimens. For those cancers only diagnosed clinically, some doubt inevitably remains as to whether the correct diagnosis was made. While diagnostic methods available in Malawi include basic microscopic examination of tumour tissues, they are inadequate to evaluate detailed morphology, immunophenotype and genetic abnormalities of tumours. Tumour tissues were not tested for viruses, which would have been important in providing more evidence for infectious cancer aetiology. This was not within the scope of the study.

An aspect of major concern was the possibility of misclassification of exposure to infections for the different cancer types. The detection of EBV and HHV-8 antibodies in young children may reflect maternal antibody status. It was also not possible to obtain information on exposure to these viruses from questionnaire data, as they do not present with distinct clinical symptoms. Diagnosis of HIV in children

younger than 18 months using antibody tests, as discussed in Section 2.8.9.1, may also lead to misclassification of HIV exposure. Despite having a positive antibody test, a young child may not be HIV infected, but antibodies detected may be maternal. This problem only applied to four (3 retinoblastoma and 1 non-malignant) of the 16 children under 18 months old, the rest were HIV negative.

Burkitt lymphoma is the most frequent child malignancy in Malawi. As discussed in Section 2.8.8, obtaining a control group large enough to detect any differences in infections prevalence was a particular challenge. Evaluating the role of infections in other specific cancer types was problematic because of the anticipated small sample sizes; consequently, using a case-control study approach was not possible. As a result, only prevalence of infections were reported for three other specific cancer types: Wilms tumour, retinoblastoma and rhabdomyosarcoma.

No formal analyses were conducted for non-Burkitt non-Hodgkin lymphoma because of the small sample size. One out of the 8 non-Hodgkin lymphoma cases recruited was HIV positive (data not shown). As discussed in Chapter 1, ALL is also thought to be associated with an infectious cause. Two out of the 6 leukaemia cases recruited were diagnosed with ALL, and none were HIV positive. It is not clear if there are few cases of leukaemia in Malawi, or if they are not being diagnosed. It is possible that leukaemia cases are not being diagnosed because they are misclassified and treated for infectious diseases like malaria (Fleming,1993).

With particular regard to the case-control studies of Burkitt lymphoma and Kaposi sarcoma, this research should be considered well designed for the Malawian setting

as the controls arose from the same population from which the cases came and had similar probability of seeking medical care. Consequently, the confounding effects of factors that would be different between cases and controls if they were drawn from different populations, such as area of residence and socio-economic status, were reduced. The intention was to select cases and controls from hospital admissions having exposure rates to the study factors, such as HIV and malaria, similar to those of the population from which the cases and controls arose. As some of the risk factors under investigation, such as malaria are highly geographically dependent, use of neighbourhood controls could have led to overmatching.

As described in Section 2.8.5, incident and prevalent cases were included in the analyses, the disadvantage being that prevalent cases may include patients who may have had the disease for some time, and may have changed their habits (or "exposures"). Children diagnosed with cancer prior to the onset of the study are also immunosuppressed and more likely to be susceptible to infectious diseases, for example, it is possible that Burkitt lymphoma may be more susceptible to malaria. In addition, these children will probably have received some form of cancer treatment which may have affected their infectious disease antibody status and levels, and consequently may have caused infectious disease exposure misclassification.

One important aspect of the study design used for the case-control component of this research, use of 'other cancers' as controls has been previously utilised both in Western and African populations (Smith et al.,1988; Newton et al.,1995; Sitas F et al.,2000; Newton et al.,2001). The advantages of using 'other cancers' as controls, include reduction of selection, recall and interviewer biases, and have been discussed

in Section 2.8.4. The important assumption when using 'other cancers' as controls for a specific cancer type is that different cancers have different causes. This is particularly important when evaluating the associations between infections such as HIV and cancer; some specific cancers are known to be associated with HIV including Kaposi sarcoma and also some lymphomas. If children diagnosed with these specific cancers had been included in the control group for the case-control studies, the risk associated with HIV would have been under-estimated. This was addressed by excluding known HIV-associated cancers (including all lymphomas and Kaposi sarcoma) from the control group for Kaposi sarcoma and Burkitt lymphoma. The resulting control group included a variety of different cancer types, as shown in Table 4.1. The advantage of this is that it diluted the potentially biasing effects of including a specific diagnostic group associated with a particular exposure e.g. excluding Burkitt lymphoma which is known to be associated with EBV, when evaluating the association of EBV and other cancer types.

Questionnaire data on past exposure to infections (some of which are asymptomatic or may be un-or misdiagnosed) is possibly of limited value. Information about exposure to certain infections, such as malaria, was obtained from self-reported data. It is particularly difficult to ascertain the reliability of these data. The Burkitt lymphoma case-control analyses demonstrated that the questionnaire data were unreliable measures of past exposure to malaria and call into to question the value of questionnaire data for this research area.

It is worth bearing in mind that when reviewing results across different cancer types, cancers occurring in children are unique because they occur at different ages. This is

well illustrated in Appendix 6.1. This may also mean that mothers of children with different cancer types will be of different ages as well. This may lead to a possible maternal age effect on factors such as number of children the mother has had or number of sexual partners, as mothers whose children present with cancer at an older age will tend to be older and more likely to have had more sexual partners or more children.

While the serological results provide substantially stronger evidence for associations between infections and cancer, it should still be acknowledged that it was not possible to determine if the raised antibody levels of infections were as a result of the tumour rather than the cause of the disease (reverse causality).

7.4. Proposed recommendations

The ultimate goal of much cancer research is identifying measures useful for prevention of cancer initiation and development. This thesis has focused particularly on the role played by infections in the aetiology of childhood cancers in Malawi. The recommendations presented here are tailored towards reducing the incidence of certain forms of childhood cancer in Malawi and other countries in sub-Saharan Africa with similar patterns of childhood cancer.

Malaria appears to play a role in the development of Burkitt lymphoma. Malaria prevention methods, such as use of insecticide treated mosquito nets, need to be made widely available. While already of public health interest because they reduce the occurrence of malaria, their potential to also reduce the incidence of Burkitt lymphoma would be an additional incentive for promotion of these measures. A

proposed evaluation method might be to determine the baseline Burkitt lymphoma incidence in a specific area, where malaria prevention strategies are being implemented and determine if the incidence of the tumour has changed. This would be best done in co-operation with malaria prevention teams.

The role of EBV in the development of Burkitt lymphoma in children is now well established. An anti-EBV vaccine, which is still in the clinical trial phases, has already been created for the prevention of another disease caused by this virus, infectious mononucleosis, which is common in students in developed countries. A potential use would be to vaccinate young children in regions with endemic Burkitt lymphoma, before they acquire EBV, with the aim of reducing the occurrence of the tumour. A country such as Malawi, where Burkitt lymphoma is relatively common, would be an ideal place to conduct a clinical trial to determine if an anti-EBV vaccine might be useful in reducing the incidence of Burkitt lymphoma, cost not withstanding. If the vaccine is shown to have a protective effect, then malaria prevention in conjunction with vaccination may ultimately lead to reduction in Burkitt lymphoma cases.

Malaria and EBV may be acting together to increase the risk of Burkitt lymphoma in children. This finding needs to be reconfirmed in other larger studies, and if real, EBV and malaria prevention strategies need to be put in place in areas at high risk for Burkitt lymphoma.

Associations between HIV and Burkitt lymphoma, and HIV and Kaposi sarcoma have been highlighted. Prevention of mother to child transmission of HIV may help

reduce the occurrence of these cancer types. No new HIV-cancer associations have been revealed from this study. This could partly be as a result of the small sample sizes available for the other cancer types examined. Larger samples of other cancer types need to be examined before reliable answers relating to associations with HIV can emerge. Recruitment for the study described in this thesis is still ongoing, and in future has the potential to provide a much larger sample of childhood cancers than that described in this thesis.

It is important to evaluate if the clinical presentation of Burkitt lymphoma in children infected with HIV presents in the same way as the tumour in children not infected with this virus. A review of Malawian children with Burkitt lymphoma suggested that there may be a difference in the site of presentation between HIV positive and negative cases (Sinfield et al.,2007). It was reported there that HIV positive children presented with disease in unusual sites (knee, brain, inguinal lymph nodes) compared with HIV negative cases who commonly presented with facial, abdominal and orbital tumours. Evaluation of the outcome of treatment between HIV positive and HIV negative patients needs to be done to assess whether Burkitt lymphoma also infected with HIV may require different treatment protocols. This would be best done in large multiple paediatric oncology units that treat a relatively large number of such Burkitt lymphoma cases. With the rolling out of paediatric HIV anti-retroviral treatment, it is imperative to evaluate mode of treatment for the tumour and the interaction with anti-retroviral drugs.

The effect of high EBV antibody titres in Kaposi sarcoma cases needs to be further examined using larger studies in varied populations. Children who present to hospital

with Kaposi sarcoma may need to be investigated to see whether they present with EBV-associated lymphomas as co-morbidities. This would ensure that the child is adequately treated. It would also be important to evaluate whether children with Kaposi sarcoma and subsequently treated with anti-retroviral therapy are at an increased risk of developing EBV-associated tumours.

The role of episiotomies in HHV-8 transmission needs to be further evaluated and verified by other studies. The hypothesis resulting from this thesis is that HHV-8 is possibly transmitted at delivery when an episiotomy is performed, and this subsequently predisposes the child to developing Kaposi sarcoma. A study of the potential role played by episiotomies in the transmission of HHV-8 at delivery would need to be done in conjunction with obstetricians. Whether pregnancy facilitates HHV-8 reactivation needs to be examined in detail in African populations. The mechanism of tumour formation in relation to HHV-8 infection in children is not well understood. It is possible that children who acquire HHV-8 at birth facilitated by an episiotomy may receive a large infective dose. It may be this which ultimately initiates Kaposi sarcoma formation. It is also be possible that, because of the immature immune system, HHV-8 acquired at birth may be more likely to lead to development of Kaposi sarcoma than if acquired at an older age. These hypotheses need to be examined in detail.

The role of HHV-8 in the aetiology of retinoblastoma requires further investigation with a larger sample size to determine if the finding is real. If indeed it is, examination of retinoblastoma tumour tissue would be essential to determine if HHV-8 DNA is present in tumour cells. It is likely that such a study would yield

negative results, as the epidemiology of HHV-8 does not fit the epidemiology of retinoblastoma globally, which also occurs in children living in developed countries who are less likely to be infected with HHV8.

In order to address the issue of childhood cancer on a wider scale, childhood cancer registration in developing countries needs to be improved. This will provide a more accurate picture of the occurrence of cancer in children and facilitate directed cancer control. Based on population structure data, there are now more children living in developing than in developed countries. Children in developing countries are also more exposed to infections than those from developed countries. It therefore seems sensible that investigations into childhood cancer and associated infections be conducted in developing countries. Data on childhood cancer from developing countries should be regularly compared to that from developed countries. Any differences or similarities identified by such routine monitoring of patterns have the potential to help determine important risk factors.

The treatment and survival of children with cancer in developed countries is very poor because appropriate life saving treatments are not available there. Multi-centre studies need to be carried out to develop appropriate and affordable treatment particularly for the African setting. Randomised control trials of this nature have been conducted for Burkitt lymphoma in Malawi, but they have involved small sample sizes (Hesseling et al.,2003; Kazembe et al.,2003).

Finally, multiple centre epidemiological studies examining the occurrence and aetiology of childhood cancers in sub-Saharan Africa (some are being conducted in

Southern Africa) are essential if the scientific evidence about the risk factors of childhood cancer in African children is to be unravelled.

7.5. Conclusions

In this research conducted in Malawi, prevalence of HIV was highest in children diagnosed with Kaposi sarcoma, with over 80% being HIV positive. Six percent of Burkitt lymphoma cases were HIV positive, and this tumour was found to be strongly associated with HIV, but Wilms tumour, retinoblastoma and rhabdomyosarcoma were not. Burkitt lymphoma cases had the highest malaria and EBV antibody prevalence. The prevalence of HHV-8 antibodies was highest in Kaposi sarcoma cases, with over 80% being HHV-8 positive. Retinoblastoma cases had an HHV-8 seroprevalence similar to that of Kaposi sarcoma.

The case-control study of Burkitt lymphoma strengthened the evidence associating this childhood cancer with EBV and malaria. This study suggests that malaria prevention may have a role to play in decreasing the risk of Burkitt lymphoma in African children. The case-control study of Kaposi sarcoma reconfirms the evidence that this tumour is associated with HIV and HHV-8 infection in children. The strength of the association with EBV was unexpected and it raises the possibility that this virus may be acting as a co-factor for disease among children. Associations found with reported use of episiotomies during delivery of the index child in mothers of children with Kaposi sarcoma may suggest a potential route of transmission of HHV-8 from mother to child, but may also be a chance finding.

Conclusions about Wilms tumour, retinoblastoma and rhabdomyosarcoma need to be treated with caution because of the small sample sizes. This study has created a valuable resource of child-mother blood sample pairs available for this study and other future projects. Questionnaire data are available for a large number of child-mother pairs, and contains a vast range of information that has been invaluable for this thesis, and maybe used for other projects as well. Strengths and weaknesses of the study have been discussed, and recommendations for further work proposed.

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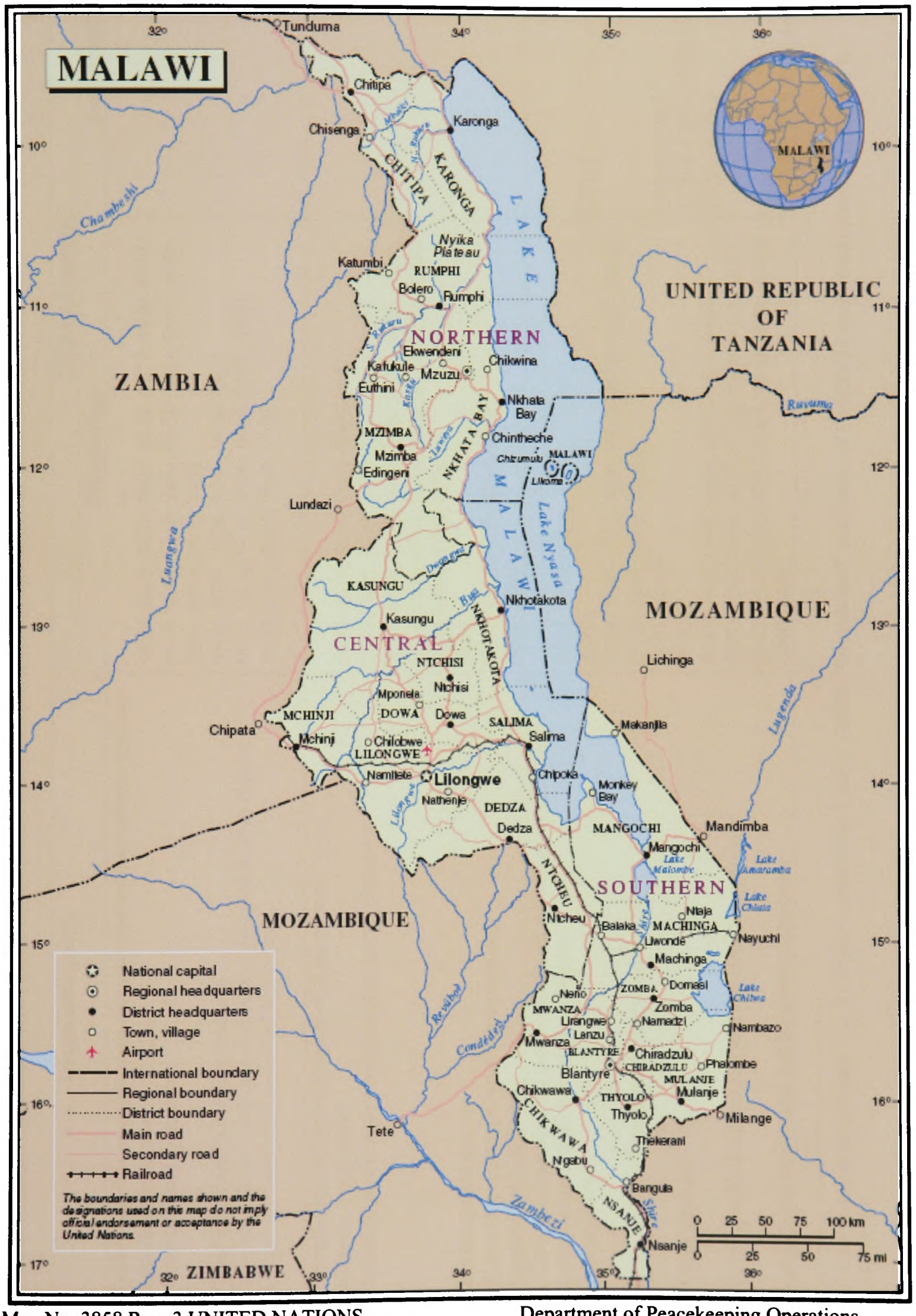
Appendix 2.1: Map of Malawi (United Nations, 2004)



Map No. 3858 Rev. 3 UNITED NATIONS
January 2004

Department of Peacekeeping Operations
Cartographic Section

Appendix 2.2: International Classification of Childhood Cancer, Third Edition-Main Classification table[1]

