

The epidemiology of Kaposi's sarcoma associated-herpesvirus in Uganda

Volume 1 of 1

by

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Thesis submitted in fulfillment of a Doctor of Philosophy

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May 2013

Abstract

Over the past two decades there has been an explosion in the number of cases of Kaposi's sarcoma (KS) in parts of sub-Saharan Africa, where Kaposi's sarcoma associated-herpesvirus (KSHV) and HIV are relatively prevalent. Currently KS is the most commonly reported cancer in Uganda causing significant morbidity and mortality. Limiting KSHV transmission or halting disease progression could prevent KS. Here, I describe an investigation of factors that might impact on transmission of KSHV and report the first prospective study of antibody titre to KSHV to determine risk of KS from Africa.

Stored samples from Medical Research Council, Uganda cohorts were tested using an ELISA to KSHV antigens. Results from a birth cohort found that among both mothers and children malaria parasitaemia was identified as a novel association with KSHV seropositivity. Among children HIV exposure and HIV infection was associated with antibodies to KSHV. A random effects meta-analysis conducted to clarify wider evidence of an association between KSHV and HIV found that HIV was associated with an increased prevalence of antibodies to KSHV in mothers and children. A case-control study nested within a longitudinal HIV cohort found among individuals who develop KS, antibody titres to KSHV are higher and increased over time compared to adults who did not.

It is plausible that control of malaria may also reduce the spread of KSHV. How malaria may interact with KSHV and if malaria control will reduce transmission are key future questions. Prevention and treatment of HIV with anti-retroviral therapy may lower KSHV transmission between mothers and children. For individuals with HIV-KSHV co-infection, increasing antibody titre to KSHV precedes development of KS. Research is required to elucidate co-factors driving progression to cancer. A clinically valid tool to screen for risk of HIV-associated KS is urgently needed.

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ABBREVIATIONS

CLR	Conditional logistic regression
ELISA	Enzyme-linked immunosorbent assays
EMaBS	Entebbe Mother and Baby Study
epg	Egg per gram
GEE	Generalized estimating equations
GPC	General Population Cohort
HHV8	Human herpesvirus 8
HIV	Human Immunodeficiency Virus
HLA	Human leukocyte antigen
IFA	Immunofluorescence assays
IV	Intravenous
KICS	KSHV-associated inflammatory cytokine syndrome
KS	Kaposi's sarcoma
KSHV	Kaposi's sarcoma-associated herpesvirus
MCD	Multicentric Castleman's disease
MRC	MRC Uganda Research Unit on AIDS
MSM	Men who have sex with men
NCI	National Cancer Institute
OR	Odds ratio
PBMC	Peripheral blood mononuclear cell
PRISMA	Preferred Reporting Items for Systematic reviews and Meta-Analyses
RCC	Rural Clinical Cohort
STD	Sexually transmitted disease
STI	Sexually transmitted infection
UHSBS	Uganda HIV-AIDS sero-behavioural survey
UVRI	Uganda Virus Research Institute
VOS	Viral Oncology Section

Preface

A Wellcome Trust Training Fellowship funded the work leading to this thesis. This PhD was designed to utilize the cohorts at the Medical Research Council (MRC) Uganda. I trained in the KSHV ELISA technique at the National Cancer Institute, USA and transferred and validated the KSHV serological assay to MRC Uganda. In preparation for the studies I organised the archiving of over 600,000 samples held at the MRC Uganda in Entebbe. I was responsible for ethical approval and annual reporting to the institutional and national (Ugandan) review boards. The management tasks for the studies included personnel and financial organisation. I conducted the statistical analysis in this thesis. Two papers have been published from this thesis and another is in draft form.

I would like to express my deepest gratitude to the individuals who made this thesis and related work possible. I hope to be able to emulate the support and kindness that I have been shown in the future.

Author's declaration

I confirm that the work presented within this thesis is my own work unless indicated by a reference and it has not been submitted for examination at this or any other institution for another award. The published work included in this thesis is as follows:

- Wakeham K, Webb EL, Sebina I, Muhangi L, Miley W, Johnson WT, Ndibazza J, Elliott AM, Whitby D, Newton R. Transmission of Kaposi's sarcoma associated herpesvirus (KSHV) in mother-child pairs in Uganda. J Acquir Immune Defic Syndr. 2013 Jun 1;63(2):228-33
- Wakeham K, Webb EL, Sebina I, Muhangi L, Miley W, Johnson WT, Ndibazza J, Elliott AM, Whitby D, Newton R. Parasites and Kaposi's sarcoma associated herpesvirus (KSHV) in Uganda. Infect Agent Cancer. 2011 Sep 30;6(1):15

Katie Wakeham May 2013.

Chapter 1: Introduction to thesis

1.1 Introduction

Kaposi's sarcoma (KS) is a neoplasm observed in individuals with immune compromise including HIV infection (HIV-associated KS) or after solid organ transplantation due to the use of immunosuppressive drugs (iatrogenic KS)¹. KS also occurs in people without overt immune suppression in the Mediterranean (classic KS) and Africa (endemic KS)². All four epidemiological forms of KS are identical histologically and characterised by pronounced lymphangio and angiogenesis¹. Substantial molecular and serological evidence supports Kaposi's sarcoma-associated herpesvirus (KSHV) as the causal agent of all forms of KS^{1, 3, 4}. Worldwide patterns of KSHV seroprevalence show a striking geographical variation⁵. The reasons underlying this remain unclear, but are important to understand as the occurrence of KS in a population is strongly correlated with KSHV seroprevalence.

Studies from sub-Saharan Africa report high KSHV prevalence⁶⁻²⁵, with primary infection beginning in childhood and increasing with age^{7, 9, 10, 12, 13, 15, 16, 22, 25}. There is considerable evidence, too, that KSHV transmission occurs via saliva^{26, 27} and in Africa transmission from mothers^{7, 13, 23, 25}, is likely to be an important route. Risk factors governing childhood vulnerability to infection with KSHV remain only partly elucidated. Exposures in childhood including HIV, other infectious diseases and socioeconomic conditions may be key. Understanding transmission dynamics is a prerequisite for the development of strategies to prevent spread and the subsequent diseases associated with KSHV.

Infection with KSHV alone does not confer a high risk of developing KS, implying that other factors are likely involved²⁸. The most important co-factor associated with KS development among KSHV infected individuals is HIV infection. KS is the most common tumour arising in HIV-infected persons, and is an AIDS-defining illness. In the United States during the nineties, KS was reported to be over 20,000 times more common in

persons infected with HIV than in the general population and over 300 times more common in people with HIV than in other immunosuppressed hosts, such as renal transplant recipients²⁹. In the era of HIV, the cancer registry in Kampala, Uganda reports KS as the commonest cancer in adults and children³⁰⁻³². Not all HIV-KSHV co-infected individuals develop KS³³ and understanding the evolution of biomarkers such as KSHV antibodies in asymptomatic KSHV-HIV co-infected individuals may be key to identifying individuals at highest risk of KS. Screening for risk of KS may be particularly important in resource poor settings where access to ART and cancer treatments are limited. While HIV is one of the strongest predictors of KS development in KSHV infected individuals, the occurrence of KS in HIV-negative individuals suggests host, viral or environmental co-factors predisposing to KSHV oncogenesis³⁴. An improved understanding of the epidemiology of KS and KSHV may give clues to additional as yet unknown factors.

1.1.2 Thesis aim

This thesis specifically addresses the following primary research questions:

1. Are HIV and other common infectious agents present in Uganda risk factors for antibodies to KSHV among mothers and their children?
2. Are measures of socioeconomic status associated with maternal and childhood KSHV seropositivity?
3. What is the level of evidence in published literature for HIV as a co-factor for KSHV seroprevalence among mother-child pairs?
4. Among HIV-KSHV co-infected adults resident in Uganda, does the pattern of antibody titre to KSHV differ between those who develop KS compared to those who do not?

Questions 1,2 and 4 outlined above were conducted within the context of existing cohorts at the MRC Uganda Research Unit on AIDS (MRC) and the Uganda Virus Research Institute (UVRI). The first cohort, the Entebbe Mother and Baby Study (EMaBS) was a large Wellcome Trust funded

double blind randomised placebo controlled trial, which was designed to determine the impact of helminth infections and their treatment on vaccine responses and infectious disease outcomes³⁵. The second was a cohort of HIV-infected adults recruited from a long standing MRC/UVRI General Population Cohort (GPC)³⁶. HIV positive individuals were followed at regular intervals in a dedicated study clinic, which provided clinical care and anti-retroviral therapy (ART). KSHV serology technology was transferred to MRC/UVRI from the National Cancer Institute (NCI), USA. Access to the rich datasets held at MRC/UVRI and the opportunity to link KSHV serology results provides the opportunity to address important primary and secondary questions on the epidemiology of KSHV in Uganda. Question 3 was addressed by a systematic literature review and meta-analysis. The intent of this study is to bring additional clarity to the evidence of the role of HIV as a co-factor for KSHV transmission between mothers and their children.

1.2 Thesis structure

First, the dramatic change in the clinico-epidemiological profile of KS in the era of HIV will be described. HIV-associated KS is an aggressive cancer with high mortality and is a major public health concern in Uganda. The association of detection and titre of antibodies to KSHV and risk of HIV-associated KS is presented. Next, the occurrence of KS in individuals without overt immune suppression is discussed. Patterns of occurrence of classic and endemic KS may suggest co-factors for oncogenesis in addition to HIV. Regional patterns of KSHV seroprevalence may suggest potential co-factors for KSHV infection. Of key interest in this thesis is KSHV seroprevalence among mothers and children in Uganda. Childhood is a major risk period for primary KSHV infection and mothers are likely the child's predominant source. HIV is potentially an important co-factor in KSHV transmission, but other local factors in Uganda may also be important including parasites. The results chapters then present; (1) a study of risk factors for KSHV seropositivity in mothers and their children in Uganda; (2) a meta-analysis of the association between HIV and KSHV in mothers, pregnant women and

children, and (3) an investigation of antibody titre against KSHV and risk of developing HIV-associated KS. The final chapter is a discussion of the results their implications.

Chapter 2: Kaposi's sarcoma

2.1 Chapter contents

The epidemiology of KS dramatically altered in the era of HIV. In Uganda KS is now the commonest cancer in HIV-infected individuals. The epidemiology of KS among individuals without evident immune deficiency may reveal clues as to potential co-factors for disease development. To potentially screen for and prevent HIV-associated KS, risk factors that precede development of this tumour are important to determine. Antibody titres to KSHV are a potential biomarker for screening for risk of KS.

Objectives:

- I. To describe the epidemiology of KS in the era of HIV.
- II. To describe the epidemiology of the forms of KS not associated with overt immune suppression (so called classic and endemic KS).
- III. To review risk factors for HIV-associated KS. A critical review of relevant literature available in PubMed on risk of KS development and antibody titres to KSHV in HIV infected adults is presented.

Summary: The presence of HIV infection is one of the strongest predictors of risk for KS in KSHV infected individuals. Detection of antibodies to KSHV precedes and is predictive of development of HIV-associated KS.

2.2 Introduction

KS was first described by Moritz Kaposi's in 1872³⁷. He reported a skin tumour on the lower limbs of older men of Mediterranean or Ashkenazi Jewish descent which he named "idiopathic multiple pigmented sarcoma"³⁸. Subsequently, in the 1950's and 1960's KS was reported on the lower limbs of older black men resident in sub-Saharan Africa³⁹. Reports described a tumour with a striking geographical distribution with highest rates in North East and Eastern Zaire (Democratic Republic of Congo), Rwanda, Burundi, Uganda, Malawi, Tanzania, Rhodesia (Zimbabwe) and Kenya³⁹⁻⁴⁷. KS presenting in older men in the

Mediterranean and Africa was considered a relatively rare indolent tumour at this time². Cases of KS subsequent to solid organ transplant have been reported in the literature since the 1970's in individuals of Mediterranean, Jewish, Arabic, Caribbean, or African descent⁴⁸. The relative rarity of KS was to change in the 1980's. In 1981 the Centers for Disease Control and Prevention (CDC) Morbidity and Mortality Weekly Review reported the occurrence of KS and pneumocystis pneumonia among men who have sex with men (MSM)⁴⁹. This report heralded the advent of the HIV epidemic⁴⁹. HIV changed both the clinical occurrence and the epidemiology of KS. HIV-associated KS became recognised as an aggressive cancer with high morbidity and mortality⁵⁰. As the HIV epidemic progressed in Africa, KS became the most commonly reported cancer in many countries in this region⁵¹. The high prevalence of KS in countries such as Uganda is a major health problem due to the lack of potential treatment options including ART and chemotherapy. In 1994 a gamma herpesvirus was identified in KS biopsies by Chang and Moore⁴. The virus was named Kaposi's sarcoma-associated herpes virus (KSHV) or human herpesvirus 8 (HHV-8). In a recent comprehensive review of the role of KSHV in the aetiology of KS, conducted by the International Agency for Research on Cancer⁵², it was concluded that KSHV is a class 1 carcinogen (defined as being 'definitely carcinogenic to man') and the principle underlying cause of the malignancy.

2.3 Clinical-epidemiological forms of Kaposi's sarcoma

KS is a low-grade tumour of endothelial origin⁵³. KS tumours are noted to have a large inflammatory and vascular component⁵⁰. Historically, the first disease associated with KSHV infection was KS. However, it soon became acknowledged that two other conditions were also linked to this virus: primary effusion lymphoma and Castleman's disease. KSHV is the aetiological agent for four clinical-epidemiological types of KS (Table 1):

HIV-associated: This type of KS is the most common tumour arising in HIV-infected persons, and is an AIDS-defining illness in the CDC guidelines. HIV-associated KS typically has an aggressive clinical

behaviour characterized by disseminated muco-cutaneous and visceral lesions.

Classic: An indolent cutaneous proliferative disease, mainly affecting the lower extremities of older men of Mediterranean and Jewish origin^{54, 55}. Apparent immune deficiency is not associated with this type of KS.

Endemic: The endemic form of KS is found in equatorial Africa and typically affects older men, but is also reported to occur in children. It is not thought to be associated with overt immune suppression⁴⁰.

Iatrogenic or transplant-associated: KS may occur after solid organ transplantation due to the use of immunosuppressive drugs such as calcineurin inhibitors. Transplant-associated KS is similar to HIV-associated KS in its clinical presentation and usually regresses with reduction in immunosuppression⁵⁶.

Table 2.1: Table of sub-types of KS, risk groups, clinical presentation, course and if overt immune suppression is commonly recognised

Type	Main at risk groups	Clinical presentation	Clinical course	Overt immune suppression
Endemic	Older men Equatorial Africa Children	Lower limbs in adults Viscera and lymph nodes in children	Rarely aggressive in adults	No
Classic	Older men 3:1 male: female Mediterranean, Israel	Lower limbs	Indolent	No
Iatrogenic	Post transplant Use of calcineurin inhibitors	Disseminated	Aggressive May regress on modification of immunosuppressive drugs	Yes
HIV-associated	MSM Heterosexual men and women in Africa	Disseminated	Aggressive May regress with ART	Yes

2.4 Worldwide occurrence of Kaposi's sarcoma

Worldwide patterns of HIV-associated KS incidence broadly mirror that of HIV-KSHV co-infection. Currently, the prevalence of HIV-associated KS greatly eclipses the occurrence of the other clinical-epidemiological types of this cancer²⁹. The HIV epidemic has significantly changed the public health importance of KS, which is reflected in the current magnitude of research effort. Iatrogenic or post-transplant KS most commonly occurs in the setting of solid organ transplant in populations where KSHV is relatively prevalent⁴⁸. KS in individuals without overt immune suppression tend to occur in specific geographical locations in equatorial Africa (endemic) and the Mediterranean (classic), with a distinct age and sex bias^{40, 57}. The pathogenesis of classic and endemic KS among KSHV infected individuals remains an enigma. Investigation of the underlying pathogenesis of KS in individuals without HIV and iatrogenic immunosuppression may reveal subtle and indeed key mechanisms of oncogenesis.

2.4.1 The epidemiology of HIV-associated Kaposi's sarcoma

HIV is the strongest risk factor for development of KS in a KSHV infected individual. In the United States, before the introduction of ART, KS was over 20,000 times more common in persons infected with HIV than in the general population and over 300 times more common in HIV infected subjects than in other immunosuppressed hosts, such as renal transplant recipients^{29, 58}. The reported relative risk of HIV-associated KS among individuals in Africa is lower than in studies from North America^{58, 59}. This is likely due to higher background occurrence of endemic KS in Africa.

In North America and Europe, KS is a cancer predominantly found in HIV-infected MSM, the risk group with highest KSHV seroprevalence. KS is much less common in HIV-infected heterosexuals, injection drug users, transfusion recipients, women, children and haemophiliac patients, all of whom tend to have a lower KSHV seroprevalence compared to MSM²⁹. In

sub-Saharan Africa, a dramatic increase in the occurrence of KS has been reported over the past 30 years^{51, 60}. In Africa, although KS is more common in men than in women, the sex-ratio is much less disparate, a consequence of similar HIV and KSHV prevalence in men and women^{51, 60}.

In Uganda, the Kampala Cancer Registry has shown a significant increase in the incidence of KS in the era of the HIV epidemic³² (Figure 2.1) with narrowing of the sex-ratio and a dramatic rise in reported cases in children. Registration of KS in the 1960's was likely "endemic" type^{39, 41, 46, 47}, an indolent tumour affecting the legs of predominantly men, with risk increasing with age. In the time period between 1971 and 1991 the surge in reported occurrence of KS reflects HIV-associated KS^{31, 32}. By the late 1990's, KS was the commonest reported cancer among men in Uganda and second only to cancer of the cervix among women^{31, 32}.

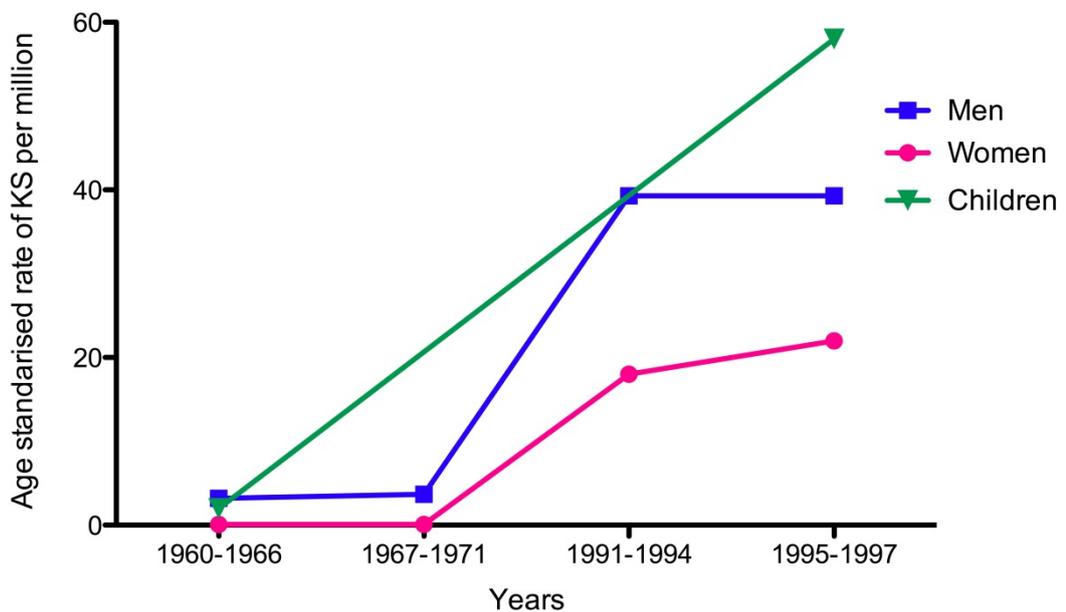


Figure 2.1: Age-standardized rates (world standard population) of KS reported by the Kampala Cancer Registry, Uganda in four time periods for men, women and children. Data taken from Trends in cancer incidence in Kyadondo County, Uganda, 1960- 1997, British Journal of Cancer (2000) 82(9), 1585, Wabinga H.R. et al.³²

In South Africa a similar increase in the incidence of KS, in men and women has been reported in the era of HIV⁵⁹. In Zambia, KS in infants and young children occurred almost exclusively after the advent of the HIV epidemic with a near equal sex-ratio⁶¹. Similar changes are reported in other African countries⁵¹.

In the Mediterranean, where classic type KS is endemic, the arrival of HIV also caused an increase in KS incidence and the ratio of male-to-female cases to drop from 10:1 to 3:1⁶².

KS is infrequently reported among HIV-infected patients in Asia. In a study of 248 patients infected with HIV in Bangkok, Thailand, no KS was reported⁶³. There is a paucity of published literature about HIV-associated KS in Asia and comprehensive cancer registry data is required to confirm KS epidemiology.

ART has led to marked declines in the incidence of HIV-associated KS. In data derived from USA population-based cancer registries, KS rates among white men rose from 0.5 per 100,000 people per year in 1973, to between 31.3 and 33.3 from 1987 through 1991, and then declined to 2.8 in 1998 with the advent of ART⁶⁴. In another North American study, the standardized incidence ratio for KS within a HIV-infected population compared to the general population fell from 22,100 to 3640 (1990-1995 to 1996-2002) with the widespread use of ART⁶⁵. The 5-year survival rates for patients with HIV-related KS in North America has improved from about 10% (1980-1995) to 50% (1996-2005)⁶⁶. Much of this improvement in survival is attributed to ART. Data on the impact of ART on KS incidence in Africa is lacking, in part due to the paucity of cancer registration. It may be expected that the impact of ART roll-out will be less in this region due to limits in ART coverage and that HIV-infected individuals commonly present with advanced AIDS defining illnesses.

2.4.2 The epidemiology of iatrogenic or post-transplant Kaposi's sarcoma

Post-transplant KS tends to occur in countries that carry out a high per capita number of organ transplants and have a significant population prevalence of KSHV: Iran, Israel, Greece, Italy⁶⁷⁻⁷⁰. A study in Iran reported KS as the commonest tumour type in solid organ transplant recipients⁶⁷, as have studies in Israel⁶⁸, Italy⁶⁹ and Greece⁷⁰. KS is seen most often in patients whose immunosuppressive treatment regime includes calcineurin inhibitors such as cyclosporine.

2.4.3 The epidemiology of Kaposi's sarcoma among individuals without overt immune suppression

Forms of KS that occur without overt immunosuppression (classic and endemic KS) have distinct geographical, age and gender influences². These factors may have profound aetiological significance, but they remain unclear at present. It is important to discriminate classic and endemic type KS from KS that occurs in individuals with profound immune deficiency (HIV and post transplantation) because the epidemiological distributions are very different. A paucity of robust worldwide seroprevalence studies and major limitations in cancer registration hampers research. This is particularly problematic in sub-Saharan Africa and parts of Asia and South America where health care systems and collections of epidemiological data are very limited.

2.4.3.1 Geographic and Ethnic variation of Classic and Endemic type Kaposi's sarcoma

The incidence rates of classic type KS in reports from Europe vary (Table 2.2)^{54, 55, 71-84}. Low rates are reported in England⁸⁴, Scotland⁸³, Denmark⁷⁵ and Sweden⁷⁶, with slightly higher rates reported from France and Spain⁵⁴. Relatively high rates are reported in Greece⁷³, North and Central Italy⁷⁸, the Faroe Islands and Iceland⁷⁷. The highest incidence rates in Europe are reported in Sardinia and Southern Italy (Sassari)⁷⁸

(Table 2.2). Outside Europe, low rates of KS are reported in older men in the USA⁸², Canada⁵⁴ and Australia⁸¹. Israel reports relatively high rates of classic KS, especially among individuals of Jewish descent^{54, 55, 71, 72}.

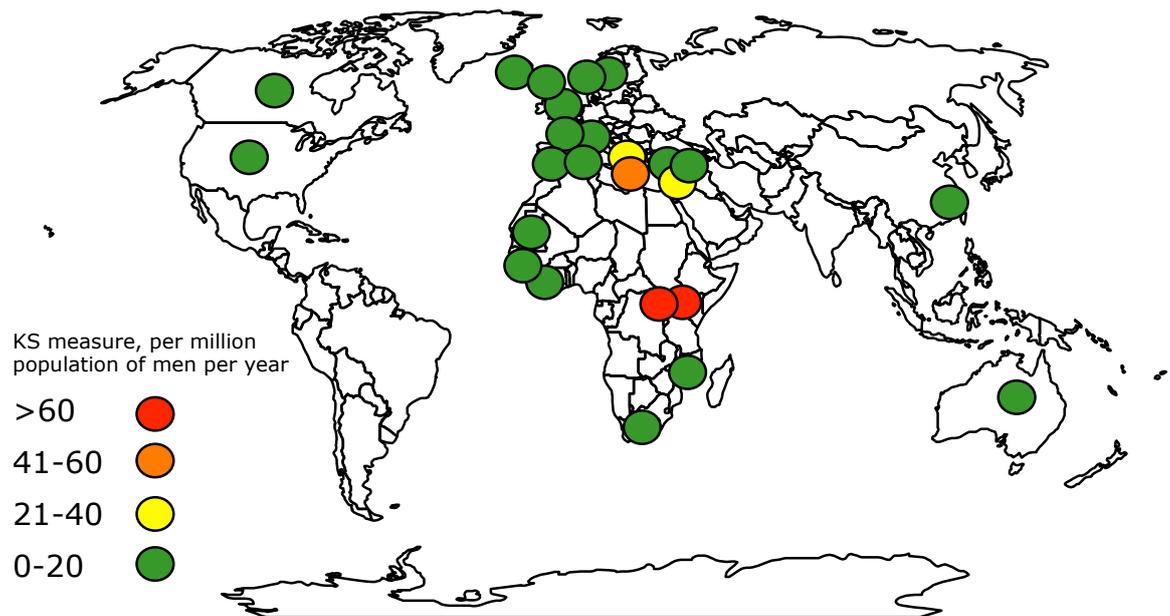


Figure 2.2: Measure of classic or endemic KS among men over the age of 65 years. The databases and studies used in this figure are tabulated in Table 2.2. The literature search is described in the general methods section.

Reports have suggested that the incidence of Classic KS may be increasing in Europe and Israel^{54, 74}. This was noted prior to the AIDS epidemic and as a separate phenomenon to the increased rate of KS after immunosuppressive therapy for solid organ transplants. It remains unclear whether this is due to greater registration of cancers due to improved access to healthcare and better diagnosis classification, changes in the general population including aging and immigration of

individuals from higher risk areas to countries in Europe with lower KS incidence, or due to an increase in factors driving KS development.

The published data on rates of endemic KS in Africa come from historical data collected in the 1960's and 70's³⁹⁻⁴⁷. More recent data on the occurrence of endemic KS in Africa can only be inferred from reports of KS in older men (Table 2.2)^{32, 85-90}. The first report was published in 1961 by Oettle who described endemic KS as being predominately found in older black men and suggested environmental factor(s) could best explain its distribution³⁹. The highest rates at this time were found in a band across central and East Africa. The rate of endemic KS appeared to fall off towards West and South Africa^{39, 46}. In Uganda, geographical restriction of the cancer over relatively small distances, with the highest occurrence along the North Western border with the Congo (West Nile) and in the South West (Ankole and Kigezi) has been reported (Figure 2.3)^{40, 41, 46}. In Kenya, the highest incidence rates were reported among the Kikuyu, Embu and Meru tribes living in areas of high altitude with moderate rainfall. Much lower incidence was observed in hot dry areas along Lake Victoria and the coastal regions^{40, 42}. In Tanzania low rates of endemic KS were found along the Coast in Dodoma and Dar a Salaam with highest rates in Mwanza, on the shores of Lake Victoria^{40, 42}.

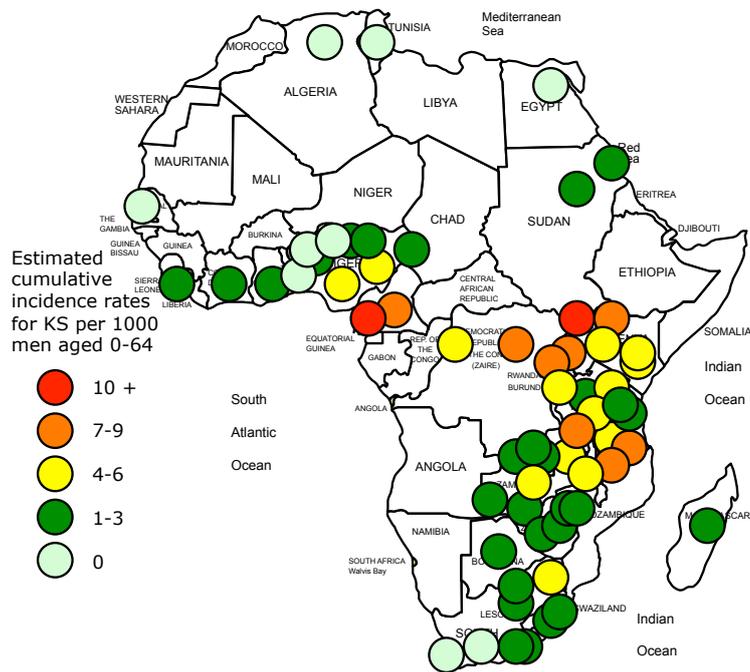


Figure 2.3: Estimated cumulative incidence rates for KS per 1000 men aged 0-64. Data taken from The geographical distribution of Kaposi's sarcoma and of lymphomas in Africa before the AIDS epidemic, *British Journal of Cancer* (1998); 78(11), 1521, Cook-Mozaffari, P. et al.⁴⁰

KS that resembles classic or endemic KS has been reported in Argentina, Colombia and Peru⁹¹⁻⁹³. Amerindian populations in the Brazilian Amazon basin have very high KSHV seroprevalence⁹⁴ but only a small number of reports of classic KS in this population are currently in published literature⁹¹⁻⁹³. Given the limitations of cancer registries in Brazil⁹⁵, it is currently difficult to interpret this information. Classic type KS is rarely reported in the Far East although case reports do exist⁹⁶.

Migrant studies suggest that risk for Classic KS may be associated with country of birth. Iscovich et al⁵⁴ reported incidence rates of classic KS among Jews born and living in Israel were different from immigrants; Jewish immigrants born in Africa have the highest incidence rates (ASR >27.0, 95% CI 1.4-2.4). Individuals born in Eastern Europe have intermediate rates (ASR between 14-18) and those born in Western Europe and Iran have the lowest rates (ASR 5.5, 95% CI 0.3-0.6). A

study conducted in the USA reported the highest incidence rate of classic KS is in Jews born in Eastern Europe and Mediterranean countries⁹⁷. Furthermore, second-generation American born Jews are at lower risk for classic KS than their parents⁹⁷. During the 1950's the rate of endemic KS in South Africa among immigrants from the Netherlands was significantly lower than Bantu people living in a similar area³⁹. The differential rates of KS may be due to variation in host or viral genetic or environmental risk factors for KSHV infection or KS development.

Table 2.2: KS per million population of men of classic or endemic KS or KS over the age of 60 years or prior to 1980.

Country	Year of data collection	Database or 1st author	KS measure	Notes on measure of KS (age standardised incidence rate, per million population among men, per year)
Americas				
Canada	1988-1992	Iscovich	0.2	Over the age of 65 years
USA	2009	SEER	0.6	Between 65-74 years
Europe				
Denmark	1970-1973	Hjalgrim	0.3	Over the age of 60 years
Faroe Islands	1974-1984	Hjalgrim	7	
France	1988-1992	Iscovich	2	Over the age of 65 years
Greece	1974-1978	Touloumi	10	
Iceland	1965-1969	Hjalgrim	6	
Italy (total)	1985-1998	Dal Maso	10	
Sassari (South Italy)	1985-1999	Dal Maso	47	Classic KS
North and central Italy	1985-2000	Dal Maso	9	Classic KS
Israel (Jews)	1988-1992	Iscovich	21	Over the age of 65 years
Israel	1961-1989	Iscovich	17	Classic KS
Sardinia	1987-1991	Cottini	25	Classic KS
Spain (Granada)	1988-1992	Iscovich	2	Over the age of 65 years
Sweden	1988-1992	Iscovich	0.3	Over the age of 65 years
United Kingdom (England)	2009	ONS	0.4	Over the age of 65 years
United Kingdom (Scotland)	2010	Scottish Cancer Registry	0	Over the age of 65 years
Australasia				
Australia	1972-1976	Kaldor	1	
China (Shanghai)	1988-1992	Iscovich	0	Over the age of 65 years
Kuwait	1988-1992	Iscovich	3	Over the age of 65 years
Africa				
Guinea	1992-1995	Koulibaly	0	Over the age of 65 years
Ivory Coast	1995-1997	Echimane	0	Over the age of 65 years
Mali	1988-1992	Iscovich	8	Over the age of 65 years
South Africa	1998-2002	Nontuthuzelo	0	Over the age of 65 years
Uganda	1988-1992	Iscovich	66	Over the age of 65 years
Uganda	1960-1971	Wahbinga	250	Over the age of 65 years
Zimbabwe	1988-1992	Iscovich	20	Over the age of 65 years

For methods and critique please see general methods chapter

SEER; United States Surveillance Epidemiology and End Results

ONS; United Kingdom's Office for National Statistics

2.4.3.2 Age and sex variations of Classic and Endemic type Kaposi's sarcoma

Classic KS has an overwhelming male predominance, with a male-to-female ratio of approximately 10:1 to 15:1⁵⁴. Endemic KS in Africa is reported to be about eight to ten times more common in men than in women⁴⁰. KS most commonly occurs in individuals over the age of 50 years⁵⁴.

2.5 The pathogenesis of Kaposi's sarcoma

Increased risk of KS among individuals with immunosuppression is well recognised. A key element for KSHV pathogenesis and KS development is T-cell immunity⁹⁸. In situations associated with loss of T-cell function such as HIV infection and after an organ transplant, the incidence of KS in those infected with KSHV dramatically increases.

HIV is associated with the absence of KSHV-specific T-cell responses^{99, 100}, increases in KSHV viral load⁹⁹ and progression of KS¹⁰⁰. Anti-retroviral therapy is associated with regression of KS and decreasing KSHV detection, and this is accompanied by restoration of numbers and functions of KSHV-specific CD4 positive^{98, 101, 102} and CD8 positive T-cells^{98, 102-104}.

KS development in KSHV infected individuals after solid organ transplant is linked to the use of calcineurin inhibitors. This class of drugs prevents organ rejection by immunosuppression largely through T-cell inhibition. Loss of KSHV-specific T-cells has been demonstrated with calcineurin inhibitors, with recovery and remission of KS on stopping medication¹⁰⁵. The majority of post-transplant KS patients are KSHV seropositive prior to transplantation, suggesting that reactivation of latent virus is responsible for the disease^{106, 107}.

While obvious immune suppression is not evident in classic and endemic

types of KS, more subtle modulation of immune function may be present. In an Italian study, patients with classic KS were found to have a significantly lower total lymphocyte count than KSHV seropositive individuals without KS¹⁰⁸. An increased risk of classic KS was associated with topical¹⁰⁹ and systemic corticosteroid¹¹⁰ use in a case control study of individuals with the cancer compared to KSHV seropositive controls. Classic and endemic KS are strongly associated with increasing age. Old age is characterized by progressive alternations to the immune system including declining effectiveness of T-cells and immune deficiency¹¹¹, that may be important in KS pathogenesis.

2.6 Risk factors for Kaposi's sarcoma

A substantial body of work shows that KSHV infection is necessary for KS development, but not all those infected develop the cancer. This points to the presence and/or inter-play of risk factors in addition to infection with KSHV. For all four clinical-epidemiological types of KS, replicating virus has an important role in the pathogenesis of the cancer^{108, 112-114}. KSHV viral replication can be estimated indirectly by detection of KSHV viral load in body fluids or antibody titration in plasma and serum. Other risk factors include host immunomodulating genes, KSHV sub-types and environmental co-factors¹.

In this section risk factors for HIV-associated KS will be discussed; first laboratory measurable markers of viral replication and then background host and other viral factors. Second, risk factors for KS in individuals without overt immune will be outlined, with emphasis placed on potentially subtle co-factors that may be revealed in the absence of HIV or immune suppression.

2.6.1 Laboratory measurable risk factors for HIV-associated Kaposi's sarcoma

The identification of laboratory measurable risk factors that precede and are predictive of the development of HIV-associated KS is important because the cancer is aggressive, highly morbid and is not curable. To be able to accurately predict individuals at the highest risk of KS may allow for screening and prevention. Antibody titre to KSHV antigens and the quantification of KSHV viral load in PBMC are obvious choices. While currently not clinically validated tools, KSHV antibody titre and viral load are relatively easy to perform measures of KSHV activity. Studies have shown that the detection of KSHV DNA in peripheral blood mononuclear cells (PBMCs) and high KSHV antibody titres are associated with the risk of development of HIV-associated KS^{33, 113, 115-117}.

2.6.2 Kaposi's sarcoma-associated herpes virus load and risk of Kaposi's sarcoma

In studies from North American and Northern European cohorts, KSHV DNA detection in PBMCs of HIV-infected individuals without KS predicts the subsequent appearance of KS lesions^{113, 114, 118-120}. In cross-sectional studies from sub-Saharan Africa HIV-infected individuals with KS have significantly higher KSHV viral loads in PBMCs¹²¹ and in plasma¹²² compared to HIV-seropositive individuals without KS. In studies from Uganda and Zimbabwe, among individuals with HIV-associated KS, increasing KSHV viral load is associated with the extent of KS lesions and the development of new KS lesions^{112, 123}. Among HIV-infected individuals undetectable KSHV viral load in PBMCs is associated with regression of KS lesions¹²⁴.

While evidence suggests that quantification of KSHV viral load is informative for detecting risk of KS, detection of KSHV viral load prior to KS development can be problematic. The technique is limited because (1) KSHV viral DNA loads in PBMCs are frequently not high enough to be detected¹²⁵, (2) assay costs are high, (3) through-put is low and (4) a

relatively sophisticated laboratory is required. These issues have limited the use of KSHV viral load assays in sub-Saharan Africa.

2.6.4 Antibodies to Kaposi's sarcoma-associated herpesvirus

Compared to assays to detect KSHV DNA, antibody assays offer potential through-put automation. KSHV serological assays are the tool used in this thesis. A comprehensive peer reviewed monograph has been published by IARC (monographs volume 100B)¹²⁶ which includes a number of tables presenting evidence for a causal association between the presence of KSHV viral load or antibodies and KS¹²⁶. This review was not repeated, but for this thesis it was essential to establish the evidence of association of the detection of antibody to KSHV and risk of HIV-associated KS. KSHV as the aetiological cause of KS was assumed. Hence, two critical literature searches were carried out in PubMed. First, the association between the detection of antibodies to KSHV and HIV-associated KS in sub-Saharan Africa was investigated^{16, 127-131} (Table 2.3). This search was to ascertain that among KSHV endemic populations the presence of antibodies to KSHV is associated with HIV-associated KS. The potential concern in regions with a high background prevalence of KSHV infection is that the detection of antibodies to KSHV may not differentiate between individuals with HIV-associated KS and HIV-infected individuals without KS. Next, a search was conducted for longitudinal studies registered on PubMed investigating the association of antibodies to KSHV preceding HIV-associated KS diagnosis and subsequent risk of development of HIV-associated KS^{33, 115-117, 127, 132-137} (Table 2.4). This search was widened to include all studies of HIV-associated KS regardless of population location. The methods and limitations of both searches are presented within the general methods chapter.

In all studies identified investigating antibodies to KSHV in HIV-infected individuals with and without KS, the prevalence of antibodies was higher in individuals with HIV associated-KS than in HIV-positive individuals without KS (Table 2.3). Two studies estimated the risk of HIV-associated

KS by increasing fluorescent intensity signal to KSHV antigens^{128, 131}. In a study by Newton et al high fluorescent intensity to a KSHV antigen was associated with about a ten-fold increase in risk of KS, although the trend in the estimated ORs was driven by the difference in KSHV seronegative individuals compared to those defined as having medium or high fluorescent intensity¹²⁸. Sitas et al, reported that KS was more frequent among those with high intensity fluorescent signals for antibodies against KSHV¹³¹.

Six studies presented analysis for the risk of prevalent antibodies to KSHV prior to development of HIV-associated KS (Table 2.4): one reported null findings³³, while five reported an increased risk^{117, 134-137}. Two studies^{117, 136} reported an increased risk of KS with increasing antibodies to KSHV. Low antibody titre to KSHV was associated with an increased risk of 25 (95% CI 3.2-192.6) of developing KS, while high titre was associated with an increased risk of 52 (95% CI 6.1-441.3), compared to being KSHV seronegative¹³⁶. Newton et al reported an increased risk of KS with increasing latent antibodies and lytic optical density measured by ELISA, although the trend was not linear¹¹⁷.

Table 2.3: Prevalence of antibodies to KSHV among HIV-infected individuals with and without KS

1st author and year	Location	Cohort description	Laboratory technique	Number samples HIV-associated KS	KSHV prevalence in individuals with HIV-associated KS (%)	Number samples HIV positive without KS	KSHV prevalence in HIV positive individuals without KS (%)	Odds or hazard ratio (95% CI) for risk of KS by fluorescent intensity of antibodies to KSHV
Gao, 1996	Uganda	Cross-sectional	IFA	18	78	35	51	
Simpson, 1996	Uganda	Cross-sectional	ORF 65 ELISA	17	82	34	47	
He, 1998	Zambia	Cross-sectional, antenatal clinic	IFA	21	90	103	51	
Sitas, 1999	South Africa	Cross-sectional	IFA	38	83	210	32	KSHV seronegative 11 (4-29) Low 48 (10-234) Medium 62 (26-148) High 1683 (545-5194)
Newton, 2003	Uganda	Case-control	Latent IFA	266	82	422	41	KSHV seronegative 1 Low 4 (3-6) Medium 11 (7-18) High 9 (4-20)
Caterino-de-Araujo, 2010	Mozambique	Cross-sectional hospital based	Latent and lytic IFA	24	83	88	33	

The methods and a critique of this table are given within the general methods chapter.

Table 2.4: Antibodies to KSHV predating the development of HIV-associated KS.