Diagnostic group	ICD-O-3 code(s)	
	Morphology	Topography
I. Leukemias, myeloproliferative diseases, and myelodysplastic diseases		
a. Lymphoid leukemias	9820, 9823, 9826, 9827, 9831–9837, 9940, 9948	
b. Acute myeloid leukemias	9840, 9861, 9866, 9867, 9870–9874, 9891, 9895–9897, 9910, 9920, 9931	
c. Chronic myeloproliferative diseases	9863, 9875, 9876, 9950, 9960–9964	
d. Myelodysplastic syndrome and other myeloproliferative diseases	9945, 9946, 9975, 9980, 9982–9987, 9989	
e. Unspecified and other specified leukemias	9800, 9801, 9805, 9860, 9930	
II. Lymphomas and reticuloendothelial neoplasms		
a. Hodgkin lymphomas	9650–9655, 9659, 9661–9665, 9667	
b. Non-Hodgkin lymphomas (except Burkitt lymphoma)	9591, 9670, 9671, 9673, 9675, 9678–9680, 9684, 9689–9691, 9695, 9698–9702, 9705, 9708, 9709, 9714, 9716–9719, 9727–9729, 9731–9734, 9760–9762, 9764–9769, 9970	
c. Burkitt lymphoma	9687	
d. Miscellaneous lymphoreticular neoplasms	9740–9742, 9750, 9754–9758	
e. Unspecified lymphomas	9590, 9596	
III. CNS and miscellaneous intracranial and intraspinal neoplasms		
a. Ependymomas and choroid plexus tumor	9383, 9390–9394a	
b. Astrocytomas	9380a	C72.3
	9384, 9400–9411, 9420, 9421–9424, 9440–9442a	
c. Intracranial and intraspinal embryonal tumors	9470–9474, 9480, 9508a	
	9501–9504a	C70.0–C72.9
d. Other gliomas	9380a	C70.0–C72.2, C72.4–C72.9, C75.1, C75.3
	9381, 9382, 9430, 9444, 9450, 9451, 9460a	
e. Other specified intracranial and intraspinal neoplasms	8270–8281, 8300, 9350–9352, 9360–9362, 9412, 9413, 9492, 9493, 9505–9507, 9530–9539, 9582a	
f. Unspecified intracranial and intraspinal neoplasms	8000–8005a	C70.0–C72.9, C75.1–C75.3

[1] From Eva Steliarova-Foucher, C. S., Brigitte Lacour, Peter Kaatsch, (2005). "International Classification of Childhood Cancer, third edition." Cancer **103**(7): 1457-1467.

Continuation of Appendix 2.2: International Classification of Childhood Cancer

Diagnostic group	ICD-O-3 code(s)	
	Morphology	Topography
IV. Neuroblastoma and other peripheral nervous cell tumors		
a. Neuroblastoma and ganglioneuroblastoma	9490, 9500	
b. Other peripheral nervous cell tumors	8680–8683, 8690–8693, 8700, 9520–9523 9501–9504	C00.0–C69.9, C73.9–C76.8, C80.9
V. Retinoblastoma	9510–9514	
VI. Renal tumors		
a. Nephroblastoma and other nonepithelial renal tumors	8959, 8960, 8964–8967	
	8963, 9364	C64.9
b. Renal carcinomas	8010–8041, 8050–8075, 8082, 8120–8122, 8130–8141, 8143, 8155, 8190–8201, 8210, 8211, 8221–8231, 8240, 8241, 8244–8246, 8260–8263, 8290, 8310, 8320, 8323, 8401, 8430, 8440, 8480– 8490, 8504, 8510, 8550, 8560–8576, 8311, 8312, 8316–8319, 8361	C64.9
c. Unspecified malignant renal tumors	8000–8005	C64.9
VII. Hepatic tumors		
a. Hepatoblastoma	8970	
b. Hepatic carcinomas	8010–8041, 8050–8075, 8082, 8120–8122, 8140, 8141, 8143, 8155,8190–8201, 8210, 8211, 8230, 8231, 8240, 8241, 8244–8246, 8260–8264, 8310, 8320, 8323, 8401, 8430, 8440, 8480–8490, 8504, 8510, 8550, 8560–8576, 8160–8180	C22.0, C22.1
c. Unspecified malignant hepatic tumors	8000–8005	C22.0, C22.1
VIII. Malignant bone tumours		
a. Osteosarcomas	9180–9187, 9191–9195, 9200	C40.0–C41.9, C76.0–76.8, C80.9
b. Chondrosarcomas	9210, 9220, 9240	C40.0–C41.9, C76.0–76.8, C80.9
	9221, 9230, 9241–9243	
c. Ewing tumor and related sarcomas of bone	9260	C40.0–C41.9, C76.0–76.8, C80.9
	9363–9365	C40.0–C41.9

Diagnostic group	ICD-O-3 code(s)	
	Morphology	Topography
d. Other specified malignant bone tumours	8810, 8811, 8823, 8830,8812, 9250, 9261, 9262, 9270–9275, 9280–9282, 9290, 9300–9302,9310–9312, 9320–9322, 9330, 9340–9342, 9370–9372	
e. Unspecified malignant bone tumours	8000–8005, 8800, 8801, 8803–8805	C40.0–C41.9
IX. Soft tissue and other extraosseous sarcomas		
a. Rhabdomyosarcomas	8900–8905, 8910, 8912, 8920, 8991	
b. Fibrosarcomas, peripheral nerve sheath tumours, and other fibrous neoplasms	8810, 8811, 8813–8815, 8821, 8823, 8834–8835	C00.0–C39.9, C44.0–C76.8, C80.9
	8820, 8822, 8824–8827, 9150, 9160, 9491, 9540–9571, 9580	
c. Kaposi sarcoma	9140	
	8587, 8710–8713, 8806, 8831–8833, 8836, 8840–8842, 8850–8858, 8860–8862, 8870, 8880, 8881, 8890–8898, 8921, 8982, 8990, 9040–9044, 9120–9125, 9130–9133, 9135, 9136, 9141, 9142, 9161, 9170–9175, 9231, 9251, 9252, 9373, 9581	
	8830	C00.0–C39.9, C44.0–C76.8, C80.9
	8963	C00.0–C63.9, C65.9–C69.9, C73.9–C76.8, C80.9
	9180, 9210, 9220, 9240	C49.0–C49.9
	9260	C00.0–C39.9, C47.0–C75.9
	9364	C00.0–C39.9, C47.0–C63.9, C65.9–C69.9, C73.9–C76.8, C80.9
	9365	C00.0–C39.9, C47.0–C63.9, C65.9–C76.8, C80.9
d. Other specified soft tissue sarcomas	8800–8805	C00.0–C39.9, C44.0–C76.8,

Continuation of Appendix 2.2: International Classification of Childhood Cancer

Diagnostic groups	ICD-O-3 code(s)	
	Morphology	Topography
X. Germ cell tumours, trophoblastic tumours, neoplasms of gonads		
a. Intracranial and intraspinal germ cell tumours	9060–9065, 9070–9072, 9080–9085, 9100, 9101a	
b. Malignant extracranial and extragonadal germ cell tumours	9060–9065, 9070–9072, 9080–9085, 9100–9105	
c. Malignant gonadal germ cell tumours	9060–9065, 9070–9073, 9080–9085, 9090, 9091, 9100, 9101	
d. Gonadal carcinomas	8010–8041, 8050–8075, 8082, 8120–8122, 8130–8141, 8143, 8190–8201, 8210, 8211, 8221–8241, 8244–8246, 8260–8263, 8290,8310, 8313, 8320, 8323, 8380–8384, 8430, 8440, 8480–8490,8504, 8510, 8550, 8560–8573, 9000, 9014, 9015,8441–8444, 8450, 8451, 8460–8473	
e. Other and unspecified malignant gonadal tumours	8590–8671 8000–8005	C56.9, C62.0–C62.9
XI. Other malignant epithelial neoplasms and malignant melanomas		
a. Adrenocortical carcinomas	8370–8375	
b. Thyroid carcinomas	8010–8041, 8050–8075, 8082, 8120–8122, 8130–8141, 8190, 8200, 8201, 8211, 8230, 8231, 8244–8246, 8260–8263, 8290, 8310, 8320, 8323, 8430, 8440, 8480, 8481, 8510, 8560–8573, 8330–8337, 8340–8347, 8350	C73.9
c. Nasopharyngeal carcinomas	8010–8041, 8050–8075, 8082, 8083, 8120–8122, 8130–8141, 8190,8200, 8201, 8211, 8230, 8231, 8244–8246, 8260–8263, 8290,8310, 8320, 8323, 8430, 8440, 8480, 8481, 8500–8576	C11.0–C11.9
d. Malignant melanomas	8720–8780, 8790	
e. Skin carcinomas	8010–8041, 8050–8075, 8078, 8082, 8090–8110, 8140, 8143, 8147, 8190, 8200, 8240, 8246, 8247, 8260, 8310, 8320, 8323, 8390–8420, 8430, 8480, 8542, 8560, 8570–8573, 8940, 8941	C44.0–C44.9

Continuation of Appendix 2.2: International Classification of Childhood Cancer

Diagnostic groups	ICD-O-3 code(s)	
	Morphology	Topography
f. Other and unspecified carcinomas	8010–8084, 8120–8157, 8190–8264, 8290, 8310, 8313–8315, 8320–8325, 8360, 8380–8384, 8430–8440, 8452–8454, 8480–8586,8588–8589, 8940, 8941, 8983, 9000, 9010–9016, 9020, 9030	C00.0–C10.9, C12.9–C21.8, C23.9–C39.9, C48.0–C48.8, C50.0–C55.9,C57.0–C61.9, C63.0–C63.9, C65.9–C72.9, C75.0–C76.8, C80.9
XII. Other and unspecified malignant neoplasms		
a. Other specified malignant tumours	8930–8936, 8950, 8951, 8971–8981, 9050–9055, 9110, 9363	C00.0–C39.9, C47.0–C75.9
b. Other unspecified malignant tumours	8000–8005	C00.0–C21.8, C23.9–C39.9, C42.0–C55.9, C57.0–C61.9, C63.0–C63.9,C65.9–C69.9, C73.9–C75.0, C75.4–C80.9

Appendix 2.3a: Study information sheet

Study Title: Infections and Childhood Cancer in Malawi

Investigators: Dr Nora Mutalima, Prof Elizabeth Molyneux, , Prof Liomba, Prof Borgstein, Prof Jaffe, Dr Mkandawire, Dr Nkume, Dr Kamiza, Dr Robert Newton, Dr Lucy Carpenter.

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully; you may discuss it with others if you wish. Please ask if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part. Thank you for reading this.

1. What is the purpose of the study?

The aim of this study is to learn more about the causes of cancer in children. For the purpose of the study, all patients aged 0-15 years will be considered children. We are particularly keen to know if there are causes of cancer that we might be able to prevent in the future (such as infections), in order to stop other children getting these diseases. We are asking the parents of every child with cancer in this hospital to take part. We hope that the findings of this research may be of help in the future, but they will not affect the care that your child receives in any way. There is no direct medical or other benefit, either to your child, or to you, in taking part.

2. Why have you been chosen?

You are being asked to take part because your child or charge has been admitted to the ward with cancer or suspicion of cancer. All children and parents or guardians of children with cancer are being asked to take part.

3. Do you have to take part?

It is up to you to decide whether to take part. It is not compulsory to do so. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. Even after you agree to take part, you are free to withdraw at any time without giving a reason. Your decisions will not affect the standard of care your child receives.

4. What do you have to do?

If you agree to participate in this study, the following things will take place:

- You will be asked to answer some questions by a trained interviewer – it will take about 30 minutes. Some of the questions asked will be private and the information you provide will be treated with the strictest confidence.
- If it has not already been done for medical reasons, you will be asked to give permission for an HIV test to be conducted on your child. You will be fully counselled pre and post-test, according to local procedures. If you do not want to know the result, then we will not tell you. As the parent, it is up to you whether to inform the child of his/her HIV status. This HIV result will be kept confidential, even the staff of the hospital will not be told. It is your decision whether to tell them or anyone else.

Your child will be required to give some spots of blood for laboratory tests. We intend to look for new infections in these samples that might cause cancer. Some of these infections have not yet been determined and so we would like to store the samples for
testing when more information becomes available. By the time this testing is done, we will not be able to link the results to a particular person by name and so we will not inform you of the results. No genetic testing will be carried out. These tests are being done for the purposes of medical research only and will not alter the care of your child in any way.

- We are interested in the risk factors that lead to childhood cancer in general. Some of these factors are exposure to infectious agents as well as life style and living conditions of both the mother and the child. Some cancers such as Kaposi sarcoma and Burkitt lymphoma often accompany viral infections. Infectious agents like viruses can be transmitted from mother to child when the child is still in the womb, during delivery, or breastfeeding, and in the course of the child's life through different practices like feeding. It is therefore important that both mother and child are tested for infectious agents. One of the important infectious agents associated with cancer is the HIV. Children may get this infection from their mother when they are still in the womb, during delivery, and breastfeeding. For this reason, it is important that an HIV test is also conducted on the mother. As the child's mother, you will be asked to give a sample of blood (about a spoonful). This will be used for laboratory tests as discussed above, including HIV. You will be counselled by a trained counsellor before this is done, and you will be given a chance to decide if you do want it done or not. You will be given the choice as to whether you wish to know the test result or not. Your HIV test result and the other test results will be kept confidential.

5. Discomforts and Risks of the study:

You will be asked some questions for about 30 minutes, and some of these will be sensitive questions about your private life. The counselling about HIV infection may be disturbing to you.

You shall also have the discomfort of a needle stick in your arm vein for a few minutes. Your child will experience the additional discomfort of a finger or heel prick for blood spots, which is relatively non-invasive, but can leave a bruise. There are no special risks involved in being a participant.

6. What are the benefits of the study?

You will be given the opportunity to know your HIV status and to learn how to protect yourself and others from becoming infected. The study aim is to understand the causes of cancer in children, and ultimately find a way of preventing it. We hope that the findings of this research may be of help in the future, but they will not affect the care that your child receives in any way. There is no direct medical or other benefit, either to your child, or to you, in taking part.

7. Will your taking part in this study be kept confidential?

All information that is collected about you during the course of the research will be kept strictly confidential. Any information about you that leaves the hospital will have your name and address removed so that you or your child cannot be recognized from it.

8. Use of research results:

The findings from this study will be analysed and written up for publication in medical journals.

The information collected about you and your child will be treated in a confidential manner, and you will not be personally identified in the reporting of the results. The results of this study may be published, but your name or medical records will not be revealed.

In case you have any questions, you can contact Dr Nora Mutalima or Dr Elizabeth Molyneux at the Paediatric Oncology ward at Queen Elizabeth Hospital. A copy of this information sheet will be given to you, and a copy of the signed consent form will be given to you if you request it.

_____	_____	
Name of Parent/Guardian print	Date	Signature/Thumb

_____	_____	_____
Name of Person taking consent	Date	Signature

Public Health
Old Road Campus
Roosevelt Drive
Headington, Oxford
OX3 7LF, UK

College of Medicine
Department of Paediatrics
Private Bag 360
Chichiri Blantyre 3
Malawi

Appendix 2.3b: Consent forms for children and mothers

Consent form for child

Child's study identification number: ☐☐☐☐☐

Study Title: Infections and Childhood Cancer in Malawi

OXTREC No: 008-04 **COMREC No: P.03/04/277**

Investigators: Dr Nora Mutalima, Prof Elizabeth Molyneux, , Prof Liomba, Prof Borgstein, Prof Jaffe, Dr Mkandawire, Dr Nkume, Dr Kamiza, Dr Robert Newton, Dr Lucy Carpenter.

To be read and filled in by child's parents/guardian *(Please tick box if yes, and cross if no)*

1. I confirm that I have read and understood the information sheet dated..... ☐ for the above named study and have had the opportunity to ask questions.

2. I understand the child's participation is voluntary and that we are free to withdraw at any time, without giving any reason, and without affecting the child's medical or legal rights. ☐

3. I understand that sections of any of the child's medical notes may be looked at by responsible individuals from the research group. I give permission for these individuals to have access to these records ☐

4. I understand that the blood sample collected from my child, and will be tested for infections. ☐

5. I understand that this child will be tested for HIV (unless this has already happened) and that the result will be confidential ☐

6. I consent for this child to take part in the above named study. ☐

Name of Parent/Guardian	Date	Signature/Thumb print
Name of Person taking consent	Date	Signature

Public Health
Old road Campus
Headington, Oxford
OX3 7LF, UK

College of Medicine
Department of Paediatrics
Private Bag 360
Chichiri Blantyre 3, Malawi

Continuation of Appendix 2.3b: Consent forms for children and mothers

Consent form for the mother

Mother's study identification number: ☐☐☐☐☐

Study Title: Infections and Childhood Cancer in Malawi

OXTREC No: 008-04 **COMREC No: P.03/04/277**

Investigators: Dr Nora Mutalima, Prof Elizabeth Molyneux, , Prof Liomba, Prof Borgstein, Prof Jaffe, Dr Mkandawire, Dr Nkume, Dr Kamiza, Dr Robert Newton, Dr Lucy Carpenter.

To be read and filled in by child's mother. (*Please tick box if yes, and cross if no)*

1. I confirm that I have read and understood the information sheet dated..... ☐ for the above named study and have had the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without affecting the child's medical or legal rights. ☐

3. I understand that the blood sample collected from me will be tested for infections. ☐

4. I understand that I will be tested for HIV (unless this has already happened) and that the result will be confidential ☐

5. I consent to take part in the above named study. ☐

Name of Mother	Date	Signature/Thumb print
Name of Person taking consent	Date	Signature

Public Health
Old road Campus
Headington, Oxford
OX3 7LF, UK

College of Medicine
Department of Paediatrics
Private Bag 360
Chichiri Blantyre 3, Malawi

Appendix 2.4a: Child cancers recruited in Malawi July 2005 to August 2006 and their basis of diagnosis (Classification based on ICCC-3)[1]

ICCC-3 diagnostic group	Number	Percentage	Percentage morphologically diagnosed
I. **Leukaemias**			
a) Lymphoid leukaemias	2	0.6%	100%
b) Acute myeloid leukaemias	2	0.6%	100%
e) Unspecified and other specified leukaemias	2	0.6%	0
II. **Lymphoma**			
a) Hodgkin lymphoma	3	1%	33%
b) Non Hodgkin lymphoma	8	2.6%	88%
c) Burkitt lymphoma	148	48.5%	74%
e) Unspecified lymphomas	9	3%	33%
III. **CNS and miscellaneous intracranial and intraspinal neoplams**			
e) Unspecified intracranial and intraspinal neoplasms	1	0.3%	0
IV. **Neuroblastoma and other peripheral nervous cell tumours**			
a) Neuroblastoma and ganglioblastoma	6	2%	100%
V. **Retinoblastoma**	18	6%	44%

[1] Eva Steliarova-Foucher, C. S., Brigitte Lacour, Peter Kaatsch, (2005). "International Classification of Childhood Cancer, third edition." Cancer **103**(7): 1457-1467.

Continuation of Appendix 2.4a: Child cancers recruited in Malawi July 2005 to August 2006 and their basis of diagnosis

ICCC-3 diagnostic group	Number	Percentage	Percentage morphologically diagnosed
VI. **Renal tumours**			
a) Nephroblastoma and other nonepithelial renal tumours	30	10%	87%
VII. **Hepatic tumours**			
a) Hepatoblastoma	1	0.3%	100%
b) Hepatic carcinomas	4	1.3%	50%
VIII. **Malignant bone tumours**			
a) Osteosarcomas	2	0.6%	100%
b) Chondrosarcomas	1	0.3%	100%
c) Ewing tumour and related sarcomas of bone	1	0.3%	100%
IX. **Soft tissue and other extraosseous sarcomas**			
a) Rhabdomyosarcomas	18	6%	56%
c) Kaposi sarcoma	22	7%	23%
e) Unspecified soft tissue sarcomas	1	0.3%	100%
X. **Germ cell tumours**			
b) Malignant extracranial and extragonadal germ cell tumours	2	0.6%	100%
c) Malignant gonadal germ cell tumours	6	1.9%	75%

Continuation of Appendix 2.4a: Child cancers recruited in Malawi July 2005 to August 2006 and their basis of diagnosis

ICCC-3 diagnostic group	Number	Percentage	Percentage morphologically diagnosed
XI. **Other malignant epithelial neoplasms and malignant melanomas**			
f) Other and unspecified carcinomas	1	0.3%	100%
Missing diagnosis	3	1%	0
Non-malignant diagnoses[2]	11	3.6%	73%
Other cancers NOS[3]	3	0.9%	0
Overall total	**305**	**100%**	**67%**

[2] See Appendix 2.4b
[3] See Appendix 2.4c

Appendix 2.4b: Non-malignant conditions and their basis of diagnosis

Non-malignant diagnoses	**Number**	**Percentage morphologically diagnosed**
Aplastic anaemia	1	100%
Benign cystic teratoma	2	100%
Benign mesenchymal tumour	1	100%
Cellular mesoblastic nephroma	1	100%
Chronic inflammation	1	100%
Downs syndrome	1	0
Haemangioma	1	0
Liver abscess	1	100%
Melanotic neuroectodermal tumour of infancy	1	100%
Ranula	1	0
Total	11	73%

Appendix 2.4c: Other tumours NOS recruited and their basis of diagnosis

Tumour	**Number**	**Percentage morphologically diagnosed**
Small round blue cell tumours	2	100%
Abdominal tumour	1	0

Appendix 2.5: Cancer diagnosis and vaccinations abstraction forms

Infection and Childhood Cancer in Malawi

OXTREC No: 008-04 **COMREC No: P.03/04/277**

Abstraction form 1: Cancer diagnosis

Child's Study ID No: ☐☐☐☐☐

1. Age of Child
Years ☐☐ Months ☐☐

2. Sex of Child
Male ☐ Female ☐

3. Date of filling in form
☐☐/☐☐/☐☐
D D / M M / Y Y

4. Child's current weight ☐☐ Kgs

5. Child's current height ☐☐☐ cms

6. Details of diagnosis:

a) **Diagnosis** _____

b) **Benign/Malignant** *(circle one)*

c) **Metastatic** Yes ☐ No ☐ Don't know ☐

d) **Primary site of tumour** _____

e) **Other tumour sites** _____

f) **Histological diagnosis** *(if available)*

_____

_____

_____

_____

_____

g) **Are tumour cells present in :**

Bone marrow a) Yes ☐ b) No ☐ c) Don't know ☐

CSF a) Yes ☐ b) No ☐ c) Don't know ☐

h) **Date of first diagnosis with this condition** ☐☐/☐☐/☐☐
D D / M M / Y Y

i) **Pathology number of biopsy specimen** *(if available)* ☐☐☐☐☐☐☐☐☐☐☐

j) **Name of pathologist:** .....

7. Basis of diagnosis *(tick one)*

a) Clinical ☐ b) Clinical investigation (e.g. ultrasound, X-rays) ☐ c) Specific biochemistry ☐

d) Specific tumour markers ☐ e) Cytology/Haematology ☐ f) Histology of metastasis ☐

g) Histology of primary tumour ☐ h) Unknown ☐

8. HIV serostatus of child

a) Positive ☐ b) Negative ☐ c) Indeterminate ☐ d) Not known ☐

Infections and Childhood Cancer in Malawi

OXTREC No: 008-04 **COMREC No: P.03/04/277**

Abstraction form 2: Vaccination record

Child's Study ID No: ☐☐☐☐☐

1.

Is the child's vaccination record (health passport) available? a) Yes ☐ b) No ☐

2.

Child Date of Birth

☐☐/☐☐/☐☐

D D / M M / Y Y

3.

Birth weight in grams

☐☐☐☐ Don't know ☐

4.

i. Is the vaccination record complete for the child's age?

a) Yes ☐ b) No ☐ c) Don't know ☐

ii. What vaccinations has the child received or not received?

BCG	a) Yes ☐	b) No ☐	c) Don't know ☐
OPV0	a) Yes ☐	b) No ☐	c) Don't know ☐
OPV1	a) Yes ☐	b) No ☐	c) Don't know ☐
OPV2	a) Yes ☐	b) No ☐	c) Don't know ☐
OPV3	a) Yes ☐	b) No ☐	c) Don't know ☐
DPT1	a) Yes ☐	b) No ☐	c) Don't know ☐
DPT2	a) Yes ☐	b) No ☐	c) Don't know ☐
DPT3	a) Yes ☐	b) No ☐	c) Don't know ☐
HepB+Hib1	a) Yes ☐	b) No ☐	c) Don't know ☐
HepB+Hib2	a) Yes ☐	b) No ☐	c) Don't know ☐
HepB+Hib3	a) Yes ☐	b) No ☐	c) Don't know ☐
Measles	a) Yes ☐	b) No ☐	c) Don't know ☐

5.

Stool/ Urine results:

Stool ova a) Positive ☐ b) Negative ☐ c) Not known ☐

Stool ova type: _____

Stool ova number a) One plus ☐ b) Two plus ☐ c) Three plus ☐ d) Four plus ☐ e) Unknown ☐

Stool parasites a) Positive ☐ b) Negative ☐ c) Not known ☐

Stool parasite type: _____

Stool parasite number a) One plus ☐ b) Two plus ☐ c) Three plus ☐ d) Four plus ☐ e) Unknown ☐

Urine ova a) Positive ☐ b) Negative ☐ c) Not known ☐

Urine ova type: _____

Urine ova number a) One plus ☐ b) Two plus ☐ c) Three plus ☐ d) Four plus ☐ e) Unknown ☐

Urine parasites a) Positive ☐ b) Negative ☐ c) Not known ☐

Urine parasite type: _____

Urine parasite number a) One plus ☐ b) Two plus ☐ c) Three plus ☐ d) Four plus ☐ e) Unknown ☐

Continuation of Appendix 2.5: Cancer diagnosis and vaccinations abstraction forms

Child's Study ID No: ☐☐☐☐☐

Ultrasound report:

Appendix 2.6: Child recruitment questionnaire

Infections and Childhood Cancer in Malawi: Questionnaire for all children

OXTREC No: 008-04 **COMREC No: P.03/04/277**

Child's study ID No: ☐☐☐☐☐

Ward	**Interviewer**	**Date of Interview**
_____	_____	☐☐/☐☐/☐☐ *D D / M M / Y Y*

1.
a) Informant's First Name _____ **Last Name** _____

b) Informant's relationship to child _____

2.
Reason for admission
a) Suspected cancer ☐ b) Review ☐ _____

3. **Child Date of Birth**	**4.** **Sex of Child**	**5.** **Child's study ID No.**	**6.** **Mother's study ID No.**
☐☐/☐☐/☐☐ *D D / M M / Y Y*	Male ☐ Female ☐	☐☐☐☐☐	☐☐☐☐☐

7.	**8.**
Child's First Name _____	**Mother's First Name** _____
Child's Last Name _____	**Mother's Last Name** _____

9.	**10.**
Child's nationality _____	**Child's tribe** _____

11.	**12.**
Father's nationality _____	**Father's Tribe** _____

13.
Where does the child normally live?
District _____ Region _____ Country _____

14.	**15.**
i. Is the child's mother alive? (*if yes go to Q. 15*)	**i. Is the child's father alive?** (*If yes go Q.16*)
a) Yes ☐ b) No ☐ c) Don't know ☐	a)Yes ☐ b) No ☐ c) Don't know ☐
ii. If no, what was the date of death? ☐☐/☐☐/☐☐ Don't know ☐ *D D /M M / Y Y*	**ii. If no, what was the date of death?** ☐☐/☐☐/☐☐ Don't know ☐ *D D /M M / Y Y*
iii. What was the cause of the death? _____ Don't know ☐	**iii. What was the cause of death?** _____ Don't know ☐

16.
What number in the family is this child? *(put 1 if firstborn 2 if second etc)* ☐☐

Continuation of Appendix 2.6: Child recruitment questionnaire

Child Study ID No: ☐☐☐☐☐

17.
Who normally looks after the child during *weekdays*?
a) Mother ☐ b) Father ☐ c) Brother/Sister ☐ d) Other relative ☐
e) Crèche/Nursery ☐ f) Grandparent ☐ h) Other*(specify)* ☐ |_____|

18.
Who normally looks after the child during *weekends*?
a) Mother ☐ b) Father ☐ c) Brother/Sister ☐ d) Other relative ☐
e) Crèche/Nursery ☐ f) Grandparent ☐ h) Other*(specify)* ☐ |_____|

19.
Who normally looks after the child at *night*?
a) Mother ☐ b) Father ☐ c) Brother/Sister ☐ d) Other relative ☐
e) Crèche/Nursery ☐ f) Grandparent ☐ h) Other*(specify)* ☐ |_____|

20.
Does anyone in the home use chemical pesticides or insecticides? a) Yes ☐ b) No ☐

21.
Do you use any powders, sprays or chemicals to kill insects around your house? a) Yes ☐ b) No ☐
If yes, which kind? _____
How many times used? a) per week ☐ OR b) per month ☐ OR c) per year ☐

22.
Do you use any powders, sprays or chemicals to kill insects when cultivating? Yes ☐ No ☐
If yes, which kind? _____
How many times used? a) per week ☐ OR b) per month ☐ OR c) per year ☐

23.
Do you use any powders, sprays or chemicals to kill weeds when cultivating? Yes ☐ No ☐
If yes, which kind? _____
How many times used? a) per week ☐ OR b) per month ☐ OR c) per year ☐

24.
Do you have any mosquito nets in your house? a)Yes ☐ b) No ☐ c) Don't know ☐
If yes how many do you have? ☐☐
Is the net/ nets insecticide treated? a)Yes ☐ b) No ☐ c) Don't know ☐
If yes when did you last treat the net/nets? ☐☐ months

25.
Has this child ever slept under a mosquito net? a)Yes ☐ b) No ☐ c) Don't know ☐
How many times does the child use a mosquito net in an average ye
a) Everyday ☐ b) 2-3 times a week ☐ c) about once a month ☐ d) Rarely ☐

Sample collected from child
a) Venous blood ☐

Appendix 2.7: Mother's questionnaire

Infection and Childhood Cancer in Malawi

OXTREC No: 008-04 **COMREC No: P.03/04/277**

Biological mother's questionnaire.

Mother's study ID No: ☐☐☐☐☐

General questions about you

1. Age of mother ☐☐ Years Unknown ☐

2. Mother Date of Birth ☐☐/☐☐/☐☐ *D D / M M / Y Y* Unknown ☐

3. Age of father ☐☐ Years Unknown ☐

4. Father Date of Birth ☐☐/☐☐/☐☐ *D D / M M / Y Y* Unknown ☐

5. What is your nationality? |_____|

6. To what tribe do you belong? |_____|

7. Your weight ☐☐☐ Kgs

8. Your height ☐☐☐ cms

9.

i. Where were you born?

District |_____| Region |_____| Country |_____|

ii. How long did you live there? a) ☐☐ Years b) All her life ☐

10.

i. Where are you normally resident?

District |_____| Region |_____| Country |_____|

ii. How long have you lived there? a) ☐☐ Years b) All her life ☐

iii. How long has the child lived at this address? ☐☐ Years

11.

How many people usually live in your house? ☐☐

12.

Where do you get your drinking water?

a) River/stream ☐ b) Well ☐ c) Borehole ☐ d) Communal tap ☐ e) Personal tap ☐

13.

Do you boil your drinking water? a) Yes ☐ b) No ☐

14.

i. Do you add any chemicals to your drinking water? a) Yes ☐ b) No ☐

If yes, specify the chemical name |_____| b) Don't know ☐

15.

Where is your toilet?

a) In the house ☐ b) In the yard ☐ c) Other *(specify)* ☐ |_____|

16.

What type of toilet is it?

a) Flush ☐ b) VIP latrine ☐ c) Latrine with San plat ☐ d) Simple Pit/Long drop ☐

d) Other *(specify)* ☐ |_____|

Mother's study ID No: ☐☐☐☐☐

17.
What level of education did you attain?
a) Crèche ☐ b) Primary school 1-4 ☐ c) Primary school 5-8 ☐ d) Secondary school ☐
e) College ☐ f) University ☐ g) None ☐

18.
Are you: *(If Single, go to Q 38)*
a) Married ☐ b) Living as married ☐ c) Single ☐ d) Separated ☐ e) Divorced ☐
f) Widowed ☐

19.
How often does your current husband /partner live with you?
a) All the time ☐ b) At least one day per week ☐ c) Less than once a week ☐

20.
i. Is your current husband /partner employed (full/part)? a) Yes ☐ b) No ☐
ii. If no, how long has he been unemployed?
a) Unemployed less than a year ☐ b) Unemployed a year or more ☐

21.
If in paid employment, what is the occupation of your current husband/partner? |_____|

22.
i. Are you employed (full/part)? a) Yes ☐ b) No ☐
ii. If no, how long have you been unemployed?
a) Unemployed less than a year ☐ b) Unemployed a year or more ☐

23.
If in paid employment, what is your current occupation? |_____|

24. **Does the house where the child normally sleeps have:** *(tick for yes, cross for no*	a) Electricity ☐	b) Piped water/ Borehole ☐	c) Phone ☐	d) Radio ☐	e) Vegetable growing land ☐

25. **Does the house where the child normally sleeps have:** *(tick for yes, cross for no*	a) TV ☐	b) Fridge ☐	c) Chickens ☐	d) Other animals *(specify)* |_____| |_____|

Questions about your family

26.
How many brothers and sisters did you have?
(same mother) ☐☐

27.
Were you the first born, second born etc? *(put 1 if first etc)* ☐☐

28.
Did you usually share a bed with other family members as a child? a) Yes ☐ b) No ☐
c)Don't know ☐

Mother's study ID No: ☐☐☐☐☐

Alcohol and Tobacco use

29.

i. Have you ever drank alcohol? a) Yes ☐ b) No ☐

ii. Do you still drink? a) Yes ☐ b) No ☐

iii. If yes, in the last year, how often did you drink alcohol?

a) Less than one day per week ☐ b) 1-3 days per week ☐ c) 4-6 days per week ☐ d) Daily ☐

30.

i. Have you ever smoked tobacco? a) Yes ☐ b) No ☐

ii. Do you still smoke? a) Yes ☐ b) No ☐

If yes, how many cigarettes each day? a) Less than 5 ☐ b) 6-9 ☐ c) 10-19 ☐ d) 20+ ☐

Your Reproductive and Sexual history

31.
How many pregnancies *(including miscarriages)* have you had? ☐☐

32.
How many children have you had? ☐☐

33.
How many of these children are alive now? ☐☐

34.

i. Do your children have different fathers? a)Yes ☐ b) No ☐

ii. If yes, how many different fathers ☐☐

35.