Ist author, year and location	Study description	Number of HIV-associated KS cases	Serological technique	KSHV seroprevalence in KS cases prior to diagnosis (%)	KSHV seroprevalence in controls (%)	Odds or hazard ratio (95% CI) for risk of KS	Adjustment for potential confounders	Notes
Gao, 1996, USA	Nested case-control, 40 HIV+MSM, 20 HIV+ haemophilia	40	Immunoblot assay for latent nuclear antigen	80	18 (HIV-infected), 0 (haemophiliacs)	Not given	CD4 count at time of diagnosis	Samples collected 6-75 months prior to diagnosis for cases. For controls samples collected once
Renwick, 1998, Netherlands	Cohort, 1459 HIV+ MSM, 1167 HIV+ IVDU	99	ELISA ORF73 and ORF65.2 Seropositivity defined as positive to one or both antigens	87	Not given	OR of association between KSHV prevalence and KS 8.9 (4.3-18.3) Hazard ratio of development of KS: KSHV prevalent 3.3 (1.9-5.8), KSHV seroconverter 5.2 (2.9-9.3)	CD4 count	Most recent sample tested. If seronegative individuals considered negative throughout study. If positive, enrolment sample tested. If positive considered sero-positive throughout. If negative samples tested at yearly intervals to determine seroconversion year.
Melbye, 1998, Denmark	Cohort 259 MSM	10	Latent nuclear antigen (IFA), ELISA ORF65 and Western blots Seropositivity defined as positive to ELISA/IFA or ELISA/WB	100	Not given	Not given	None	259 MSM, 8.9% of whom were HIV infected at enrolment and followed with yearly visits for 15 years; 41 developed AIDS (10 had KS) Of KS cases 3 seroconverted prior to HIV 5 seroconverted after HIV and 2 were diagnosed with HIV and KSHV at the same time
Chatlynne, 1998, USA	Cohort, 91 blood donors, 57 HIV + MSM	62	Whole virus assay	84	11 (blood donors), 92 (HIV-KS), 26 (HIV + no KS), 50 (HIV + MSM with no KS)	Rate ratio developing KS 4.4 (1.9-10.2); positive to either assay, 2.1 (1.1-4.1); lytic assay, 4.3 (2.0-9.2)	None	Median titre KSHV positive AIDS- KS 400, median titre KSHV positive blood donor 100. Four patients with AIDS KS serial samples prior to diagnosis; titres increased sharply with time
Gruich, 1999, Australia	Cohort, 130 MSM with AIDS	37	ELISA ORF73 mad ORF65. Seropositivity defined as positive to either	81	43	Relative hazard developing KS seropositive compared to seronegative 3.6 (1.7-9.5)	None	Time from blood sample to diagnosis: 3-5 years 37/115 developed KS
O'Brien, 1999, USA	Cohort of 245 MSM	31	IFA latent nuclear antigen	75	50	Relative hazard of KS, seropositive compared KSHV seronegative 29.56 (3.9-224.32)	CD4 count and HIV viral load	Median time from blood collection to diagnosis: 13 months; range 4-27 months
Rezza, 1999, Italy	Cohort of 366 HIV+ MSM	21	IFA latent nuclear antigen	95	35	Low titer vs. neg: 24.8 (3.2-192.6) High titer vs. neg: 51.8 (6.1-441.3)	Age	All HIV incident. Number samples per subject not stated. Median time from blood collection to diagnosis KS from HIV seroconversion: 5.6 years; range 0-10.7 years. Risk of KS increased with increasing anti-KSHV antibody titer
Jacobson, 2000, USA	Cohort 251 MSM HIV+	42	IFA lytic antigens	Not stated	Not stated	Those who seroconverted to KSHV after being infected with HIV remained at significantly increased risk for developing KS (RH = 2.6; P = .04), compared with those who became infected with KSHV before HIV	CD4 count and HIV RNA	No data on the risk of KS in KSHV positive compared to negatives. Number samples per subject not stated. 182 were KSHV seropositive before seroconverting to HIV (34 developed KS) and 69 became KSHV seropositive after HIV infection (8 developed KS).
Quinlivan, 2001, Switzerland	Nested case-control, 36 HIV+ controls	36	Latent nuclear antigen (N-IFA), latent membrane antigen (M-IFA) ELISA ORF65.2	N-IFA: 61 M-IFA: 71 Lytic ELISA: 14	N-IFA: 32 M-IFA: 61 Lytic ELISA: 14	OR for risk of KS by KSHV seropositivity N-IFA: 3.0 (1.0-11.1) M-IFA: 1.7 (0.5-6.7) Lytic: 1.2 (0.2-6.8)	None	Median time between blood sample and diagnosis 4.7 years (range 3-7 years), 5-12 samples per case. Analysis is of case-control pairs Controls matched to cases for observation time, CD4 count, sex, age and HIV risk group
Biggar, 2003, USA	Cohort, 245 HIV+ MSM	31	IFA for ORF73 ELISA K8.1 Definition of seropositivity: positive to either	94	27	Not given	None	Mean 8.1 years, mean 6.9 samples per subject Of HIV-KS cases of 15 subjects were KSHV infected before HIV, 2 seroconverted at same time and 50% seroconverted to KSHV after HIV.
Newton, 2006, UK	Case-control study nested within clinical trial	189	ELISA ORF73 and K8.1	Latent: 38 Lytic: 33	Latent: 12 Lytic: 19	OR for KS by seropositivity Latent: 4.4 (2.3-8.3) Lytic 2.0 (1.2-3.4)	CD4 count	Risk (OR) for KS increased with increasing lytic CD4; trend not linear The risk (OR) of KS increased with increasing latent anti-KSHV antibody titre Matched for age group, sex, HIV transmission group, ethnicity, length time in trial. 1 or 2 samples per subject

The methods and a critique of this table are given within the general methods chapter.

2.7 Other risk factors for the development of HIV-associated Kaposi's sarcoma

While KSHV-HIV co-infection rates explain the majority of KS occurrence, host and viral factors may influence KSHV associated disease expression, since not all HIV-KSHV infected individuals develop KS.

Measures of HIV-associated immune deficiency predict the development of KS¹¹³. The CD4 count is an important factor associated with the development of HIV-associated KS. KS risk among ART-naïve individuals with HIV resident in Europe steeply increased with decreasing CD4 cell count (HR for <50 *versus* 350 cells μl^{-1} , 12.9; 95% CI 9.6–17.2)¹³⁸. In a case series of homosexual men resident in the UK, a new diagnosis of KS while on ART, the rate ratios for developing KS for patients with CD4 counts <200, 200-349, and 350-499 cells/mm³ were 18.9 (95% CI 8.5-42.1), 3.6 (95% CI 1.4-9.0), and 4.1 (95% CI 1.7-9.7), compared to patients with ≥ 500 cells/mm³¹³⁹. Among ART users, a nadir CD4 count prior to ART initiation is also important with a greatly increased risk of KS with a CD4 count below 50 cells μl^{-1} (HR for <50 *versus* 350 cells μl^{-1} , 5.4; 95% CI, 2.1–13.8)¹³⁸. High HIV RNA level (> 5.0 log₁₀ copies/ml) is associated with an increased risk of KS development (HR adjusted for CD4, 4.0; 95% CI, 2.2–7.2 per log₁₀). Another study reported an increased risk of HIV-associated KS with increasing HIV RNA levels (relative hazard for one log₁₀ increase HIV RNA 3.3 (95% CI 1.9-5.7)).

Genetic polymorphisms in host immune genes are obvious research targets. KS plaques are associated with inflammation and angiogenesis and KS is more common among individuals with immune deficiency. The majority of work has focused on HLA and cytokine genes. Results have not been overwhelming but this could mean that the wrong genes are being studied. HLA-DRB1 was implicated in one study in the development of KS in KSHV infected individuals with HIV co-infection¹⁴⁰. Other groups identified an IL-6 promoter genotype associated with

altered gene expression as a risk factor for development of KS in HIV-infected men¹⁴¹.

In a HIV-infected cohort a haemoglobin of less than 8.5g/dl was associated with antibodies to KSHV and high KSHV viral load¹⁴². Multicentric Castleman's disease (MCD) is a frequently fatal disease associated with reactivation of KSHV. In a recent study of MCD, in the multiple regression analysis, low haemoglobin was the strongest predictor of elevated KSHV viral load¹⁴³.

Corticosteroid therapy is associated with development of HIV-associated KS^{144, 145}. This is important due to the frequent use of steroids of a number of immune and infectious disorders associated with HIV.

2.8 Risk factors for Kaposi's sarcoma among Kaposi's sarcoma-associated herpesvirus infected individuals without overt forms of immune suppression

This section briefly discusses factors that may explain development of KS without overt immune suppression. Co-factors for development of KS may set risk of disease at the time of primary KSHV infection, or later in the trajectory of infection and oncogenesis.

2.8.1 Viral factors

KSHV can be classified into major sub-types defined by the variability of the K1 open reading frame. The five major subtypes (A, B, C, D and E) of KSHV¹⁴⁶ have a distinctive worldwide geographic distribution^{94, 146-148}. These sub-types do not correlate with patterns of KS occurrence or severity¹⁴⁹. Recent research has focused on KSHV microRNA sequences. Distinct polymorphisms in KSHV microRNA sequences have been reported in individuals with MCD¹⁵⁰, HIV-associated KS¹⁵¹ and KSHV-associated inflammatory cytokine syndrome (KICS)¹⁵². Both MCD and KICS are characterised by very high KSHV viral loads and presumably

near-complete loss of viral control. It is plausible that variant microRNA sequences may be associated with a more aggressive KSHV phenotype.

2.8.2 Host factors

KS in individuals without overt immune suppression has an overwhelming male predominance. In regions where endemic and classic types of KS occur, KSHV seroprevalence is similar in men and women. Historical studies have hypothesized that female sex hormones may be protective¹⁵³ or male sex hormones promote¹⁵⁴ for KS development. Alternatively, men and women may be differentially exposed to an environmental factor or factors. Epidemiological and biological data are, however, lacking. The strong association between older age and classic and endemic KS may imply that exposure to factors over a long period is required. Alternatively, factors associated with old age, such as progressive alternations to the immune system may be important, as discussed above¹¹¹.

Classic KS in Mediterranean men has been associated with HLA-DR5 and HLA-B18¹⁵⁵, but no HLA association was found in a study of Jewish patients with classic KS¹⁵⁶. An interleukin-6-promoter polymorphism was identified in a familial case of classic KS, in which the disease occurred in four siblings who had no recognised underlying immunodeficiency¹⁵⁷. No HLA aberrations have been reported with endemic KS¹³³. Polymorphisms in the tumour suppressor gene p53 have not been reported to be a risk factor for the development of classic and endemic KS¹⁵⁸. Among KSHV-seropositive Italians, classic KS risk was associated with diplotypes of IL8RB and IL-13^{159, 160}.

Classic KS has been reported to be associated with reduced haematocrit and reduced haemoglobin (<12g/dL)¹⁰⁸. In the anaemic state the total oxygen content of the blood is reduced and the blood fails to deliver oxygen to the tissues (hypoxia). KS has a tendency to occur in relatively hypoxic tissues; the edges of wounds or on the lower extremities¹⁶¹ which has led to the hypothesis that anaemia or hypoxia may be co-

factors for KS development. Hypoxia induces reactivation and replication of KSHV *in vitro*¹⁶². Cells exposed to hypoxic conditions accumulate hypoxia-inducible factors (HIF1/2), which control transcriptional activation of a number of genes responsive to low cellular oxygen¹⁶³. The KSHV genome contains at least two lytic genes with hypoxia response elements¹⁶⁴. Hypoxia may also induce KSHV reactivation via reactive oxygen species (ROS)^{165, 166}. ROS are highly reactive molecules containing oxygen and peroxides. They are produced as a by-product of oxygen metabolism and are involved in normal cell functions. Markers of oxidative stress are key features of KS lesions^{165, 166}. It has been shown that the ROS hydrogen peroxide induces KSHV reactivation directly¹⁶⁶, and via inhibition of the NF- κ B pathway¹⁶⁷. In mouse models antioxidants inhibit KSHV lytic replication and development of KSHV-related diseases¹⁶⁸.

2.8.3 Environmental factors

An intriguing part of the clinical presentation of classic and endemic KS is the location of the cancer on the lower limbs. Areas of high incidence of endemic KS are characterised by a common geological substrate, composed of fertile reddish-brown volcanic clay soils. Volcanic mountains fringe the western branch of the Great Rift Valley in eastern Congo, in western Uganda and Tanzania, and further south in Malawi¹⁶⁹. The unusually high prevalence of endemic forms of KS in regions of intercontinental rifts and volcanism may point to prolonged exposure to indigenous iron oxide rich volcanic soils as a common aetiological risk factor². An association between classic KS and contact with soils has also been postulated; classic KS is found in areas with high iron and aluminosilicate clays¹⁷⁰. A study of the geographic association with KS⁴⁰ reported that areas where KS was relatively common were equatorial, humid and with an altitude of over 600m, where the population is mostly rural and likely to come into contact with ferrisol and ferralitic soils. However, the absence of these environmental factors was not found for many areas with a low reported incidence of KS; both the Kenyan Rift valley and

Ethiopian plateau have these environments but low KS rates. Whether these environmental factors are direct risk factors for KS or they are surrogate markers of another exposure is not known.

2.9 Chapter Conclusion

The presence of HIV infection is one of the strongest predictors of risk for KS in KSHV infected individuals. The detection of antibodies to KSHV precedes and is predictive of development of HIV-associated KS. The epidemiology of forms of KS not associated with overt immune suppression (so called classic and endemic KS) has distinct geographical, age and gender associations. The pattern of occurrence of classic and endemic KS may reveal subtle co-factors for KS oncogenesis.

Chapter 3: The epidemiology of Kaposi's sarcoma-associated herpesvirus.

3.1 Chapter contents

KSHV infection is universally found in KS tumours. KSHV infection per se, is deemed necessary but not sufficient for KS development. The majority of those infected with KSHV do not develop the neoplasm. The incidence of KS largely mirrors KSHV seroprevalence in populations. It is therefore of key importance to first understand which populations are at greatest risk of infection with KSHV. Geographical differences of KSHV seroprevalence may suggest the involvement of as yet unknown host genetic, viral or environmental factors for risk of KSHV infection.

Objectives:

- I. To describe general patterns of KSHV seroprevalence in adult populations around the world.
- II. To give a brief overview of KSHV sero-epidemiology in North America, Europe and Asia.
- III. To present a detailed description of patterns of KSHV seroprevalence in Africa and Uganda. A critical review of relevant literature will be presented.
- IV. To discuss reasons underlying any potential geographical differences in KSHV sero-epidemiology and if this points to potential co-factors for infection.
- V. To overview key risk factors for KSHV seropositivity in sub-Saharan Africa.

Summary: There is marked geographical variation in KSHV seroprevalence. This has a profound influence on worldwide differences in KS incidence. Epidemiological studies are needed to clarify potential reasons underlying this.

3.2 Serological assays for Kaposi's sarcoma herpesvirus

Epidemiological studies have used serologic tests to study patterns of infection with KSHV. KSHV encodes multiple antigenic proteins that are generally divided into two groups, depending on the phase of the cell cycle when they are produced: lytic and latent. Serological assays are commonly either immunofluorescence assays (IFA) or enzyme-linked immunosorbent assays (ELISAs). IFAs are based on primary effusion lymphoma cell lines with expression of latent and lytic phase proteins. ELISAs are commonly based on recombinant proteins or peptides.

In order to critically interpret worldwide patterns of KSHV seroprevalence it is key to understand both the limitations and strengths of the serological assays. Marked discordance of KSHV seroprevalence between different assay types and laboratories has been reported. In one study a test panel of serum samples from HIV-associated KS patients were tested for antibodies to KSHV using six assays (three different IFAs and three different ELISAs). The seroprevalence ranged from 61 to 100%. The same study also reported testing a panel of serum samples from children using the same six assays. KSHV seroprevalence ranged from 0% to 20%¹⁷¹. Other groups have reported similar disagreements between assays and laboratories^{125, 172-174}. Possible reasons for variation in results are described below.

Firstly, assay outcomes may differ if different antigenic products of KSHV have been used. The immune response to antigenic KSHV epitopes is complex and not completely understood. Individual humoral responses on exposure to KSHV antigens are highly variable (For review see Robey¹⁰²). The most consistently antigenic KSHV gene products; K8.1, ORF 73 and ORF 65, tend to be selected for epidemiological studies.

Secondly, the choice between IFA and ELISA for detection of antibodies may influence the results. IFAs have been considered the reference test for detecting antibodies to KSHV¹⁷⁵ but have a number of operational and performance issues. The process of immunostaining for IFAs is laborious

and cannot be automated for a high throughput of samples. IFA scoring is highly subjective and day-to-day variability in sensitivity is frequently high. Sensitivity of IFAs in detecting antibodies to KSHV may be as low as about 60%¹⁷².

Thirdly, it is important when selecting a KSHV antigen to consider length and conformation to maximize immunogenicity. Assays that use full length proteins expressed by a baculovirus system in insect cells have a better sensitivity and specificity compared to peptides or bacterially-expressed proteins¹⁷⁵.

Fourthly, the algorithm for testing including definition of cut-off and use of more than one assay or antigen may affect the results. The definition of seropositivity to KSHV is generally determined based on the ability to discriminate between groups with KSHV-associated disease and groups of healthy blood donors from populations where KSHV infection is rare. The majority of epidemiological studies test for only one or two KSHV antigens, which may lead to under estimation of KSHV seropositivity.

Assay limitations must be acknowledged when interpreting potential patterns of KSHV across populations and within risk groups.

While KSHV serological assays have important limitations they have allowed important observations (For review see Tedeschi¹⁷⁶) on global patterns of KSHV burden. ELISAs for KSHV antibody detection have allowed automated testing of large numbers of samples for epidemiological studies. Careful evaluation of testing algorithms in different test populations using full-length insect cell-expressed protein has increased reproducibility and performance. ELISAs based on KSHV proteins K8.1 and ORF 73 used in this thesis have been rigorously developed by the Viral Oncology Section (VOS), National Cancer Institute (NCI). They have been shown to be reproducible, with a high specificity and sensitivity¹⁷⁵ and have been used in over 40 epidemiologic studies worldwide.

3.3 Worldwide seroprevalence of Kaposi's sarcoma-associated herpesvirus

Figure 3.1 shows a world map derived from studies measuring antibodies to KSHV in "healthy" subjects without KS such as blood donors, or HIV negative subjects^{8, 11, 13, 14, 16-21, 24, 25, 28, 94, 129, 177-191}. The methods for constructing this map are presented within the general methods chapter. Figure 3.1 shows a band of very high (greater than 60%) KSHV seroprevalence across East and Central Africa. Antibodies to KSHV are also detected in a high proportion of populations studied in Southern Africa and in an indigenous population resident in the Brazilian Amazon region. KSHV seroprevalence is moderately high (16-30%) in studies in Brazil (outside of the Amazonian region), West Africa, Egypt and the Mediterranean. The remaining areas of North America, Central America, China, Europe and East and South East Asia have low KSHV seroprevalence (less than 15%).

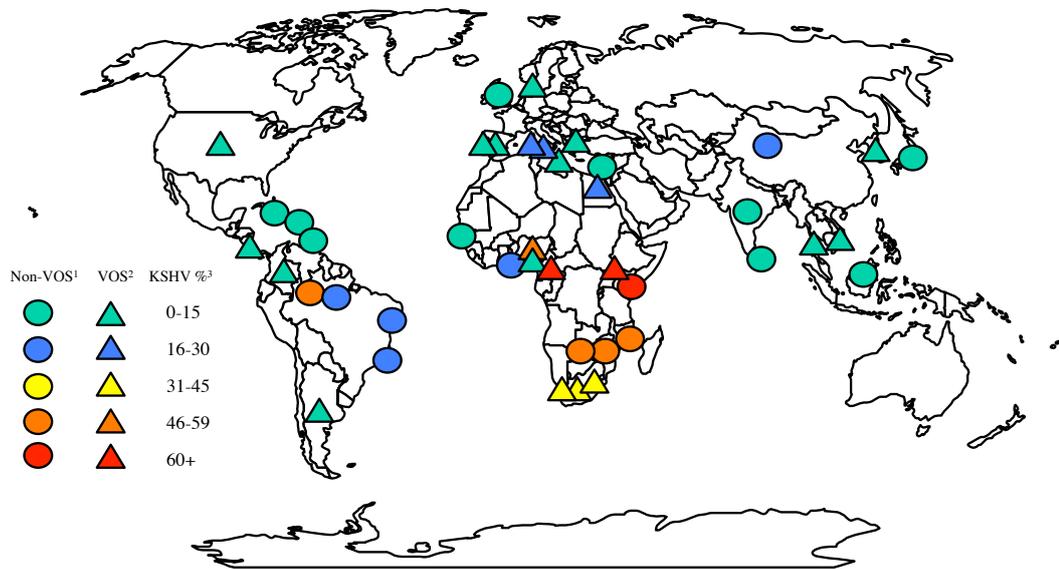


Figure 3.1: World map of KSHV seroprevalence from studies conducted between 1998 and 2012. Studies included needed to state that the population tested was without KS, “healthy”, blood donors and/or HIV negative.

¹Non-VOS: Study not conducted by Viral Oncology Section, National Cancer Institute

²VOS: Study conducted by Viral Oncology Section, National Cancer Institute

³KSHV-%: KSHV seroprevalence

Table 3.1: KSHV seroprevalence from adult populations describe as "healthy", "blood donors" and/or "HIV negative" and without KS

Continent, Country	Region	KSHV serological assay	Population	Number in study	Age	KSHV seroprevalence (%)	1st Author, year	VOS assay ¹
Europe								
Denmark		ELISA K8.1	Cross section, outpatient clinic	641	20-40+	4	Hjalgrim, 2001	VOS
Greece		ELISA K8.1 and ORF73	Cross sectional, healthy urban	955	16-69	7	Zavitsanou, 2007	VOS
Malta		ELISA K8.1	Hospital outpatients	200	65+	9	Vitale, 2000	VOS
Sardinia		ELISA K8.1	Out patients	104	65+	28	Vitale, 2001	VOS
Sicily		ELISA K8.1	Out patients	153	65+	20	Vitale, 2001	VOS
Sicily		ELISA K8.1 and ORF73	General population controls for a KS study	1154	32-92	12	Pelser, 2009	VOS
Spain		ELISA K8.1	General population	100	19-49	4	de-Sanjose, 2009	VOS
Spain		ELISA K8.1 and ORF73	Women, general population	599	20-75	4	de-Sanjose, 2009	VOS
United Kingdom		ELISA ORF65	Blood donors	174	Adults	2	Simpson, 1996	
Asia								
China	Xinjiang	ELISA K8.1, ORF65 and ORF73	Village sampling	1534	18-91	24	Wang, 2011	
Israel		Latent IFA	Relatives of individuals in a KS study	2403	25-55+	15	Davidovici, 2001	
India		Whole virus ELISA	Healthy individuals	108	20-58	4	Ablashi, 1999	
Japan		Latent IFA	HIV negative, gen population	1000	Mean age 44	0.2	Fujii, 1999	
Korea		ELISA K8.1 and ORF73	Women, general population	914	20-75	5	de-Sanjose, 2009	VOS
Malaysia		Whole virus ELISA	Healthy individuals	75	9-85	4	Ablashi, 1999	
Sri Lanka		Whole virus ELISA	Healthy individuals	59	21-78	4	Ablashi, 1999	
Thailand		ELISA K8.1 and ORF73	Women, general population	2148	20-75	9-10	de-Sanjose, 2009	VOS
Thailand		Whole virus ELISA	Healthy individuals		17-69	4	Ablashi, 1999	
Vietnam		ELISA K8.1 and ORF73	Women, general population	2263	20-75	11-15	de-Sanjose, 2009	VOS
South America								
Argentina		ELISA K8.1 and ORF73	Women, general population	967	20-75	6	de-Sanjose, 2009	VOS
Brazil		Latent IFA	Amazon Amerindians	781	30+	65	Biggar, 2000	
Brazil	Salvador	Whole virus ELISA	General population, blood donors	781	18-63	19	Nascimento, 2008	
	Sao Paulo	ELISA ORF73		631		18		
	Manaus (Amazon North)	Latent IFA		874		27		
Coasta Rica		ELISA K8.1 and ORF73	Women, general population	1001	20-75	11	de-Sanjose, 2009	VOS
Colombia		ELISA K8.1 and ORF73	Women, general population	1774	20-75	13	de-Sanjose, 2009	VOS
Cuba		Latent IFA	Blood donors	171		1	Kouri, 2004	
Jamaica		Whole virus ELISA	Blood donors	250		4	Ablashi, 1999	
Trinidad		Whole virus ELISA	Blood donors	160		1	Ablashi, 1999	
North America								
USA		ELISA K8.1 and ORF73	Cross section, national stats for USA	16,923	18 or older	2.5	Engels, 2007	VOS
Africa								
Egypt		K8.1 ELISA	Healthy women	495	15-45+	25	Mbulaiteye, 2008	VOS
Cameroon		ELISA K8.1 and ORF73	HIV negative women	321	19-50	78	Whitby, 2012	VOS
Ghana		ELISA ORF59, ORF65, K8.1 and ORF73	Blood donors	40	>40	28	Nuvor, 2001	
Malawi		ELISA K8.1 and ORF65	Adults in hospital	73	Adults	54	De-santis, 2002	
Mozambique		Lytic ELISA	Community study	166	Adults, mean age 30	50	Ceffa, 2007	
Nigeria		K8.1 ELISA	Healthy individuals from offices and factories	287	15-45	14	Eltom, 2002	VOS
Nigeria		ELISA K8.1 and ORF73	Women, general population	1085	20-75	46	de-Sanjose, 2009	VOS
Senegal		Latent and lytic IFA	Pregnant women	407	Mean age 30 years	14	Gaye-Diallo, 2001	
South Africa		K8.1 ELISA	Mothers attending vaccination clinics	2546	15-40+	35	Dedicoat, 2004	VOS
South Africa		ELISA K8.1 and ORF73	Mothers involved in paternity disputes	2103	Mean 33.2	37	Malope, 08	VOS
South Africa		ELISA K8.1 and ORF73	Mothers involved in paternity disputes	1740	Mean 26	26	Malope, 2010	VOS
Tanzania		Lytic and Latent IFA	Mothers of children with Burkitts lymphoma	42	23-50	66	de-The, 1999	
Uganda		ELISA K8.1 and ORF73	Women in RCT recruited from antenatal clinics	1915	14-35+	60	Wakeham, 2011	VOS
Zambia		Latent IFA	Women child bearing age	183	Child bearing age	47	He, 1998	

Studies included had to state that the population tested was without KS, "healthy", "blood donors and/or HIV negative. Methods in general methods chapter.

¹VOS assay: Study conducted by Viral Oncology Section, National Cancer Institute

KSHV seroprevalence will be briefly described for each major region in turn; North America and Northern Europe, South America, the Mediterranean, Asia and sub-Saharan Africa. Risk factors for KSHV seropositivity and transmission vary by geographic region and will be described under each major region. A greater emphasis will be placed on KSHV epidemiology in Africa. A discussion on the geographic variation in KSHV seroprevalence will then follow.

3.3.1 Kaposi's sarcoma-associated herpesvirus sero-epidemiology in North America and Northern Europe

KSHV seroprevalence in the North American and Northern European general population is commonly reported as being about 1-3%^{127, 179, 192}. In a USA based comprehensive study of KSHV seroprevalence using the National Health and Nutrition Examination Survey (NHNES) which sampled 13,894 adults from the US general population between 1988 and 1994, the overall seroprevalence was low (around 1-2%)¹⁷⁹.

Relatively high levels of KSHV seroprevalence are reported in men who have sex with men (MSM). In the NHNES, KSHV seropositivity in MSM was six-fold higher than in heterosexual individuals¹⁷⁹. Within MSM populations HIV infection is a major risk factor for KSHV seropositivity, with reported seroprevalence in HIV infected MSM of up to 77%^{129, 192-194}. Self-reported markers of high-risk sexual behaviour in MSM are also associated with KSHV seropositivity. In MSM cohorts KSHV seropositivity is associated with increasing numbers of male partners^{179, 193, 195}, history of a sexually transmitted infection¹⁹³, antibodies to hepatitis B¹⁷⁹ and herpes simplex virus^{179, 195}. Longitudinal studies report infection with KSHV was already highly prevalent among MSM populations when the HIV epidemic began in San Francisco, and its prevalence was maintained at a nearly constant level through the nineteen eighties and nineties¹⁹⁶.

HIV is not associated with KSHV seropositivity in other (non-MSM) HIV infected groups; heterosexuals, hemophiliacs and IV drug users, these groups have a KSHV seroprevalence similar to levels reported in the

general population^{192, 193}. Among men reporting to be exclusively heterosexual KSHV seroprevalence is low and risky sexual behaviour is not associated with KSHV infection¹⁹³.

Reports from North America and Europe consistently demonstrate that KSHV seroprevalence is lower among women than among men. Risk of KSHV infection among women in North America has less clear cut and consistent associations with HIV and sexual risk factors. A study of women in San Francisco Bay area reported a KSHV seroprevalence of 4% in HIV infected women and 1% in women at high risk of HIV¹⁹⁷. Other studies of KSHV and HIV in women have reported no or borderline associations between the two infections^{179, 198}.

Self-reported markers of high-risk sexual activity in women, such as a high lifetime number of male partners, a history of commercial sex and young age at the time of first intercourse were found to be associated with KSHV seropositivity in one study¹⁹⁸, but not in another¹⁷⁹.

In adolescents in North America antibodies to KSHV are associated with MSM, risky sexual behaviour in males and African-American descent¹⁹⁹. Children in North America and Northern Europe are at very low risk of KSHV seropositivity²⁰⁰.

Despite the consistent reports of relatively high KSHV prevalence among MSM, it remains unclear if KSHV is sexually transmitted in this population or if sexual risk factors are a surrogate marker for some other mode of KSHV transmission. The exact mechanism of KSHV transmission remains unclear. KSHV DNA is found more commonly in saliva than in semen, prostate or nasal secretions^{201, 202} and salivary contact has been postulated as the most likely route of spread. KSHV is not widely disseminated in urogenital tissue²⁰³. KSHV DNA is present at very low concentrations in semen²⁰⁴ and is often undetectable in semen²⁰⁵ and the prostate gland²⁰⁶. It has been postulated that MSM are more likely to have a large number of sexual partners and this may increase the chance of contact with infected saliva. Exposure to KSHV in heterosexual

populations is likely low. Women reporting a relatively large number of lifetime sexual partners (greater than 50) remain at low risk of KSHV seropositivity. KSHV seroprevalence in heterosexual men and women is similarly low¹⁷⁹ suggesting that heterosexual intercourse is inefficient at KSHV transmission. This may be accounted for by the fact that KSHV is found infrequently in semen and prostatic secretions and it is rarely detected in cervico-vaginal secretions²⁰⁷. MSM may be more likely to have anal intercourse than heterosexual individuals, but no significant correlation between KSHV serostatus and receptive or insertive unprotected anal sex or oral-anal contact in MSM has been shown. Furthermore women reporting anal intercourse did not have an increased risk of KSHV¹⁹⁸. However it is difficult to implicate specific sexual behaviours as they are correlated with each other. Why then MSM are at greater risk of KSHV seropositivity than heterosexual populations in the USA and Europe is unclear. In a study of HIV negative MSM in San Francisco use of amyl nitrates was strongly associated in the adjusted analysis with KSHV¹⁹⁵ and this has been reported in a number of studies^{208, 209}. Amyl nitrates increase sexual drive, cause relaxation of the anal sphincter and potentially alter T-cell function, increasing vulnerability to infectious agents such as KSHV²¹⁰. The increased seroprevalence of KSHV in MSM could also potentially be due to the fact that MSM more likely to come into contact with a HIV-positive partner¹⁹⁵. HIV-positive MSM are more likely to be infected with KSHV^{129, 192-194} and may shed the virus at higher quantities in their saliva²¹¹.

In conclusion, in North America and northern Europe, KSHV seroprevalence is generally low among both men and women, but is higher among MSM and among people whose ethnic origin is a country in which KSHV is more prevalent (such as those of African origin). Among MSM, HIV and other markers of risky sexual activity are associated with KSHV. The mode of KSHV transmission remains undefined.

3.3.2 Kaposi's sarcoma-associated herpesvirus sero-epidemiology in the Mediterranean

Countries in the Mediterranean have moderate KSHV seroprevalence in general population studies (Table 3.1). Sardinia has a KSHV seroprevalence of 28-35%^{28, 212} and one of the world's highest incidence rates of the classical sub-type of KS⁵⁴. Regional differences of KSHV seropositivity within Mediterranean countries including Italy and Greece have been reported⁵⁴. Due to the location and presence of classic KS, Israel is often considered with Mediterranean epidemiology and has similar KSHV seroprevalence rates²¹³. KSHV seroprevalence in the Mediterranean increases with age²¹⁴ and is more common in men than in women²¹⁵. This epidemiological pattern mirrors the observation that classic KS occurs more commonly in older men⁵⁴.

A high KSHV seroprevalence in the first-degree relatives of individuals with classic KS and familial clustering of KSHV seropositivity has been reported^{157, 215}. The specific relationship (spouse, offspring, sibling) has no effect on KSHV seroprevalence, which suggests a predominantly non-sexual horizontal transmission route of the virus within families. A study of KSHV seroprevalence and sexual risk factors reported that antibodies to KSHV were higher in HIV-infected MSM²¹⁴. There was no increased risk of KSHV seropositivity compared to the general population associated with risky heterosexual behaviour or intravenous drug use²¹⁴. This finding is similar to North American studies¹⁷⁹.

A series of publications on KSHV, KS and blood sucking arthropods from Italy will be discussed later in the section in Chapter 5.

3.3.3 Kaposi's sarcoma-associated herpesvirus sero-epidemiology in Asia

Studies of KSHV epidemiology in Asia are sparse and those reported often have low sample size. Seroprevalence to KSHV in populations of Asia is low to moderate (Table 3.1 and Figure 3.1), ranging from less than 1-24%^{20, 178, 188-190}. One study reported that seroprevalence in Northern Thailand amongst heterosexual couples with HIV or at high risk of HIV is about 25%²¹⁶. This is more than two-fold higher than in healthy, blood donor populations in the same region. Most couples (with HIV or at high risk of HIV) in this study were discordant for KSHV suggesting that the KSHV transmission event did not take place between husband and wife. Seroprevalence of KSHV in South and North Xinjiang, China was 23% and 26%, respectively¹⁸⁸. Older age was independently associated with higher KSHV seroprevalence¹⁸⁸. Seroprevalence of KSHV in Chinese patients with chronic hepatitis B, acquired through unsafe needle practices, is about 30%²¹⁷. This is higher than other studies based in China and raises the possibility of KSHV transmission via contaminated needles. No studies in Asia of children or MSM populations could be identified.

3.3.4 Kaposi's sarcoma-associated herpesvirus sero-epidemiology in South America and the Caribbean

KSHV seroprevalence data from South American cities range from 1-19%, which is similar to rates from North America and Northern Europe (Table 3.1 and Figure 3.1)^{20, 94, 181, 182, 190}. Reported rates of KSHV seroprevalence in Brazilian urban areas were 18-19% in a study of healthy adult participants¹⁸². KSHV seroprevalence was not associated with age, reported sexual behaviour or with markers of socioeconomic status. Women in a female general population study in Argentina had a seroprevalence of 6%²⁰; there was no association with HPV infection status, number of lifetime sexual partners or age of first sexual intercourse. The women tested were between 20 and 75 years and prevalence remained steady with age.

One might expect that South Americans of African descent and Afro-Caribbean's may have a high KSHV seroprevalence in keeping with that found in Africa. However, KSHV seroprevalence in these populations appears moderate to low. In a population-based sero-epidemiological survey in a village in French Guiana, among 1337 individuals of African origin aged 2-91 years, the overall KSHV seroprevalence was 13% with no difference according to sex²¹⁸. KSHV seropositivity increased with age and there was strong mother-child correlation but no correlation between spouses suggesting non-sexual transmission mainly between mother and child. Studies of Caribbean populations with a high proportion of African origin descendants (Cuba, Jamaica, Trinidad) have reported a low KSHV seroprevalence of between 1-4%^{20, 181, 190}. This might suggest that removal from the African environment leads to a reduction in KSHV transmission. Indeed, in a STD clinic in London KSHV seroprevalence was high in individuals born in Africa but not in second generation Africans²¹⁹.

Amerindian populations have a high reported KSHV seroprevalence. Antibodies to KSHV have been reported in nearly 60% of Amerindian populations^{182, 220}. Seroprevalence is not associated with gender or age^{94, 182}. In Brazil antibodies to KSHV were associated with indigenous descent¹⁸². The high KSHV seroprevalence in Amerindian populations has not yet been explained. In one study Amerindian and non-Amerindian people living in the same area had markedly different KSHV seroprevalence suggesting that environmental co-factors are not associated with detection of antibodies to KSHV²²⁰. There have been limited reports of classic KS (HIV uninfected individuals) in Amerindian populations in the Amazon basin⁹¹. This will be discussed in the section on disease below.

3.4 Kaposi's sarcoma-associated herpesvirus sero-epidemiology in Africa

KSHV seroprevalence varies markedly across Africa (Table 3.1 and Figure 3.1)^{8, 11, 13, 14, 16, 18-21, 24, 25, 187, 191}. Great interest lies in this variation as it may suggest as yet un-identified co-factors for infection that vary across geographical regions. In broad terms in Africa, KSHV seroprevalence increases over a wide age range and is higher in HIV-infected individuals^{8, 9, 13, 18, 191} and patients with KS^{16, 127-131}. A literature search was carried out of reports of KSHV seroprevalence in HIV negative adults without a diagnosis of KS. These limits were placed to reduce variation in KSHV seroprevalence by age, HIV and KS status (Table 3.2 shows the studies used in Figure 3.2 ordered by the year of study publication). The general pattern that emerges is a high KSHV seroprevalence band across East and Central Africa and lower KSHV seroprevalence at Africa's poles^{8, 11, 13-17, 19, 21, 24, 25, 127, 129, 131, 183, 187, 190, 191, 221-230}. The methods and critique of the literature review is in the general methods chapter.

3.4.1 Elimination of population and assay factors that may affect patterns of Kaposi's sarcoma-associated herpesvirus seroprevalence

There are potential issues in taking results from all published studies of KSHV epidemiology in Africa. Variation in KSHV seroprevalence may represent differences in the population tested, assay, sample size and year of study rather than true geographical variation. To try and eliminate some of these issues only studies of HIV negative women, tested using a KSHV ELISA developed by VOS, NCI^{231, 232} were selected from Table 3.2 (Table 3.3)^{8, 14, 18, 20, 24, 25, 191, 227}. KSHV seropositivity results from women were selected, as studies of men were sparse. Four of the eight studies only recruited mothers or pregnant women^{8, 13, 25, 191}. Results from HIV positive individuals were excluded due to the association between HIV and KSHV in some studies. All studies were conducted between 2002 and 2012, using a similar testing strategy currently used by VOS, NCI. Although the number of studies is now small, the pattern of a high equatorial band of KSHV seroprevalence remains.

Table 3.2: KSHV seroprevalence in HIV negative adults without KS in sub-Saharan Africa

Author, year	Country	Serological technique	Definition of KSHV seropositive if more than one assay used	Age of population	Number in study	KSHV seroprevalence (%)
Gao, 1996	Uganda	Latent IFA LNA-immunoblot		Adult	47	51 62
Simpson, 1996	Uganda	ORF 65 ELISA Latent IFA		Adult	17 17	35 53
He, 1998	Zambia	Latent IFA		Women child bearing age	183	47
Olsen, 1998	Zambia	Latent IFA ORF 65 WB	Positive to both	15-84	251	58
Bourboullia, 1998	South Africa	Latent IFA			107	16
Albashi, 1999	Uganda Zambia	Whole virus ELISA		18-67 17-68	62 40	39 38
de-The, 1999	Uganda Tanzania	Lytic and Latent IFA Lytic and Latent IFA	Positive to either	23-50 23-50	14 42	86 66
Sitas, 1999	South Africa	Latent IFA		Adults	309	20
Enbom, 1999	Eritrea	Latent IFA Lytic IFA		16-40 Pregnant women 18-75 Rashaidas	112 42	5 26
Kakoola, 2001	Uganda	Latent IFA ELISA ORF65 ELISA ORF73 Western blot ORF65	Positive to both the IFA and the ELISA (one or both antigens), or IFA alone or in the ELISA alone	18-54	114	74
Wawer, 2001	Uganda	Latent IFA Lytic immunoblot	Positive to both	15-29	523	40
Nuvor, 2001	Ghana	ELISA ORF59 ELISA ORF65 ELISA ORF73 ELISA K8.1	Positive to any	>40	40	28
Gaye-Diallo, 2001	Senegal	Latent IFA Lytic IFA	Positive to either	Mean age 30 years, pregnant women	407	14
De-santis, 2002	Malawi	ELISA K8.1 ELISA ORF65	Positive to either	Adults	73	54
Hladik, 2003	Uganda	ELISA K8.1 ELISA ORF65 Lytic IFA	Reactive to at least 2/3 assays or reactive IFA alone	Median 24 years, IQR 19-30		39
Newton, 2003	Uganda	Latent IFA		Adults	607	50
Dedicoat, 2004	South Africa	Latent IFA K8.1 ELISA		15-40	702 702	14 35
Kasakla, 2005	Zambia	Lytic IFA Latent IFA	Positive to either	14-43 pregnant women	3160	38
Ceffa, 2007	Mozambique	Lytic ELISA		Adults, mean age 30	166	50
Malope, 2007	South Africa	ELISA K8.1 ELISA ORF73	Positive to either	Mothers, mean age 30	824	25
Adjei, 2008	Ghana	Whole virus ELISA		18-65	3275	24
Mbulaiteye, 2008	Egypt	K8.1 ELISA		15-45	495	25
Malope, 2010	South Africa	ELISA K8.1 ELISA ORF73	Positive to either	Mean age 26	1740	26
Pfeiffer, 2010	Nigeria	ELISA K8.1 ELISA ORF73	Positive to either	Adult	718	10
Biryahwaho, 2010	Uganda	ELISA K8.1 ELISA ORF65	Positive to either	15-59	1505	55
Wakeham, 2011	Uganda	ELISA K8.1 ELISA ORF73	Positive to either	14-35+	1915	60
Whitby, 2013	Cameroon	ELISA K8.1 ELISA ORF73	Positive to either	19-50	321	78

KSHV seroprevalence in HIV negative adults for all studies identified through a comprehensive Pub-Med search

Table 3.3. KSHV seropositivity in Women in Africa using K8.1 and ORF73 ELISA developed by VOS, NCI

1st author, year	Country	Number of women	HIV prevalence (%)	Odds ratio for association of KSHV and HIV (95% CI)	Age of population	Definition of KSHV seropositivity	Overall KSHV seropositivity (%)	KSHV seropositivity in HIV negative women (%)	KSHV seropositivity in HIV positive women (%)	Association between KSHV seropositivity and age
Eltom, 2002	Nigeria	287	4	1.5 (1.0-2.1)	15-45	K8.1 ELISA	14	13	33	No
Dedicoat, 2004	South Africa	2546	28	2.1 (1.7-2.5)	15-40+	K8.1 ELISA	40	35	53	No
Malope, 2008	South Africa	2103	40	4.1 (3.1-5.2)	Mean 33	Positive to either K8.1 or ORF73	48	37	68	No
Mbulaiteye, 2008	Egypt	495	0	na	15-45+	K8.1	25	25	na	
de Sanjose, 2009	Nigeria	1143	Not given	na	Mean 41	Positive to either K8.1 or ORF73	46	Not given	Not given	No
Malope, 2010	South Africa	1740	23	Not given	Mean 26	Positive to either K8.1 or ORF73	45	26	41	No
Pfeiffer, 2010	West Nile, Uganda	329	Not given	na	Adult		70	Not given	Not given	No
	Kampala, Uganda	517	Not given	na	Adult	Positive to either K8.1 or ORF73	40	Not given	Not given	Yes
	Tanzania	97	Not given	na	Adult		45	Not given	Not given	Yes
	Nigeria	718	Not given	na	Adult		24	10	28	Yes
Wakeham, 2011	Uganda	1915	10	1.4 (1.0-1.9)	14-35+	Positive to either K8.1 or ORF73	61	60	67	No
Whitby, 2013	Cameroon	321	Not given	0.8 (0.6-1.2)	19-50	Positive to either K8.1 or ORF73	78	78	79	No

Methods given within the general methods chapter

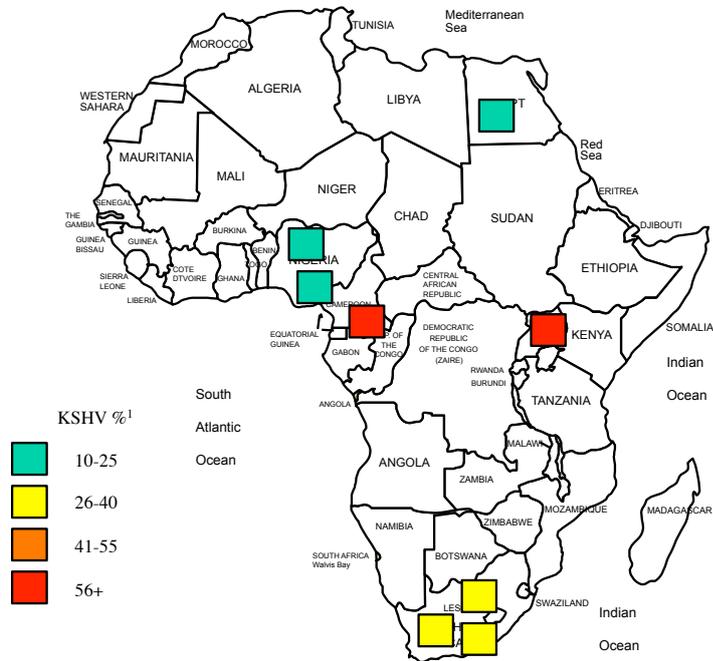


Figure 3.3: KSHV seroprevalence in HIV negative women in studies conducted by the Viral Oncology Section, National Cancer Institute. Studies used in this figure are tabulated in Table 3.3.

¹KSHV-%: KSHV seroprevalence

A KSHV or KS "belt" across Africa has been hypothesized²³³. Dollard et al used a standardized set of serological assays and a number of different algorithms to define KSHV seropositivity on samples collected from young adults in the "KS belt" (Uganda), and outside the "KS belt" (Zimbabwe and South Africa). In an analysis restricted to HIV negative adults and adjusted for age individuals in Uganda had significantly higher odds of KSHV seropositivity than Zimbabwe and South Africa.

Both the tables and figures produced for this thesis and the study by Dollard et al²³³ suffer from similar limitations: varied sampling methods of cohorts KSHV antibody tested and limited knowledge of potential confounders. Furthermore, data is lacking for many countries. Nevertheless, conservative conclusions can be drawn that a geographic pattern of KSHV seroprevalence in Africa does seem to exist.

3.5 Kaposi's sarcoma-associated herpesvirus seroprevalence in Uganda

Studies of populations in Uganda report a broad range of KSHV seroprevalence in HIV negative adults without KS: between 35 and 86%^{8, 19, 127, 129, 190, 221, 222, 224, 225, 228}. Seven studies which state the region, district or town of the population the sample was taken from were identified (Table 3.4, Figure 3.4)^{8, 19, 127, 129, 190, 221, 222, 224, 225, 228}. The map (Figure 3.4) shows areas of high KSHV seroprevalence ($\geq 60\%$) in the West Nile, Western Region bordering the Congo, and Entebbe, with lower KSHV seroprevalence in the Eastern and Southwest regions. Studies from the capital Kampala have reported a wide range of KSHV seroprevalence.

Table 3.4: KSHV seroprevalence of HIV negative adults without KS in studies conducted in Uganda

Author, year	Population residence	Study	Serological technique	Definition of KSHV seropositive if more than one assay used	Age of population	Number in study	KSHV seroprevalence (%)	Plotted on map
Gao, 1996	Not stated	Comparison of assay and samples from multiple countries and risk groups	Latent IFA		Adult	47	51	No
			LNA-immunoblot				62	No
Simpson, 1996	Not stated	Comparison of assay and samples from multiple countries and risk groups	ORF 65 ELISA		Adult	17	35	No
			Latent IFA				17	No
Albashi, 1999	Not stated	Collected in 1960's for a Burkitts Lymphoma study, stored NCI Natural Products Repository	Whole virus ELISA		18-67	62	39	No
de-The, 1999	West Nile	Serum samples Burkitts lymphoma study, collected 1970's, stored IARC	Lytic and Latent IFA	Positive to either	23-50	14	86	Yes
Kakoola, 2001	Kampala	Blood bank, Kampala Uganda	Latent IFA ELISA ORF65 ELISA ORF73 Western blot ORF65	Positive to both the IFA and the ELISA (one or both antigens), or IFA alone or in the ELISA alone followed by a positive Western blot	18-54	114	74	Yes
Wawer, 2001	Rakai	General population cohort	Latent IFA Lytic immunoblot	Positive to both	15-29	523	40	Yes
Hladik, 2003		Blood bank, Kampala Uganda	ELISA K8.1 ELISA ORF65 Lytic IFA	Reactive to at least 2/3 assays or reactive IFA alone	Median 24 years, IQR 19-30	203	39	Yes
Newton, 2003		Non-KS cancer patients presenting at main hospital, Kampala	Latent IFA		Adults	607	50	Yes
Biryahwaho, 2010	All regions	Uganda AIDS Behavioral Survey	ELISA K8.1	Positive to either	15-59	1505	55	No
	Kampala					130	47	Yes
	East Central					158	49	Yes
	Southwestern					149	51	Yes
	Central					170	56	Yes
	Eastern					164	56	Yes
	Northeastern					171	58	Yes
	North Central					158	59	Yes
Western	182	61	Yes					
West Nile	223	66	Yes					
Wakeham, 2011	Entebbe	Mother-child cohort, randomised control trial	ELISA K8.1 ELISA ORF73	Positive to either	14-35+	1915	60	Yes

Methods given within the general methods chapter

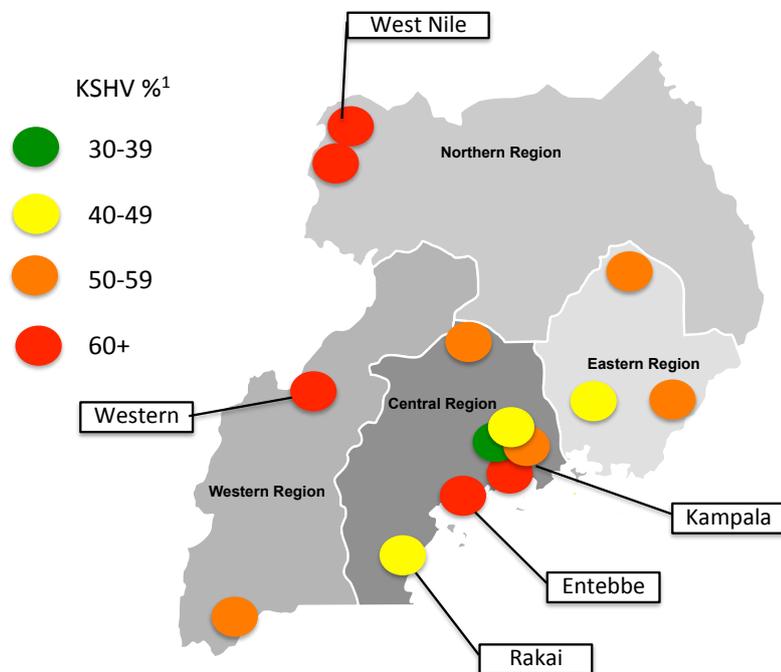


Figure 3.4: KSHV seroprevalence in seven studies conducted in Uganda with location of sample collection documented. Study participants HIV negative adults without KS. Studies used are tabulated in Table 3.4.

¹KSHV-%: KSHV seroprevalence

Published reports have suggested that the variation of KSHV seroprevalence across narrow geographic regions in Uganda exist. But in terms of the validity of results the major recurring problem for investigating potential variation in KSHV seroprevalence remain (a) details of the population studied in terms of key confounders (b) the diversity of assay types and definition of KSHV seropositivity (c) the sample size and (d) year sample collected. Confounders may include age, HIV and after co-infections.

In conclusion, the in-depth study of KSHV seroprevalence in Uganda highlights the need for caution in interpretation of serological data in terms of populations tested, assay, sample size and the year conducted. It remains unclear as to whether geographical variation in KSHV seropositivity exists. Further studies with large sample size and using a standardized assay and collection of extensive data on potential confounders for infection and local environmental factors are needed.

3.6 Factors that could underlie geographical variation of Kaposi's sarcoma-associated herpesvirus prevalence

Taking account of issues that may bias KSHV seroprevalence results, broadly speaking three factors could influence the geographical variation of KSHV sero-epidemiology. These factors are:

1. KSHV viral actors
2. Host factors
3. Environmental factors

3.6.1 Kaposi's sarcoma-associated herpesvirus viral factors

The majority of the KSHV genome is highly conserved, but both ends of the genome show marked variability. At the far left of the KSHV genome, ORF K1 encodes for a lytic transmembrane glycoprotein, which has a sequence variation of up to 30%^{234, 235}. Five major subtypes (A, B, C, D and E) of KSHV are classified on the basis of open reading frame (ORF K1)^{146, 236} and show some worldwide variation in geographic distribution^{147, 236, 237}. KSHV subtypes from North America, Europe and Asia tend to be A and C and those from Africa are predominantly the subtype B. Subtype D is rare and is found only in the Pacific Islands. Subtype E has been identified Brazilian among Amerindians⁹⁴.

At the right hand side of the KSHV genome is ORF K15, which has two forms: P and M²³⁷. The P form accounts for the majority of isolates worldwide, while the M form has been found in KS patients in Taiwan,

China and Africa. There are a number of hypotheses to explain the variability of ORF K1^{149, 238, 239}. The major consensus is that KSHV is an "ancient human virus"²³⁹, which has evolved with the human host to a point of equilibrium so that KSHV can persist as a latent infection without risk of either host clearance or overwhelming KSHV infection that may kill the host. ORF K1 is thought to have evolved very slowly and subtypes reflect human migration out of Africa over the past 60,000 years (for review see Hayward²³⁹). KSHV ORF K1 nucleotide sequences have not been found to change over time within an individual or during oncogenesis²³⁸. There is no evidence to support K1 variability correlating to KSHV seroprevalence, KSHV-related pathologies or regions that report endemic KS¹⁴⁹.

KSHV encodes microRNAs which may have a central function in viral infection and oncogenesis²⁴⁰. MicroRNAs are small (approximately 22 nucleotide) non-coding RNAs that post-transcriptionally regulate gene expression by degradation of messenger RNA and at translation through repression of protein synthesis. MicroRNAs are found in virtually all cells. KSHV microRNAs have been shown to play a critical role in KSHV cell entry, endothelial cell reprogramming, KSHV gene expression, cytokine production, immune evasion, cell survival and immortalization (For review see Qin²⁴⁰). MicroRNA-K12-1, 9, and 11 expression have been shown to be associated with macrophage and endothelial cell susceptibility to KSHV entry²⁴⁰. Increased cell vulnerability to KSHV infection may increase infection persistence and allow entry of virus to neighboring cells. MicroRNA and risk of oncogenesis have been discussed in the section on KS. Whether KSHV microRNA expression could account for geographic differences in KSHV infection rates is currently unknown, but this is an area of extensive research.

3.6.2 Host genetics

The migration of modern humans out of Africa resulted in a population bottleneck and concomitant loss of genetic diversity²⁴¹. Differences in diet, climate, and exposure to pathogens resulted in geographically distinct populations experiencing different selection pressures that resulted in genetic changes²⁴². African populations are generally older than non-Africans and exhibit a larger diversity of genetic material. Research has identified host variation in the risk of many infections. In Africa host genetic associations with malaria, tuberculosis, schistosomiasis and leprosy have been characterized²⁴³.

Studies of genetic polymorphisms and KSHV infection have been limited and disparate in their findings. Research into host genetic risk of KSHV infection or seropositivity has focused mainly on human leukocyte antigen (HLA) polymorphism. A study based in South Africa, found that carrying of HLA alleles HLA-A*6801, HLA-A*30, HLA-A*4301, and HLA-DRB1*04 was associated with increased shedding of KSHV DNA in saliva²⁴⁴. But no such associations were found in a study examining the same HLA types in children in Uganda²⁴⁵. A study in a relatively high KSHV endemic area in Sardinia found no HLA association with susceptibility to KSHV infection²⁴⁶. In Italy a study of killer cell immunoglobulin-like receptors to class I HLA found no association with 50 alleles and KSHV infection²⁴⁷.

The Amerindian population has a high reported KSHV seroprevalence^{94, 182}. This ethnic group shows a very low genetic diversity compared to other populations. The Amerindian indigenous people have gone through two tight genetic bottlenecks; the first during migration from central Asia and the second when descendants were devastated by smallpox, influenza and pneumonic plagues during the Spanish invasion in the 15th and 16th centuries²⁴⁸. Genetic polymorphisms associated with a high propensity to diabetes²⁴⁹ and autoimmune diseases²⁵⁰ are recognized in the Amerindian population, but studies of infection risk could not be identified. A study of Amerindian and non-Amerindian populations living

in close proximity along the banks of the Amazon River in Brazil reported that Amerindians had a KSHV seroprevalence of nearly 80% compared to 6% in non-Amerindians²⁵¹. It is unclear why a marked disparity in detection of antibodies to KSHV exists, but genetic characteristics of the Amerindian population may be important.

Current evidence is not overwhelming in support of genetic predisposition to KSHV infection or reactivation, but studies are limited and HLA may not be the correct genetic marker to study.

3.6.3 Environmental factors

A number of environmental co-factors for KSHV seropositivity including parasites^{7, 8} and oncoweeds²⁵² have been hypothesized. Regional maps of climate, topography and infectious diseases do not in broad terms match that of KSHV seropositivity making a single discrete environmental co-factor unlikely. It does however remain plausible that some environmental factor or factors contribute in part to conditions for primary KSHV infection or reactivation and hence worldwide patterns in epidemiology.

A search for an environmental co-factor that facilitates transmission of KSHV either by affecting how infectious an individual is, or how susceptible (or both), has drawn parallels with research into endemic Burkitt lymphoma and this malignancy's aetiological agents, Epstein-Bar Virus (EBV) and malaria. An epidemiologic link between malaria and Burkitt lymphoma infection is suggested by the number of endemic cases of Burkitt lymphoma that occur in discrete geographic climates along the malaria belt across sub-Saharan Africa²⁵³. Malaria and EBV infection are considered to be co-factors in the oncogenesis of Burkitt lymphoma²⁵³. Malaria may influence age of primary EBV infection²⁵⁴, and or control of EBV reactivation and replication²⁵³. The association between parasites and KSHV will be explored later in the thesis.

The “oncweed” hypothesis is that certain extracts of natural plant products in KS endemic regions cause frequent reactivation in people leading to increased shedding, transmission and risk of KS²⁵². It was proposed that this might provide a biological mechanism to explain geographic variations in KSHV reactivation rates. Higher reactivation rates may explain higher prevalence, viral loads and transmission frequency in KS endemic African regions.

Markers of poverty are common to regions with high KSHV seroprevalence in Africa and the Amazonian basin. The association is by no means perfect; low socioeconomic conditions are widespread in parts of Asia however and prevalence of KSHV is low, whereas in parts of Italy where socioeconomic conditions are comparatively much higher KSHV is elevated. However, poverty may be associated with one or more co-factors that could drive an increased risk of KSHV seropositivity. Poverty is associated with poor health, malnutrition, poor hygiene, environmental contamination and prevalent infections. All of these factors could potentially create a muted or abnormal immune response that could make KSHV infection more likely. Or factors associated with low socioeconomic status in Africa may make “risky” KSHV infection more likely: infection during early childhood, high cumulative lifetime exposure or high viral activity. Within African and Amerindian populations factors associated with low socioeconomic status tend to have null associations with KSHV seroprevalence^{8, 13}. This may be explained by the fact that individuals taking part in studies, even with high socio-economic markers in Africa, tend to all live under conditions of relative poverty.

It is plausible that some environmental factor or factors contribute in part to conditions for primary KSHV infection or reactivation and hence worldwide patterns in epidemiology. But, in conclusion, a large-scale study across all continents and coordinated through a single laboratory collecting extensive data on known and potential risk factors for KSHV infection, along with environmental information will be required to clarify the world geographical variation in KSHV seroprevalence and the potential reasons underlying it.

3.7 Factors associated with Kaposi's sarcoma-associated herpesvirus seropositivity in Africa.

This section will review risk factors for KSHV seropositivity in African populations.

3.7.1 Risk of Kaposi's sarcoma-associated herpesvirus seropositivity in childhood

Table 3.5 presents studies of risk of KSHV seropositivity in childhood in Africa. A significant proportion of KSHV infection occurs during childhood in sub-Saharan Africa, with seroprevalence ranging from 7-58% (Table 3.5)^{8-10, 13, 22, 25, 27, 230, 255-261}. As discussed above, comparison between studies is hampered by differences in serological assay, sample size, and population factors (age, HIV status) since regional variations in KSHV seroprevalence may also exist. Butler et al tested children in Uganda aged 1 to 9 years and South Africa aged 2-8 years for KSHV antibodies and found that KSHV seroprevalence among Ugandan children was much higher than among South African children²⁵⁶. Further, in Ugandan children KSHV seropositivity increased with age, but was static among children in South Africa. The authors proposed that the difference in relationship between age and KSHV infection among children in Uganda and South Africa might suggest differences in KSHV transmission. This is an interesting hypothesis and potentially fits with discrete geographical differences in KSHV infection. But currently this is the only study to directly compare the prevalence of antibodies to KSHV in children in and outside the "KS belt"²³³. Further the different age ranges and sample methods between the Ugandan and South African children could account for the differences. Other studies, which have investigated KSHV seropositivity among children in South Africa^{13, 25} or Uganda^{8, 258} have found KSHV seroprevalence comparable between the two countries (Table 3.5). Although differences in age ranges, structure and power within age categories could have affected the results. Despite potential assay, study and regional differences in KSHV transmission, patterns in risk of KSHV infection in childhood can be observed. KSHV seropositivity

tends to increase with age in the vast majority of studies identified and tabulated in Table 3.5^{8-10, 13, 22, 25, 27, 230, 255-261}. The young age of primary infection and age trend implies horizontal non-sexual transmission. There is no evidence to support the transfer of KSHV in breast milk^{23, 25}, and KSHV infection in neonates is uncommon, making vertical transmission unlikely²³. There is considerable evidence that KSHV transmission occurs via saliva^{27, 262}. In Africa, transmission from mothers and siblings is most likely the predominant route^{10, 25}. Out of ten studies identified of mother-child transmission^{8, 10, 13, 25, 230, 257-261} eight found the maternal KSHV serostatus was a key determinant of childhood serostatus^{8, 10, 13, 25, 230, 259-261}. Two studies did not; both of these had a sample size of about 30 mother-child pairs, and were unlikely to have the power to investigate maternal KSHV serostatus as a risk factor for childhood seropositivity^{257, 258}. A family study in rural Tanzania¹⁰ found KSHV seropositivity in the child strongly related to serological status of the mother. This finding was repeated in a study in South Africa; mothers with a high titre of antibodies to KSHV were more likely to shed KSHV virus in saliva and have a KSHV seropositive child²⁵. A study conducted in an endemic population from Cameroon reported that KSHV transmission mainly occurs from mother to child and between siblings. In this study transmission was independent of plasma antibody levels of KSHV in infected relatives²⁶⁰. It has been proposed that exposure to maternal or sibling saliva may occur during close contact, sharing of eating implements or pre-mastication of food²⁵⁶. Two studies have investigated siblings as a source of KSHV transmission and in both the KSHV status of the sibling was associated with KSHV serostatus of the child^{10, 260}. Only two studies have investigated risk of infection from fathers^{10, 260}; one reported a positive association although risk was less than that from mothers and siblings¹⁰, and one reported no association²⁶⁰.

Across studies gender has little or no effect on KSHV seroprevalence^{8-10, 13, 22, 25, 27, 256, 258, 259}. Markers of childhood socioeconomic status (household density, maternal education, maternal occupation, maternal income) have no associations with childhood KSHV seropositivity (Table 3.5). Water source, a potential marker of socioeconomic status and exposure to water borne parasites is not associated with KSHV seroprevalence in the three studies that investigated it as a potential cofactor^{8, 27, 259}. Having a blood transfusion as a child has been investigated in two studies^{22, 258}, one found none and the other a positive association. The study with a positive association was conducted within a sickle cell anaemia clinic in Uganda and children were likely to have had multiple blood transfusions²⁵⁸. It is common practice in Uganda to ask relatives to donate blood for the child, if a transfusion is required and this could account for transmission of KSHV if the donor was KSHV infected.

Table 3.5a: Description of studies of KSHV infection in children from sub-Saharan Africa

1st author, year	Study location	Number of children	Study design	KSHV assay	Definition of KSHV infection	Age of children	KSHV seroprevalence (%)	HIV prevalence of children (%)
Bourboullia, 1998	South Africa	111	Cross sectional, paternity disputes, mother-child pairs	Latent IFA		6m-14	42	0
Andreoni, 1999	Egypt	196	Cross sectional, attendees at vaccination clinic	Latent IFA Lytic IFA		1-12	41 8	0
Sitas, 1999	South Africa	112	Cross sectional, mother-child pairs	Latent IFA		6m-14		
Gessain, 1999	Cameroon	258	Cross sectional study, outpatient clinic, only 32 mother-child pairs	Lytic IFA Latent IFA	Positive to either	0-20	28	
Mbulaitweye, 2003	Tanzania	507	Family study	K8.1 ELISA		<17	58	
Mbulaitweye, 2003	Uganda	600	Cross sectional study, Sickle cell clinic, only 28 seropositive mothers	ELISA K8.1 ELISA ORF73	Positive to either	0-16	21	
Brayfield, 2003	Zambia	3150	Children 12 months postnatal, part of mother-child cohort study	Lytic IFA Latent IFA ELISA whole virus	Positive to both IFAs Seropositive cross checked with whole virus ELISA	1	49	28
Dedicoat, 2004	South Africa	2546	Cross sectional, mother-child pairs, vaccination clinics	Latent IFA K8.1 ELISA		0-6	12 7	22
Plancoulaine, 2004	Cameroon	153	Cross sectional family study, isolated village	Lytic IFA		1-19	33	
Mbulaitweye, 2004	Uganda	233	Cross sectional, sickle cell clinic	PCR	Saliva Buffy-coat	0-16		
Mbulaitweye, 2005	Uganda	600	Cross sectional, sickle cell clinic, mother-child pairs	ELISA K8.1		1-16	21	
Malope, 2007	South Africa	1287	Cross sectional paternity disputes, mother-child pairs	ELISA K8.1 ELISA ORF73	Positive to either	1.6-16	16	9
Butler, 2009	South Africa Uganda	427 989	Cross sectional, household survey Cross sectional, population malaria survey and children admitted to hospital	Lytic IFA, EIA ORF65, EIA K8.1	Positive to any two tests or the IFA alone	2-8 1-9	8 20	6 7
Minhas, 2010	Zambia	677	Cross sectional, nested in cohort	Lytic IFA Latent IFA ELISA whole virus	Positive to both IFAs Seropositive cross checked with whole virus ELISA	12 months	13	6
Wakeham, 2011	Uganda	1823	Antenatal clinic, RCT	ELISA K8.1 ELISA ORF73	Positive to either K8.1 and/or ORF73	1-5 years	11	1

Methods given within the general methods chapter

Table 3.5b: Factors associated with KSHV infection in children from sub-Saharan Africa from studies in Table 5a

1st author, year	Study location	KSHV serostatus of mother	Gender	Age	Mother HIV+, child HIV- unknown	Child HIV+	Household density	Number siblings	Sibling KSHV positive	Father KSHV positive	Source drinking water (surface)	Maternal education	Maternal occupation	Maternal income	Asymptomatic malaria parasitaemia	Malaria ever/never	Mosquito net	Ever blood transfusion	Low elevation village
Bourboulla, 2008	South Africa	Red																	
Andreoni, 1999	Egypt		Red																
Sitas, 1999	South Africa	Red																	
Gessain, 1999	Cameroon		Red																
Mbulatye, 2003	Tanzania	Red	Red					Red										Red	
Mbulatye, 2003	Uganda	Red	Red														Red		
Brayfield, 2003	Zambia				Grey												Grey		
Dedicoat, 2004	South Africa	Red	Red		Red				Red										
Plancoulaine, 2004	Cameroon	Red	Red						Red										
Mbulatye, 2004	Uganda		Red			Grey					Grey								
Mbulatye, 2005	Uganda	Red	Red				Grey												
Malope, 2007	South Africa	Red	Red		Red														
Butler, 2009	South Africa		Red																
Uganda			Red		Green														
Minhas, 2010	Zambia		Red		Red														
Zambia			Red		Red														
Wakeham, 2011	Uganda	Red	Red		Red														

Key
■ Factor associated with increase in KSHV seroprevalence
■ Factor associated with decrease in KSHV seroprevalence
■ No association between factor and KSHV seroprevalence

As mothers are the key source of KSHV infection for children in Africa, studies investigating the risk of KSHV seropositivity in African mothers are shown in Table 3.6^{8, 13, 22, 25, 191, 259}. Maternal age was not related to KSHV serostatus in any study of KSHV serostatus in mothers and pregnant women. The age range of studies of mothers tends to be narrow, reflecting the age range of women's fertility and possibly accounts for this. HIV had positive associations with risk of maternal KSHV and this is discussed in detail in a later section of this thesis. In the two most recent studies^{8, 191} higher socioeconomic status and increasing educational attainment were associated with decreased KSHV seroprevalence in mothers.

Table 3.6. Factors associated with KSHV seropositivity in mother from sub-Saharan Africa

1st author	Year	Study location	Number of women	Study design	Serological assay	Definition of KSHV seropositive	KSHV seroprevalence (%)	Age	Maternal HIV infection	Socioeconomic status	Educational attainment	Income	Occupation	Household size	Number pregnancies	Marital status	Syphilis	Number sex partners	History STDS	Ground water as source	Malaria	Hookworm	Mansonia
Brayfield,	2003	Zambia	3150	Mother-child cohort study, mothers 12, months postnatal	Lytic IFA Latent IFA ELISA whole virus	Positive to both IFAs Seropositive cross checked with whole virus ELISA	40 30																
Dedicoat,	2004	South Africa	2546	Cross sectional study mother-child pairs; vaccination clinics	K8.1 ELISA Latent IFA	40 14	28 28																
Mbulaitwe,	2005	Uganda	600	Cross sectional, sickle cell clinic, mother-child pairs	ELISA K8.1	34																	
Malope,	2007	South Africa	1179	Cross sectional paternity disputes, mother-child pairs	ELISA K8.1 ELISA ORF73	30	21																
Malope,	2010	South Africa	1740	Cross sectional study of pregnant women, antenatal clinic	ELISA K8.1 ELISA ORF73	45	23																
Wikeham,	2011	Uganda	1915	Cross section pregnant women, part of RCT, mother-child pairs	EIA K8.1, ORF73 ELISA ORF73	61	10																

Key

- Factor associated with increase in KSHV seroprevalence
- Factor associated with decrease in KSHV seroprevalence
- No association between factor and KSHV seroprevalence

3.7.2 Heterosexual intercourse and risk of Kaposi's sarcoma-associated herpesvirus in sub-Saharan Africa

Evidence to support sexual transmission mostly comes from studies of MSM in North America and findings in Africa are not consistent. Trends of increasing KSHV seropositivity with age in sub-Saharan Africa, raise the possibility of the role of sexual transmission^{8, 13, 25}. Table 3.7^{14, 18, 228, 263-267} shows studies of background and laboratory diagnosed sexually transmitted infections, self-reported markers of sexual risk and KSHV seropositivity in sub-Saharan Africa.

Associations with markers of sexual activity are not consistent between studies. For example, syphilis was associated with KSHV seropositivity in three of six identified studies (Table 3.7)^{18, 263, 264}. Within one study, Eltom et al¹⁸ reported from Nigeria that syphilis was a potential co-factor for antibodies to KSHV among men attending an STD clinic but not in women attending the same STD clinic or in female sex workers. Differences in sexual behaviour could lead to differences in results. In Western cohorts while specific sexual behaviours have not been identified, MSM appear overwhelmingly at more risk than heterosexual men¹⁹³. It has been suggested that sexual contact among MSM is a surrogate for exposure to saliva¹⁹³. The same could be true in Africa. In a study of KSHV shedding in commercial sex workers in Kenya the prevalence of detection of KSHV was about one third in saliva and mouth swab samples but only 4% in cervical swabs and 2% in vaginal swabs²⁶⁸. If saliva is the most likely source of transmitted KSHV, then anecdotal reports that kissing practices in Africa are uncommon may account for low or no levels of KSHV transmission through close contact during heterosexual sex. In conclusion, heterosexual transmission of KSHV in Africa may occur but is likely to be at low levels.

3.7.3 HIV as a risk factor for KSHV seropositivity in high-risk heterosexual cohorts in sub-Saharan Africa.

HIV is different from other STDs as it is not only a marker for sexual risk behaviour but is also immunosuppressive. Individuals infected with HIV may be more susceptible to and/or may be more likely to transmit KSHV infection. Of the eight studies identified from sub-Saharan Africa investigating heterosexual transmission of KSHV (Table 3.7) only one study set in Nigeria found HIV to be associated with KSHV seropositivity and this result was from a sub-analysis restricted to a female-only small reference population recruited predominantly from office and factory workers¹⁸. In Africa, cohorts studying HIV and sexual risk are all presumed to be heterosexual. The lack of association between HIV and KSHV in studies of heterosexuals is unsurprising and mirrors reports from North America and Europe. Here KSHV infection is predominately found in HIV infected MSM and not consistently in other HIV infected groups; male and female heterosexuals, female sex workers, hemophiliacs and IV drug users^{192, 193}. It could be that in Africa all individuals susceptible to KSHV have already been infected by early adulthood, thus reducing the effect of HIV as a risk factor for primary KSHV infection. Current data suggests that HIV is not a risk factor for KSHV seropositivity in high-risk heterosexual populations in Africa.

Table 3.7: Studies from sub-Saharan Africa investigating heterosexual transmission of KSHV in adults

1st Author, Year	Country	Number	Study design	Age	Serological assay	Definition of KSHV seropositive	KSHV seroprevalence (%)	HIV prevalence (%)	Syphilis prevalence (%)	Age	Gender	HIV	Syphilis	HSV-2	Messera gonorrhoea	Current STD
Wawer, 2001	Uganda	523	Population based study	15-59	Latent IFA Lytic immunoblot	Positive to both	39	20	5							
Baeten, 2002	Kenya	1061 (men only)	Cross section, truckers	17-64	Whole virus ELISA		43	12								
Eltom, 2002	Nigeria	287 Reference population 114 attendees STD clinic 863 female sex workers	Women	15-45+	ELISA K8.1	Positive to K8.1	14	4	1							
			Men	15-45+	ELISA ORF73		31	16	4							
Marcellin, 2002	Djibouti	479 Reference population 259 attendees STD clinic 282 in total	Women	15-45+	ELISA K8.1	Positive to K8.1	20	5	5							
			Men	15-45+	ELISA ORF73		31	16	4							
Lavrays, 2003	Kenya	736 female sex workers	Cross sectional, part of cohort	Adults	Whole virus ELISA		35	14	5							
			Cross sectional, general medical outpatient ward	Adults	Latent IFA		25	16								
Volpi, 2004	Cameroon	238	Cross sectional, general medical outpatient ward	18-40	Latent IFA		52	13	10							
Malope, 2008	South Africa	862 mineworkers 95 female sex workers 415 male township residents 731 female township residents	Cross sectional	16-63	Lytic IFA		48	40	8							
			Cross sectional	16-63	ELISA K8.1	Positive to either	49	37	4							
			Cross sectional	16-63	ELISA ORF73		51	77	19							
Shebi, 2011	Uganda	2288 sexually active adults	Cross sectional, HIV serobehavioral survey	>15	ELISA K8.1 ELISA ORF65	Positive to both	46	48	13							

Key

- Factor associated with increase in KSHV seroprevalence
- Factor associated with decrease in KSHV seroprevalence
- No association between factor and KSHV seroprevalence

3.8 Chapter conclusion

There is marked geographical variation in KSHV seroprevalence and this has a profound influence on worldwide incidence of KS. Limitations in serological assays and study design reduce clarity in understanding causes of this variation. Important risk factors for KSHV seroprevalence in Africa include having a KSHV seropositive mother, increasing age and HIV infection in mother-child pairs.

Chapter 4: General methods

4.1 Outline of chapter

This chapter outlines the two study populations detailed in this thesis and the KSHV serology method. Specifics of the design for individual studies will be given within the relevant chapters.

4.2 Socio-demographic position of Uganda

In the last Ugandan Population and Housing census in 2006^{269, 270}. The population of Uganda was estimated to be 24.4 million with a sex ratio of 95 males to 100 females. Children below 15 years constituted 49% of the population.

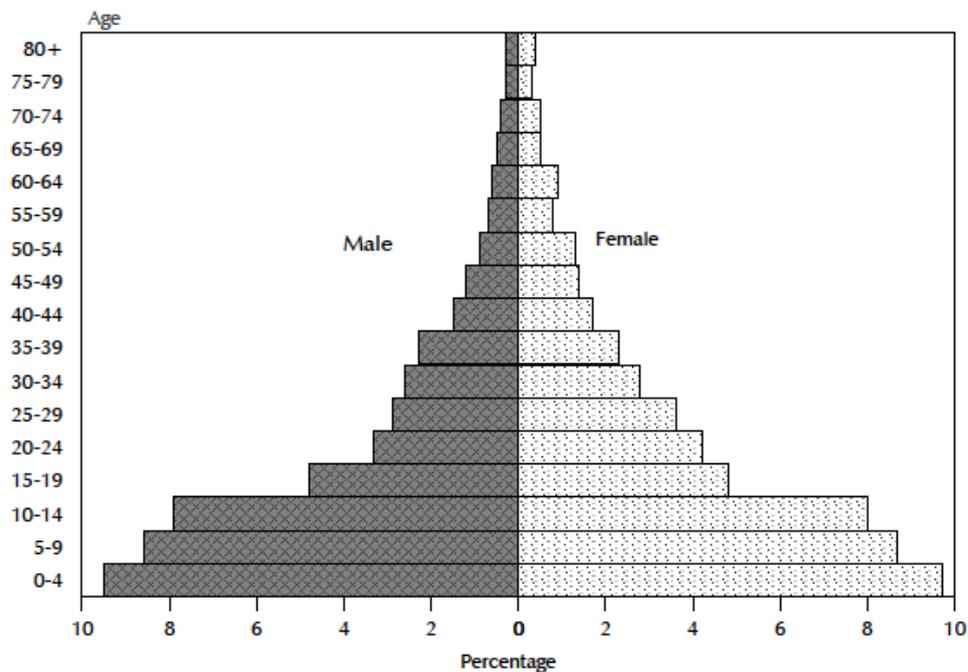


Figure 4.1: Population pyramid for Uganda (2002): A population pyramid is a pictorial representation of the age and sex distribution for the population of Uganda. Chart taken from Uganda Demographic and Health Survey, 2006²⁷⁰.

The Ugandan population has grown dramatically over the past 50 years. The high growth rate is a result of high fertility and declining mortality rates. The annual population growth rate between 1969 and 1980 was 2.7 and decreased to 2.5 between 1980 and 1991. Instability in Uganda during the early 1980s may have contributed to this decline. The annual population growth rate increased to 3.2% between the 1991 census and the 2002 census. The level of urbanisation is still low but has been increasing over time. In 2002, less than one fifth of the Ugandan population lived in urban areas and urbanisation was about 10% of the population per year.

If the annual population growth continues at its current growth of about 3% per year the population of Uganda will be over 130 million by 2050. For comparison, the United Kingdom's population growth rate is 0.60%²⁷¹, and the predicted population in 2050 is 70 million. The UK has a similar landmass to Uganda of about 90,000 square miles.

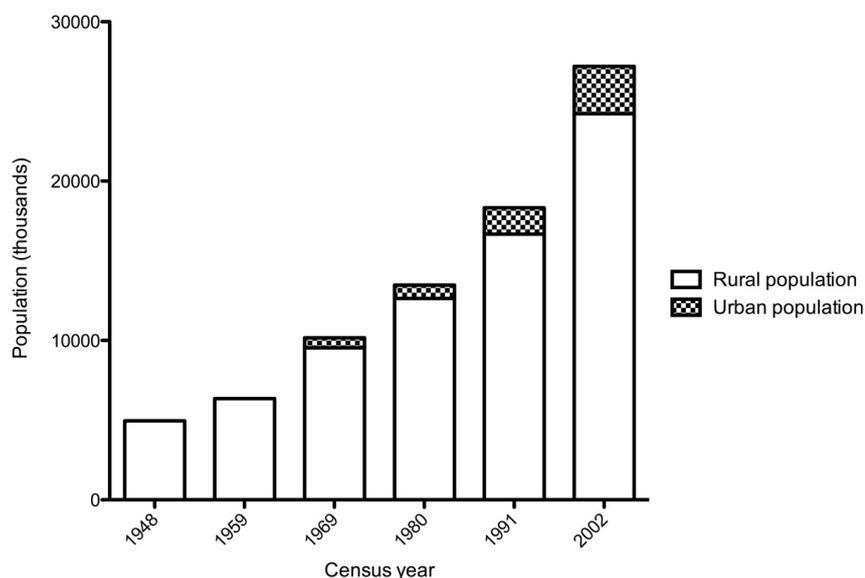


Figure 4.2: Ugandan demographic statistics from population censuses of 1948 through to 2002. In Uganda data was derived from population censuses, which started in 1948 and subsequently were held in 1959, 1969, 1980, 1991, and 2002. Over this period, the population has increased almost five-fold. Data taken from Uganda Demographic and Health Survey, 2006²⁷⁰.

Although mortality in Uganda is falling and a aging of the population is expected, mortality rates still remain relative high for key WHO indicators. The infant mortality rate was 76 per 1000 live births with an under-five mortality rate, per 1,000 live births, of 137²⁶⁹. Only about 60% of children are immunised against measles²⁷⁰. The maternal mortality rate per 100,000 live births was 880²⁶⁹. In 2006, the life expectancy in years at birth for men was 48 years and for women 51 years ²⁶⁹. The top five causes of death for all age groups were HIV (22%), malaria (11%), lower respiratory infections (not including TB) (11%), diarrheal diseases (8%) and perinatal conditions (5%) ²⁶⁹. HIV prevalence among adults (15-49 years) was 4.1% ²⁶⁹. The annual per capita total expenditure on health at an averaged exchange rate for 2006 was \$18 USD per year, and the annual per capita government expenditure on health at an averaged exchange rate for 2006 was \$5 USD per year ²⁶⁹.

In terms of markers of socioeconomic status, about 10% of the population had access to a sanitation facility (flush toilet, pit latrine, composting toilet) and only 8% of households had access to electricity. Almost all (97%), reported using firewood and charcoal for cooking. Safe access to drinking water was available in 61% of households. Only 4% of households had a television, while nearly 50% had a radio.

In the last Uganda Demographic and Health Survey (2006)²⁷⁰ the literacy rate of individuals above 10 years was 68%. About 50% of the urban population and 17% of the rural population attended secondary school. Employment in the 12 months preceding the 2006 survey was high and reported in about 85% of women and 95% of men. Occupation was reported to be in agriculture in about 80% of adult Ugandans, with the vast majority being self-employed. The percentage of the population living below the poverty line on less than 1.25 USD per day was 85% ²⁶⁹. Gross domestic product per capita 2011 was about \$450 USD per year ²⁷¹.

determine the impact of helminth infections and their treatment on vaccine responses and infectious disease outcomes in children (International Standard Randomised Controlled Trials no. 32849447); the details have been reported elsewhere³⁵.

Consenting women were recruited between April 2003 and November 2005 from the government funded antenatal clinic at Entebbe hospital. About two-thirds of women in the study area seek antenatal care²⁷². At an initial screening visit, eligibility was assessed and a questionnaire and blood sample taken. Inclusion criteria were: planned delivery in the hospital and being in the second or third trimester of pregnancy. Exclusion criteria for enrolment included a haemoglobin <8g/dL, abnormal pregnancy, clinically obvious liver disease, refusal to receive a HIV test result, and known adverse drug reaction to anti-helminth drugs. At enrolment, sociodemographic data were collected and blood samples were obtained by venepuncture, and processed for syphilis, HIV serology, CD4 count and for examination for malaria parasites and *Mansonella perstans*. A stool sample was obtained for examination for intestinal helminths. Participants were then randomised to receive albendazole (400mg) and placebo, praziquantel (40mg/kg) and placebo, both albendazole and praziquantel, or two placebos, in a 2 by 2 factorial design. During antenatal follow-up, ferrous sulphate (200mg; 60mg elemental iron) and folic acid (0.25mg) were given daily and sulfadoxine/pyrimethamine treatment for malaria was given twice in the second and third trimester. Women found to be infected with HIV were offered single-dose nevirapine to prevent vertical HIV transmission and treated for syphilis if indicated. In the early post-partum period, repeat stool and blood samples were taken and all women received a single dose of albendazole and praziquantel six weeks after delivery.

The mother, father or guardian gave consent for study participation for the child, and children were then followed from birth. At routine yearly visits, clinical data, blood for full blood count, blood slides for malaria and *M. perstans* and stool samples were collected from well children. Any helminth infections were treated. Children were seen for routine

vaccinations at 6, 10, 14 weeks and nine months. Vitamin A was given at six months and at one year. Children received routine twice-monthly visits by field workers. If unwell, attendance to the study clinic for medical treatment was encouraged. At approximately six weeks and 18 months of age blood samples were obtained from children of HIV infected mothers to ascertain the child's HIV status. At 15 months children were randomised to receive three-monthly albendazole or placebo. Visits and intervention continued quarterly from 15 months to five years. The primary outcomes were: immunological responses to BCG and tetanus immunisation, and the incidence of infectious and atopic disease events in childhood (pneumonia, diarrhoea, malaria, measles, tuberculosis and vertical HIV transmission and atopic eczema).

4.3.3 Diagnosis and treatment of infections in EMABS

Stool was prepared using the Kato-Katz^{273, 274} method for identification of Hookworm, *Schistosoma mansoni*, *Trichuris trichiura*, *Ascaris lumbricoides* and Trichostrongylus species, and by charcoal culture for *Strongyloides stercoralis*²⁷⁴. Two slides from a single stool sample were examined for each individual, within 30 minutes for hookworm, and the next day for other ova and parasites. Egg counts were measured. Blood was examined for *M. perstans* by a modified Knott's method²⁷⁵. *Plasmodium falciparum* was diagnosed by examination of thick blood films and asymptomatic malaria parasitaemia defined as the presence of parasites without fever. A *P. falciparum* parasite count per 200 white blood cells was recorded. In 6-week-old children HIV was indicated by plasma viral load. In mothers, and children 18 months of age, HIV was identified using a triple rapid test serial testing algorithm²⁷⁶. Women were screened for syphilis on a Rapid Plasma Reagin (RPR) test, with positive results being further tested for active syphilis, based on a definition of RPR titer $\geq 1:4$ and a positive Treponema Pallidum Haemagglutination Assay (TPHA) result (both kits supplied by BIOTEC Laboratories Ltd., UK). Helminth infections were treated according to study protocol, either during pregnancy (the trial intervention) or after delivery. In accordance with recommendations from the Ugandan

Ministry of Health: all women received intermittent presumptive treatment for malaria with sulphadoxine-pyrimethamine; HIV-positive women received nevirapine for prevention of mother-to-child HIV transmission, and, if they had a low CD4-count, were referred for treatment; RPR-positive women received benzathine penicillin.

4.4 Rural Clinical Cohort

4.4.1 Setting

The study population was resident in Kyamulibwa sub-county, Masaka district in South West Uganda. The study area is about 250km from the capital of Uganda, Kampala. Kyamulibwa is a rural area, the nearest large town, Masaka, is about a one hour drive away. The MRC/UVRI have a field station in Kyamulibwa, which consists of research clinics, laboratories, a surveillance field office, a data section, offices and a guesthouse. The MRC/UVRI Unit is the major provider of general health care for the study population in the area.

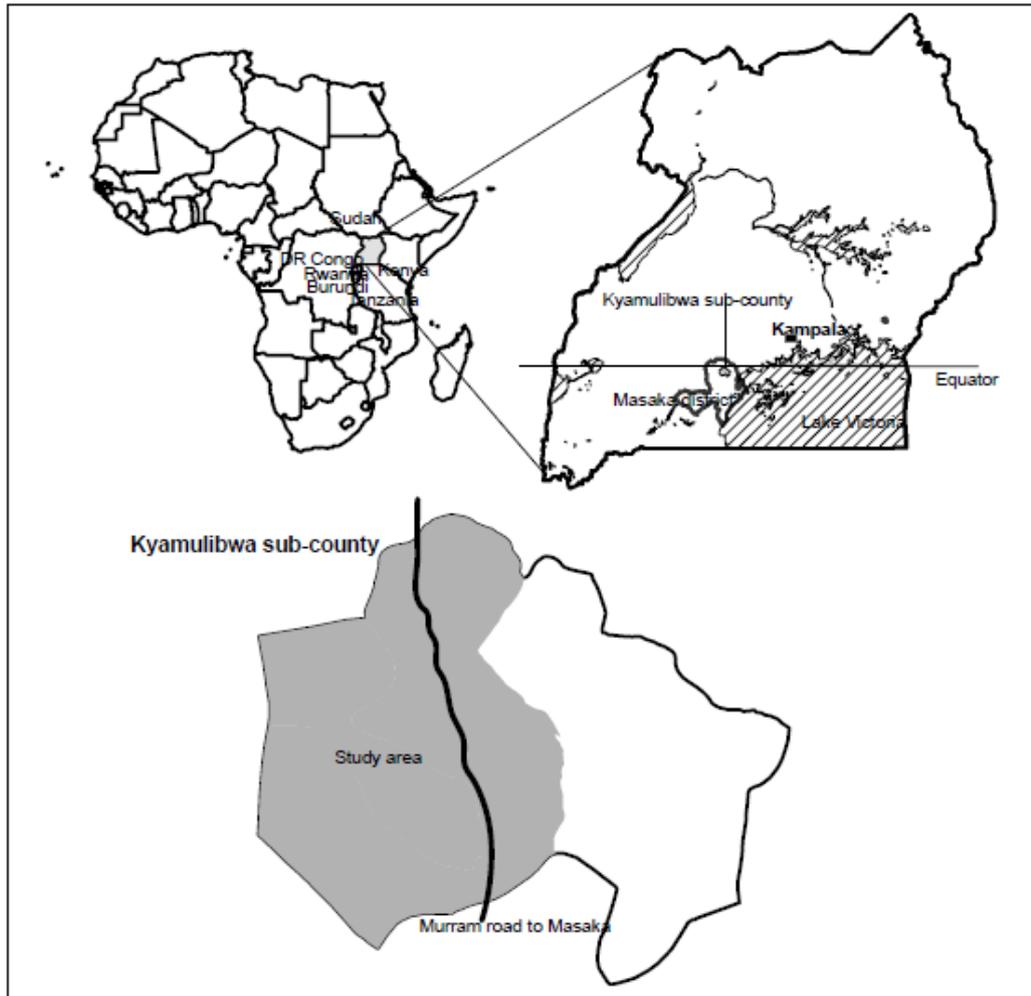


Figure 4.4: Map of rural clinical cohort study area.