How old were you when you first had sexual intercourse? ☐☐ Years **OR**

a) In primary school/ primary school age ☐ b) In secondary school/ secondary school age ☐ c) Don't know ☐

36.

How many sexual partners have you had?*(include all men you have ever had sex with)* ☐☐

37.

i. Have you ever had/been told you have had a sexually transmitted disease? a) Yes ☐ b) No ☐

ii. If yes, what was the nature of the illness and how old were you?

_____ ☐☐ Years

_____ ☐☐ Years

38.

Have you ever had sexual intercourse for money or to support your family?

a) Never ☐ b) Less than 10 times ☐ c) 11-50 times ☐ d) More than 50 times ☐

39.

Do you or your partner(s) use condoms? a) Never ☐ b) Rarely ☐ c) Often ☐ d) Always ☐

40.
Have you ever used oral contraceptives?*(If no, go to Q.63)* a) Yes ☐ b) No ☐

41.
If yes, how old were you when you first did?
☐☐ Years

42.
Are you currently using oral contraceptives?
a) Yes ☐ b) No ☐

43.
If not, how old were you when you last stopped? ☐☐ Years

Mother's study ID No: ☐☐☐☐☐

44. **Have you ever used injectable contraceptives?** *(If no go to Q.67)* a) Yes ☐ b) No ☐

45. **If yes, how old were you when you first did?** ☐☐ Years

46. **Are you currently using injectable contraceptives?** a) Yes ☐ b) No ☐

47. **If not, how old were you when you last stopped?** ☐☐ Years

Your Health

48.

i. Have you had an illness that required admission to hospital in the past 5 years?

a) Yes ☐ b) No ☐

ii. If yes, how many times have you been in hospital? a) ☐☐ b) Don't know ☐

iii. If yes, what was the illness and how old were you at the time?

|_____| ☐☐ Years

iv. About how many bowel movements do you have each week? ☐☐

49.

i. Has the father of the child had an illness that required admission to hospital in the past 5 years?

a) Yes ☐ b) No ☐ c) Don't know ☐

ii. If yes, how many times in his life has he been in hospital? a) ☐☐ b) Don't know ☐

iii. If yes, what was the illness and how old was he at the time?

|_____| ☐☐ Years

|_____| ☐☐ Years

Questions about this child

50.

i. Was this child born prematurely? a) Yes ☐ b)No ☐

ii. If yes, by how many weeks? ☐☐ Weeks

51.

i. Did you have any illnesses during this pregnancy? a) Yes ☐ b) No ☐

ii. If yes, specify nature of illness |_____|

52.

How was this child born?

a) Normal ☐ b) Normal with stitches ☐ c) Assisted e.g. forceps ☐ d) Caesarean section ☐

53.

Birth weight in grams ☐☐☐☐ grams Don't know ☐

54.

i. Where did you give birth?

a) Home ☐ b) Clinic ☐ c) Hospital ☐ d) Traditional attendant ☐ e) Other *(specify)* |____|

ii. What was the approximate duration of labour? ☐☐ Hours

iii. If the child was born in hospital, how long was the child kept there? ☐☐ Days. **Why?**

|_____|

Mother's study ID No: ☐☐☐☐☐

55.

i. Where were you living when the child was born?

District |_____| Region |_____| Country |_____|

ii. How long did the child live there? ☐☐ Months ☐☐ Years

iii. Has the child lived anywhere else since birth? a) Yes ☐ b) No ☐

iv. If yes, where? District |_____| Region |_____| **For how long**? ☐☐ Years

District |_____| Region |_____| **For how long**? ☐☐ Years

56.

i. Before now, has this child ever had an illness that required treatment in hospital? a) Yes ☐ b) No ☐

ii. If yes, how many times has he/she been in hospital? ☐☐

iii. If yes, what was the illness, and how old was the child at the time?

|_____| at age : ☐☐ Months ☐☐ Years

|_____| at age : ☐☐ Months ☐☐ Years

57.

i. Before this illness, has this child ever received an injection? a) Yes ☐ b) No ☐

ii. If yes, from whom and for what reason?

a) Hospital practitioner Yes ☐ No ☐ |_____|

b) Clinic practitioner Yes ☐ No ☐ |_____|

c) Traditional healer Yes ☐ No ☐ |_____|

d) Family member Yes ☐ No ☐ |_____|

e) Other *(specify who)* Yes ☐ No ☐ |_____|

iii. Has the child ever received a blood transfusion? a) Yes ☐ b) No ☐

iv. If yes, how many times? ☐☐

v. Has this child ever had malaria a) Yes ☐ b) No ☐ c) Don't know ☐

vi. If yes, how many times? ☐

vii. How many times has this child had severe fever? ☐☐

58.

How many people usually share the child's *bed* at night *(put 0 if the child sleeps alone)* ☐☐

59.

How many people usually share the child's *room* at night? *(put 0 if alone in the room)* ☐☐

60.

i. Did you breastfeed this child? a) Yes ☐ b) No ☐

ii. Are you still breastfeeding? a) Yes ☐ b) No ☐

iii. At what age did you start? a) At birth ☐ b) Months ☐

iv. At what age did you stop? ☐☐ Months

Mother's study ID No: ☐☐☐☐☐

61.

i. Did this child ever take formula feed? a) Yes ☐ b) No ☐

ii. If yes, at what age did the child start? ☐☐ Months

iii. At what age did the child stop? *(put 97 if still formula fed)* ☐☐ Months

62.

How old was the child when he/she started on solid food? ☐☐ Months

63.

i. Did you ever feed your child pre-chewed food? a) Yes ☐ b) No ☐ c) Don't know ☐

ii. If yes, how old was your child when they first ate pre-chewed food? ☐☐ Months

64.

i. Has your child ever shared a plate? a) Yes ☐ b) No ☐ c) Don't know ☐

ii. If yes, with whom? Mother ☐ Other(*specify*) ☐ _____

65.

Since weaning, how often does the child eat fish on average?

a) Once/ month or less ☐ b) 2-3 times/ month ☐ c) 1-2 times a week ☐ d) 3-4 times/week ☐ e) 5+ times/week ☐

66.

What type of fish does the child normally eat?

a) Fresh/Frozen ☐ b) Sun-dried ☐ c) Smoked ☐ d) Salted ☐

Samples collected from mother: Blood ☐
(tick if yes, cross if no)

Comment:

Appendix 4.1: Diagnosis characteristics, Burkitt lymphoma cases and controls

Variable	Cases 148 (%)	Controls 104 (%)
Diagnosis basis		
Clinical	36 (25)	25 (26)
Histological	109 (75)	73 (74)
Missing	3	6
Metastatic disease		
No	20 (16)	49 (64)
Yes	106 (84)	27 (36)
Missing	22	28

Appendix 4.2: General characteristics of Burkitt lymphoma cases and controls

Variable	Cases/Controls 148/104 (%)	Crude OR (95%CI)	Adjusted[1] OR (95%CI) χ^2 for heterogeneity[2]
Age			
0 to 4	19/57 (13/55)	1.0	1.0
5 to 15	126/46 (87/45)	8.2 (4.4 to 15.3)	7.7 (4.1 to 14.5)
Missing	*3/1*	χ^2 (1df)=51 P<0.0001	χ^2 (1df)= 46 P<0.0001
Sex			
Male	89/57 (60/55)	1.2 (0.7 to 2.1)	1.2 (0.7 to 2.1)
Female	59/47 (40/45)	1.0	1.0
		χ^2 (1df) =0.7 P= 0.4	χ^2 (1df) =0.3 P=0.6
Child residence			
Urban	32/30 (22/30)	1.0	1.0
Rural	111/69 (78/70)	1.5 (0.8 to 2.7)	1.5 (0.8 to 2.9)
Missing	*5/5*	χ^2 (1df)=1.9 P=0.2	χ^2 (1df) =1.5 P=0.2
Child birth order			
1 to 3	100/66 (68/65)	1.2 (0.7 to 2.0)	1.2 (0.6 to 2.1)
4 to15	46/36 (32/35)	1.0	1.0
Missing	*2/2*	χ^2 (1df)=0.4 P=0.53	χ^2 (1df)=0.2 P=0.66
***Markers of poverty* (102/80)**			
Worse off	63/41 (62/51)	1.5 (0.8 to 2.8)	1.4 (0.7 to 2.9)
Better off	39/39 (38/49)	1.0	1.0
		χ^2 (1df)=2.0 P=0.16	χ^2 (1df)=0.9 P=0.33
Type of fish			
Fresh/Frozen	30/19 (31/27)	1.0	1.0
Sun-dried	49/30 (50/43)	1.0 (0.5 to 2.2)	1.4 (0.6 to 3.4)
Smoked/salted	19/21 (19/30)	0.6 (0.2 to 1.3)	0.4 (0.2 to 1.2)
Missing	*4/10*	χ^2 (2df)=2.5 P=0.28	χ^2 (1df)= 6.4 P=0.09

[1] Adjusted for child age, sex and residence
[2] Test for trend when three or more groups

Appendix 4.3: Possible markers of infectious exposure, Burkitt lymphoma cases and controls

Variable	Cases/Controls 148/104 (%)	Crude OR (95%CI)	Adjusted[1] OR (95%CI) χ^2 for heterogeneity
Child share bed (102/80)			
0 to 1	45/28 (46/37)	1.0	1.0
2 to 7	53/48 (54/63)	0.7 (0.4 to 1.3)	1.2 (0.6 to 2.6)
Missing	*4/4*	χ^2 (1df) =1.4 *P*=0.2	χ^2 (1df)=0.3 *P*=0.6
Mother alive			
Yes	144/103 (97/99)	1.0	1.0
No	4/1 (3/1)	2.9 (0.3 to 25.9)	2.5 (0.2 to 28.3)
		χ^2 (1df)=0.9 *P*=0.33 exact=0.7	χ^2 (1df)=0.6 *P*=0.44
Father alive			
Yes	133/99 (91/95)	1.0	1.0
No	13/5 (9/5)	1.9 (0.7 to 5.6)	1.3 (0.4 to 4.3)
Missing	*2/0*	χ^2 (1df)=1.5 *P*=0.22 exact=0.3	χ^2 (1df)=0.2 *P*=0.7

Appendix 4.4: Child health (Restricted to HIV negative cases and controls), Burkitt lymphoma cases and controls

Variable	Cases/Controls 97/71 (%)	Crude OR (95% CI)	Adjusted[1] OR (95%CI) χ^2 for heterogeneity
Child admission history			
No	80/56 (83/80)	1.3 (0.6 to 2.8)	1.2 (0.5 to 2.9)
Yes	16/14 (17/20)	1.0	1.0
Missing	*1/1*	χ^2 (1df)=0.3 *P*= 0.6	χ^2(1df)=0.1 *P*= 0.8
Malaria history			
No	17/17 (18/25)	1.0	1.0
Yes	75/52 (82/75)	1.4 (0.7 to 3.1)	1.0 (0.4 to 2.5)
Missing	*5/2*	χ^2 (1df)=0.9 *P*= 0.3	χ^2 (1df)=0.0 *P*= 0.98
Malaria frequency			
0 to 3	55/46 (63/72)	1.0	1.0
4 to 10	33/18 (37/28)	1.5 (0.8 to 3.1)	1.0 (0.8 to 2.3)
Missing	*9/7*	χ^2 (1df)=1.5 *P*= 0.2	χ^2 (1df)=0.0 *P*=0.9

[1] Adjusted for child age, sex and residence

Appendix 4.5: Child mosquito net use (Restricted to HIV negative cases and controls), Burkitt lymphoma cases and controls

Variable	Cases/Controls 29/24 (%)	Crude OR (95% CI)	Adjusted[1] OR (95%CI) χ^2 for heterogeneity
Home nets			
Yes	17/19 (59/79)	0.4 (0.1 to 1.3)	0.2 (0.03 to 1.2)
No	12/5 (41/21)	1.0	1.0
		χ^2 (1df)=2.5 P= 0.11 exact=0.1	χ^2 (1df)=3.7 P= 0.08
Number of nets			
0 to 2	21/15 (84/63)	1.0	1.0
3 to 9	4/9 (16/37)	0.3 (0.1 to 1.2)	0.3 (0.1 to 1.4)
Missing	*4/0*	χ^2 (1df)=2.9 P= 0.09 exact=0.1	χ^2 (1df)=2.6 P= 0.1
Child use mosquito net?			
Yes	16/19 (57/79)	0.4 (0.1 to 1.2)	0.2 (0.03 to 0.9)
No	12/5 (43/21)	1.0	1.0
Missing	*1/0*	χ^2 (1df)=2.8 P=0.09 exact=0.1	χ^2 (1df)= 5.0 P=0.04

Appendix 4.6: Maternal characteristics, Burkitt lymphoma cases and controls

Variable	Cases/Controls 102/80 (%)	Crude OR (95%CI)	Adjusted[1] OR (95%CI) χ^2 for heterogeneity
Mother age			
18 to 29	36/42 (49/61)	1.0	1.0
30 to 55	37/27 (51/39)	1.6 (0.8 to 3.1)	1.7 (0.9 to 3.3)
Missing	*29/11*	χ^2 (1df) = 1.9 P = 0.17	χ^2 (1df) =2.3 P = 0.1
Mother education			
None	29/24 (29/30)	1.0	1.0
Primary or higher	70/55 (71/70)	1.1 (0.6 to 2.0)	1.1 (0.5 to 2.5)
Missing	*3/1*	χ^2 (1df)=0.02 P=0.88	χ^2 (1df)=0.05 P= 0.8

[1] Adjusted for mother age and residence

Appendix 4.7: Mother's sexual and reproductive characteristics (Restricted to HIV negative case and control mothers), Burkitt lymphoma cases and controls

Variable	Cases/Controls 82/58 (%)	Crude OR (95%CI)	Adjusted[1] OR (95%CI) χ^2 for heterogeneity
Marital status			
No partner	7/5 (9/9)	1.0	1.0
Has partner	72/51 (91/91)	1.0 (0.3 to 3.4)	1.7 (0.4 to 7.3)
Missing	*3/2*	χ^2 (1df)=0.0 *P*=0.9 exact=1.0	χ^2 (1df)=0.6 *P*= 0.4
Number pregnancies			
1 to 4	33/36 (41/64)	1.0	1.0
5 to 13	47/20 (59/36)	2.6 (1.3 to 5.2)	3.9 (1.2 to 12.2)
Missing	*2/2*	χ^2 (1df)= 6.9 *P*=0.01	χ^2 (1df)=3.9 *P*=0.05
Number live births			
1 to 2	7/18 (9/32)	1.0	1.0
3 to13	73/39 (91/68)	4.8 (1.9 to 12.5)	5.2 (1.6 to 16.6)
Missing	*2/1*	χ^2 (1df)=11.6 *P*=0.001 exact=0.001	χ^2 (1df)=8.7 *P*=0.01
Number children alive			
1 to 3	26/30 (33/54)	1.0	1.0
4 to10	54/26 (67/46)	2.4 (1.2 to 4.8)	4.5 (1.4 to 14.1)
Missing	*2/2*	χ^2 (1df)=6.0 *P*=0.01	χ^2 (1df)=7.4 *P*=0.01
Different fathers			
No	65/53 (81/93)	1.0	1.0
Yes	15/4 (19/7)	3.1 (1.0 to 9.8)	2.5 (0.8 to 8.5)
Missing	*2/1*	χ^2 (1df)=3.8 *P*=0.05 exact=0.08	χ^2 (1df)=2.2 *P*=0.1
Sexual partners			
1	37/39 (49/72)	1.0	1.0
2 to 4	38/15 (51/28)	2.7 (1.3 to 5.6)	3.0 (1.2 to 7.6)
Missing	*7/4*	χ^2 (1df)=6.8 *P*=0.01	χ^2 (1df)=5.8 *P*=0.02
History of STI			
No	76/55 (95/96)	1.0	1.0
Yes	4/2 (5/4)	1.4 (0.3 to 8.2)	1.5 (0.3 to 9.1)
Missing	*2/1*	χ^2 (1df)=0.2 *P*=0.67 exact=1.0	χ^2 (1df)=0.2 *P*=0.65
Mode of delivery			
Normal	73/47 (94/85)	1.0	1.0
Episiotomy	5/4 (6/7)	0.8 (0.2 to 3.2)	1.0 (0.2 to 4.4)
C-Section	0/4 (0/7)	0	0
Missing	*4/3*	χ^2 (2df)=5.9 *P*=0.05 exact=0.04	χ^2 (1df)=0.0 *P*=0.9