4.4.2 Rural Clinical Cohort Methods

The Rural Clinical Cohort (RCC)²⁷⁷⁻²⁷⁹ consists of HIV infected individuals identified through the General Population Cohort (GPC). A small number of HIV seronegative individuals are also followed through the RCC to prevent potential prejudice against people living with HIV/AIDS. The GPC was established by the MRC in 1989, to study the dynamics and natural history of HIV disease in a typical rural Ugandan community²⁸⁰⁻²⁸². A population of approximately 10,000 in a cluster of 15 villages was studied from 1989 to 1999. In 2000, the GPC was expanded to cover a further 10 villages. The cohort is open and dynamic with new births, deaths and migrations reported at each round of follow-up; the current

population under survey includes approximately 20,000 people. An annual census of the resident population collects data on births, deaths, in-migration and out-migration, age, sex, education, and relationship to household head. This is followed by an annual household survey in which, all willing residents aged 13 and above are individually recruited and includes collection of blood specimens for HIV testing and a brief behavioural questionnaire. Children (birth to 12 years) are included every third year. Every four years, starting at baseline, information is collected on socio-economic status using a list of household assets. The annual surveys have coverage of 60–70% of the resident census population in any given year. The blood specimens are obtained at each annual survey. Serum is tested for HIV-1 and the remainder is stored at -80 degrees in freezers in Entebbe. The 23rd annual survey is currently underway. The RCC consists of people living with HIV in 1989/1990 (round 1) and HIV incident cases after that. About 40% of HIV infected individuals from the GPC opt to join the RCC.

4.4.3 HIV prevalence and incidence

The Ugandan national prevalence of HIV infection reached a peak of 18% in 1992, before declining through the 1990s to about 6%^{283, 284}. HIV prevalence in the GPC has varied from 6.7 to 8.7% with an incidence, per 1000 person-years, of about 5.0²⁸⁵⁻²⁸⁷. While all participants of the serosurvey have a HIV test, uptake for receipt of the HIV test results from the annual serosurvey never exceeded 10% prior to 2004. An intervention including offering counselling and giving HIV test results in the home increased it to nearly 50%²⁸⁸. The date of HIV seroconversion is assigned to the middle of the interval between the last negative test and the first positive test. The GPC used two independent enzyme immunoassays to establish HIV-1 status (Wellcozyme HIV-1 recombinant VK 56/57, Murex Biotech, Dartford, UK; and Recombigen HIV 1/2, Trinity Biotech, Galway, Ireland). Samples discordant on enzyme immunoassay and all first time positive samples were tested by western blot (Cambridge Biotech HIV-1 western blot, Calypte Biomedical, Rockville, MA, USA).

4.4.4 HIV care within the RCC

RCC participants were reviewed in the study clinic at regular appointments. A clinical questionnaire was filled in at each visit and a full physical examination carried out and documented. CD4 cell counts were measured (FACSCount, Becton Dickinson, San Jose, USA) at baseline and every clinic visit and a serum sample was collected and stored at -80 degrees at MRC/UVRI in Entebbe. Anti-retroviral therapy (ART), available from 2004, commenced in accordance with Ugandan Ministry of Health criteria; CD4 count of 200 cells/ μ L or below; or WHO clinical stage 4; or advanced WHO clinical stage 3 with persistent or recurrent oral thrush and invasive bacterial infections; or CD4 count of 250 cells/ μ L or below during pregnancy. First-line ART regime was zidovudine (AZT), lamivudine (3TC) and nevirapine (NVP), with the possibility of switching to stavudine for AZT toxicity and to efavirenz for NVP toxicity or concurrent tuberculosis treatment. Patients on ART had a HIV viral load measurement (Amplicor MONITOR 1.5, Roche Molecular Systems, NJ, USA) at drug commencement and about every six months. HIV viral load of 400 copies/ml and above was considered detectable.

4.5 Overview of serological assays for Kaposi's sarcoma-associated herpes virus

A number of serological assays have been developed to investigate KSHV related pathology and epidemiology. KSHV encodes for multiple proteins that are classified broadly by whether they are produced during lytic and latent cycle. The most common antigens used in assays, due to relatively high immunogenicity, are latency associated nuclear antigen (LANA) encoded by ORF 73^{175, 192, 231, 232, 289}, K8.1^{125, 175, 192, 231, 232, 289} and ORF 65^{125, 129, 172}. LANA or ORF 73 is a protein expressed during latency and inhibits p53 function. ORF 65 or small viral capsid antigen (sVCA) is a lytic phase protein thought to function in virion formation and structure. ORF K8.1 is a lytic phase glycoprotein expressed on the cell surface. The presence of antibodies to KSHV is normally used to define exposure to infection rather than protective immunity.

A "gold standard" clinical reference test for KSHV is currently lacking. The reasons for this are: (1) KSHV viral DNA in biological fluids is often of low copy number^{125, 290} and cannot be used as a marker of infection; (2) not all patients with KS, who are taken to be infected with KSHV, are found to be positive on serological assays^{127, 129, 172, 231}; (3) populations taken as negative (healthy blood donors in North America or neonates) often have a seroprevalence of between 0-20%^{172, 174, 291}; (4) host responses to antigens are highly variable^{98, 116}; (5) the performance of assays tends to vary across populations, making comparative interpretations difficult^{176, 231} and (6) kinetics of appearance of KSHV antibodies are not well known and diagnosis of evidence of KSHV infection using both latent and lytic class antibodies may be necessary to achieve adequate sensitivity for detection of KSHV in individuals with and without clinical KSHV-associated disease¹⁷⁶. The concordance between single antigen assays tends to be moderate or low^{125, 171-173, 175, 231, 292}. This is a particular problem in low prevalence populations where the use of one KSHV antigen may underestimate KSHV seroprevalence. The combination of the results of several assays or the use of a combination of several peptides or proteins in a single assay has been proposed to increase sensitivity^{175, 293} of assays.

First generation KSHV serological assays were based on KSHV infected PEL cell lines and immunofluorescence assays (IFA). These techniques have major disadvantages in terms of cost, labour, low reproducibility and difficulties in automation for testing a large number of samples¹⁷⁵. Enzyme linked immunosorbent assays (ELISA) for KSHV antibodies have been developed to immunogenic recombinant proteins and peptides^{171, 175, 231}. High throughput testing can be achieved with ELISAs and they tend to be of relatively low cost and maintain high sensitivity and specificity in a large number of settings.

4.6 KSHV serology in this analysis

The KSHV assay used in this thesis is a recombinant ELISA to K8.1 and ORF 73 proteins developed by the Viral Oncology Section (VOS), National Cancer Institute (NCI), USA^{231, 232}. Both their K8.1 and ORF 73 assays have a high performance accuracy with a sensitivity of 98.78% and 89.02% respectively and specificity of 98.79% and 97.57% respectively¹⁷⁵. All ELISAs in this analysis were transferred from VOS, NCI and performed at the Uganda Virus Research Institute (UVRI).

4.6.1 K8.1 ELISA

Recombinant K8.1 protein was diluted 1/5000 in 0.05M Sodium Carbonate / 0.05M Sodium Bicarbonate, pH10.0 (Invitrogen, Carlsbad, CA) and 100 µl of diluted protein was added to each well of a flat-bottom, 96-well, non-sterile Dynex Immulon 4 HBX microtitre plate (VWR, Bridgeport,NJ). Each plate was covered with a plate sealer and incubated overnight at 4 °C. The wells were then washed 3 times with 350 µl of in-house ELISA wash (300ml PBS Ph7.4 10x, 0.005% Tween 20 (Sigma Aldrich), 2700ml distilled water) using an automated plate washer (model ELx405 Bio-Tek Instruments). Assay buffer of a volume of 280 µl was used to block each well (2.5% BSA [Sigma-Aldrich], 2.5% normal goat serum [Equitech-Bio, Kerrville, TX], 0.005% Tween 20 [Sigma-Aldrich] and 0.005% Triton-X 100 [Sigma-Aldrich] in dulbeccos PBS)) for 3h at 37 °C.

Plates containing assay buffer were stored at -80 °C for transport and before use. Plates were defrosted for two hours at room temperature. Plates were washed 3 times in 350 µl in in-house ELISA wash using an automated plate washer. Diluted test and control sera (final volume of 100 µl at 1/20 dilution in assay buffer) were pipetted into the wells and incubated at 37 °C for 90min. In order to provide an internal control, samples from patients with KS and samples from an American blood donor population were run in triplicate as positive and negative controls, respectively. Two blank wells containing assay buffer were included (see

plate layout below). Unbound antibodies were removed by washing five times with in-house ELISA wash using an automated plate washer and then tapped dry. The conjugate was prepared by diluting Goat anti-human IgG (g) alkaline phosphatase (ReserveAP™, KPL, Gaithersburg, MD) at 1:7500 in assay buffer and adding 100 µl to each well. Plates were covered and incubated at 37 °C for 30 min. After 5 washes, 100 µl of substrate, 1 step p-nitrophenyl phosphate, disodium salt (Pierce, Rockport, IL) was added to detect antigen-antibody complexes. The plates were allowed to develop at room temperature, uncovered in the dark for 25min. Absorbance was read at 405 nm using a model EL 808IU automated plate reader (Bio-Tek Instruments).

4.6.2 ORF 73 ELISA

Recombinant ORF 73 protein (0.5 mg/mL) was diluted 1/500 in phosphate buffer saline (PBS) (Invitrogen, Carlsbad, CA) and 100 µl of diluted protein was added to each well of a flat-bottom, 96-well, non-sterile Dynex Immulon 4 HBX microtitre plate (VWR, Bridgeport, NJ). Each plate was covered with a plate sealer and incubated overnight at 4 °C. The wells were then washed 3 times with 350 µl of in-house ELISA wash (300ml PBS Ph7.4 10x, 0.005% Tween 20 (Sigma Aldrich), 2700ml distilled water) using an automated plate washer (model ELx405 Bio-Tek Instruments). Assay buffer of a volume of 280 µl was used to block each well (2.5% BSA [Sigma-Aldrich], 2.5% normal goat serum [Equitech-Bio, Kerrville, TX], 0.005% Tween 20 [Sigma-Aldrich] and 0.005% Triton-X 100 [Sigma-Aldrich] in Dulbecco's PBS)) for 3h at 37 °C.

Plates containing assay buffer were stored at -80 °C for transport and before use. Plates were defrosted for two hours at room temperature. Plates were washed 3 times in 350 µl with in-house ELISA wash using an automated plate washer. Diluted test and control sera (final volume 100 µl at 1/100 dilution in assay buffer) were pipetted into the wells and incubated at 37 °C for 90min. In order to provide an internal control, samples from patients with KS, and samples from an American blood donor population were run in triplicate as positive and negative controls,

respectively. Two blank wells containing assay buffer were included (see plate layout below). Unbound antibodies were removed by washing five times with in-house ELISA wash using an automated plate washer and then tapped dry. The conjugate was prepared by diluting Goat anti-human IgG(g) alkaline phosphatase (ReserveAP™, KPL, Gaithersburg, MD) at 1:5000 in assay buffer and adding 100 µl to each well. Plates were covered and incubated at 37 °C for 30 min. After 5 washes in 350 µl in in-house ELISA wash, 100 µl of substrate 1 step p-nitrophenyl phosphate, disodium salt (Pierce, Rockport, IL) was added to every well to detect antigen-antibody complexes. The plates were allowed to develop at room temperature, uncovered in the dark for 30min. Absorbance was read at 405 nm using a model EL 808IU automated plate reader (Bio-Tek Instruments).

4.6.3 Assay intra-plate quality control

For the assay results to be considered valid, the mean of the two blanking wells was calculated and required to be between -0.05 and 0.05. The mean of the blank wells was subtracted in turn from each negative and positive control value. For K8.1, each negative control value needed to have an optical density value between 0 and 0.2, after subtracting the mean of the two blanking well's OD value. For ORF 73, each negative control value needed to have an optical density value between 0 and 0.1, after subtracting the mean of the blanking well OD values. For both K8.1 and ORF 73, each positive control value needed to have an optical density value greater than 1.5, after subtracting the mean of the two blanking well's OD value.

4.6.4 Calculation of cut-off

For the K8.1 ELISA the cut off was set as the mean of the negative controls plus 0.75 for each plate. For the ORF 73 assay, a cut-off was empirically set at the mean of the negative controls plus 0.35 for each plate.

4.6.5 Titres

Plasma or serum samples that were KSHV seropositive (optical density above cut-off) were identified. These samples were then selected for a titre ELISA. The assay protocol for titres was identical to those above. For K8.1, samples were titrated in 2-fold serial dilutions from 1:20 to 1:20480 across the plate. For ORF 73, samples were titrated in 2-fold serial dilutions from 1:100 to 1:102400. The titre value is the dilution before which the sample loses positive signal, when the optical density falls below the cut off. A negative sample was negative at the first dilution and was given a titre value of zero. A high titre positive sample may still be positive at the last dilution and was given a titre value of 1:20480 for K8.1 and 1:102400 for ORF 73.

4.7 Validation of assay transfer

The assays were transferred to the Ugandan Virus Research Institute (UVRI) from the Viral Oncology Section (VOS), National Cancer Institute (NCI), USA and analysis of the positive and negative controls showed comparable performance at NCI and UVRI (Table 4.1). Each plate was checked and passed assay intra-plate quality control as above. Each plate was set up with three positive controls and three negative controls and the mean of positive controls and negative controls was calculated. This resulted in 114 mean positive and 114 mean negative control results from VOS, NCI, and 48 mean positive and 48 mean negative control results from UVRI, Uganda. The number of samples tested in Uganda were limited by the number sent from VOS, NCI. Each plate tested (114 at VOS, NCI and 48 at UVRI) had a cut-off value calculated. For the ORF 73 assay, the cut-off was the mean of the negative controls plus 0.35 for each plate. For the K8.1 ELISA the cut off was the mean of the negative controls plus 0.75 for each plate. The results for the mean of the positive controls, the mean of the negative controls and the cut-offs were imported into StataSE11 and median and 95% confidence intervals calculated (Table 1). The performance of the assay at VOS, NCI and UVRI, Uganda was considered comparable.

Table 4.1: Comparison of median optical density results for positive and negative controls and plate cut-offs tested at Viral oncology Section (VOS), National Cancer Institute (NCI) and at UVRI, Uganda.

	K8.1 Positive controls		K8.1 Negative controls		K8.1 Cut-off	
	VOS	Uganda	VOS	Uganda	VOS	Uganda
Median	2.28	2.18	0.09	0.06	0.85	0.82
95% CI	1.70-	1.86-	0.06-	0.05-0.08	0.81-	0.80-
	2.82	2.83	0.15		0.86	0.83

	ORF 73 Positive controls		ORF 73 Negative controls		ORF 73 Cut-off	
	VOS	Uganda	VOS	Uganda	VOS	Uganda
Median	2.47	2.61	0.04	0.06	0.39	0.41
95% CI	2.03-	2.25-	0.02-	0.04-0.09	0.37-	0.40-
	3.09	3.14	0.07		0.42	0.44

Each positive or negative control tested in triplicate. The mean of the three positive or three negative controls used in the analysis.

Analysis based on 114 positive and 114 negative control means tested at VOS, NCI and 48 positive and 48 negative control means tested at UVRI, Uganda.

4.8 Assay quality control

For the first run of study samples at MRC Uganda, two hundred samples were repeated randomly across both K8.1 and ORF 73 plates to check for consistency of ELISA results. All 25 K8.1 ELISA plates and 25 ORF 73 ELISA plates included in the analysis passed quality control. For K8.1, the mean optical density of the negative controls was 0.04, range 0.01-0.06, SD 0.02. For ORF 73, the mean optical density of the negative controls was 0.02, range 0.01 to 0.05, SD 0.01. For K8.1, all blank optical density values were less than 0.19, and for ORF 73, all blank optical density values were less than 0.11. The mean cut-off for K8.1

(mean negative controls plus 0.75) was 0.81, 95% CI 0.81-0.82 and for ORF 73 the mean cut-off (mean negative controls plus 0.35) was 0.41, 95% CI 0.40-0.41. The Kappa score for agreement for the 200 duplicate samples for positive/negative outcome for K8.1 was 0.82 and for ORF 73 was 0.91. These scores represent almost perfect agreement^{294, 295}. After this run it was standard practice to run 10% of samples tested in duplicate. No issues were identified with quality control.

4.9 Methods for literature reviews

4.9.1 The epidemiology of Kaposi's sarcoma among individuals without overt immune suppression; methods for section 2.4.3

To aid description of the potential geographical variation in endemic and classic KS a critical literature search was undertaken of this cancers occurrence. First, data available from GLOBOCAN 2008, but the required information was not available²⁹⁶. A Google search was then carried out for online cancer registry databases available in English on the 11th June 2012. Search terms "cancer registry" or "cancer data" and each "country name" as listed by the Centers for Disease Control and Prevention, Atlanta, USA, (<http://wwwnc.cdc.gov/travel/destinations/list.htm/>) were used. Two were identified: United States Surveillance Epidemiology and End Results (SEER)⁸² and the United Kingdom's Office for National Statistics (ONS)^{83, 84}. Next, a PubMed search was made for "cancer registry" or "cancer" or "classic Kaposi's sarcoma" or "endemic Kaposi's sarcoma" or "Kaposi's sarcoma". Review articles were searched for relevant publications. Only articles published in English were considered. Due to the influence of age on crude incident rate, publications were first searched for age-standardised incidence rates in a population of a million in men, for "classic" KS or "endemic" KS. After the initial results, data was sparse and the search was widened to include age-standardised incidence rates per million population for any sub-type of KS in men diagnosed either over the age of 60 or prior to the HIV epidemic (1980). A date prior to 1984 was accepted for the Faroe Islands. The results of

this search are tabulated in Table 2 in Chapter 2 by continent and in alphabetical order. The results from Table 2 were plotted on a world map (Figure 2.2, Chapter 2)^{8, 13, 14, 16-21, 24, 25, 28, 94, 129, 178-191}.

This search has many limitations in the quality and quantity of data available in the public domain. Indeed, there was not a great amount of cancer registry data in the public domain. Although, limiting the search to publications in English may have affected this. Only two countries (Israel and Italy) had available data specifically about KS without overt immune suppression. An age cut-off of 65 or 60 years and a calendar cut-off of 1980 may not be sufficient to exclude HIV-related KS. KS, compared to other cancers tends to be easier to diagnose on visual inspection but misclassification cannot be ruled out. Information on diagnosis made by histology was not available in the majority of publications. The population at risk used in the calculation of incidence may be unreliable for census data from older time periods (1970's) and from Africa. Reports from sub-Saharan Africa all suffered from limited population coverage of cancer registration and it is difficult to ascertain how representative the cancer data is of true population risk. A numbers of studies identified from Africa only presented prevalence data, representing burden and were not included in this review.

4.9.2 Antibodies to Kaposi's sarcoma-associated herpesvirus among individuals with HIV-associated KS; methods for section 2.6.4

In order to present available evidence of the association between the presence of antibodies to KSHV and risk of HIV-associated KS in sub-Saharan Africa, a literature search was undertaken in PubMed up to an including 24th October 2012. Terms searched for were "Africa" and "Kaposi's sarcoma" or "KSHV" or "HHV-8". The tables published by the International Agency for Research On Cancer were cross-referenced. Inclusion criteria were (1) study population in sub-Saharan Africa, (2) KSHV serological technique (rather than viral load assessment), (3)

study subjects or cases were HIV-positive individual with KS, (4) comparison or control group were HIV-positive persons without KS. Studies measuring KSHV viraemia or investigating endemic (HIV-negative KS) or transplant-associated KS were excluded.

Thirteen studies of antibodies to KSHV and HIV-associated KS from sub-Saharan Africa were identified. Five studies were excluded as they measured KSHV DNA by a polymerase chain reaction technique^{122, 297-300}. One study was excluded as it investigated endemic KS³⁰¹. One was excluded as the comparison group was described as a "general population" and HIV status was not given³⁰².

Studies investigating the presence of KSHV antibodies and KS in sub-Saharan Africa are tabulated in Table 2.3, Chapter 2 in order of publication date^{16, 127-131}. All studies were cross-sectional or case-control in design. Only one study included children¹⁶. In all studies the prevalence of antibodies was higher in individuals with HIV-associated KS than in HIV-positive individuals without KS. Two studies estimated the risk of HIV-associated KS by increasing fluorescent intensity signal to KSHV antigens^{128, 131}; in one the comparison group included both HIV positive and HIV negative individuals¹³¹. The majority of studies (five of six) had small study size, particularly in respect to the number of samples from HIV-positive individuals with KS. Five of six studies solely used an IFA technique to estimate antibodies to KSHV, which is potential prone to poor sensitivity and specificity. No study reported repeat testing of samples or using a range of cut-off for definition of KSHV seropositivity. No study presented formal titres to KSHV antigens. Information of the whether HIV-associated KS was diagnosed clinically and/or confirmed histologically was limited in all studies. No study presented results by age; given that KSHV seroprevalence in sub-Saharan Africa increases with age this may be important.

4.9.3 Antibodies to Kaposi's sarcoma-associated herpesvirus predating the development of HIV-associated Kaposi's sarcoma; methods for section 2.6.4

A PubMed search was carried out for longitudinal studies using KSHV antibody titre to determine risk of HIV-associated KS prior to diagnosis. Due to a prior knowledge of the literature the search included studies conducted worldwide and was not restricted to Africa. The search included all listed studies on PubMed up to and including the 12th October 2012. Terms searched for in PubMed were "Kaposi's sarcoma" or "KSHV" or "HHV-8". Tables published by IARC were cross-referenced¹²⁶. Inclusion criteria included (1) presenting data on prevalence or titre of antibodies to KSHV prior to a diagnosis of KS, and (2) control of comparison group that were HIV-positive and without KS. Studies investigating KS among transplant recipients or HIV-negative KS (endemic or classic) and studies of MCD and primary effusion lymphoma were excluded.

Twenty-eight studies were identified. Studies were excluded on the basis of investigating KSHV viraemia (3)^{113, 114, 118}, KS among transplant recipients (9)^{106, 303-310} and individuals with MCD or primary effusion lymphoma (5)³¹¹⁻³¹⁵. Eleven studies were included investigating the presence of antibodies to KSHV and risk of KS prior to KS diagnosis, and are tabulated in Table 2.4, Chapter 2 in order of year of publication^{33, 115-117, 127, 132-137}. Four studies were case-control in design and the remaining seven were cohort studies. No studies were found that included children.

Only one study had more than 100 cases of HIV-associated KS, the remainder had relative small sample sizes¹¹⁷. Three of eleven studies used only an IFA assay, which may be prone to false positives and false negatives^{115, 134, 136}. No longitudinal studies were identified for HIV-associated KS in sub-Saharan Africa or any country considered endemic for KSHV infection. This may be important if repeated exposure to the virus affects KSHV viral reactivation and antibody titre. Five of the eleven studies did not adjust for any potential confounders^{33, 116, 132, 133, 137}; age

and sexual risk factors may be important. Only two of eleven studies reported a quantitative analysis of risk of KS^{33, 117}; one study by titre and one by fluorescent intensity to KSHV antigens.

4.9.4 Worldwide seroprevalence of Kaposi's sarcoma-associated herpesvirus, methods for section 3.3

To aid description of worldwide patterns of KSHV seroprevalence a review of published literature was carried out. The critical review had two phases: first a literature search of studies conducted by the VOS, NCI was conducted. This restriction was placed to limit issues with directly comparing seroprevalence results between IFA and ELISA and between assays from different laboratories. Next, countries without a VOS, NCI study were added.

PubMed was searched up to and including 31st July 2012. The search terms used were "KSHV" or "HHV8" each "country name" as listed by the Centers for Disease Control and Protection, Atlanta, USA. (<http://wwwnc.cdc.gov/travel/destinations/list.htm/>). Articles displayed in PubMed were reviewed and links to related articles and reference lists were checked. All published literature investigating KSHV seroprevalence were eligible for identification and screening. The first screening process consisted of removing duplicates and selecting studies published by VOS, NCI. Once a list of studies by VOS, NCI was compiled the all identified publications were re-screened to identify KSHV seroprevalence data in countries without a VOS, NCI study. If more than one publication available the most recent in terms of publication date was included. Inclusion criteria were; (1) KSHV seroprevalence data estimated using ELISA, Western Blot or IFA, and (2) report had to state that the populations tested were without KS, "healthy", "blood donors" and/or HIV negative. These limits were chosen to limit the potential impact of KS, HIV and sexual risk group on KSHV seroprevalence. Due to the potential increased risk of KSHV seropositivity and KS in HIV infected individuals in sub-Saharan Africa, studies for this region had to present data on laboratory defined HIV negative individuals. The major exclusion

criteria were (1) reviews, tutorials, letters, and editorials; (2) duplicate data; and (3) publications that were not written in English.

Twenty-eight publications were identified (Table 3.1, Chapter 3) containing results from 36 countries^{8, 13, 14, 16-21, 24, 25, 28, 94, 129, 178-191}. Two studies were included for Brazil as KSHV infection rates may significantly differ between Amerindian communities and other Brazilian populations^{94, 182, 251}. Study populations were varied and methodological details of selection and enrolment of study participants were largely absent. Five of the studies had a small sample size with 100 or fewer participants. These studies tended to have been published prior to the year 2000 and used an IFA tool. No general population studies that fitted criteria for this search could be identified for Ireland, Canada, Russia, Pakistan, Indonesia, Australia, New Zealand or any country in Central Asia. The only country in Western Asia with KSHV seroprevalence data was Israel.

4.9.5 Kaposi's sarcoma-associated herpesvirus sero-epidemiology in Africa, methods for section 3.4

To allow a description of potential regional variation in KSHV infection across Africa a comprehensive literature search of studies presenting KSHV seroprevalence in HIV negative adults without a diagnosis of KS was conducted (Table 3.2, Figure 3.2, Chapter 3)^{8, 13-17, 19, 21, 24, 25, 127, 129, 131, 183, 187, 190, 191, 221-230}. The search was conducted in PubMed using "KSHV" or "HHV8" and "Africa" and Google Scholar using "KSHV" and "Africa" up to and including 6th July 2012. Links were then followed to related articles and reference lists in identified articles and reviews were also checked for KSHV seroprevalence data. To be included; (1) the study had to use a IFA, ELISA or Western blot assay for KSHV serology, (2) test and state that the population was HIV negative, and (3) be conducted among individuals described as adults, "childbearing" or over the age of 12 years. Only articles published in English were assessed for inclusion and conference proceedings, abstracts, theses, dissertations or national or local vital statistics data not published as peer reviewed articles were not included.

All studies were cross-sectional. Study design was variable. Selection of study populations and limited reporting of methods makes assessment of comparability between studies difficult. The majority used the antibody results of more than one KSHV antigen to define KSHV seropositivity. As discussed above comparison of assay results between laboratories may be problematic. Studies conducted prior to 2000 (Table 3.2, Chapter 3) had small samples size (≤ 100) and used an IFA, which may be prone to false positives and false negatives. Age ranges in the studies were wide and may have confounded the KSHV seroprevalence results. Studies have been conducted in relatively few countries and prevalence is unknown for most countries.

The use of different serological assays and testing populations of different age, HIV and KS status has made accurate mapping of potential variation of KSHV occurrence problematic. Previous descriptions of KSHV seroprevalence in Africa have not taken these factors into account^{5, 227}. To eliminate issues that may cause variation in KSHV seropositivity only studies conducted using an assay originating from VOS, NCI among HIV negative women were selected (Table 3.3, Chapter 3). KSHV seropositivity results from women were selected, as studies of men were sparse. Four of the eight studies only recruited mothers or pregnant women^{8, 13, 25, 191}. Results from HIV positive individuals were excluded due to the association between HIV and KSHV in some studies (Table 3, Chapter 3, included odds ratio of association between antibodies to KSHV and women HIV infection compared to HIV negative).

4.9.6 Kaposi's sarcoma-associated herpesvirus seroprevalence in Uganda; methods for section 3.5

Reports of KSHV seropositivity among HIV negative adult populations in Uganda vary widely between 35 and 86%^{8, 19, 127, 129, 190, 221, 222, 224, 225, 228}. To investigate potential reasons for these wide-ranging results, studies conducted in Uganda were tabulated (Table 3.4, Chapter 3)^{8, 19, 127, 129, 190, 221, 222, 224, 225, 228}. Eleven studies of KSHV seroprevalence in HIV negative

adults without KS conducted in Uganda were identified (Table 4, Chapter 3) from the search in the section above. Figure 3.4, Chapter 3 pictorially represents KSHV seroprevalence of the seven studies shown in Table 3.4, which state the region, district or town of the population the sample was taken from were resident.

Only one identified study²²² reports an analysis of samples from defined geographical regions across Uganda and these found moderate regional KSHV variation. This study utilized archival plasma samples from the Uganda HIV-AIDS sero-behavioural survey (UHSBS) conducted in 2004–2005. The UHSBS is reported to be a nationally representative population-based sample of people in Uganda. It has important potential limitations in that the sample size from each district was small and no clinical information on KS was collected. There is marked variation in KSHV seroprevalence in the Ugandan capital Kampala. Each of the four studies based in Kampala used a different assay and definition of KSHV seropositivity. Two studies^{222, 224} were based on a relatively small sample size. Two of the four studies conducted in Kampala use samples from the blood bank^{221, 222} and one used samples collected at the main hospital²²⁵. Kampala hosts the only tertiary referral Unit in Uganda and hospital and blood bank based studies in Kampala likely contain samples from individuals resident across the country. Neither source is likely to represent the general population.

Sample size is important for precision of an estimate. To pictorially represent this, KSHV seroprevalence was plotted against decade in which the samples were collected with dot size proportional to the study sample size (Figure 4.5 below). It can be more clearly seen in that the older studies had low sample sizes and low power.

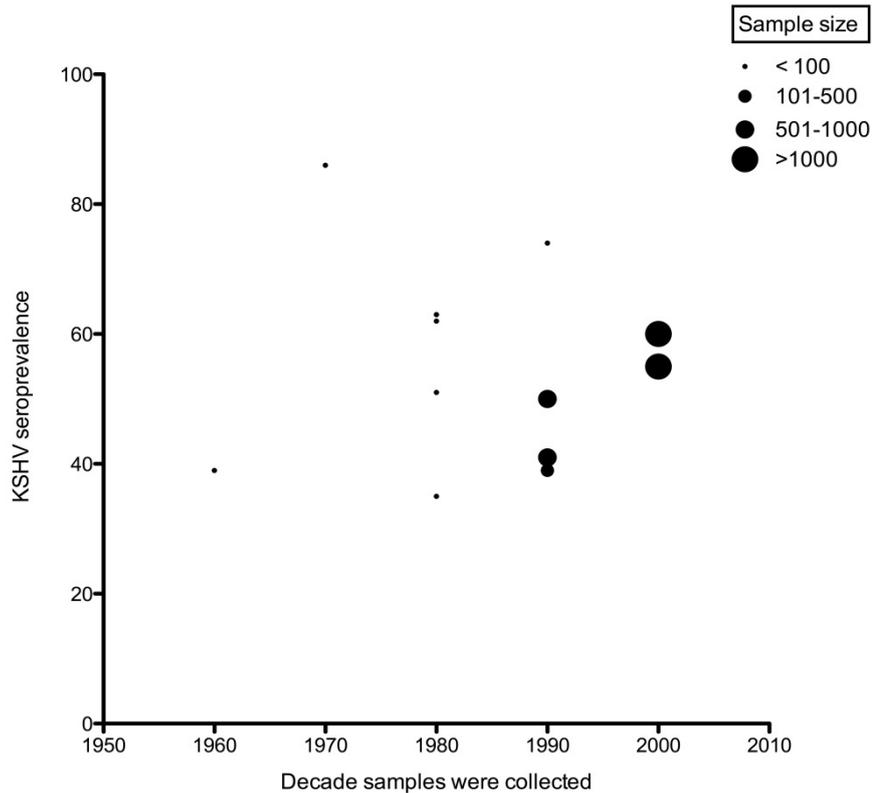


Figure 4.5: KSHV seroprevalence in HIV negative adults without KS in eleven studies identified through a comprehensive PubMed search. KSHV seroprevalence results from each study were plotted against the decade the samples were collected. The size of dot for each study represents the study sample size. Studies included in this figure are tabulated in Table 3.4, Chapter 3.

For the study conducted by Biryahwaho et al²²² KSHV seroprevalence for all regions combined was plotted. In addition to sample size, the decade the sample was collected may effect KSHV seroprevalence in a number of ways: (1) all serological assays were conducted between 1996 and 2011, samples collected in the 1960's and 1970's may have undergone a number of freeze thaw cycles which can degrade protein and hence antibody levels, (2) first generation KSHV immunofluorescence assays (IFA) tend to have issues with reproducibility and cross reactivity with other herpesviruses^{171, 175, 292}, they are labor intensive and so prevent high through-put of samples and (3) the AIDS epidemic commonly taken to have commenced in the 1980's may have effected KSHV seroprevalence in some way (cohort effect). In terms of the most valid estimate of KSHV seroprevalence in Uganda, the two most recent studies which both use two ELISAs (one to a lytic protein and one to a latent

protein) and have study sizes over 1500 are probably the most reliable estimates.

4.10 Ethics

Approval was given by the Science and Ethics Committee, Uganda Virus Research Institute; the Uganda National Council for Science & Technology; the London School of Hygiene & Tropical Medicine and the University of York.

Chapter 5: Risk factors for Kaposi's sarcoma associated herpesvirus seropositivity in mother-child pairs in Uganda

5.1 Chapter abstract

Background: Studies from Uganda report high Kaposi's sarcoma associated herpesvirus (KSHV) seroprevalence. In sub-Saharan Africa, primary KSHV infection often occurs in childhood. Transmission from mothers during childhood is likely a predominant route. This study investigates common infectious agents including HIV and parasites as potential co-factors for KSHV transmission.

Methods: We measured antibodies against KSHV, by ELISA, in 1823 paired plasma samples from pregnant women and their subsequent children in Uganda. We examined the association KSHV antibody positivity and exposures including between HIV, current parasite infections, and sociodemographic markers. and antibodies against KSHV, measured by ELISA.

Results: KSHV seroprevalence was 61 % in mothers and 11 % among their children. The risk of KSHV seropositivity was marginally higher in children of KSHV seropositive mothers compared with those of KSHV-seronegative mothers (OR 1.4, 95% CI 1.1-2.0, $p=0.04$). In both mothers and children seroprevalence of KSHV was higher among individuals with malaria parasitaemia compared to those without (mothers; OR 1.9, 95% CI 1.4-2.7, $p<0.0001$ and children; OR 4.1, 95% CI 2.4-7.0, $p<0.0001$). Children of HIV infected mothers had a higher prevalence of antibodies to KSHV than children of HIV negative mothers ($p=0.03$). This was so whether or not the child was HIV infected. These effects were not explained by age or socioeconomic status.

Conclusion: HIV infection was associated with a higher KSHV seroprevalence among children. Malaria in both pregnant women and their children is associated with the presence of antibodies against KSHV, perhaps mediated via effects on immune function.

5.2 Background

Studies from sub-Saharan Africa report high Kaposi's sarcoma associated-herpesvirus (KSHV) seroprevalence. KSHV seroprevalence exhibits a marked worldwide variation²⁰. Risk factors for seropositivity appear to vary depending upon the population^{192, 193, 233, 256}. This may reflect regional differences in modes of transmission, or suggest local co-factors for KSHV reactivation. Studies suggest that multiple characteristics and exposures in a population are important²²².

In sub-Saharan Africa, primary KSHV infection often occurs in childhood and KSHV seroprevalence increases with age²⁵. KSHV infection among newborns is uncommon, suggesting vertical transmission is unlikely^{22, 23}. There is evidence that transmission occurs via saliva^{27, 262} and in sub-Saharan Africa transmission during childhood, from mothers and siblings, is likely the predominant route^{10, 25}. A key component of this study was to investigate potential associations between KSHV serostatus and common infectious agents in a typical community in Uganda. The influence of HIV on KSHV transmission in sub-Saharan Africa is unclear. Among mothers and pregnant women in sub-Saharan Africa, HIV seropositivity is associated with a small increase in KSHV seroprevalence^{8, 13, 22, 25}. Studies of the impact of HIV infection on KSHV seroprevalence in children reveal conflicting results, with HIV having a protective²⁵⁶, null²², or positive effect^{7, 13, 25}. Parasites are potential co-factors for KSHV infection and disease because they modulate the immune system³¹⁶ and are endemic in regions where high seroprevalence of KSHV is also found^{317, 318}. While ecological associations between malaria and KSHV or KS have been reported in Italy, studies in Africa are inconclusive⁴⁰. One study from sub-Saharan Africa has attempted to measure helminth burden among cases with KS and controls; KS patients had a higher carriage of certain intestinal helminths than controls³¹⁹. Studies of KSHV in Africa have identified risk factors for KS that might be common to being at risk of certain parasites, such as exposure to surface water, high rainfall and walking barefoot^{40, 41, 301, 320}. The depth of data collected through the Entebbe Mother and Baby Study (EMaBS) allows

other key risk factors identified in the literature to also be investigated. These include the following for mothers: markers of socioeconomic status and sexual risk. And in children in markers of socioeconomic status and maternal KSHV serostatus.

5.3 Aims of chapter

This chapter describes the association between antibodies against KSHV in pregnant women in Uganda and their children with common local exposures. The primary aims are to:

- (1) Investigate the association between common infectious agents including HIV and parasites with antibodies to KSHV in mothers and their children
- (2) To investigate maternal socioeconomic factors associated with maternal and childhood KSHV seropositivity

5.4 Methods

The investigation was conducted within an existing study in Uganda - the Entebbe Mother and Baby Study (EMaBS) - a large double blind randomised placebo controlled trial designed to determine the impact of helminth infections and their treatment on vaccine responses and infectious disease outcomes³⁵. Detailed information about the study design has been reported in the general methods chapter. The mothers' enrolment plasma sample was selected for testing. Of the samples collected from children at each annual visit, the sample from the oldest available age was selected. KSHV serologic testing was based on ELISA for recombinant proteins to K8.1, a KSHV structural glycoprotein expressed during lytic infection, and for ORF 73, a nuclear antigen expressed during latency^{13, 175}. Details of the assay protocol and the quality assurance from the assay transfer and set-up are given in the general methods chapter. Randomised lists of samples were created using Stata11SE (StataCorp LP, College Station, Texas, USA). The ELISAs were performed at the Uganda Virus Research Institute (UVRI) by the PhD student and two technicians, all of whom were blinded to patient

clinical and sociodemographic details at the time of testing. Each plate was checked for internal validity as described in the general methods section.

5.4.1 Statistical analysis

Data were analysed using Stata11SE (StataCorp LP, College Station, Texas, USA). Since individual responses to KSHV antigens are complex and no gold standard assay exists, to simplify the presentation, the results for K8.1 and ORF 73 were combined to define evidence of KSHV infection as “positive” if either of the two assays were positive and “negative” if both assays were negative. The agreement between K8.1 and ORF 73 ELISA assays was assessed by calculating Kappa statistic values. The interpretation of the Kappa values was set as follows: less than zero as indicating no agreement and zero to 0.20 as slight, 0.21-0.40 as fair, 0.41-0.60 as moderate, 0.61-0.80 as substantial, and 0.81-1 as almost perfect agreement^{294, 295}. Twenty sets of twins were excluded from all analyses.

5.4.2 Analysis of risk factors for maternal KSHV seropositivity

A composite variable for socioeconomic status was derived based on home building materials, number of rooms and items collectively owned at the mothers home²⁷⁶. Anaemia was categorised in accordance to WHO criteria into three groups: normal (haemoglobin ≥ 11.3 g/dl), mild anaemia (haemoglobin 9.3-11.2 g/dl) and moderate anaemia (haemoglobin ≤ 9.2 g/dl)³²¹. CD4 counts in HIV seropositive women were categorised into three groups: ≤ 200 cells/ μ L, 201-499 cells/ μ L and ≥ 500 cells/ μ L. A high eosinophil count was defined as above the normal range cut-off value $\geq 0.45 \times 10^9$ /L. For parasite intensities, hookworm was measured by egg counts in stool and categorised as negative, light (limit of detection to $< 1,000$ egg per gram [epg] of stool), moderate (1,000 to 3,999 epg) and heavy infection ($\geq 4,000$ epg)^{35, 322, 323}. The intensity of malaria infection was categorised as being uninfected (negative), below

("low") or above ("high") the median parasite count (of positive slides), per 200 white blood cells. For microfilariae, tertiles of the number of filaria per millilitre of blood were used to categorise into low, medium and high infection intensity.

Risk factors considered for maternal KSHV seropositivity were (a) background and sociodemographic variables: age, marital status, maternal education, occupation, household socioeconomic status, number of people living in the home and number of times pregnant including current pregnancy, (b) laboratory factors: anaemia, (c) HIV, sexual and non-sexual risk factors for HIV: HIV serostatus, active syphilis infection, reporting having ever had a sexually transmitted disease, having ever had a partner known to be HIV positive, having ever received a blood transfusion and CD4 count in HIV infected women, (d) parasite infections and risk factors for malaria and helminth infections: walking distance to Lake Victoria, type of toilet, use of mosquito nets in the home, spraying home for mosquitoes, water collection source (lake, well, bore hole, stand pipe, tap), walking barefoot, wading, swimming, fishing or washing in Lake Victoria, having ever taken worm medication and received treatment for malaria in this pregnancy and eosinophil count. The outcome of interest was KSHV serostatus of the mother.

5.4.3 Analysis of risk factors for KSHV seropositivity in children

Children were categorised into three HIV status groups: HIV unexposed, HIV exposed to maternal HIV but not infected and HIV infected. As the prevalence of helminth infections in children was low, a variable for current infection with any helminth was created indicating infection with *Trichostrongylus* sp., *A. lumbricoides*, hookworm, *M. perstans*, *S. mansoni*, *S. stercoralis* or *T. trichiura*. The intensity of malaria infection was divided at the median value and categorised into three groups: uninfected (negative), below ("low") or above ("high") the median parasite count, per 200 white blood cells.

Risk factors for childhood KSHV seropositivity considered were (a) age and sex, (b) laboratory diagnosed infections: HIV, asymptomatic malaria parasitaemia, number of previous symptomatic malaria episodes and the composite variable for helminth infection, (c) maternal and household factors: KSHV serostatus of mother, maternal age at birth of child, maternal educational attainment, marital status, household socioeconomic status and number of people or children living in the home, (d) HIV exposure, (e) risk factors for malaria and helminth infections: walking distance to Lake Victoria, type of toilet, use of mosquito nets, spraying home for mosquitoes and water collection source (lake, well, bore hole, stand pipe, tap). The outcome of interest was KSHV serostatus of the child.

Potential bias in the sample of mother-child pairs was investigated by comparing the covariate distributions of mother-child pairs included, to the distributions of those in the cohort who had no samples available for inclusion. Furthermore, mother-child pairs where the child was lost to follow-up at an early age were compared to pairs with longer child follow-up.

Separately for mothers and children, potential associations between KSHV seropositivity and each potential risk factor were estimated using the Pearson chi-squared test or Fisher's exact test, where expected numbers were small. For analysis of outcome in both mothers and children, odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using logistic regression modeling and statistical significance was assessed using the likelihood-ratio test. It is of note that, for children, the initial analysis was based on a prior hypothesis that the age of the child would be a major determinant of risk of infection. Therefore two models were generated. In model 1 all ORs were adjusted for age of child. In model 2 all risk factors that were associated with the outcome at the 5% significance level in model 1 were included in a multivariable logistic model to calculate ORs. Departure from linear trend was considered for all ordered categorical exposure variables by calculation of

a likelihood-ratio test. No adjustment was made for multiple comparisons. All p-values were 2-sided and we considered a p-value of less than or equal to 0.05 to be statistically significant.

To assess factors that potentially modify the risk of KSHV in pregnant women, interactions between HIV, helminth infections and asymptomatic malaria parasitaemia were added to the regression model. To assess factors that potentially modify the risk of a KSHV seropositive mother having a KSHV seropositive child, interactions between maternal KSHV serostatus and HIV status or asymptomatic malaria parasitaemia in the child were added to the regression model.

5.5 Results

5.5.1 Background of study participants

Plasma samples were identified for 78% (1823/2345) live-born children in the cohort. The median age of women in the study was 23 years (IQR 19-27). At enrolment to the study, most were in the third trimester of pregnancy (54%) with 46% in the second. The seroprevalence of HIV among pregnant women was 10% and the median CD4 count among those who were HIV seropositive was 551 (IQR 368-796). Active syphilis was identified in 4% of women. The highest level of educational attainment reached by the majority of women was primary (50%) with 22% stating they were not able to read. The majority of women (62%) described themselves as unemployed or housewives and 85% reported a monthly income below the World Bank poverty line of 1.25 USD per day. At the time of sampling, 30% (524/1772) of the pregnant women were parasite free, 37% had one parasite, 22% had two parasites, 9% had three parasites, 2% had four parasites and <1% had five parasites. Parasite infections among the pregnant female participants were hookworm (44%), *M. perstans* (21%), *S. mansoni* (18%), *S. stercoralis* (12%), asymptomatic *P. falciparum* parasitaemia (10%), *T. trichiura* (9%), *A. lumbricoides* (3%) and *Trichostrongylus* sp. (1%).

In children, the prevalence of asymptomatic malaria parasitaemia was 5% and HIV prevalence was 1%. Helminth infections were rare among children compared to mothers: 3% prevalence of *Trichostrongylus sp.* and 1% prevalence of *A. lumbricoides* infection, hookworm, *M. perstans*, *S. mansoni*, *S. stercoralis* and *T. trichiura* were all detected in less than 1%. The majority of children (91% [1250/1375]) had no parasite infections.

Mother-child pairs with no samples available for inclusion were more likely to be from a HIV seropositive mother, a mother with malaria and less education. Mothers of children whose last available sample was at one year were younger and more likely to be HIV seropositive, compared to mothers of children who were followed for a longer period.

5.5.2 Determination of KSHV seropositivity in mother-child pairs

In pregnant women the prevalence of antibodies to K8.1, ORF 73, both antigens and either antigen was 41%, 52%, 32% and 61% respectively. There was moderate concordance between K8.1 and ORF 73 ELISA assays in detecting KSHV seropositivity ($\kappa=0.43$). The seroprevalence of KSHV among children was 9% to K8.1, 6% to ORF 73, 4% to both antigens and 11% to either K8.1 or ORF 73. There was moderate concordance between lytic K8.1 and latent ORF 73 and assays in detecting KSHV seropositivity in children ($\kappa=0.46$).

5.5.3 Risk factors for KSHV seropositivity in pregnant women in Uganda

KSHV seroprevalence, crude and adjusted odds ratios between KSHV seropositivity, sociodemographic factors and anaemia are presented in Table 5.1. Factors significant at the 5% level and included in the multivariate model were maternal age, maternal educational attainment, household socioeconomic status, anaemia, HIV status, asymptomatic malaria parasitaemia, hookworm, *M. perstans*, use of spray in the home to kill mosquitoes, water source and having ever taken worm medication.

Table 5.1. KSHV seroprevalence, unadjusted and adjusted associations between KSHV serostatus and sociodemographic factors and anaemia in pregnant women in Uganda

Factor		KSHV seropositive ¹				
		Prevalence	Crude OR (95%CI)	p ²	Adjusted OR (95% CI) ³	p ²
Background and sociodemographic factors						
Age	14-19 years	64% (272/423)	1		1	
	20-24 years	63% (434/686)	1.0 (0.7-1.2)		1.1 (0.8-1.5)	
	25-29 years	57% (243/426)	0.7 (0.6-1.0)		1.2 (0.8-1.6)	
	30-34 years	59% (119/202)	0.8 (0.6-1.1)	0.01	1.1 (0.8-1.7)	0.5
	>=35 years	55% (47/86)	0.7 (0.4-1.1)	[trend]	0.9 (0.5-1.6)	[trend]
Marital status (1 mv⁴)	Single	59% (133/226)	1		1	
	Married	32% (472/1457)	1.1 (0.8-1.5)		1.3 (0.9-1.8)	
	Widowed	72% (8/11)	1.9 (0.5-7.2)		5.5 (1.3-24.2)	
	Separated or divorced	65% (27/41)	1.3 (0.7-2.7)	0.6	1.2 (0.5-2.7)	0.7
Education (4 mv)	None	74% (50/67)	1		1	
	Primary	64% (593/920)	0.6 (0.4-1.1)		0.6 (0.4-1.1)	
	Senior	59% (398/670)	0.5 (0.3-0.9)	<0.001	0.6 (0.3-1.1)	0.09
	Tertiary	45% (72/162)	0.3 (0.1-0.5)	[trend]	0.5 (0.2-0.9)	[trend]
Occupation (3 mv)	None/at home	63% (720/1142)	1		1	
	Nurse	50% (3/6)	0.6 (0.1-2.9)		0.8 (0.1-8.1)	
	Student	50% (16/32)	0.6 (0.3-1.2)		0.1 (0.01-0.7)	
	Unskilled	65% (67/103)	1.1 (0.7-1.7)		0.7 (0.4-1.2)	
	Skilled manual	42% (11/26)	0.4 (0.2-1.7)		1.0 (0.4-2.6)	
	Farming	63% (35/56)	1.0 (0.5-2.2)		1.1 (0.6-2.0)	
	Fishing	65% (35/56)	0.4 (0.2-0.6)		0.9 (0.4-1.8)	
	Office work/teacher	38% (26/67)	1.1 (0.7-2.0)		1.1 (0.6-1.9)	
	Bar/hotel worker	57% (128/225)	0.8 (0.6-1.0)		0.8 (0.5-1.1)	
	Business (self employed)	66% (35/53)	1.1 (0.6-2.0)	0.08	1.1 (0.6-2.1)	0.7
Household SES⁵ (62 mv)	1 (lowest)	69% (72/104)	1		1	
	2	70% (104/149)	1.0 (0.6-1.8)		0.8 (0.4-1.3)	
	3	64% (358/559)	0.8 (0.5-1.2)		0.6 (0.4-1.0)	
	4	59% (298/503)	0.6 (0.4-1.0)		0.7 (0.4-1.1)	
	5	57% (211/369)	0.6 (0.4-0.9)	<0.001	0.8 (0.5-1.3)	0.1
	6 (highest)	50% (52/103)	0.5 (0.3-0.8)	[trend]	0.3 (0.2-0.7)	[trend]
Number people living at home (2 mv)	1-2	62% (337/544)	1		1	
	3-5	62% (593/947)	1.0 (0.8-1.3)		1.1 (0.8-1.4)	
	6-8	57 (153/269)	0.8 (0.6-1.1)		0.8 (0.5-1.2)	
	>9	51% (32/63)	0.6 (0.4-1.1)	0.1	0.9 (0.5-1.8)	0.3
Number of times pregnant⁶	1	61% (290/475)	1		1	
	2-4	61% (638/1048)	1.0 (0.8-1.3)		1.0 (0.7-1.5)	
	5 or more	62% (187/300)	1.1 (0.9-1.6)	0.5	0.9 (0.5-1.7)	0.9
Laboratory factors						
Anaemia⁷	11.3>	32% (337/1066)	1		1	
	9.3-11.2	31% (171/547)	1.2 (1.0-1.5)	0.001	0.9 (0.6-1.4)	
	<9.2	39% (41/105)	2.2 (1.4-3.4)	[trend]	0.9 (0.6-1.5)	0.4

¹ Pregnant women were considered KSHV positive if they had a positive ORF 73 and/or K8.1 ELISA. Participants were considered negative if both ELISAs were negative.

² All estimated using Chi-squared test. P values for heterogeneity unless stated as P value for trend.

³ Adjusted odds ratio of KSHV seropositivity. Adjusted for: maternal age, maternal education, household socioeconomic status, malaria, hookworm, mansoniella perstans, use of mosquito spray in the home to kill mosquitoes, water source and having ever taken worm medication

⁴ mv=missing values

⁵ Socio-economic status.

⁶ Number of times pregnant including current pregnancy

⁷ Haemoglobin at time of main study enrolment

In antenatal maternal samples KSHV seroprevalence decreased with increasing maternal age, educational attainment and socioeconomic status in the crude, but not in the adjusted analysis. Antibodies to KSHV were more prevalent in women with anaemia (haemoglobin <9.2g/dl) compared to women with haemoglobin above 11.3g/dl, before but not after adjusting for relevant factors. Marital status, occupation, number of people living at home and number of times pregnant were not associated with KSHV seroprevalence.

In pregnant women HIV seropositivity was associated with a marginal increase in prevalence of antibodies to KSHV, but this association was lost in the adjusted analysis (Table 5.2). There was no association between sexual and non-sexual behavioural risk factors for HIV acquisition and KSHV seroprevalence. There was no association between antibodies to KSHV and CD4 count in HIV seropositive women.

In pregnant women current infection with asymptomatic malaria, hookworm and *M. perstans* were associated with antibodies to KSHV in the crude analysis (Table 5.2). In the adjusted model the association between asymptomatic malaria parasitaemia and KSHV seropositivity was marginally strengthened. The results for five other parasites were null. Use of mosquito spray to kill mosquitoes in the home was associated with a lower KSHV seroprevalence in the crude but not in the adjusted analysis. Having ever taken worm medication was associated with a decreased prevalence of antibodies to KSHV in pregnant women in both unadjusted and adjusted models, although in the adjusted model the confidence limits included one. Reporting water source directly from Lake Victoria had a marginal positive association with risk of maternal KSHV compared to use of water from a well bore hole, stand pipe or tap. The adjusted ORs for the presence of antibodies to KSHV in women who reported using water from Lake Victoria compared to water from another source (well, bore hole, standpipe and tap) was 1.7 (95% CI 1.0-2.7,

$p=0.04$). Other factors associated with contact with surface water (distance walk from Lake Victoria, wading, swimming, fishing or washing in the Lake) were not associated with KSHV seroprevalence. Potential surrogate markers for helminth infections (walking barefoot and type of toilet at home) were also not associated with KSHV seropositivity in pregnant women.

Table 5.2. KSHV seroprevalence, unadjusted and adjusted associations between KSHV serostatus and HIV, risk factors for HIV, parasite infections and risk factors for exposure to parasites in pregnant women in Uganda

Factor		KSHV seropositive ¹				
		Prevalence	Crude OR (95%CI)	P ²	Adjusted OR (95% CI) ³	P ²
HIV, sexual and non-sexual risk factors for HIV						
HIV status	Negative	60% (989/1638)	1		1	
	Positive	68% (126/185)	1.4 (1.0-1.9)	0.03	1.0 (0.7-1.3)	0.8
Active syphilis (3 mv⁴)	No	61% (1066/1750)	1		1	
	Yes	70% (49/70)	1.5(0.9-2.5)	0.1	1.1 (0.6-1.8)	0.8
Ever had a STI⁵? (8 mv)	No	60% (355/595)	1		1	
	Yes	62% (756/1220)	1.1 (0.9-1.3)	0.3	0.9 (0.7-1.1)	0.4
Ever had a partner who was HIV positive? (3 mv)	No	61% (1094/1789)	1		1	
	Yes	64% (20/31)	1.1 (0.6-2.4)	0.7	0.8 (0.3-2.1)	0.7
Ever had a blood transfusion? (3 mv)	No	61 (1080/1766)	1		1	
	Yes	63% (34/54)	1.1 (0.6-1.9)	0.8	1.3 (0.7-2.4)	0.4
CD4 count in HIV seropositive women (mv=35)	>500	39% (29/75)	1		1	
	201-499	26% (15/57)	0.6 (0.3-1.2)		0.2 (0.1-0.6)	
	<200	17% (3/18)	0.3 (0.1-1.2)	0.4	0.1 (0.2-1.8)	0.07
Parasite infections and risk factors for malaria and helminth infections						
Asymptomatic malaria parasitaemia (32 mv)	No	60% (967/1611)	1		1	
	Yes	73% (131/180)	1.8 (1.3-2.5)	0.001	1.9 (1.4-2.7)	<0.0001
Hookworm (7 mv)	No	56% (568/1018)	1		1	
	Yes	68% (541/798)	1.7 (1.4-2.0)	<0.001	1.2 (1.0-1.5)	0.1
Mansonella perstans (4 mv)	No	59% (846/1434)	1		1	
	Yes	69% (267/385)	1.6 (1.2-2.0)	<0.001	1.1 (0.8-1.4)	0.7
Schistosoma mansoni (7 mv)	No	61% (914/1494)	1		1	
	Yes	61% (195/322)	1.0 (0.8-1.2)	0.8	0.9 (0.7-1.2)	0.5
Strongyloides stercoralis (18 mv)	No	61% (963/1580)	1		1	
	Yes	63% (141/225)	1.1 (0.8-1.4)	0.6	0.8 (0.5-1.1)	0.1
Trichuris trichiura (7 mv)	No	61% (1003/1656)	1		1	
	Yes	66% (106/160)	1.3 (0.9-1.8)	0.2	1.0 (0.7-1.4)	0.9
Ascaris lumbricoides (7 mv)	No	61% (1079/1770)	1		1	
	Yes	65% (30/46)	1.2 (0.6-2.2)	0.6	1.0 (0.5-1.9)	0.9
Trichostrongylus species (7 mv)	No	61% (1102/1798)	1		1	
	Yes	39% (7/18)	0.4 (0.2-1.0)	0.06	0.4 (0.1-1.3)	0.1
Use mosquito spray in the home (4 mv)	No	63% (904/1438)	1		1	
	Yes	55% (209/381)	0.7(0.6-0.9)	0.005	0.8 (0.6-1.1)	0.1
Use of bed net (3 mv)	No	61% (547/904)	1		1	
	Yes	62% (566/916)	1.1 (0.9-1.3)	0.68	0.9 (0.7-1.1)	0.4
Walking barefoot (2 mv)	Yes	61% (889/1440)	1		1	
	No	59% (224/381)	1.15 (0.92-1.44)	0.2	1.3 (1.0-1.7)	0.08
Water source (5 mv)	Lake	72% (59/82)	1		1	
	Well	66% (74/112)	0.8 (0.4-1.4)		0.5 (0.3-1.0)	
	Bore hole	70% (87/124)	0.9 (0.5-1.7)		0.4 (0.2-0.8)	
	Stand pipe	61% (328/537)	0.6 (0.4-1.0)	0.01	0.4 (0.3-0.7)	0.02
	Tap	58% (563/963)	0.5 (0.3-0.9)	[trend]	0.5 (0.3-0.8)	[trend]
Minutes walk to lake (7 mv)	<30	61% (721/1176)	1		1	
	>30	61% (391/640)	1.0 (0.8-1.2)	0.9	1.0 (0.8-1.3)	0.9
Wade, swim, fish, wash in lake (1 mv)	No	61% (428/706)	1		1	
	Yes	62% (686/1116)	1.0 (0.9-1.3)	0.7	0.9 (0.7-1.1)	0.2
Type toilet at home (1 mv)	Toilet	65% (17/26)	1		1	
	Latrine	54% (68/125)	0.6 (0.3-1.5)		0.9 (0.3-2.5)	
	Neither	62% (1029/1671)	0.9 (0.4-1.9)	0.3	0.9 (0.4-2.3)	0.9
Ever taken worm medicine (187 mv)	No	68% (548/811)	1		1	
	Yes	55% (457/825)	0.6 (0.5-0.8)	<0.0001	0.8 (0.6-1.0)	0.04
Malaria treatment in this pregnancy (3 mv)	No	62% (164/263)	1		1	
	Yes	61% (950/1557)	0.9 (0.7-1.2)	0.6	1.0 (0.1-11.6)	0.9
High eosinophil count (>0.45) (23 mv)	No	62% (787/1269)	1		1	
	Yes	59% (312/531)	0.9 (0.7-1.0)	0.2	1.0 (0.8-1.2)	0.8

¹ Pregnant women were considered KSHV positive if they had a positive ORF 73 and/or K8.1 ELISA. Participants were considered negative if both ELISAs were negative.

² All estimated using Chi-squared test, except for Trichostrongylus species which was estimated using a Fishers exact test. P-values for heterogeneity unless stated as p-value for trend.

³ Adjusted odds ratio of KSHV seropositivity. Adjusted for: maternal age, maternal education, household socioeconomic status, malaria, hookworm, mansonella perstans, use of mosquito spray in the home to kill mosquitoes, water source and having ever taken worm medication

⁴ mv=missing values

⁵ STI=Sexually transmitted infection

In pregnant women the prevalence of antibodies to KSHV increased with increasing intensity of hookworm infection in the crude ($p < 0.001$ [trend]) and adjusted analysis ($p=0.03$ [trend]) as measured by egg counts in stool; from 56% in those with no infection to 67% in those with light/moderate infection (limit of detection - 3,999 eggs per gram (epg) of stool) to 72% in those with heavy infection ($\geq 4,000$ epg), although confidence limits were wide in the adjusted analysis. No consistent trends were observed for malaria parasite density or *M. perstans* intensity, but most infections were light (Table 5.3).

Table 5.3. Intensity of parasite infection and odds of antibodies to KSHV in pregnant women in Uganda

Infection	KSHV seroprevalence ¹	Crude OR (95%CI)	p ²	Adjusted OR (95% CI) ³	p ²
Hookworm (7 mv⁴)					
Uninfected	56% (568/1018)	1		1	
Light	67% (460/682)	1.6 (1.3-2.0)		1.3 (1.0-1.6)	
Moderate	67%(60/87)	1.8 (1.1-2.8)	<0.001[trend]	1.4 (0.8-2.3)	0.03 [trend]
Heavy	72% (21/29)	2.1 (0.9-4.7)		1.6 (0.6-4.2)	
Malaria parasites (89 mv)					
No	60% (967/1611)	1		1	
Low	70% (44/62)	2.1 (1.3-3.3)	0.04 [trend]	1.7 (1.0-2.8)	0.1 [trend]
High	70% (43/61)	1.7 (1.0-2.5)		1.3 (0.8-2.2)	
Mansonella perstans (4 mv)					
Uninfected	59% (846/1434)	1		1	
Light	69% (91/133)	1.5 (1.0-2.2)		1.2 (0.7-1.8)	
Moderate	74% (67/91)	2.0 (1.2-3.1)	0.001[trend]	1.6 (1.0-2.7)	0.1 [trend]
Heavy	68% (109/161)	1.5 (1.0-2.0)		1.2 (0.8-1.8)	

¹ Pregnant women were considered KSHV positive if they had a positive ORF 73 and/or K8.1 ELISA. Participants were considered negative if both ELISAs were negative.

² All estimated using Chi-squared test. Fit of categorical trend tested using likelihood-ratio test

³ Adjusted odds ratio of KSHV seropositivity. Adjusted for: maternal age, maternal education, household socioeconomic status, malaria, hookworm, *Mansonella perstans*, use of mosquito spray in the home to kill mosquitoes, water source and having ever taken worm medication

⁴ mv=missing values

5.5.4 Risk factors for Kaposi's sarcoma-associated herpesvirus seropositivity in children in Uganda

KSHV seroprevalence and associations between KSHV serostatus and factors in Ugandan children aged one to five years are shown in Table 5.4. In children there was no association between antibodies to KSHV and male or female sex. Increasing age of the child had a statistically significant association with increasing seroprevalence of KSHV from 4% in one year olds to 14% in five year olds in the adjusted analysis (model 2).

In the model adjusted for age of the child, KSHV seroprevalence was increased in children exposed to but uninfected with maternal HIV compared to children not exposed to maternal HIV. Children infected with HIV had an even greater risk of KSHV seropositivity, compared to children not exposed to maternal HIV. Adjustment for potential confounders reduced but did not negate the increased odds of KSHV seropositivity in children exposed to HIV and children infected with HIV compared to children not exposed to HIV. In a sub-group analysis restricted to HIV seropositive mothers, being a HIV seropositive child was associated with a two and a half-fold increase in being KSHV seropositive compared to being a HIV negative child on model 1 (OR 2.6, 95% CI 1.0-6.9, $p=0.05$), and in model 2 (OR 2.4, 95% CI 0.8-9.3, $p=0.09$).

In both model 1 and 2, antibodies to KSHV were associated with infection with asymptomatic malaria in children (Table 5.4). High malaria intensity was associated with antibodies to KSHV in children, compared to children with no malaria infection (Table 5.5). In Ugandan children, there was no association with number of previous episodes of malaria or with current helminth infection (Table 5.4). Children born to KSHV seropositive mothers had an increased prevalence of antibodies to KSHV; adjusting for potential confounders in model 2 marginally weakened the association. KSHV seropositivity in the child decreased with increasing maternal educational attainment, although the association lost statistical significance in the adjusted analysis (model 2). In the analysis, use of a

mosquito net in the home was associated with a decreased seroprevalence of antibodies to KSHV in children in model 1 (adjusted for age of child) but not in model 2. None of the other factors examined (Table 5.4) were associated with antibodies to KSHV in children.

Table 5.4: KSHV seroprevalence and associations between KSHV serostatus and factors for children in Uganda

Factor		KSHV seropositive ¹				
		Prevalence	OR (95%CI) ²	P ⁴	Adjusted OR (95% CI) ³	P ⁴
Gender (1 mv ⁵)	Male	11% (99/925)	1		1	
	Female	11% (101/897)	1.1 (0.8-1.4)	0.7	1.0 (0.7-1.3)	0.8
Age of child	1 year	4% (5/129)	1		1	
	2 years	7% (13/182)	1.9 (0.7-5.5)		2.1 (0.7-6.9)	
	3 years	10% (70/677)	2.9 (1.1-7.2)		3.6 (1.3-10.3)	
	4 years	13% (76/585)	3.7 (1.5-9.3)	<0.0001	5.0 (1.8-14.4)	0.0003
	5 years	14% (36/250)	4.2 (1.6-10.9)	[trend]	6.0 (2.0-17.7)	[trend]
Laboratory diagnosed infections						
HIV exposure for child (18 mv)	Not exposed	10% (169/1635)	1		1	
	Exposed but uninfected	15% (22/143)	1.5 (1.9-2.5)		1.6 (1.0-2.7)	
	Infected	29% (8/27)	4.3 (1.8-10.2)	0.004	3.1 (1.2-8.3)	0.03
Asymptomatic malaria parasitaemia (128 mv)	No	10% (164/1617)	1		1	
	Yes	30% (23/78)	3.9 (2.3-6.6)	<0.0001	4.1 (2.4-7.0)	<0.0001
Number of previous symptomatic malaria episodes	None	10% (91/899)	1		1	
	1-2	11% (65/583)	1.1 (0.8-1.5)		1.1 (0.8-1.6)	
	2-5	12% (30/242)	1.2 (0.8-1.8)	0.2	1.1 (0.7-1.8)	0.4
	6 or more	14% (14/99)	1.3 (0.7-2.5)	[trend]	1.4 (0.7-2.6)	[trend]
Current helminth infection (247 mv)	No	10% (154/1490)	1		1	
	Yes	17% (14/86)	1.6 (0.9-2.9)	0.2	1.3 (0.7-2.4)	0.5
Maternal and household factors						
Maternal KSHV seropositivity	Negative	9% (61/708)	1		1	
	Positive	12% (139/1115)	1.6 (1.1-2.2)	0.0001	1.4 (1.1-2.0)	0.04
Marital status (1)	Single	8% (19/226)	1		1	
	Married	11% (177/1544)	1.3 (0.8-2.2)		1.2 (0.7-2.0)	
	Widowed	9% (1/11)	1.0 (0.1-8.2)		1.0 (0.1-9.5)	
	Divorced or Separated	7% (3/41)	0.8 (0.2-2.7)	0.8	0.5 (0.1-2.1)	0.8
Maternal education (4 mv)	None	16% (11/67)	1		1	
	Primary	12% (110/920)	0.6 (0.3-1.2)		0.6 (0.3-1.1)	
	Senior	10% (68/670)	0.5 (0.3-1.0)	0.006	0.5 (0.2-1.0)	0.08
	Tertiary	7% (11/162)	0.3 (0.1-0.8)	[trend]	0.3 (0.1-0.8)	[trend]
Number of people who live at home (2 mv)	0 to 2	10% (53/544)	1		1	
	3 to 5	11% (107/947)	1.1 (0.8-1.6)		1.1 (0.8-1.7)	
	6 to 8	13% (34/269)	1.3 (0.8-2.1)		1.4 (0.9-2.3)	
	more than 9	10% (6/63)	0.9 (0.4-2.4)	0.5	1.1 (0.5-2.8)	0.3
Number of children at home (2 mv)	0	11% (14/123)	1		1	
	1	11% (91/815)	1.0 (0.5-1.8)		1.2 (0.5-2.0)	
	2	12% (24/196)	1.0 (0.5-2.1)		1.0 (0.5-2.0)	
	3	20% (4/20)	2.1 (0.6-7.2)	0.5	2.2 (0.6-8.1)	0.8
Maternal age group	14-19	9% (36/423)	1		1	
	20-24	12% (83/686)	1.4(0.9-2.1)		1.2 (0.8-1.9)	
	25-29	12% (51/426)	1.4 (0.9-2.2)		1.2 (0.8-2.0)	
	30-32	9% (18/202)	1.0 (0.6-1.8)	0.5	0.9 (0.5-1.7)	0.7
	35+	14% (12/86)	1.6 (0.8-3.2)	[trend]	1.5 (0.7-3.2)	[trend]
Household SES (36 mv)	1 (lowest)	11% (11/104)	1		1	
	2	15% (22/149)	1.6 (0.7-3.4)		1.9 (0.8-4.2)	
	3	11% (64/559)	1.1 (0.6-2.3)		1.3 (0.6-2.6)	
	4	10% (52/505)	1.0 (0.5-2.1)		1.2 (0.6-2.4)	
	5	10% (37/369)	1.0 (0.5-2.0)	0.3	1.1 (0.5-2.3)	0.4
	6 (highest)	11% (11/103)	1.1 (0.4-2.6)	[trend]	1.2 (0.5-3.0)	[trend]
Risk factors for parasite infections						
Time to walk to the lake (7 mv)	<30 minutes	11% (133/1176)	1		1	
	>30 minutes	10% (66/640)	0.9 (0.7-1.3)	0.7	1.0 (0.7-1.4)	0.8
Type of toilet at home (1 mv)	Neither	11% (14/125)	1		1	
	Latrine	11% (184/1671)	1.5 (0.3-7.1)		1.4 (0.3-7.0)	
	Toilet	8% (2/26)	1.5 (0.4-6.5)	0.8	1.4 (0.3-6.3)	0.8
Use of mosquito net in home (3 mv)	No	12% (112/904)	1		1	
	Yes	10% (87/916)	0.7 (0.6-1.0)	0.04	0.8 (0.5-1.1)	0.3
Home sprayed for mosquitoes (4)	No	11% (152/1438)	1		1	
	Yes	12% (47/381)	1.1 (0.8-1.5)	0.8	1.1 (0.7-1.6)	0.7
Water collection source (5 mv)	Lake	17% (14/82)	1		1	
	Well	15% (17/112)	0.9 (0.4-1.9)		0.9 (0.4-2.1)	
	Bore hole	12% (15/124)	0.7 (0.3-1.6)		0.6 (0.3-1.4)	
	Stand pipe	10% (51/537)	0.5 (0.3-1.0)		0.5 (0.3-1.0)	
	Tap	11% (102/963)	0.6 (0.3-1.1)	0.2	0.7 (0.3-1.2)	0.2

¹ Children were considered KSHV positive if they had a positive ORF 73 and/or K8.1 ELISA. Participants were considered negative if both ELISAs were negative.

²All adjusted for age of child (model 1)

³ Adjusted odds ratio of KSHV seropositivity. Adjusted for: age of child, malaria, HIV exposure, maternal KSHV serostatus, maternal education and use of mosquito net in the home (model 2)

⁴ All estimated using Chi-squared test. P values for heterogeneity unless stated as p-value for trend.

⁵ mv=missing values

Table 5.5: The association of malaria intensity and KSHV seropositivity in Ugandan children aged one to five years

Malaria intensity	KSHV seroprevalence¹	OR² (95%CI)	P⁴	Adjusted OR (95% CI)³	P⁴
No malaria	10% (164/1617)	1		1	
Low	14% (22/156)	1.4 (0.9-2.3)		1.4 (0.9-2.3)	
High	28% (14/50)	3.7 (2.0-7.1)	<0.0001	4.0 (2.0-7.7)	<0.0001

¹ Children were considered KSHV positive if they had a positive ORF 73 and/or K8.1 ELISA. Participants were considered negative if both ELISAs were negative

²All adjusted for age of child (model 1)

³ Adjusted odds ratio of KSHV seropositivity. Adjusted for: age of child, malaria, HIV exposure, maternal KSHV serostatus, maternal education and use of mosquito net in the home (model 2)

⁴ All estimated using Chi-squared test. P values for trend

Table 6 shows the relationship between KSHV serostatus in mothers and children by child's age. The point estimate for odds of infection in children peaked three-fold at two years, but power was small. At three years of age, KSHV seropositivity in children of KSHV seropositive versus KSHV seronegative mothers was two-fold higher and statistically significant. If a difference in the association between mother and child KSHV status by age exists, it is not strong (p value for interaction=0.7).

Table 5.6: Percentage of KSHV seropositive children by mothers' KSHV serostatus

		KSHV seropositive children by age of child¹					
Mothers KSHV serostatus¹		1 year	2 years	3 years	4 years	5 years	1-5 years
Seronegative		2% (1/43)	3% (2/60)	7% (19/267)	10% (23/224)	14% (16/114)	9% (61/708)
Seropositive		5% (4/86)	9% (11/122)	12% (51/410)	15% (53/410)	15% (20/136)	12% (139/1115)
OR (95% CI)²		2.0 (0.2-19.0)	2.9 (0.6-13.4)	1.9 (1.1-3.2)	1.5 (0.9-2.5)	1.1 (0.5-2.1)	1.6 (1.1-2.2)
P value³		0.5	0.2	0.02	0.1	0.8	0.0001

¹KSHV seropositive to either K8.1 and/or ORF73

² Odds ratios for seropositivity in children of seropositive Vs seronegative mothers: All adjusted for age of child (model 1)

³P value for association of exposure with outcome of KSHV seropositivity. All estimated using Chi-squared test.

5.5.5 Associations between parasites and socioeconomic status

For pregnant women, associations between household socioeconomic status and eight parasite infections and HIV serostatus were examined. Higher household socioeconomic status was associated with a reduced prevalence of infection with hookworm and *M. perstans*. No association with socioeconomic status was seen for HIV, malaria parasitaemia or any of the five other parasites examined. The association between hookworm and antibodies to KSHV was observed when each household socioeconomic stratum was examined separately. For *M. perstans*, the association between infection and antibodies to KSHV also held when each household socioeconomic stratum was examined in turn (Figure 5.1). Among children, household SES was not associated with any of the infectious exposures examined (malaria, helminth or HIV infection).

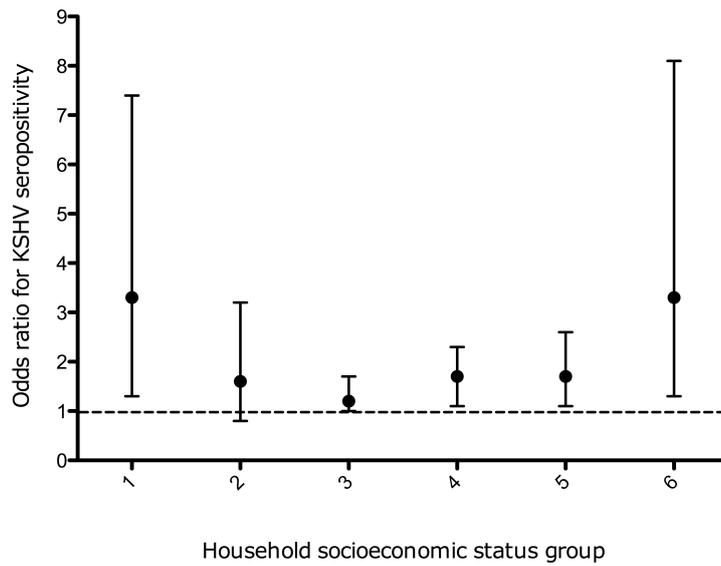


Figure 1a.
Odds ratio and 95% confidence limits for KSHV seropositivity among women with hookworm compared to women without in each household socioeconomic category

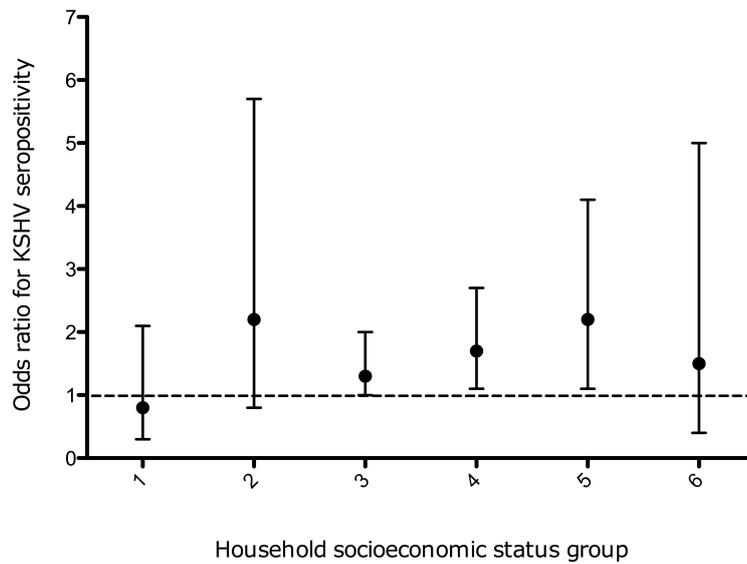


Figure 1b.
Odds ratio and 95% confidence limits for KSHV seropositivity among women with *Mansonella perstans* compared to women without in each household socioeconomic category

5.5.6 Associations between infectious agents and reported risk of parasite exposure

For mothers, all parasite infections were examined for associations with reported risk factors for parasite exposure. The following significant associations between the factor and parasite (outcome) were identified: use of mosquito net (OR 1.5, 95% CI 1.1-2.1, $p=0.008$) was associated with increased odds of asymptomatic malaria parasitaemia; wading, swimming, washing or fishing in Lake Victoria was associated with increased odds of *S. mansoni* (OR 3.3, 2.4-4.6, $p=0.0001$) compared to pregnant women who never or only sometimes swam, fished, waded or washed in the lake; having ever taken worm medication was associated with a decreased odds of hookworm (OR 0.4, 95% CI 0.4-0.5, $p>0.0001$), *T. trichiura* (OR 0.7, 95% CI 0.5-0.9, $p=0.02$), *S. stercoralis* (OR 0.6, 95% CI 0.4-0.8, $p=0.001$) and *M. perstans* (OR 0.6, 95% CI 0.5-0.8, $p<0.0001$); use of Lake Victoria as the main water source was associated with infection with *S. mansoni* (OR 2.6, 95% CI 1.6-4.2, $p<0.0001$), hookworm (OR 2.0, 95% CI 1.2-3.0, $p=0.005$), *A. lumbricoides* (OR 4.2, 95% CI 1.8-9.7, $p=0.001$) and *T. trichiura* (OR 0.3, 95% CI 1.3-4.2, $p=0.007$). In pregnant women there was a borderline association between asymptomatic malaria parasitaemia and HIV serostatus (OR 1.7, 95% CI 1.0-2.6, $p=0.03$). All associations remained significant with no material difference after adjustment for household socioeconomic status. Among children, malaria and current helminth infection were examined for associations. No associations were found.

5.5.7 Effect modification

No modifiers of the relationship between KSHV serostatus of the mother and child were detected. In pregnant women no interaction was found between HIV, malaria parasitaemia, hookworm, *M. perstans* and KSHV infection.

5.6 Discussion

The findings reported here provide evidence of an association between antibodies to KSHV and asymptomatic malaria parasitaemia in pregnant women in Uganda. Borderline associations in pregnant women between KSHV seroprevalence and using water directly from Lake Victoria as well as having ever taken worm medication were also identified; but for both these variables the confidence limits included one. The risk of KSHV seropositivity was marginally higher in children of KSHV seropositive mothers compared with those of KSHV-seronegative mothers. Among children detection of antibodies to KSHV was higher among individuals with malaria parasitaemia compared to those without. In children, HIV exposure and infection status was associated with antibodies to KSHV compared to children unexposed and not infected with HIV. Increasing age in children was confirmed as a significant risk factor for antibodies to KSHV.

5.6.1 Kaposi's sarcoma-associated herpesvirus seropositivity in mother-child pairs

KSHV seroprevalence in both mothers and their children in this study is consistent with published reports from sub-Saharan Africa^{8-10, 13, 22, 25, 27, 258-260}. The moderate concordance between latent K8.1 and ORF 73 assays in detecting KSHV seropositivity was consistent with previous studies^{13, 324}.

In pregnant women, the prevalence of antibodies to KSHV did not change significantly with age in the adjusted analysis, but the age range of study participants was relatively narrow. Previous studies of women in a similar age range in Africa have shown little or no association with age^{13, 25, 259, 324}. In sub-Saharan Africa, KSHV is endemic and prevalence of infection in children increases with age, suggesting non-sexual horizontal transmission¹⁴. The seroprevalence of KSHV in this study increased from 4% in children aged one year to 15% in five-year-old children. The mode of KSHV transmission is not fully understood. There is little

evidence for vertical KSHV transmission^{22, 23}. Antibodies to KSHV that are detected in infants less than one year tend to decrease in prevalence, possibly indicating decay in maternal antibodies acquired in-utero^{22, 23, 25}. Transmission of KSHV via breast milk has been studied. One study identified the KSHV genome in about 30% of 43 breast milk samples from mothers with high titres of antibodies against a lytic KSHV antigen²⁵. In contrast, in a longitudinal study of KSHV seropositive women in Zambia, breast milk samples did not contain KSHV DNA in any sample collected in the first six months after delivery²³. Identifying antibodies to KSHV in infants (one to twelve months) during the period they are most likely to be breastfed is uncommon. If transmission of KSHV via breast milk occurs, then it is likely rare. The majority of evidence suggests that KSHV is transmitted through saliva^{27, 262}.

Differences in the prevalence of antibodies to KSHV in men and women have been subject to some interest as endemic KS (or KS in HIV-negative individuals) is a cancer predominantly in men. In Uganda children aged one to five years were investigated in this study; there was no association between antibodies to KSHV and the sex of the child. This is consistent with other studies from this region^{8, 22, 25} and suggests that infection with KSHV per se does not account for the male predominance of endemic KS.

5.6.2 Maternal Kaposi's sarcoma-associated herpesvirus serostatus as a risk factor for antibodies to Kaposi's sarcoma-associated herpesvirus among children

KSHV infection status of mothers was positively associated with evidence of infection risk in children consistent with other studies from sub-Saharan Africa,^{13, 25}. Adjustment for potential confounders reduced the strength of association but it remained statistically significant. The analysis of odds of childhood KSHV seropositivity by maternal KSHV serostatus and child's age may suggest that the younger children are at higher risk of acquiring KSHV infection from their mothers than older children. Unfortunately the power in this analysis was too low to draw

firm conclusions, but one might expect that younger children have closer contact with their mothers than older children and are thus more likely to be exposed to maternal KSHV. In older children KSHV infection from other sources may dilute the effect of the mothers KSHV serostatus. It is of note that, of the KSHV seropositive children, nearly a third had a KSHV antibody negative mother and this figure is in keeping with published literature^{13, 25}. It is possible in the study presented in this thesis, that these mothers became infected with KSHV after the antenatal enrolment sample or that the child was infected from another source. This may reduce the effect of maternal KSHV serostatus on childhood KSHV seropositivity. KSHV transmission within families in sub-Saharan Africa has been documented with infection risk for the child showing associations, in decreasing order of importance, with infection in the mother, father and next oldest sibling¹⁰.

5.6.3 The relationship between Kaposi's sarcoma-associated herpesvirus serostatus and socioeconomic factors

The association between antibodies to KSHV in mothers and their children and marital status was null, consistent with other published reports^{22, 191}. In samples from pregnant women, potential markers of socioeconomic status, namely household socioeconomic status, maternal education, maternal occupation, number of people living at home and number of times pregnant, were not associated with antibodies to KSHV in the adjusted analysis. The composite variable and other markers of socioeconomic status were similar among women in this study, which may have made a difference difficult to detect. Other studies from sub-Saharan Africa also report an absence of an association between maternal KSHV seropositivity and socioeconomic markers including maternal educational attainment and occupation^{22, 259}. In contrast, in a report from Uganda of socioeconomic factors in mothers, the risk of being KSHV seropositive was low in the high-income group, although the trend in risk with increasing income was not linear²⁵⁹. Among children in this study, markers of socioeconomic status were not associated with antibodies to KSHV. The absence of an association between childhood

KSHV seropositivity and maternal educational attainment and markers of social status is similar to other studies from Africa ^{22, 259}. Given this general lack of association with markers of socioeconomic status, the key factor for seropositivity to KSHV in mother-child pairs may potentially be an aspect of behaviour or exposure not strongly related to socioeconomic status.

5.6.4 The impact of HIV on Kaposi's sarcoma-associated herpesvirus seropositivity

HIV is an important candidate for influencing KSHV transmission rates between KSHV infected mothers and their children. HIV infected subjects may be more likely to shed KSHV in saliva and HIV-associated immune deficiency may increase susceptibility to KSHV infection. In studies of women in sub-Saharan Africa ^{13, 22, 25}, the impact of HIV on KSHV seropositivity in mothers has been shown to be positive^{13, 22, 25, 191}. Although in one study there was a positive association between lytic K8.1 antibodies and maternal HIV, but a null association with antibodies to the latent antigen ORF 73²⁵. In the analysis for this thesis a borderline association between HIV and KSHV seropositivity in pregnant women was observed in the unadjusted analysis, but was lost when potential confounders were adjusted for. Women in this study were relatively immune competent with high CD4 counts. This could reduce the detection of antibodies to KSHV and could limit the chance of finding an association with HIV. There was no association between KSHV seropositivity and CD4 count in HIV infected women, but the numbers in each CD4 group category was small, the range was narrow and counts high.

Among children this study found some evidence that having an HIV-infected mother was a risk factor for KSHV seropositivity if the child was HIV negative, and stronger evidence that if the child was HIV positive the odds of KSHV seropositivity were increased compared to HIV unexposed, uninfected children and to HIV exposed uninfected children. Among HIV seropositive mothers the impact of the child being HIV infected compared

to HIV negative was about a two and a half-fold increase in odds of having antibodies to KSHV. This was a sub-group analysis and power was small, but may suggest that HIV infected children are at increased risk of having antibodies to KSHV. This study confirms the findings of others^{13, 22, 25}, that HIV infection is associated with an increased seroprevalence of KSHV in children, although this finding has been challenged in one study²⁵⁶. The marginal association when the child is exposed to maternal HIV but is not infected is also consistent with published literature^{13, 22, 25}. Whether HIV is acting to increase vulnerability to infection or causing reactivation in children infected with KSHV in the past, is unclear.

5.6.5 maternal sexual and non-sexual behavioural risk factors for HIV acquisition

The lack of association with syphilis and reporting having ever had a sexually transmitted disease is consistent with previous studies reporting no association between KSHV and markers of sexual behaviour in studies of mothers in Africa^{13, 14, 20, 225}. Mode of KSHV transmission is yet to be fully elucidated, but high acquisition rates during childhood imply a non-sexual route^{13-15, 25, 27, 131, 226, 228, 256, 259, 325-327}. Acquiring KSHV infection through a blood transfusion in Uganda has been reported³²⁸. This was not the case in this study, but the number of women who had had a transfusion was small and re-call bias may be a contributing factor.

5.6.6 Kaposi's sarcoma-associated herpesvirus seroprevalence and association with anaemia

The association with anaemia was lost in the adjusted model, but the number of women with severe or moderate anaemia was small due to trial entry criteria. Pregnant women were excluded from enrolment into EMaBS if they had a haemoglobin level <8g/dl. Hypoxia has been shown to cause KSHV reactivation^{162, 164}. As discussed in the introduction section, anaemia may potentially be associated with tissue hypoxia.

5.6.7 Antibodies to Kaposi's sarcoma-associated herpesvirus and parasites

5.6.7.1 Kaposi's sarcoma-associated herpesvirus and laboratory diagnosed parasite infections

In both mothers and their children asymptomatic malaria parasitaemia was significantly associated with antibodies to KSHV in the crude and adjusted analysis. The point estimate for odds of KSHV infection in both mothers and children increased in the multiple logistic models when potential confounders were included. Concurrent malaria appeared more important than previous episodes. But data on previous episodes of malaria was not available for mothers and was limited for children,

Factors associated with potential prevention of malaria infection were not protective for KSHV, but this is not surprising given that the use of mosquito spray and nets, did not prevent malaria among mothers and children. Among pregnant women, statistically significant associations with the presence or absence of hookworm or *M. perstans* and antibodies to KSHV were presented in the crude analysis. In the adjusted analysis, increasing intensity of hookworm infection was associated with the detection of antibodies to KSHV, driven by a difference between women uninfected with hookworm and women with heavy hookworm infection.