[1] Adjusted for mother age and residence

Appendix 4.8: Child serological measures of infections (Restricted to HIV negative cases and controls)[1], Burkitt lymphoma cases and controls

Variable	Cases/Controls 137/91 (%)	Crude OR (95%CI)	Adjusted[2] OR (95%CI) χ^2 for heterogeneity[3]
Child HIV status [4]***(146/93)***			
Negative	137/91 (94/98)	1.0	1.0
Positive	9/2 (6/2)	2.9 (0.6 to 14.2)	12.4 (1.3 to 116.2)
Missing	*1/10*	χ^2 (1df)=2.1 P = 0.15 exact=0.21	χ^2 (1df)=7.2 P=0.03
[5]***Child malaria antibodies***			
Negative/Low	38/48 (29/53)	1.0	1.0
Medium/High	91/42 (71/47)	2.7 (1.6 to 4.8)	2.4 (1.2 to 4.4)
Missing	*8/1*	χ^2 (1df)=12.7 P=0.0	χ^2 (1df)= 6.9 P= 0.01
[6]***Child EBV antibodies***			
Low	12/38 (9/43)	1.0	1.0
Medium	39/32 (31/36)	3.9 (1.7 to 8.6)	4.1 (1.6 to 10.1)
High	77/19 (60/21)	12.8 (5.6 to 29.2)	14.8 (5.8 to 38.5)
Missing	*9/2*	χ^2 (2df)=43.7 P = 0.0	χ^2 (1df)=37.8 P = 0.0
[7]***Child HHV-8***			
Positive	51/32 (40/36)	1.2 (0.7 to 2.1)	0.7 (0.4 to 1.4)
Negative	76/56 (60/64)	1.0	1.0
Missing	*9/1*	χ^2 (1df)=0.3 P = 0.6	χ^2 (1df)= 1.1 P = 0.3

[1] Except for HIV variable
[2] Adjusted for child age, sex and residence
[3] Test for linear trend when three or more groups
[4] Excludes HIV discrepant mother-child pairs (mother negative, child positive)
[5] Malaria antibody categories: Negative/low (OD < 0.2), Medium/high (OD ≥ 0.2)
[6] EBV antibody categories: Low (≤ 1:640), Medium (1:1280 to 1:2560), High (1:5120 to 1:20480)
[7] HHV-8 antibody categories: Positive (OD >1.25), Negative (OD < 0.85)

Appendix 4.9: Maternal serological measures of infections (Restricted to HIV negative mothers)[1], Burkitt lymphoma cases and controls

Variable	Cases/Controls 82/58 (%)	Crude OR (95%CI)	Adjusted[2] OR (95%CI) χ^2 for heterogeneity
[3]Mother HIV status (101/79)			
Negative	81/57 (84/76)	1.6 (0.8 to 3.4)	1.3 (0.6 to 2.9)
Positive	16/18 (16/24)	1.0	1.0
Missing	*4/4*	χ^2 (1df)=1.5 *P*=0.2	χ^2 (1df)=0.4 *P*=0.5
[4]Mother malaria antibodies			
Low	32/28 (39/49)	1.0	1.0
High	50/29 (61/51)	1.5 (0.8 to 2.9)	1.2 (0.5 to 2.7)
Missing	*0/1*	χ^2 (1df)=1.4 *P*=0.2	χ^2 (1df)=0.2 *P*=0.6
[5]Mother EBV			
Low	17/17 (21/30)	1.0	1.0
Medium/High	65/40 (79/70)	1.6 (0.7 to 3.5)	2.1 (0.8 to 5.7)
Missing	*0/1*	χ^2 (1df)= 1.5 *P*= 0.2	χ^2 (1df)= 2.3 *P*= 0.1
[6]Mother HHV-8			
Positive	53/35 (69/62)	1.3 (0.6 to 2.7)	1.1 (0.5 to 2.6)
Negative	24/21 (31/38)	1.0	1.0
Missing	*0/1*	χ^2 (1df)= 0.6 *P*= 0.4	χ^2 (1df)= 0.1 *P*= 0.8
Syphilis			
Negative	71/52 (87/90)	1.0	1.0
Positive	11/6 (13/10)	1.3 (0.5 to 3.9)	1.2 (0.4 to 3.8)
		χ^2 (1df)=0.3 *P*= 0.58	χ^2 (1df)=0.1 *P*= 0.75

[1] Except for HIV variable
[2] Adjusted for mother age and residence
[3] Excludes HIV discrepant mother-child pairs (mother negative, child positive)
[4] Mother malaria antibody categories: Low (OD < 1), Medium/high (OD ≥ 1)
[5] EBV antibody categories: Low (≤ 1:640), Medium/high (1:1280 to 1:20480)
[6] HHV-8 antibody categories: Positive (OD >1.25), Negative (OD < 0.85)

Appendix 4.10: Child general characteristics including markers of poverty adjustments, Burkitt lymphoma cases and controls

Variable	Cases/Controls 148/104 (%)	Crude OR (95%CI)	Adjusted[1] OR (95%CI) χ^2 for heterogeneity[3]	Adjusted[2] OR (95%CI) χ^2 for heterogeneity
Age				
0 to 4	19/57 (13/55)	1.0	1.0	1.0
5 to 15	126/46 (87/45)	8.2 (4.4 to 15.3)	7.7 (4.1 to 14.5)	7.7 (4.1 to 14.5)
Missing	*3/1*	χ^2 (1df)=51 *P*<0.0001	χ^2 (1df)= 46 *P*<0.0001	χ^2 (1df)= 46 *P*<0.0001
Sex				
Male	89/57 (60/55)	1.2 (0.7 to 2.1)	1.2 (0.7 to 2.1)	1.2 (0.6 to 2.1)
Female	59/47 (40/45)	1.0	1.0	1.0
		χ^2 (1df) =0.7 *P*= 0.4	χ^2 (1df) =0.3 *P*=0.6	χ^2 (1df) =0.3 *P*=0.6
Child residence				
Urban	32/30 (22/30)	1.0	1.0	1.0
Rural	111/69 (78/70)	1.5 (0.8 to 2.7)	1.5 (0.8 to 2.9)	1.5 (0.8 to 2.8)
Missing	*5/5*	χ^2 (1df)=1.9 *P*=0.2	χ^2 (1df) =1.5 *P*=0.2	χ^2 (1df) =1.2 *P*=0.3
People in house				
0 to 5	62/48 (62/61)	1.1 (0.6 to 1.9)	1.4 (0.7 to 2.9)	1.4 (0.7 to 2.9)
6 to 11	38/31 (38/39)	1.0	1.0	1.0
Missing	*2/1*	χ^2 (1df)=0.03 *P*=0.87	χ^2 (1df)=1.0 *P*=0.3	χ^2 (1df)=0.7 *P*=0.4
Child bed room occupants				
1 to 2	56/39 (57/52)	1.0	1.0	1.0
3 to 7	42/36 (43/48)	0.8 (0.4 to 1.5)	1.0 (0.5 to 2.1)	1.0 (0.5 to 2.1)
Missing	*4/5*	χ^2 (1df)=0.5 *P*=0.5	χ^2 (1df)=0.0 *P*=1.0	χ^2 (1df)=0.0 *P*=1.0

[1] Adjusted for child age, sex and residence
[2] Adjusted for child age, sex, residence and markers of poverty
[3] Test for linear trend when three or more groups

Continuation of Appendix 4.10: Child general characteristics including markers of poverty adjustments, Burkitt lymphoma cases and controls

Variable	Cases/Controls 148/104 (%)	Crude OR (95%CI)	Adjusted[1] OR (95%CI) χ^2 for heterogeneity[3]	Adjusted[2] OR (95%CI) χ^2 for heterogeneity
Child share bed				
1 to 2	70/62 (78/82)	1.0	1.0	1.0
3 to 7	20/14 (22/18)	1.3 (0.6 to 2.7)	2.3 (0.9 to 6.0)	2.3 (0.9 to 5.9)
Missing	*4/4*	χ^2 (1df) =0.4 *P*=0.55	χ^2 (1df)=3.1 *P*=0.09	χ^2 (1df)=3.1 *P*=0.08
Child birth order				
1 to 3	100/66 (68/65)	1.2 (0.7 to 2.0)	1.2 (0.6 to 2.1)	1.1 (0.6 to 2.1)
4 to15	46/36 (32/35)	1.0	1.0	1.0
Missing	*2/2*	χ^2 (1df)=0.4 *P*=0.53	χ^2 (1df)=0.2 *P*=0.66	χ^2 (1df)=0.1 *P*=0.8
Type of fish				
Fresh/Frozen	30/19 (31/27)	1.0	1.0	1.0
Sun-dried	49/30 (50/43)	1.0 (0.5 to 2.2)	1.4 (0.6 to 3.4)	1.4 (0.6 to 3.5)
Smoked/salted	19/21 (19/30)	0.6 (0.2 to 1.3)	0.4 (0.2 to 1.2)	0.4 (0.1 to 1.1)
Missing	*4/10*	χ^2 (2df)=2.5 *P*=0.28	χ^2 (1df)= 6.4 *P*=0.09	χ^2 (1df)= 7.6 *P*=0.02

[1] Adjusted for child age, sex and residence
[2] Adjusted for child age, sex, residence and markers of poverty
[3] Test for linear trend when three or more groups

Appendix 4.11: Possible markers of infectious exposure, including markers of poverty adjustments, Burkitt lymphoma cases and controls

Variable	Cases/Controls 148/104 (%)	Crude OR (95%CI)	Adjusted[1] OR (95%CI) χ^2 for heterogeneity	Adjusted[2] OR (95%CI) χ^2 for heterogeneity
Father alive				
Yes	133/99 (91/95)	1.0	1.0	1.0
No	13/5 (9/5)	1.9 (0.7 to 5.6)	1.3 (0.4 to 4.3)	1.3 (0.4 to 4.3)
Missing	*2/0*	χ^2 (1df)=1.5 *P*=0.22 exact=0.3	χ^2 (1df)=0.2 *P*=0.7	χ^2 (1df)=0.2 *P*=0.7
Mother alive				
Yes	144/103 (97/99)	1.0	1.0	1.0
No	4/1 (3/1)	2.9 (0.3 to 25.9)	2.5 (0.2 to 28.3)	2.2 (0.2 to 25.2)
		χ^2 (1df)=0.9 *P*=0.33 exact=0.7	χ^2 (1df)=0.6 *P*=0.44	χ^2 (1df)=0.4 *P*=0.5

[1] Adjusted for child age, sex and residence
[2] Adjusted for child age, sex, residence and markers of poverty

Appendix 4.12: Child health (Restricted to HIV negative cases and controls), including markers of poverty adjustments, Burkitt lymphoma cases and controls

Variable	Cases/Controls 97/71 (%)	Crude OR (95% CI)	Adjusted[1] OR (95%CI) χ^2 for heterogeneity	Adjusted[2] OR (95%CI) χ^2 for heterogeneity
Malaria history				
No	17/17 (18/25)	1.0	1.0	1.0
Yes	75/52 (82/75)	1.4 (0.7 to 3.1)	1.0 (0.4 to 2.5)	1.1 (0.4 to 2.8)
Missing	*5/2*	χ^2 (1df)=0.9 *P*= 0.3	χ^2 (1df)=0.0 *P*= 0.98	χ^2 (1df)=0.1 *P*= 0.8
Malaria frequency				
0 to 3	55/46 (63/72)	1.0	1.0	1.0
4 to 10	33/18 (37/28)	1.5 (0.8 to 3.1)	1.0 (0.8 to 2.3)	1.1 (0.5 to 2.4)
Missing	*9/7*	χ^2 (1df)=1.5 *P*= 0.2	χ^2 (1df)=0.0 *P*=0.93	χ^2 (1df)=0.02 *P*= 0.9

[1] Adjusted for child age, sex and residence
[2] Adjusted for child age, sex, residence and markers of poverty

Appendix 4.13: Child mosquito net use (Restricted to HIV negative cases and controls), including markers of poverty adjustments, Burkitt lymphoma cases and controls

Variable	Cases/Controls 29/24 (%)	Crude OR (95% CI)	Adjusted[1] OR (95%CI) χ^2 for heterogeneity	Adjusted[2] OR (95%CI) χ^2 for heterogeneity
Home nets				
Yes	17/19 (59/79)	0.4 (0.1 to 1.3)	0.2 (0.03 to 1.2)	0.3 (0.04 to 1.8)
No	12/5 (41/21)	1.0	1.0	1.0
		χ^2 (1df)=2.5 *P*= 0.11 exact=0.1	χ^2 (1df)=3.7 *P*= 0.08	χ^2 (1df)=1.9 *P*= 0.2
Number of nets				
0 to 2	21/15 (84/63)	1.0	1.0	1.0
3 to 9	4/9 (16/37)	0.3 (0.1 to 1.2)	0.3 (0.1 to 1.4)	0.4 (0.06 to 3.1)
Missing	*4/0*	χ^2 (1df)=2.9 *P*= 0.09 exact=0.1	χ^2 (1df)=2.6 *P*= 0.1	χ^2 (1df)=0.7 *P*= 0.4
Child use mosquito net?				
Yes	16/19 (57/79)	0.4 (0.1 to 1.2)	0.2 (0.03 to 0.9)	0.2 (0.03 to 1.3)
No	12/5 (43/21)	1.0	1.0	1.0
Missing	*1/0*	χ^2 (1df)=2.8 *P*=0.09 exact=0.1	χ^2 (1df)= 5.0 *P*=0.04	χ^2 (1df)=3.3 *P*= 0.07

[1] Adjusted for child age, sex and residence
[2] Adjusted for child age, sex, residence and markers of poverty

Appendix 4.14: Mother's general characteristics including markers of poverty adjustments, Burkitt lymphoma cases and controls

Variable	Cases/Controls 102/80 (%)	Crude OR (95%CI)	Adjusted[1] OR (95%CI) χ^2 for heterogeneity	Adjusted[2] OR (95%CI) χ^2 for heterogeneity
Mother age				
18 to 29	36/42 (49/61)	1.0	1.0	1.0
30 to 55	37/27 (51/39)	1.6 (0.8 to 3.1)	1.7 (0.9 to 3.3)	1.7 (0.9 to 3.4)
Missing	*29/11*	χ^2 (1df) = 1.9 P = 0.17	χ^2 (1df) =2.3 P = 0.13	χ^2 (1df) =2.6 P = 0.1
Mother education				
None	29/24 (29/30)	1.0	1.0	1.0
Primary or higher	70/55 (71/70)	1.1 (0.6 to 2.0)	1.1 (0.5 to 2.5)	1.1 (0.5 to 2.6)
Missing	*3/1*	χ^2 (1df)=0.02 P=0.88	χ^2 (1df)=0.05 P= 0.83	χ^2 (1df)=0.09 P= 0.8
Marital status				
No partner	9/13 (9/17)	1.0	1.0	1.0
Has partner	90/65 (91/83)	2.0 (0.8 to 4.9)	3.2 (1.04 to 9.8)	3.1 (1.0 to 9.7)
Missing	*3/2*	χ^2 (1df)=2.3 P=0.13 exact=0.17	χ^2 (1df)= 4.5 P= 0.04	χ^2 (1df)= 4.1 P= 0.04

[1] Adjusted for child age, sex and residence
[2] Adjusted for child age, sex, residence and markers of poverty

Appendix 4.15: Mother's sexual and reproductive characteristics (Restricted to HIV negative case and control mothers), including markers of poverty adjustments, Burkitt lymphoma cases and controls

Variable	Cases/Controls 82/58 (%)	Crude OR (95%CI)	Adjusted[1] OR (95%CI) χ^2 for heterogeneity	Adjusted[2] OR (95%CI) χ^2 for heterogeneity
Number pregnancies				
1 to 4	33/36 (41/64)	1.0	1.0	1.0
5 to 13	47/20 (59/36)	2.6 (1.3 to 5.2)	3.9 (1.2 to 12.2)	3.9 (1.2 to 12.4)
Missing	*2/2*	χ^2 (1df)= 6.9 *P*=0.01	χ^2 (1df)=3.9 *P*= 0.05	χ^2 (1df)=5.9 *P*= 0.02
Number children born				
1 to 2	7/18 (9/32)	1.0	1.0	1.0
3 to13	73/39 (91/68)	4.8 (1.9 to 12.5)	5.2 (1.6 to 16.6)	5.1 (1.6 to 16.4)
Missing	*2/1*	χ^2 (1df)=11.6 *P*=0.001 exact=0.001	χ^2 (1df)=8.7 *P*= 0.01	χ^2 (1df)=8.5 *P*= 0.003
Number children alive				
1 to 3	26/30 (33/54)	1.0	1.0	1.0
4 to10	54/26 (67/46)	2.4 (1.2 to 4.8)	4.5 (1.4 to 14.1)	4.8 (1.5 to 15.3)
Missing	*2/2*	χ^2 (1df)=6.0 *P*=0.01	χ^2 (1df)=7.4 *P*= 0.01	χ^2 (1df)=7.8 *P*= 0.01
Different fathers				
No	65/53 (81/93)	1.0	1.0	1.0
Yes	15/4 (19/7)	3.1 (1.0 to 9.8)	2.5 (0.8 to 8.5)	2.5 (0.7 to 8.5)
Missing	*2/1*	χ^2 (1df)=3.8 *P*=0.05 exact=0.08	χ^2 (1df)=2.2 *P*= 0.1	χ^2 (1df)=2.2 *P*= 0.1