Parasites are a potential co-factor for primary KSHV infection or KSHV pathogenesis. Areas of high KSHV seroprevalence tend, in broad terms, to have a climate suitable for high parasitic burden and transmission^{317, 318}. Biologically, parasites rely on immunomodulation of the host for survival³²⁹, and it is conceivable that this could affect an individual's immune response to KSHV. Immune perturbations associated with parasite infections may impede viral control during KSHV infection, and affect the transmission events or immune surveillance required for eliminating abnormal cells with oncogenic potential.

5.6.7.2 A review of parasites as co-factors for Kaposi's sarcoma-associated herpesvirus infection and pathogenesis

In the era prior to the HIV epidemic and the discovery of KSHV in 1994, a link between parasites and KS was first suggested by Williams and published in 1966 as an observation of the occurrence of both endemic KS and onchocerciasis in the West Nile region of Uganda⁴⁷. Onchocerciasis, or river blindness, is caused by the infection, *Onchocerca volvulus*, a roundworm that is transmitted through the bite of a black fly of the genus *Simulium*. Williams proposed that the *Simulium* fly might transmit a yet unidentified infectious agent that might act as a co-factor for KS. In a follow-up paper examining the same cohort of individuals with endemic KS³³⁰, an association was observed between KS and reported bites from blood sucking insects or drinking water directly from rivers. The author's conclusion was that a microorganism may cause KS or that an arthropod vector may spread the aetiological agent. Still in the era prior to the discovery of KSHV, geographical restriction of classic KS in the Mediterranean was reported^{79, 331}, with high incidence rates, compared to surrounding areas of classic KS, in the island of Sardinia (2 per 100,000 males) and Southern Italy (3 per 100,000 men). Geddes et al, used cancer registry data and reported that individuals with Classic KS resident in Italy were more likely to be born in areas that were endemic for malaria³³². Agricultural occupations with potential exposure to environmental toxins or infectious agents were also reported to be association with classic KS in the Mediterranean region³³³.

After the recognition of KSHV as the aetiological agent for KS in 1994, there was keen interest in the geographical variation in KSHV infection and differing incidence of KS across narrow regions in the Mediterranean and potential associations with parasites. In Italy, a marked reduction in KSHV seroprevalence was observed in subjects born after the peak of DDT house-spraying to kill the malaria vector *Anopheles labranchiae*³³⁴. Subsequently, as numbers of *Anopheles* larvae rose again, parallel increases in KSHV seroprevalence were reported³³⁴. Ascoli et al have

published the “promoter-arthropod” hypothesis, which postulates that exposure to the bites of certain species of haematophagous arthropods may be a co-factor linked to KSHV infection and/or development of classic KS in the Mediterranean³³⁵. This theory hypothesised that certain species of arthropod may “promote” viral reactivation in the biting process; during blood feeding, piercing the skin and injecting insect saliva reactivates KSHV, leading to increased viral shedding in human saliva and an increased risk of KS development³³⁶⁻³³⁸. The incidence of classic KS in the Po Valley in Italy³³⁹ and Sardinia³⁴⁰ was reported to correlate with density of “promoters”, predominantly *Aedes vexans* (Meigen) and *Aedes caspius* (Pallas). One Mediterranean study reported a null association between classic KS and malaria; this study was conducted in Sardinia and examined a correlation between malaria prevalence in 1934, and the standardized KS morbidity ratio from 1977 to 2003 in eight historical sub-areas⁷⁹.

In HIV infected populations reports investigating potential associations between KSHV, KS and parasites are sparse; an AIDS cohort of MSM in North America reported an increase in gastrointestinal amoebiasis in individuals with KS³⁴¹. In sub-Saharan Africa an association with KS and certain helminth infections as been reported³¹⁹.

Table S.7: Studies identified through PubMed exploring potential association between parasites and KSHV or KS

1st author, year	Study location	Study type	Population	HIV	Outcome of interest	Results	Proposed co-factor for KSHV or KS
Williams, 1966	Uganda, West Nile	Ecological	28 cases of KS	Prior	Geographic distribution of KS	Geographic distribution of onchocerciasis and femoral hernia	Simulium fly
Mchardy, 1984	Uganda, West Nile	Ecological and case-control	72 KS cases	Prior	KS cases	KS associated with bites from insects, drinking water from rivers	Mosquitos, Heaematopota insect
Abrams, 1990	USA	Cohort	Individuals with HIV associated KS	Yes	MSM with a higher frequency of KS	High rate of asymptomatic amebiasis	GI parasites
Geddes, 1995	Italy	Ecological and case-control	Italian cancer registry	No	Birth place and Classic KS	KS cases born in areas endemic for malaria	Malaria
Cottoni, 1997	Italy, Sardinia	Case-control	40 cases CKS	No	Classic KS	Association CKS and farming cereals. No association with history of malaria.	
Coluzzi, 2003	Italy, Sardinia	Cohort, ecological	434 plasma samples from blood donors.	No	Number of anopheles larvae and number of DDT treated households	Drop in KSHV seroprevalence mirrors eradication of Anopheles.	Anopheles labranchiae (malaria vector) or biting midges (Culicoides)
Serraino, 2003	Italy, Central region	Cross sectional, ecological	200 samples randomly sampled from a population survey	No	Seroprevalence and incidence of CKS in a region formerly endemic for malaria	KSHV seroprevalence comparable to those from an area at low risk for KS where malaria is not endemic	
Cottoni, 2006	Italy, Sardinia	Prospective cohort	332 CKS	No	Incidence of CKS and prevalence of malaria in 1934	No association with malaria prevalence and standardized morbidity ratio for CKS	
Ascoli, 2006	Italy, Po Valley	Ecological	3910 adult female mosquitos, 5 species named "promoters" due to irritation with bites	No	Density of promoter with incidence of CKS	Density of promoters associated with CKS	Promoter arthropods
Ascoli, 2009	Italy, Sardinia	Ecological	11,030 adult female mosquitos, 5 species named "promoters" due to irritation with bites	No	Density of promoter with incidence of CKS	Density of promoters associated with CKS	Promoter arthropods
Lin, 2008	Uganda	Cross-sectional	943 KS patients and 1042 with other cancer type	Not reported	Frequency of intestinal parasites among KS patients	KS patients more like to have Strongyloides stercoralis. Marginal associations with Schistosoma mansoni, Giardia Lamblia, Entamoeba histolytica and Trichomonas hominis	Strongyloides and/or other parasites
Wakeham, 2011	Uganda, Entebbe	Cross-sectional	1915 women in a RCT	10%	Association of KSHV with co-factors	Individuals with KSHV more likely to have asymptomatic malaria parasitaemia, hookworm or mansonella	Parasites

5.6.7.3 Kaposi's sarcoma-associated herpesvirus and self-reported or surrogate markers of parasite exposure

Reports of the associations between KSHV, KS and potential surrogate markers of parasite exposure have also been published, such as residence in areas of high rainfall and walking barefoot^{40, 41, 301, 320}. Previously reported risk factors for KSHV, such as use of surface rather than piped water, may also be consistent with increased exposure to parasites²⁵⁹. In this study, pregnant women reporting using surface water from Lake Victoria rather than water from a borehole, stand-pipe or tap was marginally associated with antibodies to KSHV in the adjusted analysis. While use of water from Lake Victoria is linked to socioeconomic status, adjustment for markers of socioeconomic status in this analysis did not fully explain this association. In Italy "closeness to rivers"³⁴² has been associated with risk of classic KS. KSHV seropositivity has also been reported to be higher in people living in areas surrounded by several rivers³⁴³. In Africa "drinking water from rivers" has been associated with risk of endemic KS³³⁰. Fast flowing rivers are not typical breeding grounds for mosquitoes or other parasites but are often associated with surrounding areas of still water and swampland that may be. Water masses may also be outlets for human sewage, which convey a risk of certain helminth infections.

Soil types that are potentially the most suitable for arthropod and sand-fly breeding³⁴⁴ have been associated with KS. Areas with high incidence of classic KS in Italy have landscapes on limestone, acid volcanic rocks and alluvial deposits³⁴⁰. Soil type has also been implicated in the pathogenesis of endemic KS in Africa. The "soil" hypothesis put forward by Zeigler to explain the pattern of endemic KS in Africa implicates highly absorptive volcanic kaolin clays laden with iron as the cause of local inflammation and immunologic disturbance leading to development of KS³⁴⁵. Walking barefoot was associated with endemic KS in Uganda³⁰¹, a region rich in iron-laden soils. In the study walking barefoot had a borderline association with the presence of antibodies to KSHV among pregnant women. Whether soil types may impact insect or parasite

diversity in Africa has not been reported, but would be of interest.

In this study, having ever taken worm medication was associated with a reduced KSHV seroprevalence in pregnant women. If helminth infections increase the risk of detection of antibodies to KSHV then eliminating parasite infections may be associated with a decrease. Drug effect on the detection of antibodies to KSHV cannot be ruled out, nor can an unmeasured confounder.

Eosinophilia was of interest due to its association with parasitic infection, but no association with KSHV seropositivity was found.

5.6.7.4 Biological plausibility for an association between parasites and Kaposi's sarcoma-associated herpesvirus

Helminths have adapted through evolution to overcome the host immune environment and as a consequence many infections are asymptomatic. Immunological consequences of helminth infections are recognized. In a simplified model of immunological response to helminth infection, helper T cell responses are described as predominantly TH1 or TH2. TH1 responses are associated with production of interferon-gamma, which activates cell-mediated immunity to kill intracellular pathogens. TH2 responses are associated with the production of interleukin-4, which activates B cell and humoral immunity. Host responses to helminth infections are complex but broadly biased towards TH2 and regulatory responses³⁴⁶ with the activation of eosinophils and mast cells and production of immunoglobulin E. Chronic helminth infection may lead to down-regulation of TH1 activity and the associated dampening of cell-mediated immunity may impair immune responses to co-infections with bacterial, viral, and protozoal pathogens³⁴⁷. Co-infections with helminths and viruses are common, especially in Africa, but the clinical and biological consequences are largely unknown. No biological research on the immunological impact of co-infection with KSHV and helminth infections could be identified in the literature. Co-infection with hepatitis B or hepatitis C and *S. mansoni* suggests that the outcome of viral

infection may be influenced by the balance between Th1 and Th2 responses³⁴⁸. Studies on the impact of helminths on HIV acquisition and progression have proved conflicting³⁴⁹. Loss of KSHV-specific T cells allows KSHV infected cells to proliferate and KSHV related cancers to develop¹⁰²; if helminth infections dampen T-cell mediated immunity it is reasonable to hypothesize that they may affect KSHV control and disease outcome.

Hookworm has attracted interest as a co-factor for endemic KS due to the observation that endemic KS often begins on the lower limbs in a similar location to the place where hookworm larvae enter human skin. Upon dermal penetration, hookworm secretes enzymes that facilitate migration through tissues and a multitude of immune-stimulating molecules are released leading to inflammation (for review see Loukas and Prociw³⁵⁰). Chronic hookworm infections are associated with diminished hypersensitivity reactions in the skin³⁵¹ and this type of immune reaction has also been reported in the lower limbs of people with classic KS³⁵² and in endemic KS in Africa³⁵³. It is pure speculation whether larval penetration or the immune perturbation associated with loss of hypersensitivity reactions in hookworm infections may have a role in KS development in KSHV infected individuals.

In malaria endemic regions, repeated infections lead residents to develop immunity that improves clearance of parasites and limits the inflammatory response to malaria that causes acute symptoms. Young children who have yet to develop protective immunity are at greatest risk of clinical symptoms, severe disease and death³⁵⁴. Presumed mechanisms for host immune response to malaria include the production of interferon-gamma, and activation of both TH1 and TH2 effector responses (For review see Stevenson and Riley³⁵⁵).

The impact of *P. falciparum* malaria on EBV, a herpesvirus closely related to KSHV is recognised. EBV is an oncovirus linked to a number of malignancies³⁵⁶ including Burkitt lymphoma which accounts for 30 to 50% of all childhood cancer in equatorial Africa³⁵⁷. There are a number

of theories to explain the interaction of EBV and malaria in the pathogenesis of Burkitt lymphoma:

- (1) *P. falciparum* may provide a chronic stimulus for poly-clonal B-cell expansion thereby increasing the chance of genetic translocations that immortalise cells²⁵³.
- (2) *P. falciparum* may impair EBV-specific T-cell immunity, which leads to a loss of viral control²⁵³.
- (3) *P. falciparum* may facilitate EBV infection early in childhood and lead to subsequent poor viral immune control, potentially due to an immature immune system. More than 95% of African children are infected with EBV by age three, whereas, in affluent countries, primary infection is often delayed until adolescence³⁵⁸.

Many parallels can be drawn between the effects of malaria and EBV outcome. Like EBV, KSHV latently infects B cells and can cause clonal expansion and two B cell malignancies – primary effusion lymphoma and MCD. T-cell immunity is important in the control not only of EBV but also of KSHV infection and KS disease¹⁰². Losses of KSHV-specific T-cells are associated with high replication of KSHV and KS pathogenesis and/or disease progression. Restoration of KSHV-specific T-cells is associated with KS regression. Age is also a key factor in KSHV epidemiology. Countries where classic or endemic KS occurs have a high prevalence of childhood infection with KSHV, associated with mother to child transmission^{8, 13, 22, 25, 359}, compared with countries where classic and endemic KS are rare. It is very tempting to hypothesise a similar role for malaria in KSHV disease.

Acute *P. falciparum* is also associated with another herpesvirus; herpes simplex virus-1³⁶⁰. The mechanism by which acute malaria infection triggers the reactivation of latent herpes simplex infection is not clear, but it has been proposed that interleukin-6 (IL-6), produced by sequestered malaria parasites, may result in viral reactivation^{361, 362}. IL-6 is also implicated in driving KSHV reactivation and pathogenesis of MCD. (Review see Polizzotto³⁶³). This invites the speculative question as to whether IL-6 produced in response to malaria could affect KSHV

control.

In conclusion, if parasites impact the control and pathogenesis of KSHV, the mechanism is likely to be complex. The immunological interactions between parasites and KSHV are likely to be governed by the timing of the infections in relation to each other, the intensity of duration of the infections, host genetics and environmental factors. Biological plausibility does exist but research is required to investigate potential mechanisms.

5.7 Conclusion

Co-factors for KSHV transmission and disease have been sought to explain the elevated prevalence of KSHV and incidence of KS in Uganda. Exposure to HIV infection and having a KSHV seropositive mother in childhood is associated with an increased risk of KSHV seropositivity. Data presented here also suggests that parasites such as malaria may constitute one such co-factor. Further epidemiological and laboratory studies are needed to fully understand the role of parasites as a risk factor for infection with KSHV.

5.8 Study comments, strengths and limitations

This is the first study of KSHV seroprevalence in sub-Saharan Africa that investigates direct measures of parasite burden, rather than surrogate markers of potential infection. This study benefited from being conducted within the context of a randomised control trial. The data collected was of high quality and the datasets had been extensively cleaned. Many of the associations identified in this study do not rely on human judgment or recall and use automated laboratory data. The nursing and field teams were highly trained and followed standard protocols. Lost to follow up rates were low. Stringent steps were taken to ensure ELISA assay quality control. The ELISA samples were run blind and agreement in the ELISA results for control and duplicated samples was consistently high. Given the novel finding of an association between

malaria, helminths and KSHV seropositivity a study recruiting more cases would increase confidence. To investigate an exposure-response relationship a sample with a higher number of individuals with high intensity parasite infections is needed. It would be most ideal to prospectively calculate a power-based estimate of the sample size needed. A sample containing more cases of HIV and parasite infections would reduce sampling error.

5.8.1 Generalisability

Investigations have been carried out by EMaBS to ascertain if women recruited were representative of the general population of Entebbe and Katabi and that trial enrolment was not linked to health status. Results showed biases towards higher socioeconomic status. This may mean that some of the women and children most vulnerable to parasites and KSHV infection were not included. The potential importance of the findings of this thesis may be conservative. The study was conducted retrospectively on stored samples collected by the EMaBS trial. It was not design prospectively to investigate exposures on KSHV seropositivity. The sample size was overall relatively large, but the number of some exposures including HIV and some parasites was small and this increased sampling error. The wide confidence intervals surrounding the point estimates for childhood HIV and KSHV seropositivity shows the lack of precision. All of the women included in the study were pregnant.

5.8.2 Bias, confounding and other reasons for caution

Pregnant women were recruited through a government antenatal clinic after extensive community education about the EMaBS study. Differences between women who chose to participate in the study and those who did not may have impacted the results. Selection bias occurred as HIV seropositive mothers, or mothers with malaria and less education had no plasma samples available for KSHV serology. This may have led to an underestimation of the effect of HIV or malaria on antibodies to KSHV.

Misclassification of KSHV serostatus could have affected cases and controls. Limitations in the KSHV ELISA assay used have been discussed in Chapters 3 and 4. The repeatability of KSHV ELISA results as tested by a Kappa statistic was good and using a range of cut-off values for KSHV seropositivity did not materially change the main results. The diagnosis of intestinal helminths may have been affected when only one stool sample was used. In the main EMaBS trial the sensitivity for diagnosis of parasites by stool samples was greater when three samples were used compared to one³⁶⁴. Malaria blood slides have been shown to under-estimate malaria prevalence by about 10%³⁶⁵, and this may have led to an underestimate of the strength of association between malaria and KSHV.

Recall and reporting bias, as with all studies, may have been a potential issue with markers of socioeconomic status and surrogate markers for parasite infections. It is difficult to predict, but depending on the situation, individuals may over or under report an exposure. It is perhaps reassuring that many of the "soft" surrogate markers for parasites are associated with actual parasite burden as measured in the laboratory. Although this was not the case in use of mosquito nets and use of spray to kill mosquitoes in the home, which were both associated with an increased risk of malaria. It is possible this could represent reverse causality in that individuals with multiple episodes of malaria illness start using preventative measures. Or individuals are living within areas with many mosquitos. It is of further reassurance that markers of socioeconomic status show consistency of direction with, for example, increasing household socioeconomic groups being associated with increasing wage groups³⁶⁶.

Adjustment for markers of socioeconomic status had little material effect on associations between factors and antibodies to KSHV. One cannot rule out the possibility of residual confounding by unmeasured factors. Known confounders that may explain the association between the exposure and KSHV seroprevalence have been considered. Age as a

confounder to children's KSHV status was a prior hypothesis and all analysis for children was adjusted for age. Socioeconomic status is probably the leading candidate for confounding a relationship between HIV and parasites. Socioeconomic status was associated with hookworm and *M. perstans* infection. Attempts were made to control for potential confounders by including them as covariates in the multivariate analysis.

The cross-sectional design makes it difficult to differentiate between risk factors for primary infection and reactivation, leading to a boost in antibody titres. The mothers had relatively high CD4 counts and this may have impacted on our ability to assess the role of HIV. The mother's plasma sample was collected remotely in time from the child's sample. Power to detect potential associations between antibodies to KSHV in children and helminth infections or HIV was small. The analysis was cross-sectional and associations identified should be confirmed in longitudinal studies.

The study participants were pregnant and pregnancy itself may modulate immune function. However, since all comparisons were internal within the study (i.e. comparing one group of pregnant women with another), it is difficult to see how this could have impacted on the comparisons.

5.9 Further directions

The novel finding in the analysis for this thesis is a potential association between antibodies to KSHV and malaria. Evidence from HIV-infected and renal transplant cohorts suggests that KSHV-specific T-cell function is key to control of KSHV viral replication. A birth cohort is an ideal setting because age is a determinant of immune function, risk of malaria and primary infection with KSHV. This type of study would allow the exploration of the impact of concurrent versus previous episodes of malaria to be investigated. It has been reported that poor viral immune control of EBV may be facilitated by *P. falciparum* early in childhood²⁵⁴. In addition to samples for KSHV serology, it would be advantageous to collect suitable material to study KSHV viral load in PBMCs and KSHV

shedding in saliva. KSHV viral load may be a better marker of KSHV control than antibody responses. PBMCs would also be required for an ELISpot or FACS assay to determine KSHV-specific T-cell function. If an association were identified between malaria and poor control of KSHV viral load it would be of interest to investigate the impact of malaria treatment. A prospective powered study could be designed to compare KSHV DNA loads in PBMCs and saliva in individuals with falciparum parasitaemia at the time of malaria diagnosis and after anti-malarial treatment. If KSHV were cleared from PBMCs or saliva after anti-malaria treatment a relationship between the viral control and malaria may be suggested.

Chapter 6. A meta-analysis of the association between HIV and Kaposi's sarcoma associated herpesvirus among mothers and children in sub-Saharan Africa

6.1 Chapter abstract

Background: The results presented in Chapter 5 of this thesis suggest an association between HIV and KSHV seropositivity in mother-child pairs in sub-Saharan Africa. To explore current literature a systematic review and meta-analysis was conducted.

Methods: A comprehensive search was conducted in PubMed, Medline, Web of Science and EMBASE. Relevant material was evaluated. Published adjusted odds ratios and 95% confidence intervals were transferred into Stata SE11. Due to anticipated heterogeneity between studies, a random-effects model was used for all analyses.

Results: The summary analysis suggests that: (1) HIV infection in mothers and pregnant women is associated with an increased risk of KSHV seropositivity compared to HIV-negative individuals; (2) HIV-negative children of KSHV-HIV seropositive mothers are not at increased risk of detection of antibodies to KSHV compared to HIV negative children of mothers with antibodies to KSHV alone, and (3) childhood HIV infection is associated with increased KSHV seroprevalence when compared to HIV negative children.

Conclusion: HIV infection is associated with an increased prevalence of antibodies to KSHV in pregnant women, mothers and children.

6.2 Rationale

In sub-Saharan Africa KSHV is a common infection in children and adults. Mother to child transmission of KSHV is thought to be important in the spread of infection. In this region, HIV is endemic, increasing the risk of KSHV-HIV co-infection. HIV-associated immunosuppression can cause KSHV reactivation³⁶⁷ and increase KSHV viral load in bodily fluids¹¹³. HIV-infected individuals may therefore act as highly infectious reservoirs, facilitating KSHV transmission. Furthermore, people with HIV are potentially more susceptible to infection due to the associated immune suppression. Despite this, the effect of HIV infection on KSHV transmission in mothers and their children is not well understood.

Some challenges in the interpretation of the current literature potentially stem from the HIV testing strategy employed; in particular the way the HIV status of the child is determined and the definition of the child's HIV status – HIV unexposed and uninfected, HIV exposed but uninfected, and HIV infected. A child's HIV status is not necessarily the same as maternal status and a further problem is that HIV status in children can change due to breast-feeding. In an attempt to bring clarity to the literature, a meta-analysis of epidemiological studies that determined KSHV and HIV status of mothers and their children living in sub-Saharan Africa was carried out.

6.3 Objectives

In this meta-analysis the aim was to pool results from all relevant studies to test the following hypotheses:

- (1) HIV infected mothers and pregnant women will have a higher KSHV prevalence compared to mothers and pregnant women without HIV infection,
- (2) HIV negative children of KSHV-HIV co-infected mothers will be more likely to have KSHV compared to children whose mothers are infected with KSHV alone, and
- (3) HIV infected children will have a higher KSHV prevalence compared

to children who are not infected with HIV.

6.4 Methods

Research methods and reporting are in accordance with the Cochrane Collaboration and PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses)³⁶⁸ guidelines.

6.4.1 Eligibility criteria

All studies of KSHV seropositivity in mothers and/or children in sub-Saharan Africa were considered. Publications did not have to contain results for both mothers and children. KSHV infection could be defined by the presence of antibodies to KSHV proteins in plasma or serum or by detection of the KSHV genome in saliva, plasma, serum or PBMCs. Childhood was defined as ≥ 1 year (to avoid detection of maternal antibodies) and ≤ 12 years. Mothers and children had to have had a HIV test; a child's HIV status could not be defined by maternal HIV status). Studies could be cross sectional or longitudinal in design.

6.4.2 Exclusion criteria

The exclusion criteria were designed to keep between study heterogeneity to a minimum and were as follows:

1. Individuals with KS,
2. Women part of sex-worker cohorts,
3. Studies of heterosexual transmission of KSHV,
4. Studies where recruitment took place within a hospital setting,
5. Studies set outside of sub-Saharan Africa,
6. All individuals HIV infected,
7. HIV status of participants not reported and
8. Duplicate published datasets.

6.4.3 Information source and search

PubMed was electronically searched up to and including the 17th April 2012 and the search updated on the 9th May 2013. Initial searches were conducted using "KSHV" or "HHV8" and "HIV". Due to the large number of publication returned a search using "KSHV" or "HHV8" and "Africa" in all fields of the PubMed advanced search builder was used. Followed by "KSHV" or "HHV8" and "children", and then "KSHV" or "HHV8" and "mother"/"women". Medical subject headings (MESH) and terms of relevant articles were reviewed and searches carried out using ("herpesvirus 8, human" [MeSH Terms] OR "human herpesvirus 8" [All Fields] OR "kshv"[All Fields]) AND ("hiv"[MeSH Terms] OR "hiv"[All Fields]) AND ("africa"[MeSH Terms] OR "africa"[All Fields]) AND ("child"[MeSH Terms] OR "child"[All Fields] OR "children"[All Fields]) OR ("mother"[MeSH Terms] OR "child"[All Fields] OR "mother-child"[All Fields]). Links were followed to related articles and reference lists in identified articles and reviews were also checked. For completeness, searches were run in Medline, Web of Science and EMBASE, up to and including the 9th May 2013. Only articles published in English were used. Conference proceedings, abstracts, theses, dissertations and national or local vital statistics data not published as peer reviewed articles were not included.

6.4.4 Data collection process

Articles were reviewed and the following variables recorded: first author; year of publication; location; cohort description; age; study size; method of determination of HIV status; HIV prevalence in mothers and/or children; KSHV prevalence in mothers and/or children; type of assay used to determine KSHV status; definition used to define KSHV positivity; if analysis was restricted to KSHV positive mothers, prevalence of KSHV-HIV co-infection, HIV alone, KSHV alone and neither KSHV or HIV; unadjusted odds ratios (OR) and 95% confidence intervals (CI) for KSHV; adjusted OR and 95% CI for KSHV; adjustment factors.

When published datasets were duplicated or overlapped, only the latest study was included. All first authors were contacted to provide information on missing information, including HIV testing protocol, whether analysis was restricted or not restricted to KSHV positive mothers, adjusted OR and adjustment factors. No additional information was returned.

6.4.5 Statistical analysis

Adjusted ORs and 95% CI were extracted. If a publication did not provide an adjusted OR, an unadjusted OR was sought or an OR was estimated from the raw data. ORs and 95% CI were recorded to one decimal place. Due to anticipated heterogeneity between studies, a random-effects model was used for all analyses. The OR calculated in the random effects model provides an estimate of average effect. Statistical heterogeneity was inspected graphically with a forest plot and assessed by calculating the degree of heterogeneity between study estimates using the I^2 statistic. The interpretation of I^2 was 0%, no heterogeneity, 25% low heterogeneity, 50% moderate heterogeneity, 75% high heterogeneity, where an I^2 value of 50% indicates that half the total variability is caused by true heterogeneity between studies, and not by sampling error³⁶⁹. Prediction intervals set at 95% were also calculated. Funnel plots were examined for bias and the Beggs and Eggers test conducted³⁷⁰. CIs were set at 95% and two-sided P values of less than 0.05 were considered statistically significant. All statistical analyses were performed using the StataSe version 11.0 software (Stata Corporation, College Station, TX).

6.5 Results

In PubMed the search terms yielded in English the following results: 268 articles for “KSHV” or “HHV8” and “Africa”, 275 articles for “KSHV” or “HHV8” and “children” and 178 articles for “KSHV” or “HHV8”. Searching Medline, Web of Science and EMBASE did not yield any additional publications. The search results for 721 articles were exported into Microsoft Excel. Duplicates were deleted leaving 529 unique records for screening. The published articles were screened for relevance and 247 were dropped from further analysis. The abstracts of 282 articles were read and the full text of 71 articles was read (Appendix 1).

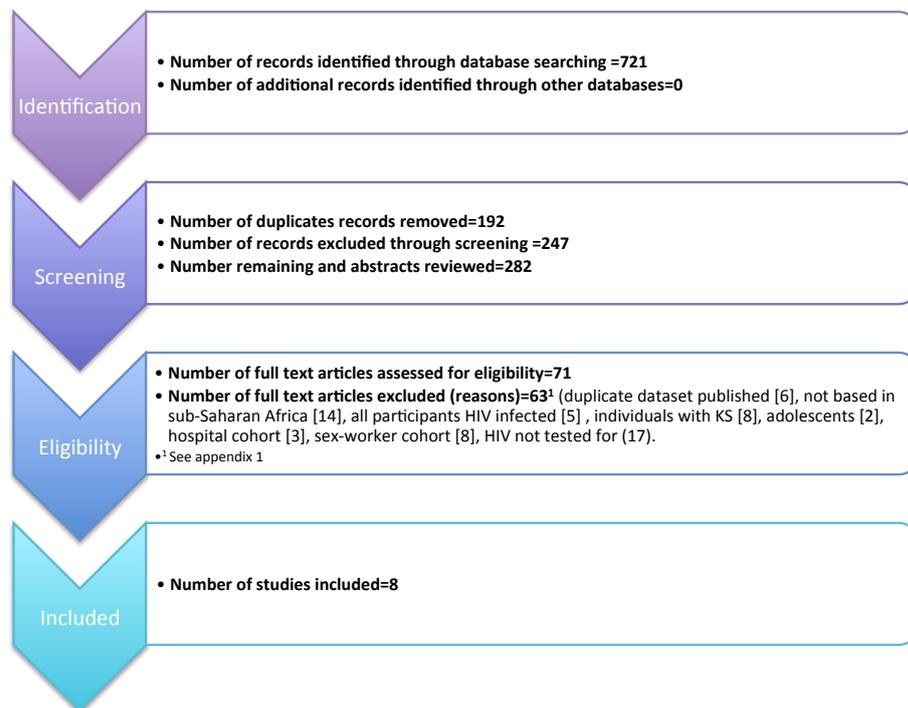


Figure 61: Flow of information through phases of systematic review and meta-analysis.

Seven studies were identified that satisfied eligibility criteria^{8, 9, 13, 22, 25, 191, 256,7}. All studies determined KSHV status by detection of antibodies to KSHV in serum or plasma. No study used KSHV viral load.

6.5.1 Objective 1: Kaposi’s sarcoma-associated herpesvirus seroprevalence in HIV infected mothers and pregnant women compared to mothers and pregnant women without HIV

Four cross-sectional studies conducted in Zambia, South Africa and Uganda met eligibility criteria (Table 6.1)^{8, 22, 25, 191}. HIV seropositivity among women was between 10 and 30%. HIV testing strategy varied between studies. KSHV seroprevalence ranged from 40 to 61%. Malope¹⁹¹ and Wakeham⁸ used the same KSHV serological assay. All four studies reported that HIV co-infection was associated with an increased point estimate in the odds of KSHV seropositivity, compared to HIV-negative mothers and pregnant women.

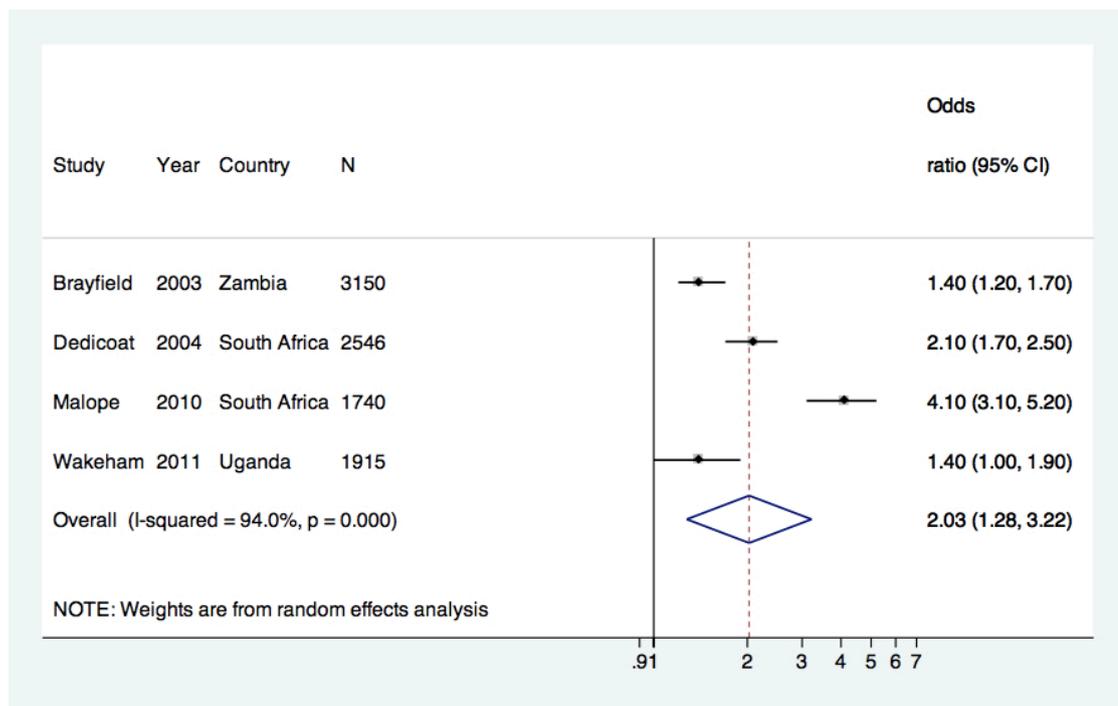


Figure 6.2: Random effect forest plot of studies identified that investigated the odds of KSHV seropositivity among HIV seropositive mothers compared to HIV seronegative mothers. Studies ordered by year of publication. Studies included are shown in Table 6.1.

The random effects summary OR for KSHV seropositivity in HIV seropositive mothers, compared to seronegative mothers was 2.0 (95% CI 1.3-3.2). The proportion of the total variation between study estimates that was due to heterogeneity (I^2 statistic) was 94.0%. The 95% prediction interval (the range within which future observations might be expected to fall) was 0.2-18.6.

A funnel plot was constructed. A large amount of heterogeneity was suggested but no conclusion could be drawn due to the small number of published studies (results not shown). The Beggs and Egger test did not indicate any evidence of bias but power to demonstrate a relationship was very low due to data only being available from four studies (results not shown).

Table 6.1: Studies identified to investigate KSHV prevalence in mothers and pregnant women with HIV co-infection compared to mothers without

Study first author	Year of publication	Location	Study size	HIV test	HIV prevalence (%)	KSHV assay	KSHV seroprevalence (%)	Definition of KSHV seropositivity	Adjusted ORs (95% CI)	Adjustment factors
Brayfield	2003	Zambia	3150	Commercial kit serology, confirmed by Western blot	30	Lytic IFA Latent IFA ELISA whole virus	40	Positive to both IFAs Seropositivity cross checked with whole virus ELISA	1.4 (1.2-1.7) ¹	Nil
Dedicoat	2004	South Africa	2546	Serology, confirmed using 2nd ELISA	28	K8.1 ELISA	40	K8.1	2.1 (1.7-2.5)	Maternal age
Malope	2010	South Africa	1740	HIV ELISA	23	ELISA K8.1 ELISA ORF73	45	Positive to either K8.1 and/or ORF73	4.1 (3.1-5.2)	Maternal age, region, education and syphilis
Wakeham	2011	Uganda	1915	Commercial kit serology, confirmed by Western blot	10	ELISA K8.1 ELISA ORF73	61	Positive to either K8.1 and/or ORF73	1.4 (1.0-1.9)	Maternal age, education, household socioeconomic status, malaria, hookworm, Mansonella

OR, odds ratio; CI, confidence interval.

¹ Adjusted odds ratio not quoted in publication

6.5.2 Objective 2: The odds of Kaposi’s sarcoma-associated herpesvirus seropositivity among HIV negative children of KSHV-HIV co-infected mothers compared to children of mothers with Kaposi’s sarcoma-associated herpesvirus alone

Four publications were included (Table 6.2)^{7, 9, 13, 25}. All studies were cross-sectional and conducted in South Africa, Zambia and Uganda and had samples sizes between 677 and 1823. The HIV testing strategy, KSHV assay and definition of KSHV seropositivity varied (Table 6.2). Malope et al. presented an adjusted OR that was not restricted to KSHV seropositive mothers¹³; exclusion or inclusion of this study did not materially change the summary OR (results not shown). Dedicoat et al. presented results for antibodies to both K8.1 and ORF 73²⁵; the forest plot and summary OR below are for K8.1 results, in order to keep the analysis comparable with objective 1 (where only K8.1 results were available).

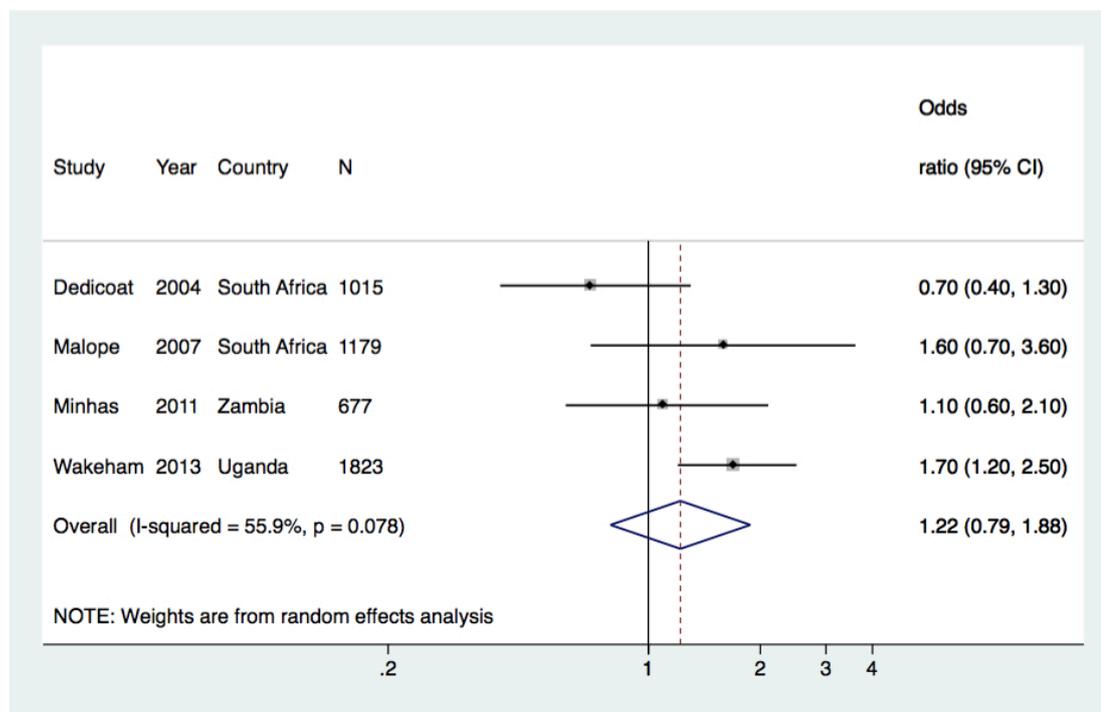


Figure 6.2: Random effect forest plot of studies identified that investigated the odds of KSHV seropositivity among HIV negative children of KSHV-HIV co-infected mothers compared to children of mothers with KSHV alone

Three of the four studies reported no increased risk of antibodies to KSHV among HIV negative children of KSHV-HIV co-infected mothers (Table 6.2).

The random effects summary OR for KSHV seropositivity in HIV negative children of HIV-KSHV seropositive mothers compared to KSHV seropositive HIV negative mothers was 1.2 (95% CI 0.80-1.9). Between studies, heterogeneity was 56% (I^2 statistic). The 95% prediction interval was 0.2-6.6.

Funnel plots, and the Beggs and Egger test, did not indicate any evidence of publication bias (results not shown). But due to the small number of studies conclusions could not confidently be drawn.

Table 6.2: Studies identified to investigate risk of KSHV prevalence in HIV negative children of KSHV-HIV co-infected mothers compared to mothers infected with KSHV alone

Study first author	Year of publication	Location	Study size	HIV prevalence (%)	HIV test	KSHV assay	Definition of KSHV sero-positive	Childhood KSHV seroprevalence (%)	Age of children (years)	Restricted to KSHV+ mothers	Adjusted ORs (95% CI)	Adjustment factors
Malope	2007	South Africa	1179	21	HIV antibody	K8.1 and ORF73 ELISA	Positive to either K8.1 and/or ORF73	16	1.6-10	No	1.6 (0.7-3.6)	Age of mother and age of child
Minhas	2011	Zambia	677	Not given	Sample at 18 and 24 months, confirmed with Western blot	BC3 MIFA and Sf9 MIFA (ORF65, K8.1, and one Sf9 ORF73)	Positive to both BC3 and one Sf9 antigen	13	1	Yes	1.1 (0.6-2.1)	HIV mother, ever rash, HIV child, EBV infection
Dedicoat (A)						EIA K8.1	K8.1	7			0.7 (0.4-1.3)	
Dedicoat (B)	2004	South Africa	1015	28	HIV RNA	IFA LANA	ORF 73	12	1-6	Yes	0.7 (0.3-2.2)	Maternal age
Wakeham	2013	Uganda	1823	10	PCR births, Western blot 18 months	K8.1 and ORF73 ELISA	Positive to either K8.1 and/or ORF73	11	1-5	Yes	1.7 (1.2-2.5)	Age of child, asymptomatic malaria parasitaemia, maternal KSHV serostatus, maternal education and use of mosquito net in the home

OR, odds ratio; CI, confidence interval.

6.5.3 Objective 3: Kaposi's sarcoma-associated herpesvirus seroprevalence among children infected with HIV compared to HIV negative children

Five cross-sectional studies containing seven sets of results were eligible^{7, 9, 13, 25, 256} and they were conducted in South Africa, Zambia and Uganda. The sample sizes ranged from 427 to 1823 (Table 6.3)^{7, 9, 13, 25, 256}. The definition of KSHV seropositivity varied and antibodies to KSHV were detected among 7 to 20% of children. The results for Malope et al. are an unadjusted OR¹³. The study by Butler and co-workers contained results from a population in Uganda and a population in South Africa²⁵⁶. One study (Dedicoat) contained results for two KSHV antigens separately²⁵. Butler et al. did not report the HIV testing strategy and it could not be obtained from other related publications. Among the Ugandan and South African populations presented by Butler et al., HIV was associated with a reduced prevalence of antibodies to KSHV²⁵⁶. In the other studies HIV was associated with increased odds of detection of antibodies to KSHV.

First, all seven populations were included in the estimation of the summary OR (Figure 6.3). Next, the analysis was repeated dropping the ORF 73 results from the Dedicoat et al. study (Figure 6.4) and one or both of the results for the Butler et al. study (Figure 6.5 and 6.6). The decision to omit the Butler et al. study results was because selection bias within the study was potentially high (see below). The Dedicoat ORF 73 results were omitted to keep the analysis in keeping with objectives 1 (where only K8.1 outcome data was available) and 2.