[1] Adjusted for child age, sex and residence
[2] Adjusted for child age, sex, residence and markers of poverty

Continuation of Appendix 4.15: Mother's sexual and reproductive characteristics (Restricted to HIV negative case and control mothers), including markers of poverty adjustments, Burkitt lymphoma cases and controls

Variable	Cases/Controls 82/58 (%)	Crude OR (95%CI)	Adjusted[1] OR (95%CI) χ^2 for heterogeneity	Adjusted[2] OR (95%CI) χ^2 for heterogeneity
Number sexual partners				
1	37/39 (49/72)	1.0	1.0	1.0
2 to 4	38/15 (51/28)	2.7 (1.3 to 5.6)	3.0 (1.2 to 7.6)	2.9 (1.2 to 7.5)
Missing	*7/4*	χ^2 (1df)=6.8 *P*=0.01	χ^2 (1df)=5.8 *P*= 0.02	χ^2 (1df)=5.7 *P*= 0.02
History of STI				
No	76/55 (95/96)	1.0	1.0	1.0
Yes	4/2 (5/4)	1.4 (0.3 to 8.2)	1.5 (0.3 to 9.1)	1.5 (0.3 to 9.2)
Missing	*2/1*	χ^2 (1df)=0.2 *P*=0.7 exact=1.0	χ^2 (1df)=0.2 *P*= 0.7	χ^2 (1df)=0.2 *P*= 0.6
Mode of delivery				
Normal	73/47 (94/85)	1.0	1.0	1.0
Episiotomy	5/4 (6/7)	0.8 (0.2 to 3.2)	1.0 (0.2 to 4.4)	1.0 (0.2 to 4.9)
C-Section	0/4 (0/7)	0	0	0
Missing	*4/3*	χ^2 (2df)=5.9 *P*=0.05 exact=0.04	χ^2 (1df)=0.0 *P*= 0.9	χ^2 (1df)=0.0 *P*= 0.9

[1] Adjusted for child age, sex and residence
[2] Adjusted for child age, sex, residence and markers of poverty

Appendix 4.16: Child serological measures of infections including markers of poverty (Restricted to HIV negative children[1]), Burkitt lymphoma cases and controls

Child general variables	**Cases/Controls 148/104 (%)**	**Crude OR (95%CI)**	**Adjusted[2] OR (95%CI) χ^2 for heterogeneity**	**Adjusted[3] OR (95%CI) χ^2 for heterogeneity**
Child HIV status [4](147/103)				
Negative	137/91 (94/98)	1.0	1.0	1.0
Positive	9/2 (6/2)	2.9 (0.6 to 14.2)	12.4 (1.3 to 116.2)	11.2 (1.2 to 103.8)
Missing	*1/10*	χ^2 (1df)=2.1 P = 0.15 exact=0.21	χ^2 (1df)=7.2 P =0.03	χ^2 (1df)=6.7 P =0.01
[5]Child malaria antibodies				
Negative/Low	38/48 (29/53)	1.0	1.0	1.0
Medium/High	91/42 (71/47)	2.7 (1.6 to 4.8)	2.4 (1.2 to 4.4)	3.6 (1.2 to 10.8)
Missing	*8/1*	χ^2 (1df)=12.7 P=0.0	χ^2 (1df)= 6.9 P= 0.01	χ^2 (1df)= 5.4 P= 0.02
[6]Child EBV antibodies				
Low	12/38 (9/43)	1.0	1.0	1.0
Medium	39/32 (31/36)	3.9 (1.7 to 8.6)	4.1 (1.6 to 10.1)	4.1 (1.6 to 10.2)
High	77/19 (60/21)	12.8 (5.6 to 29.2)	14.8 (5.8 to 38.5)	14.9 (5.7 to 38.6)
Missing	*9/2*	χ^2 (2df)=43.7 P = 0.0	χ^2 (1df)=37.8 P = 0.0	χ^2 (1df)=37.6 P = 0.0
[7]Child HHV-8				
Positive	51/32 (40/36)	1.2 (0.7 to 2.1)	0.7 (0.4 to 1.4)	0.7 (0.4 to 1.4)
Negative	76/56 (60/64)	1.0	1.0	1.0
Missing	*9/1*	χ^2 (1df)=0.3 P = 0.6	χ^2 (1df)= 1.1 P = 0.3	χ^2 (1df)= 1.1 P = 0.3

[1] Except HIV variable
[2] Adjusted for child age, sex and residence
[3] Adjusted for child age, sex, residence and markers of poverty
[4] Excludes HIV discrepant child-mother pairs (mother negative, child positive)
[5] Malaria antibody categories: Negative/low (OD < 0.2), Medium/high (OD ≥ 0.2)
[6] EBV antibody categories: Low (≤ 1:640), Medium (1:1280 to 1:2560), High (1:5120 to 1:20480)
[7] HHV-8 antibody categories: Positive (OD >1.25), Negative (OD < 0.85)

Appendix 4.17: Maternal serological measures of infections, including markers of poverty (Restricted to HIV negative mothers[1])

Mother variables	Cases/Controls 102/80 (%)	Crude OR (95%CI)	Adjusted[2] OR (95%CI) χ^2 for heterogeneity	Adjusted[3] OR (95%CI) χ^2 for heterogeneity
[4]***Mother HIV status (101/79)***				
Negative	81/57 (84/76)	1.6 (0.8 to 3.4)	1.3 (0.6 to 2.9)	1.3 (0.6 to 2.9)
Positive	16/18 (16/24)	1.0	1.0	1.0
Missing	*4/4*	χ^2 (1df)=1.5 *P*=0.2	χ^2 (1df)=0.4 *P*=0.5	χ^2 (1df)=0.4 *P*=0.6
[5]***Mother malaria antibodies***				
Low	32/28 (39/49)	1.0	1.0	1.0
High	50/29 (61/51)	1.5 (0.8 to 2.9)	1.2 (0.5 to 2.7)	1.2 (0.5 to 2.8)
Missing	*0/1*	χ^2 (1df)=1.4 *P*=0.2	χ^2 (1df)=0.2 *P*=0.6	χ^2 (1df)=0.3 *P*=0.6
[6]***Mother HHV-8***				
Positive	53/35 (69/62)	1.3 (0.6 to 2.7)	1.1 (0.5 to 2.6)	1.1 (0.5 to 2.6)
Negative	24/21 (31/38)	1.0	1.0	1.0
Missing	*0/1*	χ^2 (1df)= 0.6 *P*= 0.4	χ^2 (1df)= 0.1 *P*= 0.8	χ^2 (1df)= 0.1 *P*= 0.8
[7]***Mother EBV***				
Low	17/17 (21/30)	1.0	1.0	1.0
High	65/40 (79/70)	1.6 (0.7 to 3.5)	2.1 (0.8 to 5.7)	2.3 (0.8 to 6.5)
Missing	*0/1*	χ^2 (1df)= 1.5 *P*= 0.2	χ^2 (1df)= 2.3 *P*= 0.1	χ^2 (1df)= 2.7 *P*= 0.1
[4]***Syphilis***				
Negative	71/52 (87/90)	1.0	1.0	1.0
Positive	11/6 (13/10)	1.3 (0.5 to 3.9)	1.2 (0.4 to 3.8)	1.2 (0.4 to 3.8)
		χ^2 (1df)=0.3 *P*= 0.6	χ^2 (1df)=0.1 *P*= 0.8	χ^2 (1df)=0.1 *P*= 0.7

[1] Except HIV variable
[2] Adjusted for child age, sex and residence
[3] Adjusted for child age, sex, residence and markers of poverty
[4] Excludes HIV discrepant mother-child pairs (mother negative, child positive)
[5] Mother malaria antibody categories: Low (OD < 1), Medium/high (OD ≥ 1)
[6] HHV-8 antibody categories: Positive (OD >1.25), Negative (OD < 0.85)
[7] EBV antibody categories: Low (≤ 1:640), Medium/high (1:1280 to 1:20480)

Appendix 5.1: General characteristics of Kaposi sarcoma cases and controls

Variable	Cases/Controls 22/104 (%)	Crude OR (95%CI)	Adjusted[1] OR (95%CI) χ^2 for heterogeneity
Age			
0 to 4	6/57 (27/55)	1.0	1.0
5 to 15	16/46 (73/45)	3.3 (1.2 to 9.1)	2.9 (1.0 to 8.2)
Missing	*0/1*	χ^2 (1df)=5.7 *P*=0.02 exact= 0.02	χ^2 (1df)= 4.2 *P*=0.05
Sex			
Male	14/57 (64/55)	1.4 (0.6 to 3.7)	1.5 (0.5 to 4.2)
Female	8/47 (36/45)	1.0	1.0
		χ^2 (1df) =0.6*P*= 0.45 exact=0.5	χ^2 (1df) =0.6 *P*=0.46
Child residence			
Urban	12/30 (57/30)	3.1 (1.2 to 8.0)	2.8 (1.0 to 7.5)
Rural	9/69 (43/70)	1.0	1.0
Missing	*1/5*	χ^2 (1df)=5.5 *P*=0.02 exact= 0.03	χ^2 (1df) =4.1*P*= 0.05
Diagnosis basis			
Clinical	17/25 (77/26)	9.9 (3.3 to 29.7)	10.9 (3.3 to 35.9)
Histological	5/73 (23/74)	1.0	1.0
Missing	*0/6*	χ^2 (1df)= 21 *P* <0.001 exact=0.0	χ^2(1df)= 19 *P*< 0.001
People in house			
0 to 5	9/48 (90/61)	1.0	1.0
6 to 11	1/31 (10/39)	0.2 (0.02 to 1.4)	0.2 (0.02 to 1.6)
Missing	*0/1*	χ^2 (1df)=3.3 *P*=0.07 exact = 0.09	χ^2 (1df)=3.4 *P*= 0.12

Appendix 5.2: Possible markers of infectious exposure, Kaposi sarcoma cases and controls

Variable	Cases/Controls 22/104 (%)	Crude OR (95%CI)	Adjusted [1] OR (95%CI) χ^2 for heterogeneity
Mother alive			
Yes	12/103 (55/99)	1.0	1.0
No	10/1 (45/1)	85.8 (10.1 to 730.1)	77.7 (8.3 to 723.9)
		χ^2 (1df)=45 *P*<0.001 exact=0.0	χ^2 (1df)=27*P*<0.0001
Father alive			
Yes	14/99 (64/95)	1.0	1.0
No	8/5 (36/5)	11.3 (3.2 to 39.5)	9.1 (2.4 to 34.9)
		χ^2 (1df)=19*P*< 0.001 exact=0.0	χ^2 (1df)=11 *P*=0.001

[1] Adjusted for child age, sex and residence

Appendix 5.3: Mother's general characteristics, Kaposi sarcoma cases and controls

Variable	Cases/Controls 10/80 (%)	Crude OR (95%CI)	Adjusted[1] OR (95%CI) χ^2 for heterogeneity
Mother age			
18 to 29	4/42 (50/61)	1.0	1.0
30 to 55	4/27 (50/39)	1.6 (0.4 to 6.8)	1.5 (0.3 to 6.8)
Missing	*2/11*	χ^2 (1df) = 0.4 *P*= 0.55 exact = 0.71	χ^2 (1df) =0.3 *P* =0.58
Mother education			
None	2/24 (20/30)	1.0	1.0
Primary/higher	8/55 (80/70)	1.7 (0.3 to 8.8)	1.8 (0.2 to 17.2)
Missing	*0/1*	χ^2 (1df)=0.5 *P*=0.49 exact = 0.72	χ^2 (1df)=0.3 *P*= 0.6

Appendix 5.4: Mother's sexual and reproductive characteristics, Kaposi sarcoma cases and controls

Variable	Cases/Controls 10/80 (%)	Crude OR (95%CI)	Adjusted [1] OR (95%CI) χ^2 for heterogeneity
Marital status			
No partner	4/13 (40/17)	3.3 (0.8 to 13.5)	4.9 (1.0 to 23.9)
Has partner	6/65 (60/83)	1.0	1.0
Missing	*0/2*	χ^2 (1df)=3.1 *P*=0.08 exact = 0.1	χ^2 (1df)=3.8 *P*=0.05
Number lifetime sexual partners			
1	5/49 (50/66)	1.0	1.0
2 to 4	5/25 (50/34)	1.9 (0.5 to 7.4)	2.1 (0.4 to 10.7)
Missing	*0/6*	χ^2 (1df)=1 *P*=0.32 exact = 0.32	χ^2 (1df)=0.9 *P*=0.35
Mode of delivery			
Normal	7/68 (70/89)	1.0	1.0
Episiotomy	3/4 (30/5)	7.2 (1.3 to 39.4)	9.2 (1.4 to 58.5)
C-Section	0/5 (0/6)	0	0
Missing	*0/3*	χ^2 (2df)=8 *P*=0.02 exact = 0.05	χ^2 (1df)=5.1 *P*=0.02

[1] Adjusted for mother age and residence

Appendix 5.5: Child serological measures of infections, Kaposi sarcoma cases and controls

Variables	Cases/Controls 22/104 (%)	Crude OR (95%CI)	Adjusted[1] OR (95%CI) χ^2 for heterogeneity[2]
[3]***Child HIV status***			
Negative	4/91 (19/98)	1.0	1.0
Positive	17/2 (81/2)	193.4 (32.8 to 1140.5)	762.7 (44 to 13376)
Missing	*1/10*	χ^2 (1df)=77 *P*< 0.001 exact=0.0	χ^2 (1df)=62 *P*< 0.001
[4]***Child HHV-8 antibodies***			
Positive	17/36 (77/38)	5.5 (1.9 to 16.1)	4.8 (1.4 to 16.4)
Negative	5/58 (23/62)	1.0	1.0
Missing	*0/8*	χ^2 (1df)=10.9 *P*=0.001 exact=0.0	χ^2 (1df)=7.5 *P*=0.01
[5]***Child EBV antibodies***			
Low	2/50 (9/53)	1.0	1.0
Medium/High	20/45 (91/47)	11.1 (2.5 to 50.2)	10.9 (2.3 to 52.2)
Missing	*0/9*	χ^2 (1df)= 13.7 *P*= 0.0 exact=0.0	χ^2 (1df)= 13.9 *P*< 0.001
[6]***Child malaria antibodies***			
Negative/Low	15/50 (68/52)	1.9 (0.7 to 5.3)	2.1 (0.7 to 6.1)
Medium/High	7/46 (32/48)	1.0	1.0
Missing	*0/8*	χ^2 (1df)= 1.9 *P*= 0.2 exact=0.2	χ^2 (1df)= 1.9 *P*= 0.2

[1] Adjusted for child age, sex and residence
[2] Test for linear trend when there are three groups
[3] Excludes HIV discordant mother-child pairs (mother negative, child positive)
[4] HHV-8 antibody categories: Positive (OD >1.25), Negative (OD < 0.85)
[5] EBV antibody categories: Low (≤ 1:1280), Medium/High (≥ 1:2560)
[6] Malaria antibody categories: Negative/low (OD < 0.2), Medium/high (OD ≥ 0.2)

Appendix 5.6: Maternal serological measures of infections, Kaposi sarcoma cases and controls

Variables	Cases/Controls 10/80 (%)	Crude OR (95%CI)	Adjusted[1] OR (95%CI) χ^2 for heterogeneity[2]
[3]***Mother HIV***			
Negative	2/57 (22/76)	1.0	1.0
Positive	7/18 (78/24)	11.3 (2.1 to 59.2)	19.5 (2.1 to 181.7)
Missing	*1/4*	χ^2 (1df)=11 *P*=0.001 exact = 0.002	χ^2 (1df)=10 *P*=0.01
[4]***Mother HHV-8 antibodies***			
Positive	6/50 (67/66)	1.0 (0.2 to 4.5)	0.7 (0.1 to 3.4)
Negative	3/26 (33/34)	1.0	1.0
Missing	*1/3*	χ^2 (1df)=0.003 *P*= 0.9 exact=1.0	χ^2 (1df)=0.2 *P*= 0.7
[5]***Mother EBV antibodies***			
Low	1/17 (11/22)	1.0	
Medium/High	8/60 (89/78)	2.3 (0.3 to 19.4)	
Missing	*1/1*	χ^2 (1df)=0.6 *P*= 0.4 exact=0.7	
[6]***Mother malaria***			
High	1/35 (11/45)	0.2 (0.02 to 1.3)	
Low	8/42 (89/55)	1.0	
Missing	*1/3*	χ^2 (1df)=3.9 *P*= 0.05 exact= 0.05	
Mother syphilis			
Positive	1/10 (11/13)	0.8 (0.1 to 7.4)	1.3 (0.1 to 13.9)
Negative	8/67 (89/87)	1.0	1.0
Missing	*1/3*	χ^2 (1df)=0.0 *P*= 0.87 exact=1.0	χ^2 (1df)=0.04 *P*= 0.84

[1] Adjusted for mother age and residence
[2] Test for linear trend when there are three groups
[3] Excludes HIV discordant mother-child pairs (mother negative, child positive)
[4] HHV-8 antibody categories: Positive (OD >1.25), Negative (OD < 0.85)
[5] EBV antibody categories: Low (1:640 or less), Medium/high (1:1280 to 1:20480)
[6] Mother malaria antibody categories: Low (OD < 1), Medium/high (OD ≥ 1)

Appendix 7.1a: HIV and cancer case-control analyses

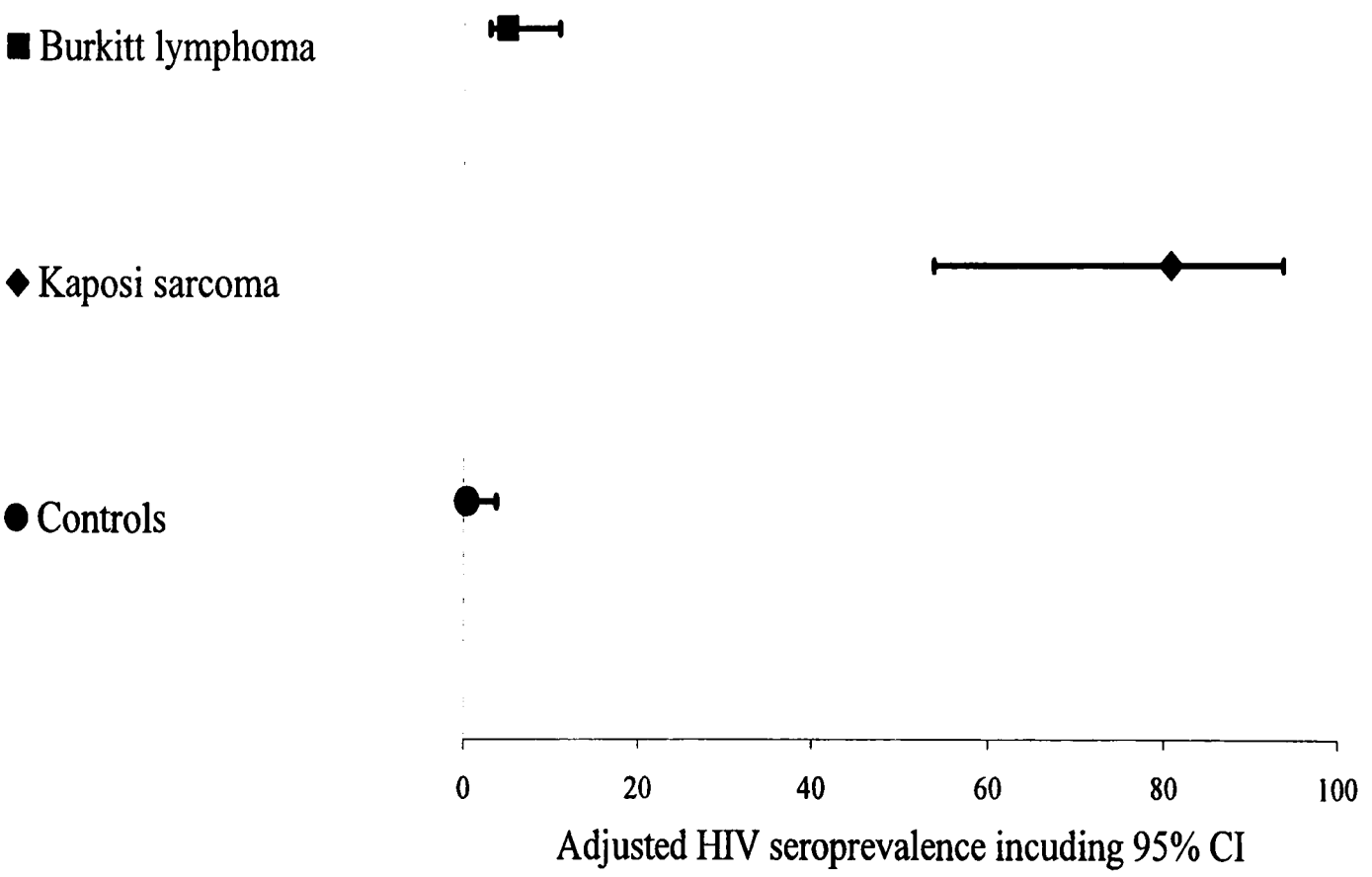
Diagnosis	**[1]Child HIV status**	**Cases/Controls[2] n (%)**	**Crude OR (95%CI)**	**[3]Adjusted OR (95%CI) χ^2 for heterogeneity**
Kaposi sarcoma (18/103)	Positive	17/2 (81/2)	193.4 (32.8 to 1140.5)	762.7 (44 to 13376)
	Negative	4/91 (19/98)	1.0	1.0
	Missing	*1/10*	χ^2 (1df)=77 $P<0.001$ exact=0.0	χ^2 (1df)=62 $P<0.001$
Burkitt lymphoma (146/103)	Positive	9/2 (6/2)	2.9 (0.6 to 14.2)	12.4 (1.3 to 116.2)
	Negative	137/91 (94/98)	1.0	1.0
	Missing	*1/10*	χ^2 (1df)=2.1 $P=0.15$ exact=0.21	χ^2 (1df)=7.2 $P=0.03$

[1] Excludes HIV discrepant mother-child pairs (mother negative, child positive)
[2] See Table 4.1
[3] Adjusted for child age, sex and residence

Appendix 7.1b: Adjusted seroprevalence child HIV status[1]

Cancer type	Seroprevalence of HIV antibodies (%) (n/total)	[2]Adjusted seroprevalence of HIV antibodies (%)	Lower 95%CI	Upper 95% CI
Burkitt lymphoma	6 (9/146)	5	3	11
Kaposi sarcoma	81 (17/21)	81	54	94
Wilms tumour	0 (0/29)	-	-	-
Retinoblastoma	0 (0/12)	-	-	-
Rhabdomyosarcoma	0 (0/18)	-	-	-
		Test for heterogeneity χ^2 (1df)=49.9 $P = 0.0001$		
[3]Controls	2 (2/93)	0.4	0.04	3.8

Graph 7.1 Adjusted child HIV seroprevalence for all children

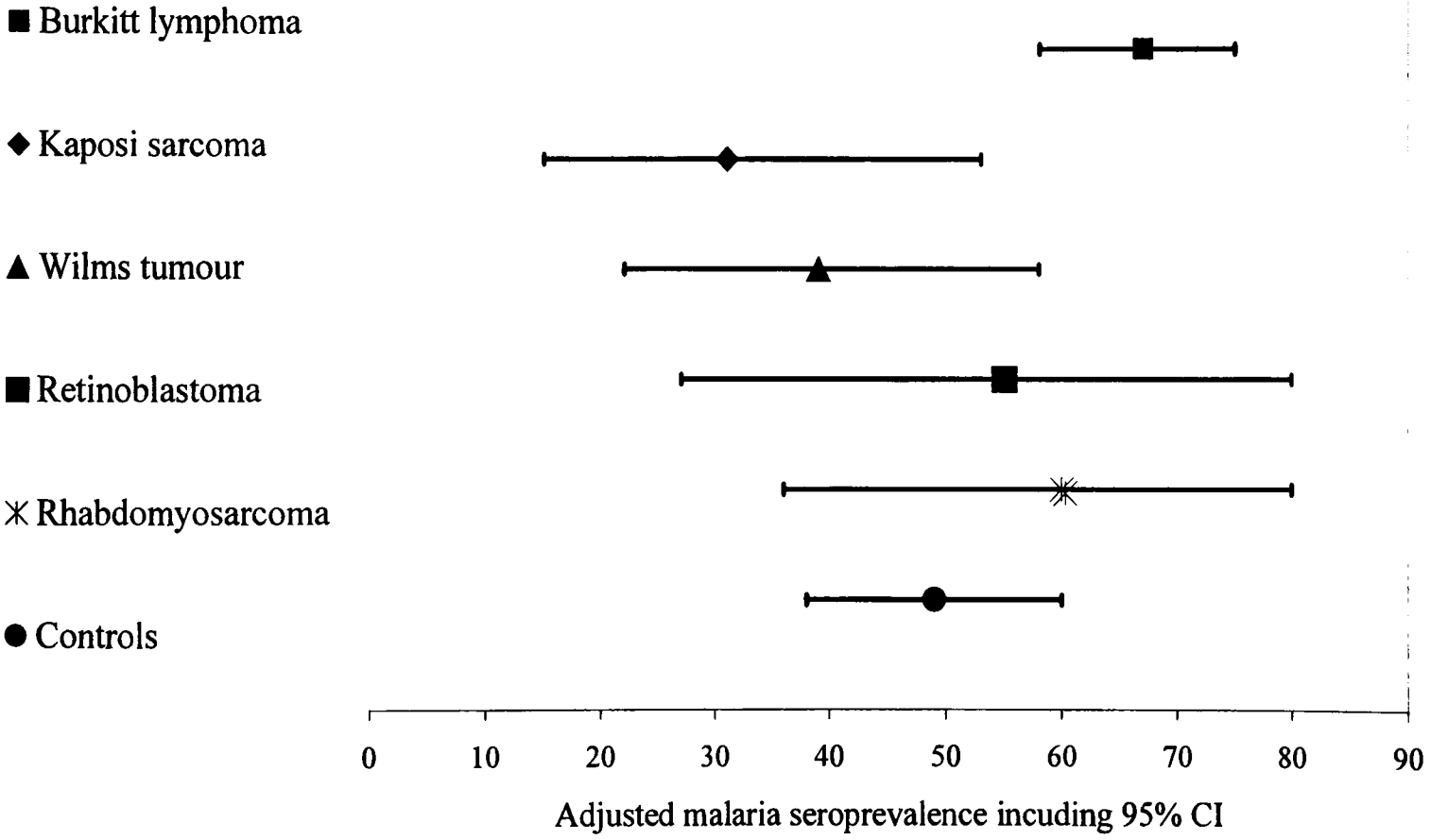


[1] Excludes HIV discrepant mother-child pairs (mother negative, child positive)
[2] Adjusted for child age, sex and residence
[3] See Table 4.1

Appendix 7.2: Adjusted seroprevalence child malaria ([1]All children)

Cancer type	Seroprevalence of malaria antibodies (%) (n/total)	[2]Adjusted seroprevalence of malaria antibodies (%)	Lower 95%CI	Upper 95% CI
Burkitt lymphoma	68 (95/139)	67	58	75
Kaposi sarcoma	32 (7/22)	31	15	53
Wilms tumour	33 (10/30)	39	22	58
Retinoblastoma	50 (7/14)	55	27	80
Rhabdomyosarcoma	61 (11/18)	60	36	80
		Test for heterogeneity χ^2 (4df)= 13.6 P = 0.009		
[3]Controls	48 (46/96)	49	38	60

Graph 7.2 Adjusted malaria seroprevalence for all children

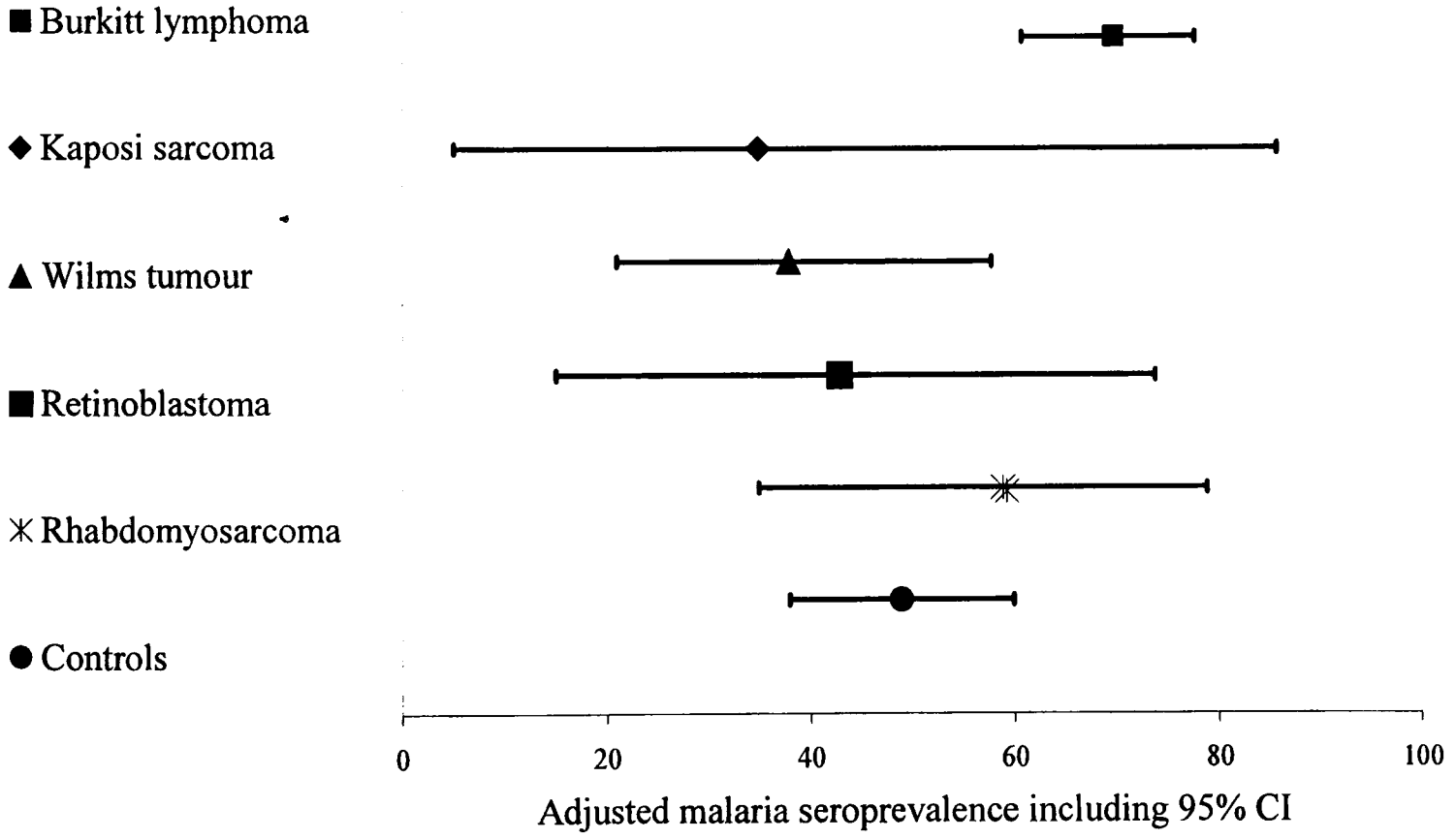


[1] All categories of HIV status
[2] Adjusted for child age, sex and residence
[3] See Table 4.1

Appendix 7.3: Adjusted seroprevalence child malaria (Restricted to HIV negative children)

Cancer type	Seroprevalence of malaria antibodies (%) (n/total)	[1]Adjusted seroprevalence of malaria antibodies (%)	Lower 95%CI	Upper 95% CI
Burkitt lymphoma	71 (91/129)	70	61	78
Kaposi sarcoma	25 (1/4)	35	5	86
Wilms tumour	34 (10/29)	38	21	58
Retinoblastoma	42 (5/12)	43	15	74
Rhabdomyosarcoma	61 (11/18)	59	35	79
		Test for heterogeneity χ^2 (4df)=8.9 P = 0.06		
[2]Controls	47 (42/90)	49	38	60