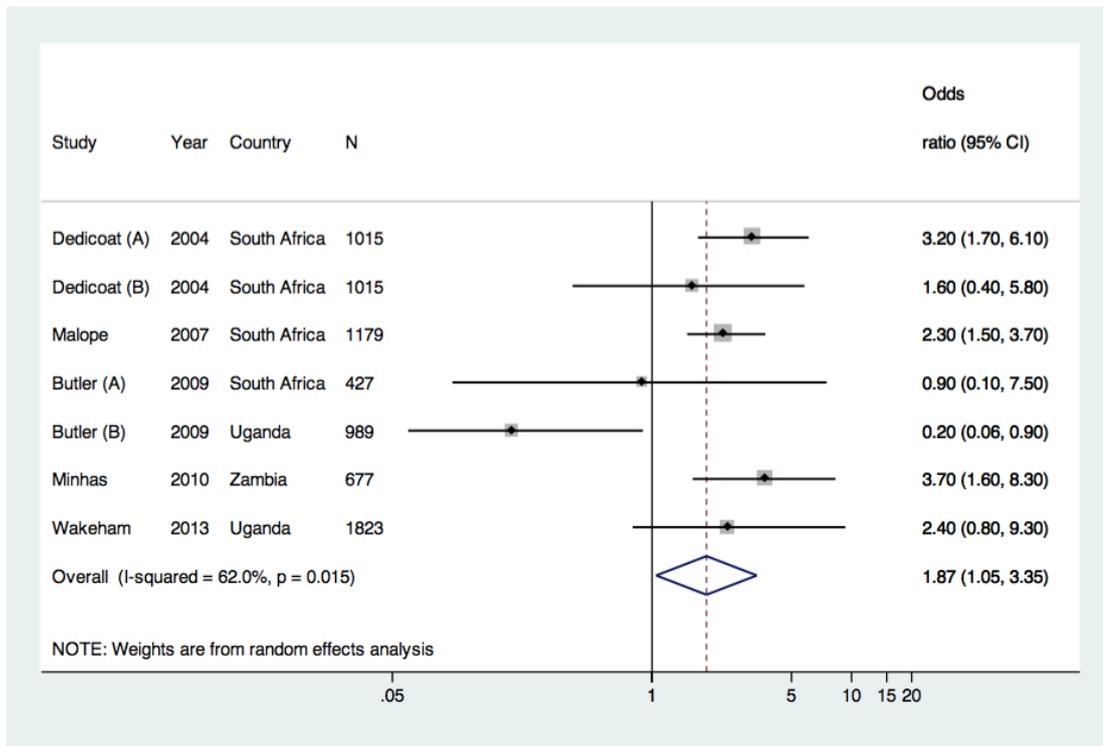


Figure 6.3: Random effect forest plot of studies identified that investigated the odds of KSHV seropositivity among HIV-infected children, compared to HIV negative children. Studies are ordered by year of publication.

The random effects summary OR for KSHV seropositivity among HIV-infected children compared to HIV-negative children was 1.9 (95% CI 1.1-3.4). The total variation in study estimates due to heterogeneity (I^2 statistic) was 62.0%. The 95% prediction interval was 0.4-9.7.

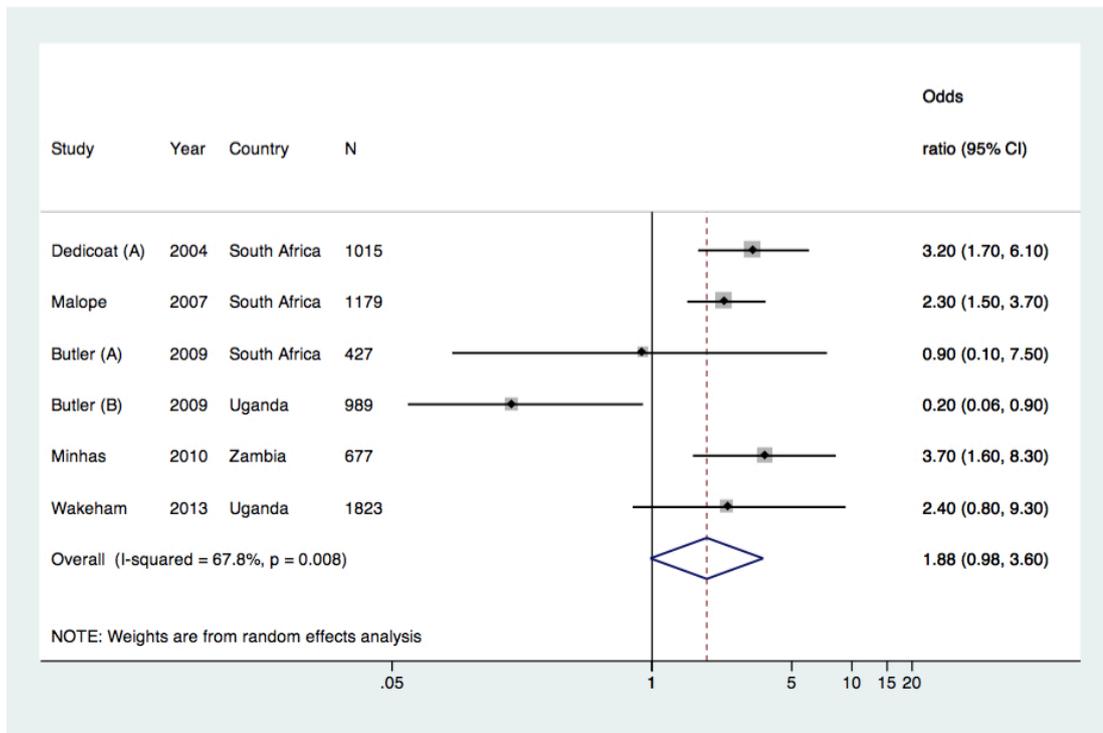


Figure 6.4: Random effect forest plot of studies identified that investigated the odds of KSHV seropositivity among HIV-infected children, compared to HIV negative children. ORF 73 results from the Dedicoat et al. paper have been omitted. Studies are ordered by year of publication.

The random effects summary OR (with the ORF 73 results from the Dedicoat et al. study omitted) for KSHV seropositivity among HIV-infected children compared to HIV-negative children was 1.9 (95% CI 1.0-3.6). The total variation in study estimates due to heterogeneity (I^2 statistic) was 67.8%.

The summary OR results for the association between HIV and KSHV in children, in turn omitting the Butler et al. South African population, the Butler et al. Ugandan population, or both, were OR 2.0 (95% CI 1.0-3.9), I^2 statistic=73.0% and 2.6 (95% CI 2.0-3.6) I^2 statistic=0%, and 2.7 (95% CI 2.0-3.7) I^2 statistic=0% (Figure 6.5), respectively.

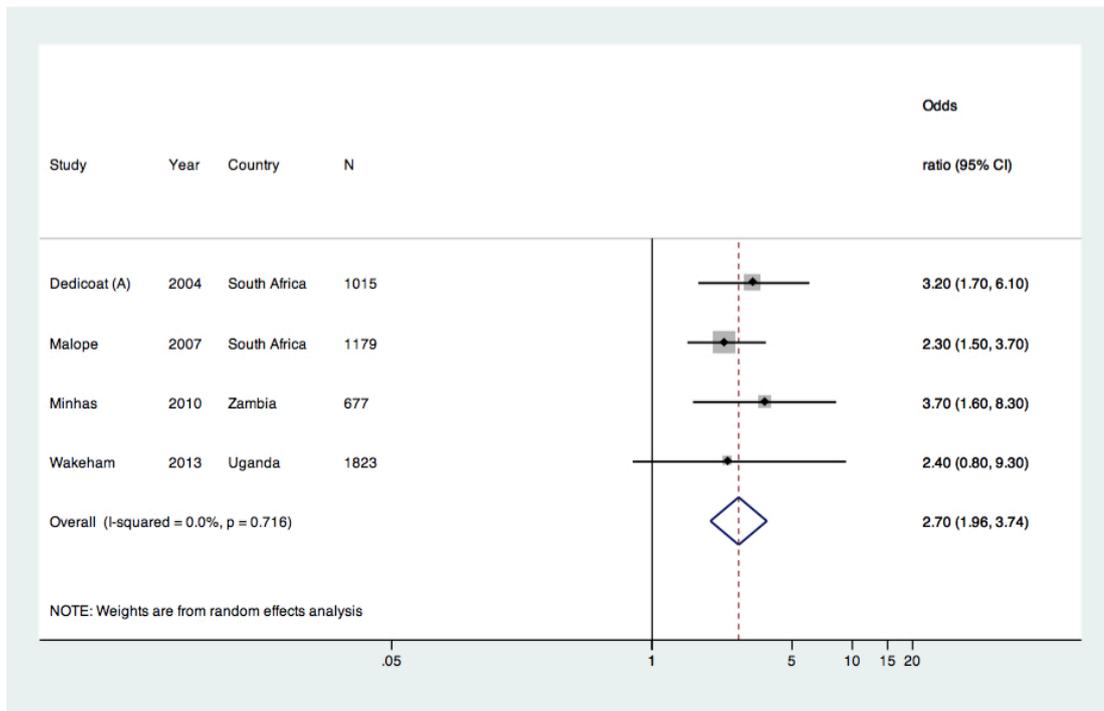


Figure 6.5: Random effect forest plot of studies identified that investigated the odds of KSHV seropositivity among HIV-infected children, compared to HIV negative children. ORF 73 results from the Dedicoat et al. study and both populations are presented but Butler et al. have been omitted. Studies are ordered by year of publication.

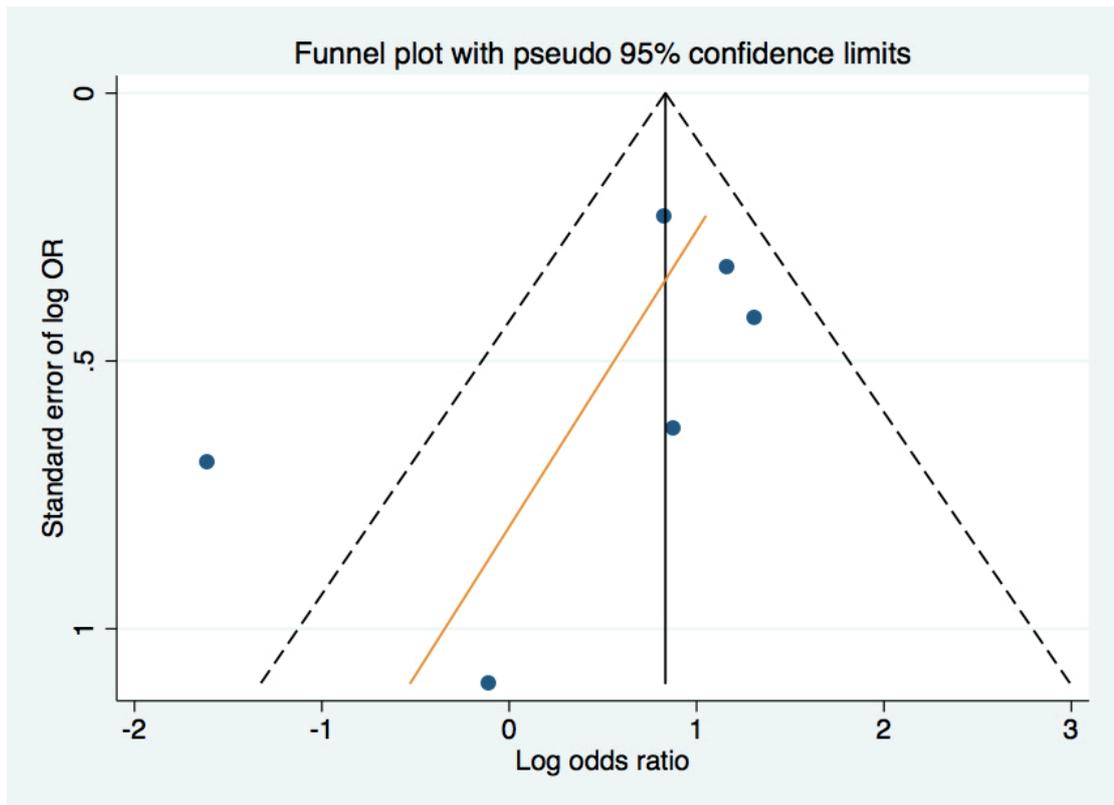


Figure 6.6: Funnel plot, using data from six studies of the association between antibodies to KSHV and HIV infection in children. Both the Ugandan and South African populations from Butler et al. are included. Only the K8.1 results from the Dedicoat et al study are included. Eggers regression line for funnel plot asymmetry is shown in orange.

The funnel plot (Figure 6.6) for examining the risk of bias in studies to investigate KSHV seroprevalence among children infected with HIV compared to HIV negative children, suggest possible publication bias. But the small number of published studies makes a firm conclusion impossible to draw. Neither Beggs' nor Egger's test suggested any evidence of small study effect, but the small number of studies means there is very low power to detect a relationship.

Table 6.3: Studies identified to investigate risk of KSHV prevalence in HIV infected children compared to children without HIV

Study first author	Year of publication	Location	Study size	HIV test	HIV prevalence among children (%)	KSHV assay	Definition of KSHV sero-positive	Childhood KSHV seroprevalence (%)	Age KSHV sample for children (years)	Restricted KSHV+ mothers	Adjusted ORs (95% CI)	Adjustment factors
Butler (A)	2009	South Africa	427	Not stated	6	Lytic IFA, EIA ORF65, EIA K8.1	Positive to any two tests or the IFA alone	8	1.5-9	na	0.9 (0.1-7.5)	Age, sex, study site
Butler (B)	2009	Uganda	989	Not stated	7	Lytic IFA, EIA ORF65, EIA K8.1	Positive to any two tests or the IFA alone	20	1.5-9	na	0.2 (0.06-0.9)	Age, sex, study site
Minhas	2010	Zambia	677	Sample at 18 and 24 months, confirmed with Western blot	6	BC3 MIFA and Sf9 MIFA (ORF65, K8.1, ORF73)	Positive to both BC3 and Sf9 (one antigen)	13	1	No	3.7 (1.6-8.3)	HIV mother, ever rash, HIV child, EBV infection
Dedicoat (A)	2004	South Africa	1015	HIV RNA	22	EIA K8.1	K8.1	7	1-6	Yes	3.2 (1.7-6.1)	Child age
Dedicoat (B)						IFA LANA	ORF 73	12			1.6 (0.4-5.8)	
Malope	2007	South Africa	1179	HIV antibody	10	K8.1 and ORF73 ELISA	Positive to either K8.1 and/or ORF73	16	1.6-10	No	2.3 (1.5-3.7) ¹	Nil
Wakeham	2013	Uganda	1823	PCR births, Western blot 18 months	1	K8.1 and ORF73 ELISA	Positive to either K8.1 and/or ORF73	11	1-5	No	2.4 (0.8-9.3)	Age of child, asymptomatic malaria parasitaemia, maternal KSHV serostatus, maternal education and use of mosquito net in the home

OR, odds ratio; CI, confidence interval.

¹Odds ratio calculated from raw data

6.6 Risk of bias within and across studies

The author's judgment of risk of bias in the studies including in the meta-analysis is presented in Table 6.4^{7-9, 13, 22, 191, 256}. All studies were considered to have moderate to high selection bias. Five of the studies rated with moderate selection bias were recruited through government run maternal-child health services (four antenatal wards and one vaccination clinic). Women attending local government services may differ in sociodemographic or clinical factors, including health status and income, from the general population. Malope et al. was rated as having high risk of selection bias¹³. This study used left over blood samples from paternity suits in South Africa. Butler et al. was also rated as high for selection bias; information on the selection of the South African population was limited and results for the Butler's Ugandan population were pooled from a household survey and a study conducted in a transfusion clinic. Children attending a transfusion clinic may be a biased population, either because they are unwell or because they are worried about a particular medical issue²⁵⁶. Attrition of individuals and/or loss of samples was judged as high in one study (Minus)⁹ because over half of the children did not return for a HIV test and no information was available on the children who did not re-attend.

The laboratory exposure (HIV) and outcome (KSHV) measures were judged to be at low and moderate risk of measurement bias respectively. Butler et al. could not be judged on measurement of HIV status as neither the paper or associated publications described the HIV testing strategy²⁵⁶. KSHV serological assays have potential problems with misclassification due to false positive and false negative rates. One study (Butler) sent samples for independent verification of KSHV antibody results²⁵⁶. Only one study (Malope) described the KSHV serology quality control steps and adjustment of cut-off values¹³. Two studies (Wakeham, Malope) stated that the KSHV serological assay was run blind^{7, 13}. No study presented analysis from a range of cut-offs or checked assay repeatability.

Confounding was deemed high in two studies (Butler and Brayfield)^{22, 256}. In one, Butler et al., no prior hypothesis was stated but results from both South African and Ugandan populations were adjusted for age, sex and study site. The odds of KSHV seropositivity in HIV-infected children compared to HIV-negative children in Butler et al. are not in keeping with the other published literature. The papers authors do not explore the reasons behind this. Brayfield et al. and Malope et al. only presented an unadjusted OR^{13, 22}. A criticism of all studies was the limited data on potential confounders.

Table 6.4: Authors judgement on risk of bias

Study first author	Year of publication	Cohort description	Selection bias	Attrition bias	Exposure (HIV status)	Outcome measure (KSHV serology)	Selective reporting	Confounding	Notes
Brayfield	2003	Cohort of healthy women admitted to a government labour ward. First born child recruited.	Moderate	Low	Low	Moderate	Low	High	No adjustment for confounders. KSHV assay not run blind.
Butler	2009	Household survey of mothers and their children in South Africa and pooled data from two studies in Kampala, Uganda; population-based cohort originally assembled for a malaria study and a clinic-based study of KSHV transmission by blood transfusion.	High	Unclear	Unclear	Moderate	Low	High	A prior hypothesis was not stated but for both South African and Ugandan populations ORs were adjusted for age, sex and study site. No information on HIV testing. KSHV assay not run blind. Results not in keeping with other published literature and this was not explored.
Dedicoat	2004	Mothers and their children attending a government vaccination programme in rural South Africa.	Moderate	Low	Low	Moderate	Low	Moderate	Recruitment strategy not in methods. Not stated if KSHV and HIV assay run blind.
Malope	2007	South African mothers and their children in paternity disputes.	High	Low	Low	Moderate	Low	Moderate	Laboratory staff running KSHV assay blinded. Comprehensive details on KSHV assay. Limited data on potential confounders.
Malope	2010	Black pregnant women attending a government antenatal clinic, recruited as part of national South Africa HIV study.	Moderate	Low	Low	Moderate	Low	Moderate	Not stated if KSHV and HIV assays conducted blind. Data on potential confounders limited.
Minhas	2010	Cohort of healthy women admitted to a government labour ward. First born child recruited.	Moderate	High	Low	Moderate	Low	Moderate	740/1240 children did not return for HIV test and were excluded from analysis. Limited data on potential confounders.
Wakeham	2011	Women attending a government antenatal clinic recruited into a RCT.	Moderate	Moderate	Low	Moderate	Low	Moderate	87% of mother-child pairs recruited had a samples for testing for KSHV. HIV seropositive mothers and mothers with malaria and less education more likely to have no sample. Laboratory staff running KSHV assay blinded.
Wakeham	2013	Cohort mother-child pairs. Women attending a government antenatal clinic recruited into a RCT.	Moderate	Moderate	Low	Moderate	Low	Moderate	Mother-child pairs with no samples available for inclusion more likely to include a HIV seropositive mother with less education. Mothers of children whose last available sample was at one year were generally younger and more likely to be HIV seropositive, compared to mothers of children followed up for a longer period. Laboratory staff running KSHV assay blinded.

6.7 Between study heterogeneity and publication bias

Heterogeneity in the summary estimate random effects models was moderate to high. Clinical and statistical heterogeneity between studies investigating potential associations between HIV and KSHV could account for this. Participants differed in age, a known risk factor for KSHV seropositivity in Africa. KSHV assay type varied, and false positive or false negative results could lead to differences in KSHV seroprevalence between studies. Misclassification of HIV test results are unlikely as studies used validated clinical assays. Studies varied in the prevalence of HIV-KSHV positive participants, and hence the power to detect a relationship. Studies differed in the factors used to adjust the multivariate analysis; some studies had a prior hypothesis and others adjusted for all factors significant at the 5% level in the multivariate analysis. Two studies only presented un-adjusted ORs. Study location also varied; epidemiological patterns of KSHV transmission may vary across Africa^{233, 256}. Geographical differences that may affect KSHV transmission include KSHV viral factors, host genetic susceptibility, environmental co-factors and human behaviours. It is notable that Uganda has a relatively high prevalence of endemic KS compared to South Africa, which may suggest underlying differences in population baseline risk for KSHV infection or KS development. Due to the small number of studies identified, formal statistical investigation of heterogeneity was not carried out. Unmeasured factors could also account for heterogeneity.

The prediction interval width depends on the heterogeneity or uncertainty between studies. Its advantage over calculating a summary estimate and 95% CI is that it indicates effect in an individual setting and may make the analysis more useful in clinical practice and for policy making³⁷¹. The prediction intervals calculated in this study were wide and in all cases crossed one, indicating that in some settings HIV may have no (or even a protective) effect on KSHV seroprevalence outcome. The large width is in part due to the small number of studies and large heterogeneity in this meta-analysis. A larger number of studies and

better knowledge of causes of bias are needed to calculate more robust prediction intervals.

Funnel plots to investigate publication bias were created for each objective, but the small number of studies made interpretation difficult. The Beggs and the Egger test for each outcome did not indicate any evidence of publication bias, but due to the relatively small number of studies, power was very small. Begg and colleagues propose that 25 studies or more are required for moderate power³⁷².

6.8 Within study limitations

KSHV serological assays are potentially prone to issues of misclassification and poor repeatability. No study presented data using a range of KSHV assay cut-offs or repeated measures to test robustness of associations. The studies were cross-sectional in design, and changes in associations with time were not investigated. Longitudinal data could potentially be important given the limitations of the KSHV serological assays and the potential for antibody titres to change biologically over time. Little or no data was available for length of time individuals had HIV or KSHV infection and immune status (CD4 count and HIV viral load) or ART status. One cross sectional study reported that KSHV saliva viral load is increased at higher CD4 counts in HIV positive individuals³⁷³, raising the possibility that KSHV may be more easily transmitted early in HIV disease. Long-term ART is associated immune reconstitution is associated with reduced KSHV viral activity, and lower KSHV antibody titres^{102, 104}. While all the studies were relatively large in overall size, the number of HIV-KSHV co-infected individuals for each of the three analyses was small. This can be noted in the relatively wide confidence of intervals for each study. In particular, Wakeham et al. had a small population of HIV infected children⁷.

6.9 Discussion

HIV is potentially an important co-factor for KSHV transmission. High prevalence areas of KSHV seropositivity in sub-Saharan Africa overlap with areas where HIV is endemic. In these regions dual infection with HIV and KSHV occurs, making the role of co-infection in transmission an important issue.

Within the context of the limitations of this analysis:

1. HIV seropositivity in mothers and pregnant women is associated with an increased prevalence of antibodies to KSHV,
2. Maternal HIV seropositivity was not associated with increased risk of detection of antibodies to KSHV among HIV-negative children, and
3. Children with HIV infection had an increased seroprevalence of antibodies to KSHV

HIV is implicated in the transmission of viral co-infections. Some viral co-infections such as HCV and HBV have common routes of transmission with HIV, increasing the risk of dual infection. Others, such as herpes simplex virus, increase the risk of acquiring HIV, and may also increase the risk of transmitting HIV³⁷⁴{Celum, 2004 #3746}. There is no current evidence to suggest that KSHV may impact HIV transmission. It is more plausible that HIV infection may increase an individual's risk of being infectious or vulnerable to KSHV. In this analysis HIV infection in mothers and children was associated with increased odds of detection of antibodies to KSHV compared to individuals without HIV. The progressive immunological decline associated with HIV infection makes individuals more vulnerable to infections. HIV could potentially cause an increase in antibodies to KSHV by increasing the risk of primary KSHV infection. But HIV infection may make antibodies to KSHV simply easier to detect by causing KSHV reactivation and a subsequent boost in antibody titre in a latently infected individual. The cross-sectional design of the studies makes it impossible to differentiate between these two

possible explanations of the observed association.

In this analysis, HIV infection did not appear to increase maternal transmission of KSHV. This is not in keeping with the original hypothesis. HIV infection is associated with KSHV reactivation and replication and increased oral, cervical and vaginal shedding of KSHV^{268, 375, 376}, which could increase the infectiousness of an individual. The small number of studies used in the calculation of the summary estimate makes conclusions difficult to draw with any degree of certainty.

6.10 Conclusion

There are clear limitations in the summary estimates for this meta-analysis but all relevant published studies have been included. With these limitations in mind this analysis suggests that HIV infection is associated with increased detection of antibodies to KSHV in mothers and children in sub-Saharan Africa. Large longitudinal studies are required to investigate KSHV transmission in mother-child pairs. Collection of KSHV viral load data may help investigate if HIV is facilitating transmission of KSHV either by effects on infectiousness or susceptibility, or both.

Chapter 7: Antibodies against Kaposi's sarcoma associated herpesvirus and the risk of developing HIV-associated Kaposi's sarcoma in a rural population cohort in Uganda

7.1 Chapter abstract

Background: In Uganda, KSHV is endemic and the HIV epidemic has resulted in marked increases in incidence of KS. No prospective studies of the evolution of KSHV antibody titres in relation to risk of HIV-associated KS have previously been conducted in this population. Consequently we conducted a case-control study nested within an existing longstanding population-based cohort to investigate whether the pattern of KSHV antibody titres in individuals who subsequently develop KS, differs over time, when compared to individuals who do not.

Methods: We identified 30 individuals who developed KS, from a cohort of HIV-infected people in rural Uganda. For each case, four individually matched controls were selected from among HIV and KSHV seropositive individuals not diagnosed with KS. Matching factors were sex, age band, and CD4 count at time of (pseudo)diagnosis. Serum samples were tested for antibodies against KSHV using two quantitative ELISAs: one to lytic phase K8.1 and the other to latent phase ORF 73. Antibodies measured in samples from two time periods were considered: after HIV seroconversion in the six years before KS diagnosis (using a pseudo date of diagnosis for controls), and prior to HIV seroconversion. Antibody titres were converted to doubling dilutions for the analysis. Data were analysed using linear regression with generalized estimating equations (GEE) to allow for repeated measures.

Results: In the six years prior to KS (pseudo)diagnosis, both K8.1 and ORF 73 antibody levels were higher in cases than in controls: the GEE regression estimate for the difference in K8.1 doubling dilutions between cases and controls was about four doubling dilutions ($p < 0.0001$); the estimated difference of ORF 73 antibody between cases and controls was about 3 doubling dilutions ($p < 0.0001$). Levels of antibodies for both K8.1 and ORF 73 in KS cases increased over time towards KS diagnosis, compared with controls ($p < 0.0001$). Prior to HIV seroconversion, the

level of K8.1 antibody was already higher in KS cases compared to controls.

Conclusion: In this nested case-control study of HIV-infected individuals from rural Uganda: (1) individuals who develop KS have a higher K8.1 and ORF 73 antibody titre, which is evident for years prior to diagnosis compared to those without KS; (2) K8.1 and ORF 73 antibody titre in KS cases tend to increase towards diagnosis and (3) K8.1 antibody titre was higher in those who went on to develop KS compared to those who did not, even prior seroconversion to HIV.

7.2 Background

KSHV is the aetiological agent responsible for KS⁴. HIV infection is the strongest predictor of risk for KS among KSHV infected individuals^{29, 136}. Worldwide, HIV-associated KS is the most common tumour arising in HIV-infected persons. The risk of developing KS in HIV-KSHV co-infected individuals in the USA, approaches 50 % in ten years¹⁹³. However, not all HIV-KSHV co-infected individuals develop KS³³. Understanding the evolution of biomarkers such as KSHV viral load or antibodies in asymptomatic KSHV-HIV co-infected individuals may be key to identifying individuals at highest risk of KS. Anti-retroviral therapy (ART) is associated with a decreased incidence of KS¹³⁸. Individuals who develop KS while receiving ART tend to have less extensive disease¹²⁴. Identifying those at greatest risk of KS may be particularly important for prioritizing ART treatment and cancer treatments in resource limited settings.

The detection of the KSHV genome in peripheral blood mononuclear cell (PBMC) samples taken prior to KS diagnosis has been associated with as much as a 10-fold increase in the risk of developing KS¹¹³, but viral DNA in biological fluids is often of low copy number, particularly in pre-clinical or early stage clinical disease^{125, 262}. Consequently the use of viral DNA as a predictive marker for development of KS is limited. Serological assays may offer an alternative marker for the risk of developing KS. Serology also has added benefits in being substantially cheaper than

techniques to detect DNA, such as the polymerase chain reaction (PCR), and is, relatively speaking, more easily transferable to a variety of environments. KSHV antibody tests could be simplified into a point-of-care test using lateral flow technology.

In studies from South Africa and Uganda, increasing antibody titres against KSHV were associated with an increased risk of developing KS^{128, 131}. There is some evidence, from longitudinal cohorts in North America and Northern Europe, that antibody titres against KSHV are increased preceding or at the onset of clinically evident KS^{33, 115-117, 127, 132-137}. Data from longitudinal studies of serological markers of KS risk in sub-Saharan Africa are lacking, and there remains substantial uncertainty about patterns of antibodies prior to diagnosis. It is unclear whether antibody patterns may differ in KSHV-HIV co-infected individuals in sub-Saharan Africa compared to North America and Northern Europe. In sub-Saharan Africa, KSHV is endemic in the general population^{16, 127-131}; it is often an infection in healthy children^{7, 9, 10, 13, 22, 256, 259, 260} and repeated exposure to KSHV is presumably a common occurrence. By contrast, in North America and Northern Europe, KSHV is rare in the general population and occurs predominantly in MSM¹⁹³, often at the time of or after HIV seroconversion¹¹⁶. Titres to antibodies to KSHV in an endemic setting, such as in Uganda, may be higher and patterns more evident.

7.3 Aims of chapter

In Uganda, KSHV is endemic^{8, 19, 222, 228} and the HIV epidemic has resulted in KS being the commonest reported cancer³². No longitudinal study of the evolution of KSHV antibody titres as a predictor of the risk of KS has been carried out in this population. Therefore, this study investigates:

- (1) whether, prior to diagnosis of HIV-associated KS, antibody titres to KSHV are higher in those who develop KS compared to HIV-infected individuals who do not develop KS;
- (2) whether the pattern of antibody titres in individuals who develop KS differs over time when compared to individuals who do not; and
- (3) whether the titre and pattern of antibody titres prior to HIV

seroconversion differs between those who become KS cases compared to those who do not.

7.4 Methods

Data from the RCC^{277, 279, 377}, based in Kyamulibwa, South-West Uganda, was used to identify 30 individuals with HIV-associated KS diagnosed between 2nd October 1990 and 5th July 2010. All cases were confirmed by a review of clinic notes by two clinicians. Five cases had histological verification of diagnosis and the remainder had clinical documentation only. Controls were selected using the following procedure: each case was classified by sex, age band (≤ 37 years or > 38 years), and CD4 count band (≤ 200 cells/ μ L, 201-500 cells/ μ L, ≥ 501 cells/ μ L) at the time of their KS diagnosis. Potential controls were then examined to find the time points at which their characteristics (sex, age and CD4 count band) matched those of the case. This time point was then taken as the pseudo-diagnosis date for that control and they were included in the dataset as matched to the corresponding case. Serum samples taken at the (pseudo)diagnosis time point, along with all available serum samples prior to (pseudo)diagnosis for cases and controls, were tested for antibodies against KSHV using a quantitative lytic K8.1 ELISA and latent ORF 73 ELISA as previously described in the general methods section^{13, 175}. Initially, 222 controls fulfilling the inclusion criteria were tested and 149 were found to be KSHV seropositive to either K8.1 and/or ORF 73 on the KS pseudo-diagnosis serum sample. For each case four individually matched controls were selected from HIV and KSHV seropositive people without a diagnosis of KS during the study period.

In a sub-study of patterns of K8.1 and ORF 73 antibodies prior to HIV seroconversion, 12 KS cases and 29 controls with a date of HIV seroconversion and available serum samples, were identified. All samples available prior to HIV seroconversion were selected for K8.1 and ORF 73 serology.

The K8.1 and ORF 73 ELISAs were performed at the Uganda Virus Research Institute (UVRI) by the study lead and a technician, both of whom were blinded to patient details. Randomised lists of samples were produced. Serum samples positive to antibodies against K8.1 at a 1:10 dilution were subsequently tested at doubling dilutions from 1:20 to 1:20,480 to measure titre. Serum samples positive to antibodies to ORF 73 at a 1:10 dilution were tested at doubling dilutions from 1:100 to 1:102,400. The titre value was the dilution before which the sample lost positive signal.

7.4.1 Statistical analysis

Data were analysed using Stata11SE (StataCorp LP, College Station, Texas, USA). Two time periods were of interest in this study. The main study analysed data from a time period up to 6 years prior to KS (pseudo)diagnosis when all study participants were HIV seropositive. The sub-study investigated a time period around and prior to HIV seroconversion.

The distribution of K8.1 and ORF 73 titres was skewed. To simplify the analysis and presentation of results, titres were converted to the number of dilutions. This number, from 0-10 for K8.1 and 0-11 for ORF 73, was used for all analyses and will be referred to throughout as "K8.1 doubling dilution" or "ORF 73 doubling dilution" (Table 7.1). Samples KSHV seronegative were coded zero.

Table 7.1: Antibody titres to KSHV and doubling dilution equivalents

Panel a: K8.1 titre and doubling dilutions

K8.1 Titre	Doubling dilutions
Negative	0
1:20	1
1:40	2
1:80	3
1:160	4
1:320	5
1:640	6
1:1280	7
1:2560	8
1:5120	9
1:10240	10

Panel b: ORF 73 titre and doubling dilutions

ORF 73 Titre	Doubling dilutions
Negative	0
1:100	1
1:200	2
1:400	3
1:800	4
1:1600	5
1:3200	6
1:6400	7
1:12800	8
1:25600	9
1:51200	10
1:102400	11

Results for HIV viral load were considered a suitable representation of an individual's viral load at KS diagnosis if within 100 days of (pseudo)diagnosis date. The characteristics of cases and controls were compared using the Mann-Whitney test, t-test and chi-squared test.

Data from the time period in the six years prior to (pseudo)diagnosis were analysed in two ways. Firstly, Odds Ratios (ORs) and 95% confidence intervals (CI) were calculated using conditional logistic regression, maintaining the case-control matching. For each individual, their mean doubling dilution, based on data from all time points within the six years, was calculated. These were then considered separately as predictors for case-control status in conditional logistic regression models. In order to investigate whether change in doubling dilution was associated with a subsequent diagnosis of KS, the rates of change (gradient) in each individual's doubling dilution over the time preceding (pseudo)diagnosis date were estimated using linear regression modeling of samples from all time points. These gradients were then included, along with the mean doubling dilution for each individual, in conditional logistic regression models. Secondly, linear regression models corrected using a generalized estimating equation (GEE) that ignores matching and fits a population averaged regression line correcting within individual correlations, were used. In the GEE analysis repeated measures of doubling dilution and time elapsed were modeled with an exchangeable correlation structure. Interaction terms between time elapsed and case control status were examined in order to assess whether doubling dilution changed across time differently in cases and in controls.

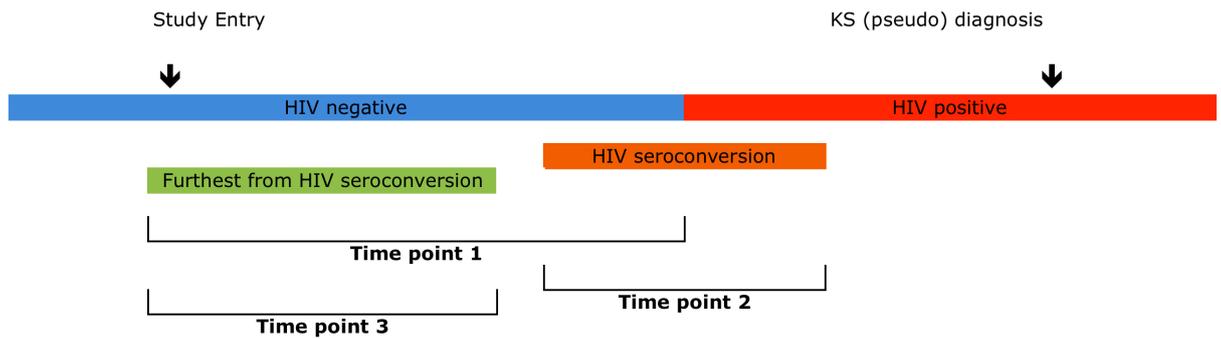


Figure 7.1: Time periods for study

For the sub-study of data from the period prior to HIV seroconversion, three time periods were considered (Figure 7.1):

Time point 1 - The mean doubling dilution for K8.1 and ORF 73 was estimated for the time period prior to HIV seroconversion when all individuals were HIV-negative (blue band from study entry).

Time point 2 - The mean doubling dilution for both antibodies was estimated at HIV seroconversion (orange band). The HIV seroconversion time period was defined as being within 6 months of the first HIV seropositive sample. Time points just before and just after seroconversion were included.

Time point 3 - In order to investigate any potential impact HIV seroconversion may have on antibodies to KSHV, the mean doubling dilution for samples taken at HIV seroconversion for cases and controls was compared to the mean doubling dilution of samples collected at least two years prior to HIV seroconversion (furthest from HIV seroconversion, green band).

Finally, the analysis was restricted to only those cases and controls K8.1 or ORF 73 seropositive at the (pseudo)diagnosis time point. Tests for statistical significance were derived from likelihood ratio test statistics. All p values were 2 sided and significance was considered at the 5% level.

7.5 Results

Results were available for 30 cases and 108 matched controls. All cases and controls were seropositive to either K8.1 or to ORF 73 at KS (pseudo) diagnosis, but not necessarily to both. At the KS diagnosis time point 93% (28/30) of the cases were seropositive to K8.1, as were 69% (74/108) of the controls at the pseudo-diagnosis time point. For ORF 73, at the KS (pseudo)diagnosis time point, 100% (30/30) of the cases were seropositive, as were 88% (95/108) of the controls. The median number of samples available from each individual was 6 (range 2-12). ART regimes for the KS cases were as follows: zidovudine(AZT)/lamivudine(3TC)/nevirapine(NVP) - five cases, stavudine/3TC/NVP - two cases; and for the controls: AZT/3TC/NVP - 16 controls, stavudine/3TC/NVP - two controls, AZT/3TC/efavirenz- two controls and unknown-one control.

7.5.1 Results from the basic analysis

Table 7.2 presents the characteristics of the study population. K8.1 and ORF 73 titre was higher in KS cases compared to controls at the (pseudo)diagnosis time point. Figure 7.3 and Figure 7.2 depict the distribution of titres and doubling dilutions in cases and controls at the (pseudo)diagnosis time point.

Table 7.2. Characteristics of study participants

Factor	KS case n=30	KS control n=108	P value
Sex, n(%)			Matching factor
Female	14 (47%)	52 (48%)	
Male	16 (53%)	56 (52%)	
Age, n(%)			Matching factor
Less than 37 years	14 (47%)	56 (52%)	
Older than 37 years	16 (53%)	52 (48%)	
CD4 count class, n (%)			Matching factor
Less than 200	16 (53%)	60 (56%)	
200-500	8 (27%)	24(22%)	
Greater than 500	6 (20%)	24 (22%)	
Median CD4 count at diagnosis, (IQR)	131 (40-468)	177 (109-491)	0.09 ^c
Detectable HIV VL at diagnosis, (%) ^φ	3 (15%)	12 (20%)	0.6 ^λ
Mean Log ₁₀ HIV VL at diagnosis, (SD) ^φ	4.16 (2.04)	4.15 (2.24)	0.9 ^β
On ART at time of diagnosis, n (%)	7 (23%)	21 (19%)	0.6 ^λ
Median time on ART in days, (IQR)	90 (89-176)	356 (181-720)	0.8 ^α
Comparison of K8.1 results between cases and controls			
Mean K8.1 optical density at (pseudo)diagnosis (SD)	2.4 (1.1)	1.5 (1.0)	<0.0001 ^α
Mean K8.1 optical density of all samples (SD)	2.5 (0.8)	1.4 (0.8)	<0.0001 ^α
Median K8.1 titre at (pseudo)diagnosis (IQR)	2640 (660-10240)	40 (0-80)	<0.0001 ^α
Median K8.1 titre of all samples (IQR)	320 (80-2560)	40 (0-80)	<0.0001 ^α
Mean K8.1 log titre at (pseudo)diagnosis (SD)	7.3 (2.7)	2.1 (2.1)	<0.0001 ^α
Mean K8.1 log titre of all samples (SD)	5.4 (1.8)	2.0 (1.9)	<0.0001 ^α
Comparison of ORF 73 results between cases and controls			
Mean ORF 73 optical density at (pseudo)diagnosis (SD)	1.8 (0.8)	1.5 (0.9)	0.005 ^α
Mean ORF 73 optical density of all samples (SD)	1.8 (0.9)	1.6 (0.9)	0.005 ^α
Median ORF 73 titre at (pseudo)diagnosis (IQR)	3200 (200-102400)	800 (0-25600)	<0.0001 ^α
Median ORF 73 titre of all samples (IQR)	3200 (100-102400)	800 (0-12800)	<0.0001 ^α
Mean ORF 73 log titre at (pseudo)diagnosis (SD)	3.6 (1.0)	2.6 (1.2)	0.0005 ^α
Mean ORF 73 log titre of all samples (SD)	3.4 (1.3)	2.7 (1.1)	<0.0001 ^α

^φ Missing data; 49 control, 10 case

^β Missing data; 1 control, 1 case

^α Ranksum test

^β TTest

^λ Chi-squared test

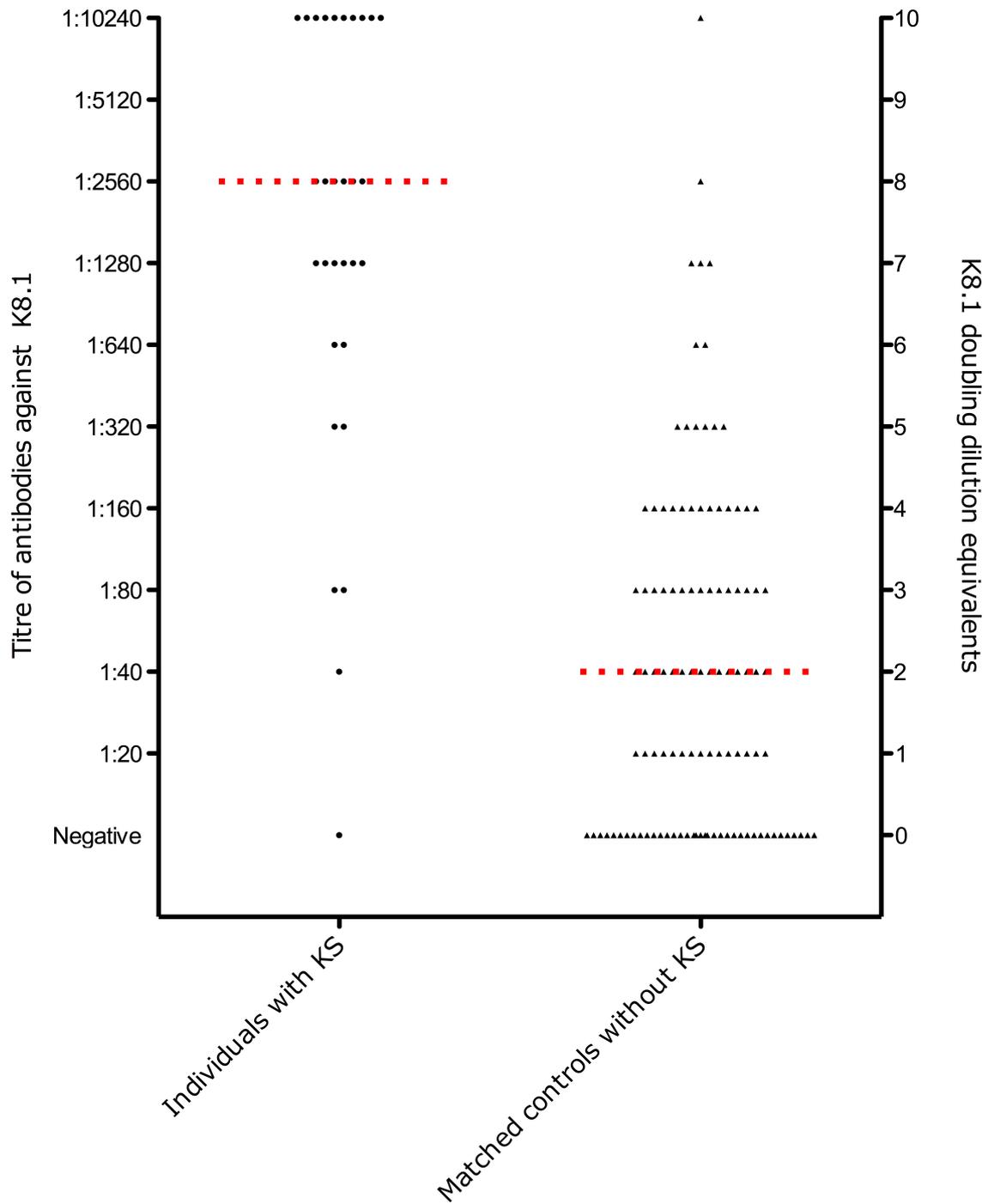


Figure 7.2: Distribution of titres of antibodies to K8.1 and doubling dilution equivalents in 30 HIV-positive individuals with Kaposi's sarcoma (KS) and 108 matched controls, at time of (pseudo)diagnosis. For each patient with KS, four matched controls without KS were selected and matched for sex, CD4 count group and age. The median titre in each group is indicated by a horizontal red line.