Graph 7.3 Adjusted malaria seroprevalence for HIV negative children

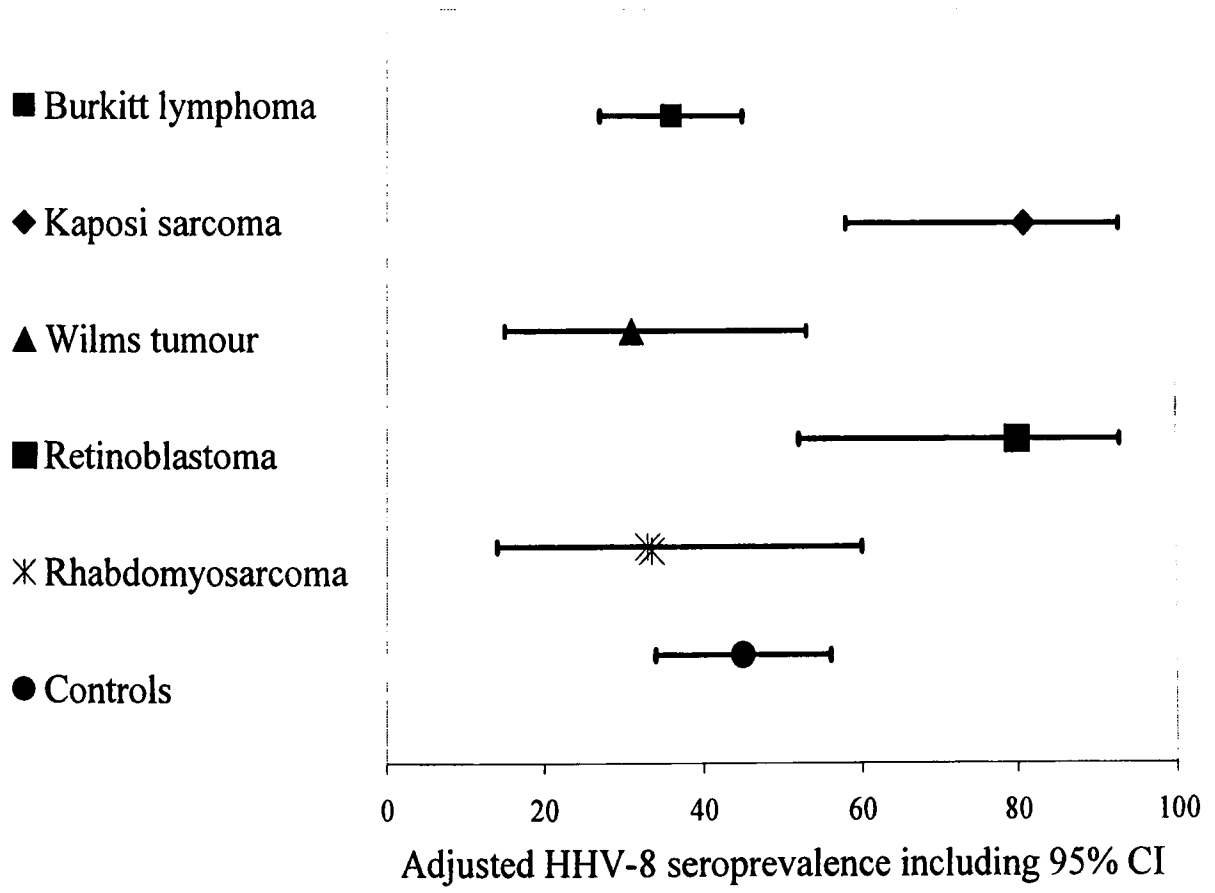


[1] Adjusted for child age, sex and residence
[2] See Table 4.1

Appendix 7.4: Adjusted seroprevalence child HHV-8 ([1]All children)

Cancer type	Seroprevalence of HHV-8 antibodies (%) (n/total)	[2]Adjusted seroprevalence of HHV-8 antibodies (%)	Lower 95%CI	Upper 95% CI
Burkitt lymphoma	42 (57/137)	36	27	45
Kaposi sarcoma	77 (17/22)	81	58	93
Wilms tumour	24 (7/29)	31	15	53
Retinoblastoma	57 (8/14)	80	52	93
Rhabdomyosarcoma	29 (5/17)	33	14	60
		Test for heterogeneity χ^2 (4df)= 21.9 $P = 0.0002$		
[3]Controls	38 (36/94)	45	34	56

Graph 7.4 Adjusted HHV-8 seroprevalence for all children

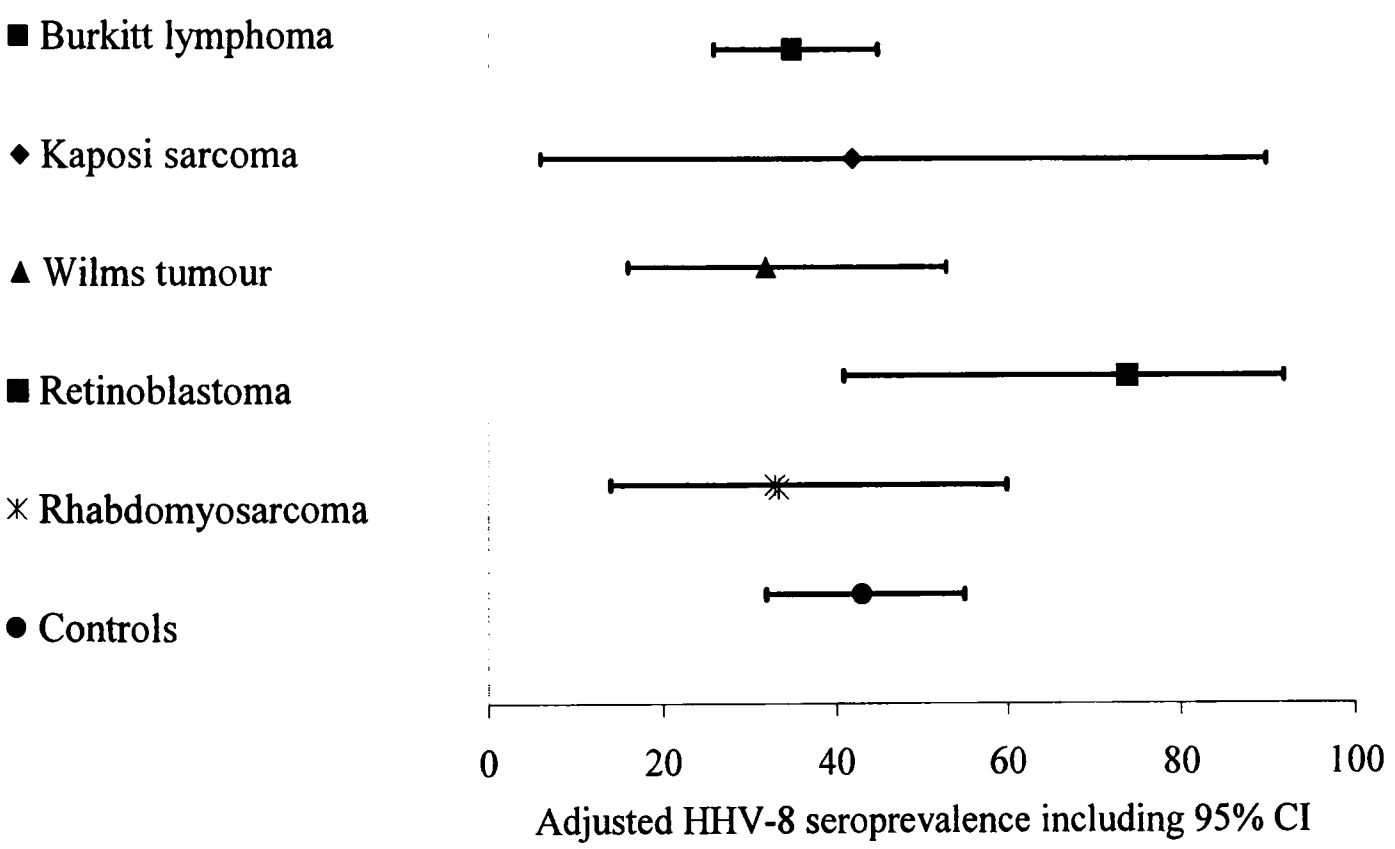


[1] All categories of HIV status
[2] Adjusted for age, sex and residence
[3] See Table 4.1

Appendix 7.5: Adjusted seroprevalence child HHV-8 (Restricted to HIV negative children)

Cancer type	Seroprevalence of HHV-8 antibodies (%) (n/total)	[1]Adjusted seroprevalence of HHV-8 antibodies (%)	Lower 95% CI	Upper 95% CI
Burkitt lymphoma	40 (51/127)	35	26	45
Kaposi sarcoma	25 (1/4)	42	6	90
Wilms tumour	25 (7/28)	32	16	53
Retinoblastoma	50 (6/12)	74	41	92
Rhabdomyosarcoma	29 (5/17)	33	14	60
		Test for heterogeneity χ^2 (4df)=5.6 $P = 0.2$		
[2]Controls	36 (32/88)	43	32	55

Graph 7.5 Adjusted HHV-8 seroprevalence for HIV negative children

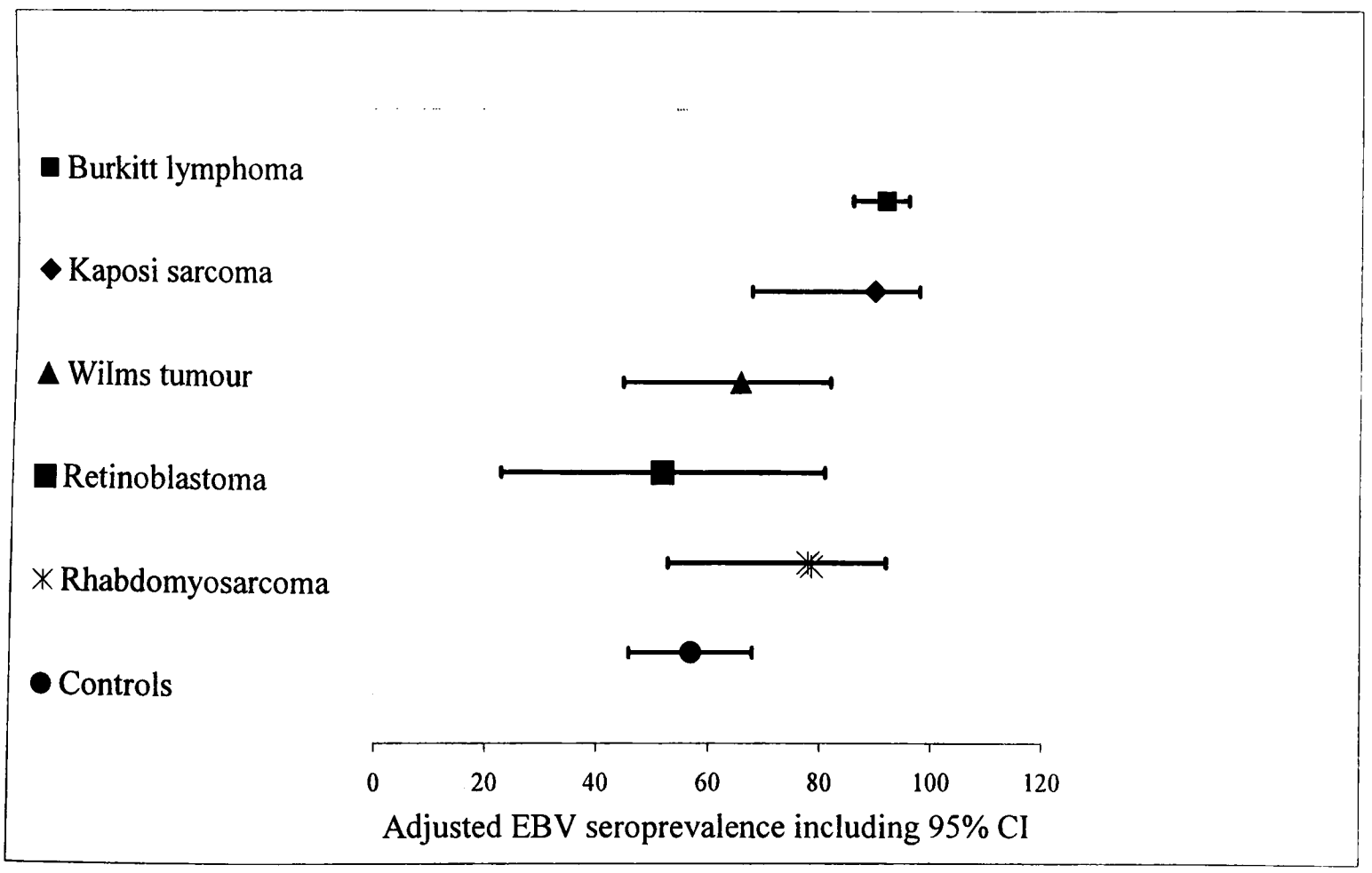


[1] Adjusted for childe age, sex and residence
[2] See Table 4.1

Appendix 7.6: Adjusted seroprevalence child EBV ([1]All children)

Cancer type	Seroprevalence of EBV antibodies (%) (n/total)	[2]Adjusted seroprevalence of EBV antibodies (%)	Lower 95%CI	Upper 95% CI
Burkitt lymphoma	91 (126/138)	92	86	96
Kaposi sarcoma	91 (20/22)	90	68	98
Wilms tumour	67 (20/30)	66	45	82
Retinoblastoma	57 (8/14)	52	23	81
Rhabdomyosarcoma	72 (13/18)	78	53	92
		Test for heterogeneity χ^2 (4df)=14.1 $P = 0.006$		
[3]Controls	57 (54/95)	57	46	68

Graph 7.6 Adjusted EBV seroprevalence for all children

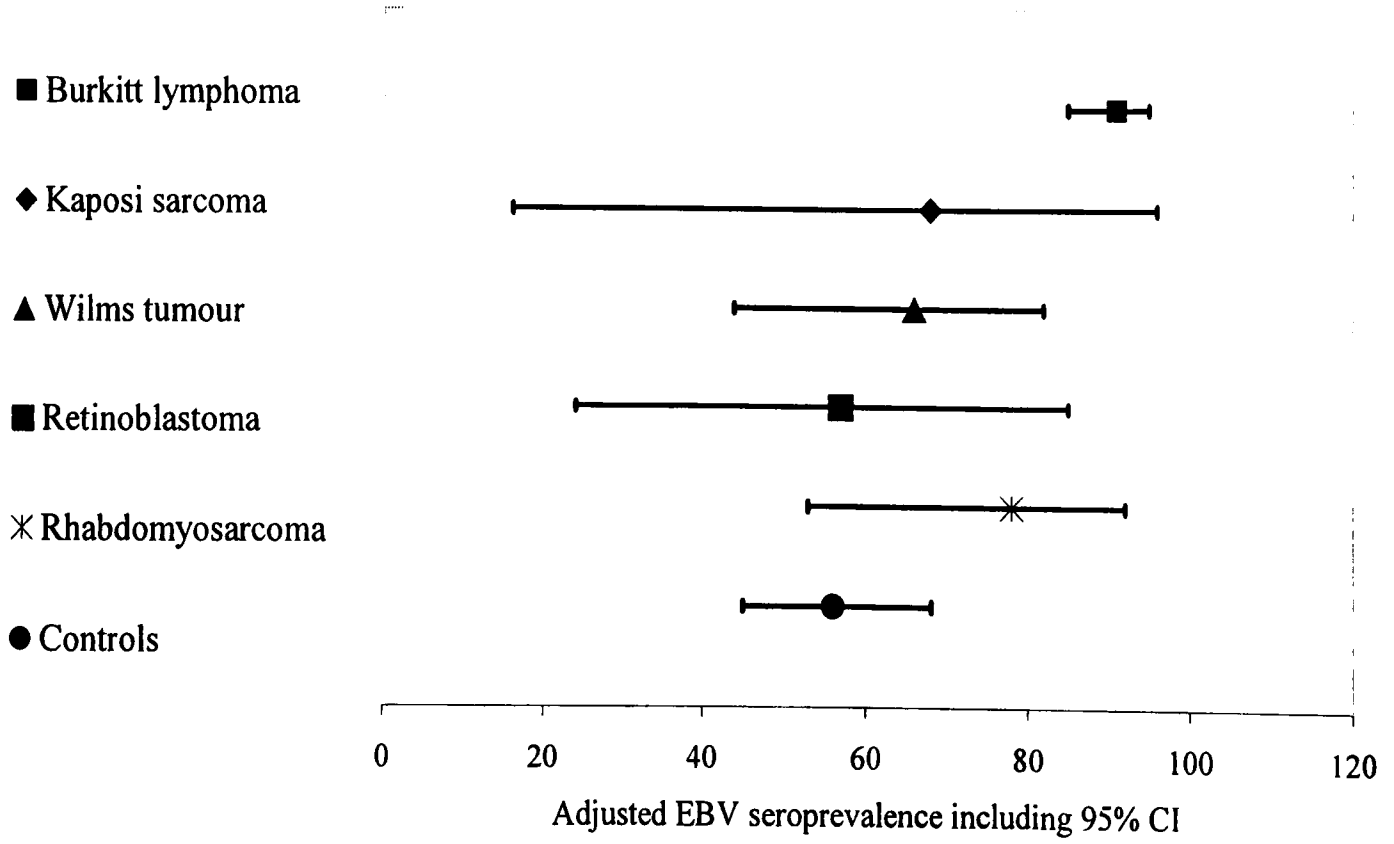


[1] All categories of HIV status
[2] Adjusted for child age, sex and residence
[3] See Table 4.1

Appendix 7.7: Adjusted seroprevalence child EBV (Restricted to HIV negative children)

Cancer type	Seroprevalence of EBV antibodies (%) (n/total)	[1]Adjusted seroprevalence of EBV antibodies (%)	Lower 95%CI	Upper 95% CI
Burkitt lymphoma	91 (116/128)	91	85	95
Kaposi sarcoma	75 (3/4)	68	16	96
Wilms tumour	66 (19/29)	66	44	82
Retinoblastoma	58 (7/12)	57	24	85
Rhabdomyosarcoma	72 (13/18)	78	53	92
		Test for heterogeneity χ^2 (4df)=10.7 P =0.03		
[2]Controls	57 (51/89)	56	45	68

Graph 7.7 Adjusted EBV seroprevalence for HIV negative children



[1] Adjusted for child age, sex and residence
[2] See Table 4.1