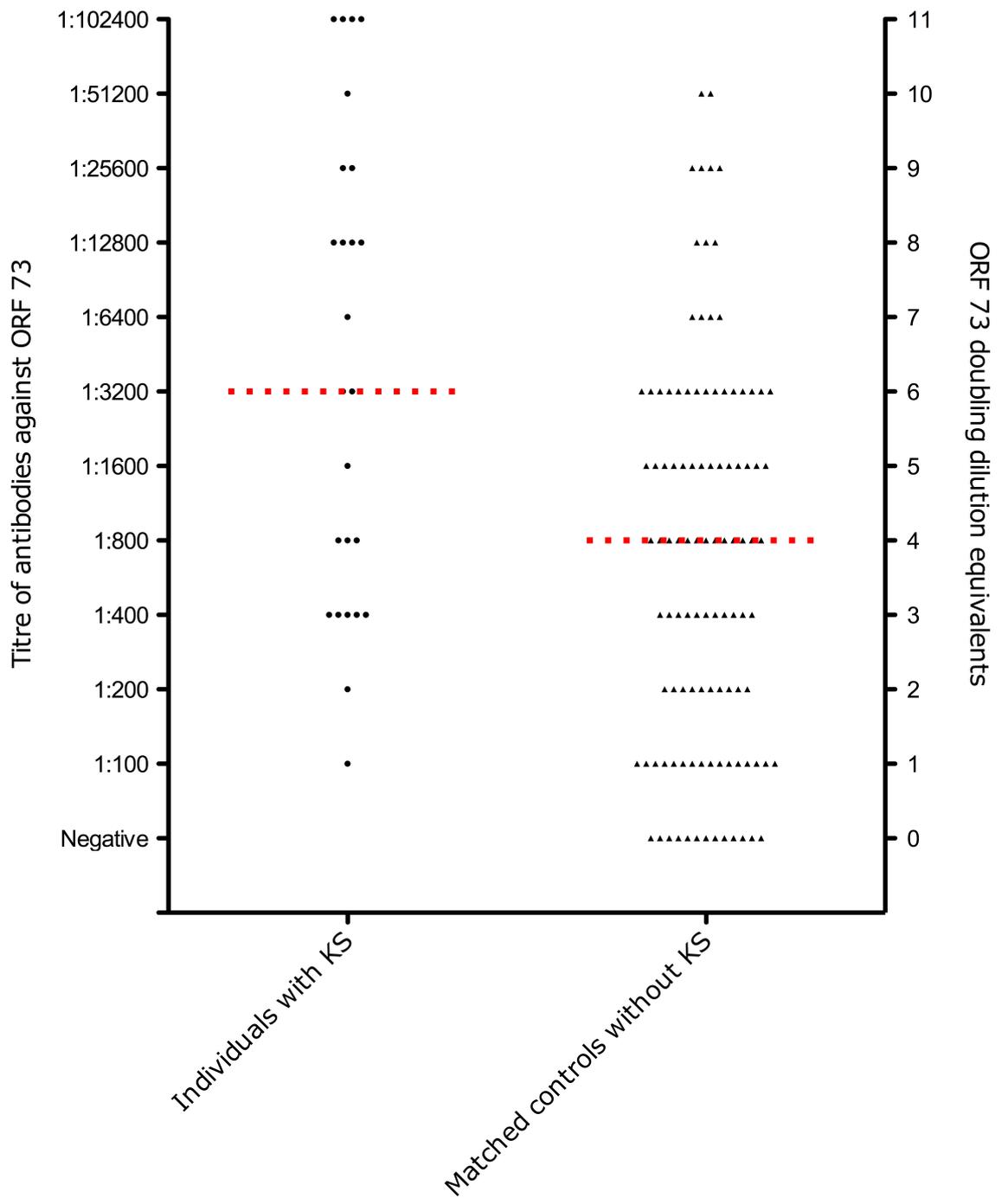


Figure 7.3: Distribution of titres of antibodies to ORF 73 and doubling dilution equivalents in 30 HIV-positive individuals with Kaposi's sarcoma (KS) and 108 matched controls at time of (pseudo)diagnosis. For each patient with KS, four matched controls without KS were selected and matched for sex, CD4 count group and age. The median titre in each group is indicated by a horizontal red line.

7.5.2 Results from conditional logistic models

The conditional logistic models investigating whether K8.1 or ORF 73 titre dilution was predictive of KS status are presented in Table 7.3, showing the odds of having KS approximately doubled for each additional doubling dilution (OR 2.2, 95% CI 1.5-3.1, $p < 0.0001$). Adjusting for ART status at the time of KS (pseudo)diagnosis did not materially change the results. The rate of change of K8.1 antibody level between the first and last time points for a case was approximately double the rate for a control (OR 2.1, 95% CI 1.0-4.3, $p = 0.04$). For ORF 73, each additional doubling dilution was associated with a one and a half-fold increase in the odds of developing KS (OR 1.5 95% CI 1.2-1.8, $p < 0.0001$). As with K8.1, adjusting for ART status did not change the results. The rate of change in ORF 73 antibody titre between the first and last time points was borderline (OR 1.0, 95% CI 0.6-1.9, $p = 0.9$).

Table 7.3: Odd ratio for being a case compared to being a control for each unit increase in doubling dilution

KSHV antibody	OR (95% CI) CLR crude	P value	OR (95% CI) CLR over time	P value	OR (95% CI) CLR over time adjusted	P value
K8.1	2.2 (1.5-3.1)	<0.0001	2.2 (1.5-3.1)	<0.0001	2.2 (1.5-3.1)	<0.0001
ORF 73	1.5 (1.2-1.8)	<0.0001	1.5 (1.2-1.8)	0.001	1.4 (1.2-1.8)	0.001

CLR, conditional logistic regression; CI, confidence interval; OR, odds ratio.

The CLR crude OR analyses all samples in the 6 years prior to (pseudo)diagnosis, does not take time into account nor control for any potential confounding factors.

The CLR over time OR takes into account time but does not control for any potential confounding factors.

The CLR over time adjusted OR takes into account time and is adjusted for on ART at time of (pseudo)diagnosis.

7.5.3 Linear regression model corrected using a generalized estimating equation

The GEE model accounts for the lack of independence in the repeated measurements by assuming within-patient correlation. For K8.1 doubling dilution the GEE crude estimate for the difference in cases and controls was four doubling dilutions, or a 16-fold increase (4.1, 95% CI 3.3-5.0, $p < 0.0001$). The interaction term for time was significant ($p < 0.0001$), indicating that K8.1 doubling dilution changes across time differently in cases and in controls. This is illustrated in Figure 7.4. For cases K8.1 increases with time towards KS diagnosis, but no time trend is apparent for controls. The difference in estimated K8.1 doubling dilution between cases and controls is greater nearer to the time of (pseudo)diagnosis. Adjusting for ART status at time of (pseudo)diagnosis did not materially change the results (3.9, 95% CI 2.8-5.0, $p < 0.0001$).

The estimated difference of ORF 73 doubling dilution from the GEE model between cases and controls was about 3 doubling dilutions or 8-fold (2.8 95% CI 1.5-3.9, $p < 0.0001$). The interaction term for the time was significant ($p < 0.0001$), signifying that ORF 73 doubling dilution changes across time differently in cases compared to controls (Figure 7.5). For cases titre increases with time towards KS diagnosis, but no time trend is present for controls. The estimated difference in ORF 73 doubling dilution is greater nearer to the time of (pseudo)diagnosis between cases and controls. Adjusting for ART status at time of (pseudo)diagnosis did not materially change the results (2.7, 95% CI 1.5-3.9, $p < 0.0001$).

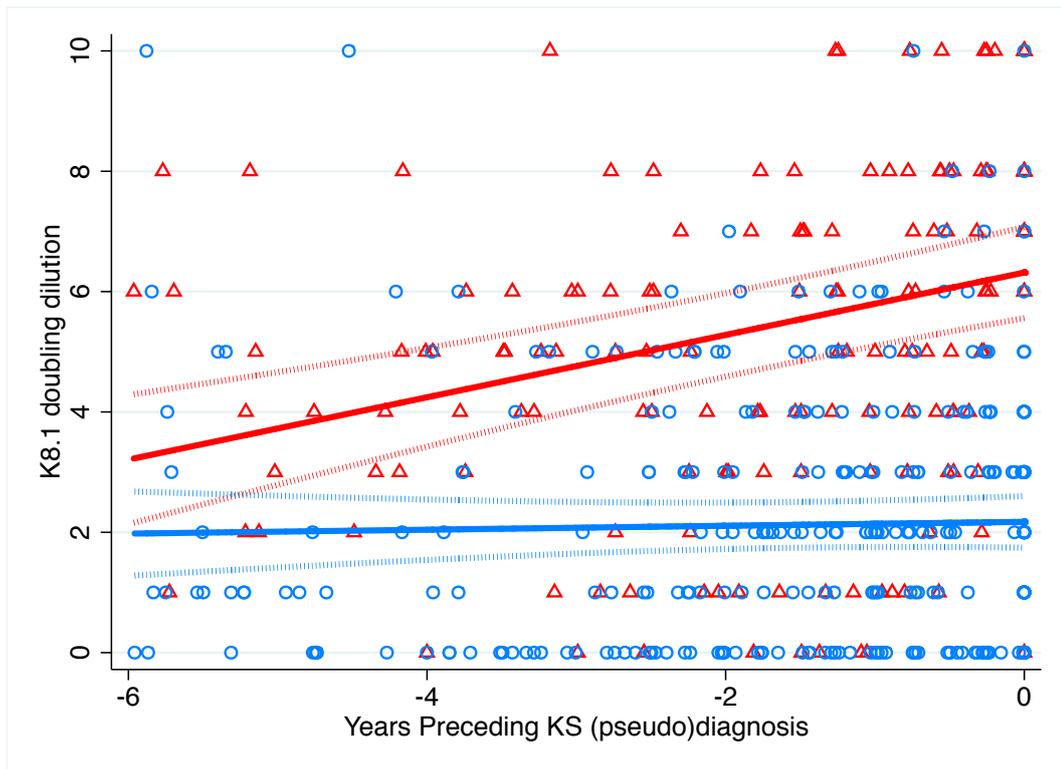


Figure 7.4: Graphical representation of the difference between K8.1 doubling dilution for KS cases and non-KS controls using a linear regression model corrected by a generalized estimating equation (GEE). Red, KS cases. Blue, KS controls. Dashed lines represent 95% CI.

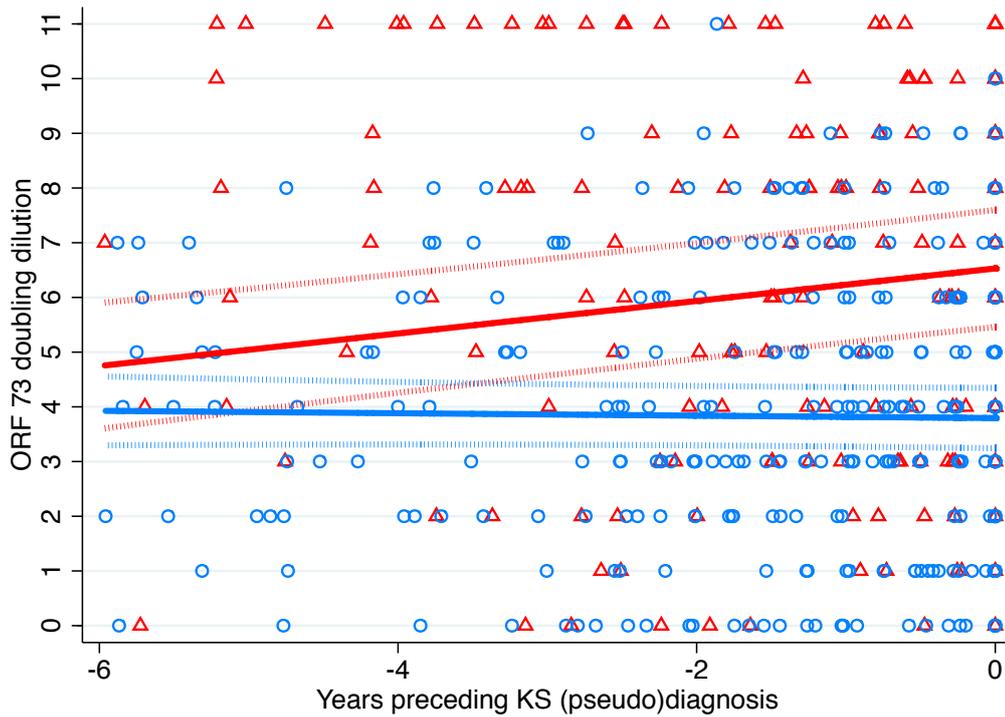


Figure 7.5: Graphical representation of the difference between ORF 73 doubling dilution for KS cases and non-KS controls using a linear regression model corrected by a generalized estimating equation (GEE). Red, KS cases. Blue, KS controls. Dashed lines represent 95% CI.

7.5.4 Antibodies to K8.1 and ORF 73 prior to HIV seroconversion

The median number of days from HIV seroconversion to KS was 1956 (range 432-5454) [or 5.4 years, range 1.2-15.0 years]. All except four cases (13 % [4/30]) were K8.1 seropositive at HIV seroconversion and all seroconverted prior to KS diagnosis. All cases were ORF 73 seropositive at the serum sample closest to HIV seroconversion. The characteristics of the 12 cases and 29 controls analysed to investigate the impact of HIV seroconversion on antibodies to KSHV are presented in Table 7.4.

The mean K8.1 doubling dilution was higher in KS cases compared to controls in both the time period furthest from HIV seroconversion and in the HIV-negative period (Table 7.5). In the peri-HIV time period (within 6 months of HIV seroconversion) samples from individuals who went on to develop KS had a higher point estimate of K8.1 mean doubling dilution when compared to those who did not develop KS, but the difference did not reach statistical significance. The mean doubling dilution for ORF 73 for cases compared to controls was not statistically different at the period furthest from HIV seroconversion, in the HIV-negative period or at HIV seroconversion. There was no statistical difference in mean doubling dilutions for KS cases between samples furthest from HIV seroconversion and samples at HIV seroconversion for either K8.1 ($p=0.5$) or ORF 73 ($p=0.3$).

Table 7.4. Characteristics of study participants in time period prior to HIV seroconversion

Factor	KS case n=12	Control n=29	P value
Sex, n (%)			
Female	5 (42%)	13 (45%)	0.6 ^λ
Male	7 (58%)	16 (55%)	
Age, n (%)			
Less than 37 years	8(67%)	20 (69%)	0.5 ^λ
Older than 37 years	4 (33%)	9 (31%)	
CD4 count class, n (%)			
Less than 200	6 (50%)	16 (55%)	0.2 ^λ
200-500	2(17%)	10 (34%)	
Greater than 500	4 (33%)	3(10%)	
Median CD4 count at diagnosis, (IQR)	186 (35-509)	159 (115-444)	0.9 ^α
Detectable HIV VL at diagnosis, (%) ^φ	3(27%)	3(13%)	0.3 ^λ
Mean Log ₁₀ HIV VL at diagnosis, (SD) ^φ	3.4 (2.4)	4.5 (2.0)	0.2 ^θ
On ART at time of diagnosis, n (%)	5 (42%)	4(14%)	0.05 ^λ
Median time on ART in days, (IQR)	89 (89-90)	273 (89-736)	0.09 ^α
Time			
Median number days between first sample available and HIV seroconversion (IQR)	2661 (934-3701)	1480 (554-2103)	0.6 ^α
Range of days between first sample available and HIV seroconversion	172-4830	98-5303	

^α Ranksum test

^θ TTest

^λ Chi-squared test

Table 7.5: Comparison of mean doubling dilutions for different time periods for 12 KS cases and 29 controls

KSHV antibody	Time period							
	Period furthest from HIV seroconversion		HIV negative		HIV seroconversion		HIV positive	
	KS case (number samples=19)	Control (number samples=29)	KS case (number samples=24)	Control (number samples=40)	KS case (number samples=11)	Control (number samples=18)	KS case (number samples=133)	Control (number samples=171)
Mean K8.1 doubling dilution (SD)	4.7 (2.2)	3.7 (1.9)	4.8 (2.1)	3.6 (2.0)	4.7 (2.3)	2.8 (2.0)	5.2 (2.8)	2.1 (2.2)
P value ^a	0.02		0.02		0.09		<0.0001	
Mean ORF 73 doubling dilution (SD)	4.4 (3.2)	4.1 (2.3)	4.9 (3.0)	4.0 (2.2)	5.2 (2.7)	3.5 (2.3)	6.0 (3.4)	3.9 (2.6)
P value ^a	0.6		0.2		0.2		<0.0001	

SD, standard deviation

^a Ranksum test

Restricting the results to K8.1 or ORF 73 seropositive individuals at (pseudo)diagnosis did not change the results (data not shown).

7.6 Discussion

7.6.1 K8.1 and ORF 73 antibody titre in individuals with HIV-associated Kaposi's sarcoma

In this nested case-control study of HIV-infected individuals from rural Uganda, doubling dilutions have been used to investigate potential differences in K8.1 and ORF 73 antibody titre between individuals who develop HIV-related KS and HIV seropositive individuals who do not. The study has shown that individuals who develop KS: (1) have a higher K8.1 and ORF 73 antibody titre compared to those without KS up to six years prior to (pseudo)diagnosis; and (2) that K8.1 and ORF 73 antibody titre tends to increase with time towards KS diagnosis. Individuals who did not develop KS had relatively stable titres over time.

Increasing antibody titres against KSHV have been associated with an increased risk of developing KS both in HIV infected and uninfected people^{117, 128, 131}, although the absolute risk for a given median KSHV antibody titre was much greater in HIV-infected patients compared to HIV-negative patients; the respective risk ratios for HIV-associated versus HIV-negative KS compared to KSHV seronegative individuals were 48 versus 2 at titres of 1:200, 62 versus 6 at titres of 1:51,200, and 1683 versus 12 at titres of 1:204,800¹³¹. The results presented in this study are also in keeping with studies of KSHV antibodies prior to KS diagnosis from North America and Northern Europe. In these regions high titre to KSHV antibodies are associated with risk of development of KS in renal transplant recipients and in HIV-infected individuals¹¹⁷. A study from Italy investigated the risk of KS in a HIV-KSHV co-infected cohort using an immunofluorescence assay; bright cytoplasmic staining at a dilution of 1:5 or more were considered positive and further dilutions for titres of 1:25 and 1:125 were carried out. The risk of KS increased with increasing anti-K8.1 KSHV antibody titres, reaching more than 40% 10 years after HIV seroconversion for individuals with an antibody titre of 1:125¹³⁶. A Swiss study determined change in proportion of samples seropositive to KSHV between KS cases and controls: the reactivity in KS

cases steadily increased throughout the observation time of about five years compared to controls³³.

Due to the paucity of longitudinal studies and the variation in serological techniques it is not possible to compare antibody titres to KSHV between sub-Saharan Africa, North American and Northern European cohorts. Yet difference in titre and patterns of titres between KS cases and controls in this HIV seropositive Ugandan cohort were certainly evident and are consistent with data from elsewhere.

7.6.2 K8.1 antibody titres and HIV seroconversion

The median time from the point of infection with both HIV and KSHV, to development of KS, was similar in this cohort to Western cohort studies. In an Italian cohort, predominantly comprising men who have sex with men, the median time to develop KS was 5.6 years (range, 2.2–9.9 years) from the time of HIV seroconversion and 4.2 years (range, 1.4–7.6 years) from the diagnosis point of KSHV-HIV co-infection¹³⁶. In Western cohorts, KSHV seroconversion is reported to be more common at the same time as HIV infection or in individuals who are already HIV-positive^{115, 116, 378}. In the study reported here all cases were ORF 73 seropositive at or before HIV seroconversion. All but four KS cases in this study were K8.1 seropositive at or before HIV seroconversion and all seroconverted prior to KS diagnosis. This may be because in the West the at-risk group for KSHV-HIV co-infection is men who have sex with men, and risk behaviours for KSHV are closely related to those of HIV; whereas in this study the majority of individuals were KSHV infected prior to HIV seroconversion and non-sexual factors including those in early childhood are likely the important determinants of primary KSHV infection.

In the smaller sub-set of samples analysed prior to HIV seroconversion, KS cases had higher antibodies to K8.1 prior to contracting HIV and at HIV seroconversion, compared to controls. For ORF 73 there was no difference between cases and controls mean doubling dilutions at HIV

seroconversion, or at the two HIV-negative time periods (“HIV-negative” and “period furthest from HIV seroconversion”). However, the small number of individuals in this sub-set analysis limits the power of the study. But the results suggest that HIV seroconversion has little if any impact on KSHV antibody titre, since the results at the time period furthest from HIV seroconversion and the time period around HIV seroconversion are similar. In contrast, a cohort study from Amsterdam reported that HIV seroconversion was associated with a significant boost in antibody titre³⁷⁸. KS cases had a higher antibody titre to K8.1 prior to HIV seroconversion than controls. The KSHV glycoprotein K8.1 is expressed during the lytic cycle³⁷⁹. In the lytic phase KSHV is reactivated with the initiation of extensive viral DNA replication and gene expression, resulting in production of new viral particles³⁸⁰. Viral reactivation is crucial for KSHV pathogenesis and contributes to the progression of KS^{381, 382}. Risk of tumour development is associated with increasing KSHV antibody titres to the lytic protein K8.1^{225, 33}. High antibody titres to K8.1, are evident in this cohort prior to HIV seroconversion. A potential explanation for high antibodies to K8.1 and the limited impact on this of HIV is that in KSHV endemic countries such as Uganda viral reactivation is being driven by other co-factors. Viral reactivation and lytic activity of KSHV could also be due to repeated exposure to the virus in this prevalent setting. Alternatively, antibody titres may be an inadequate indicator of KSHV viral activity; a marker such as KSHV viral load may be required.

7.6.3 Methods of statistical analysis

A number of statistical techniques were used in the analysis of this study. It is reassuring to consider that whether using simple methods, for example comparing median K8.1 antibody titres, or more complex techniques such as conditional logistic regression or linear regression adjusted for correlation with individuals, the results remain consistent with each other. The potential advantage of the conditional logistic regression (CLR) model is that it explicitly allows for the individual case-control matching. It may also be more biologically intuitive model

because in this model case/control status is the outcome, which is a consequence of antibody level. The disadvantage of the CLR approach (compared to linear regression adjusted using GEE) used in this thesis is that fitted changes in antibody level over time only used the first and last time points as a gradient and did not take into account intermediate data points. This may account for the null result for the rate of change analysis for ORF 73 in the CLR model. The model presented of linear regression adjusted using GEE uses the antibody dilution as the outcome rather than case-control status. It allows for the fact that each individual has multiple time points and does not make assumptions about patterns of antibody change between time points. It also allows examination of trend in titre over time, in addition to allowing investigation of differences in the trend between cases and controls. This model also allowed production of a graph, an aid to interpretation. A potential disadvantage of this model is that it does not allow for the matching.

The only small difference in results between conditional logistic regression model and GEE is that the estimate of change in antibody level over time is stronger (smaller p-value) for the GEE. Possible reasons for this are that the GEE takes into account all time points, if there is a large intermediate value then this is taken into account in the GEE analysis but not in the CLR analysis, or that GEE does not allow for the matching.

As an alternative to using linear regression adjusted using GEE a full random effects model (random intercept linear regression) was also used to estimate KSHV antibody level (results not shown). The results were similar between the two approaches. The full random effects model has the advantage when inference about individual variability in a population is of major interest whereas the GEE approach models group or population differences.

7.6.4 Importance of determining risk of Kaposi's sarcoma in sub-Saharan Africa

In HIV-infected individuals ART is associated with a reduction in the incidence of KS¹³⁸, and among those who do develop KS the disease is less extensive³⁸³. Targeting individuals in need of ART may be particularly important in resource-poor settings where drugs to treat HIV are in short supply and clinicians may have to prioritize individuals to receive treatment. Furthermore, modalities to treat or palliate more bulky symptomatic tumours, including radiotherapy and chemotherapy, may not be available. Currently no ART guidelines for early intervention in preventing KS are in general practice; in part this is due to the inability to predict those at greatest risk of KS. This is in contrast to other co-infections such as the hepatitis B virus (HBV) and hepatitis C virus (HCV). ART is recommended in HBV infection in HIV co-infected individuals, regardless of CD4 cell count as treatment prevents liver fibrosis, reduces risk of cirrhosis, end-stage liver disease, and hepatocellular carcinoma (HCC)³⁸⁴. Recent guidelines recommend treatment of HIV in HCV-infected patients, regardless of HIV RNA and CD4 cell counts as ART slows liver fibrosis progression rates³⁸⁵ and development of HCC³⁸⁶.

The use of anti-herpesvirus treatment to prevent or influence the course of KSHV infection and KS might also be considered but has been disappointing. Reduction in KSHV replication with Valganciclovir has been reported³⁸⁷. Small reductions in the risk of KS in a UK based HIV-positive cohort were reported with both foscarnet and ganciclovir; acyclovir had no effect³⁸⁸. But this potential benefit has not been replicated^{389, 390}. The null findings are also supported by in-vitro studies that continued to detect KSHV in the PBMCs of patients receiving foscarnet and/or ganciclovir³⁹¹. New agents that may target specific KSHV proteins involved in angiogenesis for prevention of KS are currently under development.

7.7 Conclusion

HIV-infected individuals in Uganda who develop KS have high antibody titres prior to diagnosis compared to those who do not develop KS. Antibody titres increase with time towards KS diagnosis in individuals that develop KS compared to those who do not. Prior to HIV seroconversion K8.1 antibodies may already be higher in those who go on to develop HIV-associated KS compared to those who do not.

7.8 Study comments, strengths, and limitations

This is the largest longitudinal study to date examining patterns of antibody titres to both K8.1 and ORF 73 in individuals with HIV-associated KS, in a KSHV endemic population in sub-Saharan Africa. CD4 count was well matched and this was of particular importance since the CD4 count is the most important factor associated with the development of KS. In a case series, for a new diagnosis of KS while on treatment with ART, the rate ratios for developing KS in patients with CD4 counts less than 200, and 200-499 cells/mm³ were about 19 and 4, compared to those with ≥ 500 cells/mm³³¹³⁹. Given that the development of KS is closely associated with CD4 count it is not surprising that low CD4 count is associated with increased KSHV viral loads and elevated KSHV antibody titres¹¹³. A likely consequence of matching CD4 count was that cases and controls were similar for HIV viral load and whether or not they were on ART. It is perhaps surprising, given the CD4 counts of this cohort, that relatively few had a detectable HIV viral load. While CD4 counts are a more important prognostic indicator in late stage HIV disease compared to HIV viral load³⁹², the rate of CD4 cell decline correlates with the HIV viral burden³⁹³.

The definition of case-control status in this study relied in the majority of cases on clinical diagnosis made at the time of clinical check-ups. While large dermal KS lesions are relatively easy to diagnose, lack of histological confirmation may have lead to misclassification of cases and controls and small dermal lesions may have been missed. Further, no

diagnostic tests were available to ensure controls did not have visceral involvement. Date of KS diagnosis was based on the clinical review date, which may have been affected by how regularly the HIV clinic was attended.

The measure of exposure within the GEE analysis was antibody titre to KSHV antigens K8.1 and ORF 73. The strengths and weaknesses of the serological assay have been discussed in the introductory chapters of this thesis. The result of this study relies on the validity of measured antibody titre.

The relatively small numbers of individuals on ART at KS (pseudo)diagnosis, in spite of low CD4 counts, most likely reflects local practices and limited access to ART in Uganda at the time these cases were diagnosed. Further studies will be needed to explore ART and risk reduction of developing KS in resource poor settings. There is a theoretically stronger argument for the potential need for early intervention with ART to prevent KS in sub-Saharan Africa, compared to other regions, because: (1) KSHV infection is endemic; (2) individuals present with low CD4 counts³⁹⁴; (3) access to ART may be limited; and (4) interventions to control or palliate KS are largely lacking.

Unfortunately no data on staging KS in terms of the extent of the tumour, immune status and systemic illness was available. Oral lesions are the initial site in about 15% of cases in European and North American populations³⁹⁵, while the gastrointestinal tract is involved in approximately 40% of patients with KS at initial diagnosis and in up to 80%, at autopsy³⁹⁶. These types of lesions can cause problems with nutrition, which may additionally impact on immune function and hence risk of KS and/or antibodies to KSHV.

Information on any potential immune reconstitution inflammatory syndrome (IRIS) would also be of interest and was not collected in this cohort. IRIS is used to describe a collection of host responses that can occur following the initiation of ART. In addition to worsening symptoms

from preexisting infections, the initiation of ART has been associated with progression of KS³⁹⁷. It may be important to investigate whether clinical worsening of KS is accompanied by loss of KSHV viral control, increased anti-KSHV antibodies and KSHV viral load.

Other limitations of this study include the fact that some individuals had few samples available for testing and the timing of samples was not at regular intervals. Furthermore, HIV seroconversion data was only available in a small number of individuals and the number of samples available for K8.1 titre was small. Due to limitations in data collection it was not possible to investigate mortality or response of KS to ART or other treatments.

7.9 Further directions

Recent advances in biotechnology offer the hope of KS screening and prevention, along with improved drug development. Unfortunately neither a biomarker to accurately predict individuals at greatest risk of KS, nor a preventative therapy is currently available. At present, once KS is clinically evident the main aims of treatment are symptom palliation, prevention of disease progression, and reducing psychological stress. The median survival for advanced KS in first world centers using ART in combination with systemic chemotherapy is about one year^{398, 399}. Where available, treatment is primarily centered on ART, and systemic chemotherapy is normally reserved for individuals with advanced disease. Advanced disease is defined by the AIDS Clinical Trial Group and includes presence of gastrointestinal or extensive oral disease, a CD4 cell count <200/ μ L and a history of opportunistic infections⁴⁰⁰. Liposomal anthracycline agents in Western settings have response rates of about 50% in advanced KS⁴⁰¹ and may marginally increase median survival³⁹⁹. Biological indicators specific for detecting early risk of developing HIV-associated KS are a key area for future research and are discussed below.

Increasing KSHV viral load is associated with risk of KS occurrence. Further studies with HIV viral load data would be of interest to clarify its role as a predictive marker of the risk of KS in sub-Saharan Africa and to clarify the relationship between HIV viral load and antibodies to KSHV in a cohort in this setting. In a study of HIV treatment-naïve adults with HIV-associated KS in Zimbabwe, KSHV DNA levels in PBMCs of <660 copies/ml prior to ART treatment were associated with a greater survival and tumour regression⁴⁰². Whether KSHV DNA levels could be used as a clinical tool to stratify individuals with KSHV-HIV co-infection into KS risk groups is currently not known. Guidelines for treatment to prevent pathology caused by other oncogenic infections are well recognized; anti-viral therapy based on HBV DNA levels is well validated⁴⁰³.

The results from this study and others suggest that antibody titre to KSHV proteins may help characterise increased KS risk. Current serological assays have limitations and are only deemed valid for epidemiological studies rather than those in clinical settings¹⁷³. Testing for antibodies to KSHV in addition to K8.1 and ORF 73 may improve clinical validity. The transition from KSHV latency into the lytic cycle is a critical step in disease pathogenesis. The expression of lytic genes has been defined for immediate-early (IE) and late genes. IE genes include ORF K8.1 and ORF 50. The KSHV ORF 50 gene product is known as the 'replication and transcriptional activator' or Rta, and plays a critical role in the switch from latency to lytic replication⁴⁰⁴. Transcript expression of IE genes or their protein products could be prime candidates for markers of initial KSHV pathogenesis. Testing for a panel of multiple KSHV antibodies may also improve utility. Studies are underway by VOS, NCI to define a panel of antigenic epitopes that may refine serology into a more predictive tool. Multiplex platforms could be used to detect multiple antibodies in a single biological sample.

Whether changes in KSHV viral load and/or antibody titres could be used as a risk assessment tool remains unclear. It seems likely that a clinically valid test will require the combination of molecular or immunological measures. Viral parameters may be required in

conjunction with host factors. Important viral measures could be viral microRNA profiles^{151, 152} or viral IL-6 levels⁴⁰⁵. Potential host molecular markers could include activation events of nuclear factor kappa B (NFκB) pathways⁴⁰⁶. NFκB, involved in cellular responses to stress and dysregulation, has been linked to KS development. Vascular endothelial growth factor (VEGF) is a protein produced by cells that stimulates vascular development and angiogenesis. Expression of VEGF has been linked to poor prognosis in a number of cancers. Early immunological changes that may herald KSHV pathogenesis include changes in the profile of KSHV-specific T cell responses. Human cytokine levels are measurable in disease; human IL-6 has an important role in pathogenesis of MCD⁴⁰⁷ and the chemokine receptor CCR5 has been implicated in HIV-associated cancers⁴⁰⁸.

In conclusion, clinical tools to screen for risk of KSHV pathogenesis and therapies to prevent KS in those at greatest risk are urgently required. Given recent advances in technology and our understanding of KSHV oncogenesis, much progress is hoped for in the coming decade.

Chapter 8. Conclusion

8.1 Introduction

Infectious agents are responsible for over 20% of cancer deaths in the developing world⁴⁰⁹. These cancers are potentially preventable. The scale of the problem is significant. Nearly 60 000 people in sub-Saharan Africa alone died in 2002 from cancers caused by co-infection with KSHV and HIV⁴¹⁰. In Uganda, KS is the highest registered cancer and the attributed morbidity and mortality are significant³². With the ultimate goal of reducing the burden of KS, there is an opportunity to prevent KS at a number of points on the trajectory of KSHV infection from transmission to frank malignancy. This thesis potentially contributes knowledge in two key areas of KS prevention: preventing transmission and preventing progression to KS.

8.2 Chapter outline

In this final chapter the key results from this thesis are discussed within the context of plausibility, potential public health impact and future research directions. The first section relates to *KSHV transmission* and includes the following:

1. hypotheses for the influence of malaria on KSHV infection;
2. the potential impact of public health measures to control malaria on KSHV transmission;
3. the interaction between HIV and KSHV and how preventative HIV interventions may affect the spread of KSHV;
4. the co-factors that may be important for KSHV transmission; and
5. future research directions.

The second part of this chapter deals with *KS prevention* and has the following sections:

1. the search for risk factors for HIV-associated KS oncogenesis;
2. speculation on malaria as a co-factor for KS and potential research directions; and

3. the development of a risk assessment tool for KS.

This, the final chapter of this thesis closes with an overall final conclusion.

8.3 Kaposi's sarcoma associated-herpesvirus transmission

8.3.1 Hypotheses for the influence of malaria on Kaposi's sarcoma associated-herpesvirus infection

One of the novel findings presented is that malaria parasitaemia is associated with antibodies to KSHV. If this result reflects a true interaction between malaria and KSHV it may be due to a number of mechanisms including the following: (1) polyclonal activation of B-cells leading to an increase in antibody titre above the cut-off for defining seropositivity; (2) an increase in primary KSHV infection; and (3) lytic reactivation of KSHV, with the resulting higher KSHV viral load leading to an increase in antibodies. In contrast to the first scenario, it is plausible that scenarios two and three could increase the spread of KSHV (Table 8.1). Lytic reactivation of KSHV infection is known to be key to the maintenance of lifelong KSHV infection within the host and for transmission to new hosts⁴¹¹. KSHV transmission is likely via saliva^{26, 326}. A potential hypothesis is that malaria could cause reactivation of latent KSHV, increased oral shedding of KSHV and thus increase the chance that a host will transmit to a new individual (Figure 8.1). Indeed, *P. falciparum* is known to trigger reactivation of other herpesviruses, EBV²⁵³ and herpes simplex³⁶⁰. One could further postulate that malaria could increase vulnerability to secondary infection with KSHV, possibly by altering the mucosal barrier or immune response.

Table 8.1: Knowledge and questions relating to malaria and KSHV infection

Widely acknowledged facts relating to KSHV	Proposed questions
Reactivation of KSHV is important for dissemination within the host and maintenance of latency	Does malaria increase KSHV reactivation within the host?
Reactivation of KSHV is important for transmission between hosts	Does malaria increase KSHV transmission?
KSHV is likely transmitted via saliva	Does malaria increase KSHV oral shedding?
KSHV is restricted to particular populations	Does malaria account in some part for the distribution of KSHV infection?
Co-factors influencing vulnerability to KSHV infection are largely unknown	Does malaria increase vulnerability to KSHV infection?
Reactivation of KSHV from latency is a critical pathogenic step during the progression to KS	Does malaria inhibit host immune control of KSHV replication?

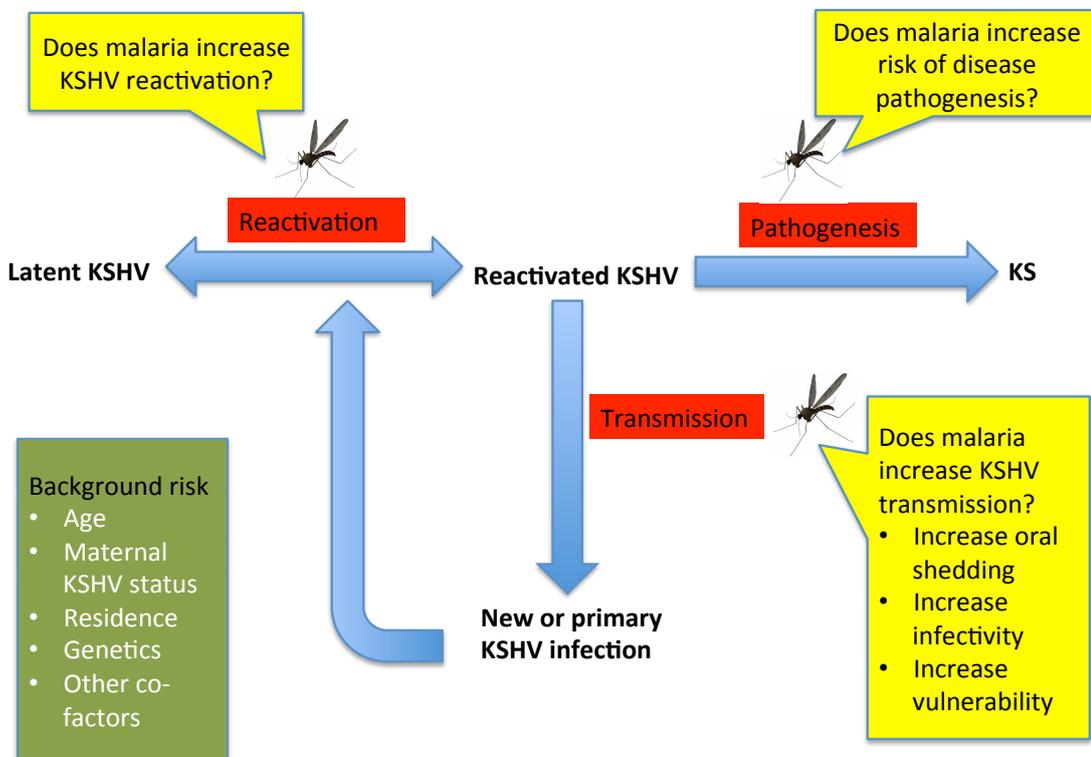


Figure 8.1: Proposed pathway for KSHV infection dynamics and KS pathogenesis and points at which malaria may modify the process.

8.3.2 The potential impact of public health measures to control malaria on Kaposi's sarcoma associated-herpesvirus transmission

Extensive efforts are underway to control malaria by mosquito eradication, prophylactic drugs, vaccination and prevention of mosquito bites. This raises the question if a by-product of these efforts will be to reduce KSHV transmission. Indeed, reduced KSHV seroprevalence related to suppression of Anopheles density in Italy has been reported³³⁴. In this thesis, the use of bed nets and spraying insecticide in the home were not associated with reduction in detection of antibodies to KSHV, but neither were they associated with reduction in malaria. This should be repeated with home checks for use of nets and sprays.

8.3.3 Interaction and preventative interventions for HIV and Kaposi's sarcoma associated-herpesvirus co-infection

The study of mother-child pairs reported that children infected with HIV were at an increased risk of KSHV seropositivity⁷. This result was supported by the meta-analysis. Among mothers the evidence for an association between HIV and antibodies to KSHV was weaker⁸. The biological mechanism for this was outside the scope of this thesis. However, within the context of the limitations addressed in chapters 5 and 6, it is interesting to speculate that HIV may be associated with vulnerability to KSHV infection. If this is true, HIV prevention or early ART intervention could prevent or delay primary KSHV infection. In 2012 MRC Uganda reported that among HIV-infected children in the General Population Cohort, mortality was extremely high in the first two years of life compared to children not infected with HIV⁴¹². HIV disease progression among children tends to be rapid, especially in resource-limited settings. With the introduction of paediatric anti-retroviral therapy to Uganda, prevention of HIV-associated disease will become more important. While preventing HIV infection would always be the public health priority, interventions to prevent primary KSHV infection or reactivation may become important as the HIV trajectory shifts to one of a chronic illness.

8.3.4 Additional co-factors for Kaposi's sarcoma associated-herpesvirus transmission

Risk factors for KSHV transmission are likely to be multifactorial and differ between geographic areas. Certainly, the co-spatial distribution of KSHV and *P. falciparum* is not exact, implying that if malaria is a co-factor for KSHV transmission or KS pathogenesis, it acts in combination with other factors. Background risk factors for KSHV infection, reactivation or disease pathogenesis may include age^{7, 8, 13, 22, 25, 191, 259}, exposure to KSHV^{7, 218, 257}, residence²⁵⁶ and genetic profile^{244, 245} (Figure 8.1). Other factors that impact the host immune function may also be important, including nutritional status, parasites and other infectious agents.

To reach the goal of reducing KSHV transmission and prevalence, further research is needed to increase current knowledge (Table 8.2). This thesis highlights parasites including malaria in addition to HIV as potential co-factors for KSHV transmission but broad searches for other co-factors are still required. There is good evidence to support age^{7, 8, 13, 22, 25, 191, 259} and KSHV status of close relatives^{7, 218, 257} as key determinants of risk of KSHV seropositivity in sub-Saharan Africa. Other co-factors for KSHV infection are less clearly defined. This is in part due to the fact that KSHV research has sometimes been limited by studies not being specifically designed to investigate KSHV and so can lack the breadth and power to study risk factors. There is a particular paucity of data collection on co-infections. This is important as simultaneous infection by KSHV and other pathogens may impact the detection of KSHV antibodies in a number of ways. While financially costly, KSHV viral load and potentially, information on KSHV-specific T-cell responses will be key to determine the biological interaction between KSHV and "factor".

Future studies can be approached a number of ways (Table 8.2) and if risk factors are to be clarified diligent collection of data on potential

confounders along with biological samples for KSHV assays are required. A cross-sectional survey of KSHV prevalence among populations with and without malaria control could be relatively easily done and results may inform the plausibility of malaria as a co-factor for KSHV. A case-control study of individuals with and without KSHV would allow multiple exposures to be studied while keeping cost and time to a minimum. Information from a study of this type could then be used to inform a cohort study which would allow the study of the natural evolution of KSHV infection and allow the complexities of timing and duration of exposure to co-factors to be investigated.

KSHV activity (antibody titre, viral load and specific T-cell responses) could be measured among individuals before and after treatment for certain parasite infections. It would be of particular interest to determine if drugs that clear *P.falciparum* also reduce markers of KSHV reactivation. A birth cohort study would allow the temporal relationship between primary infections with KSHV and other infectious agents to be investigated. The age of the host is an important determinant of outcome for *P.falciparum*. Any potential interaction between malaria and KSHV may also be governed by age. Quantitative laboratory based studies will be needed to investigate biological mechanisms of factors on KSHV control. Perturbations in adaptive T-cell function could affect KSHV transmission and/or KSHV disease pathogenesis and are a good candidate for investigation¹⁰². If malaria is involved, the order, timing and frequency of *P.falciparum* and KSHV is likely to be important. Acute and chronic *P.falciparum* malaria infections may preferentially impact T-cell immunity and hence KSHV infection²⁵³. Finally, a review of the literature will be required to pool results and review potential co-factors for KSHV transmission.

Table 8.2: Suggested studies to increase level of evidence for co-factors for KSHV transmission

Research question	Proposed study design
Does malaria control reduce KSHV prevalence?	Ecological survey
Are certain co-factors more common in individuals with evidence of KSHV infection?	Case control
Does treatment for certain parasite infections reduce markers of KSHV reactivation?	Cohort
What co-factors are associated with transmission of KSHV?	Cohort
Does timing and duration of co-infections dysregulate KSHV control?	Birth cohort
What is the biological mechanism for the co-factors effect on KSHV?	Quantitative studies
What is the level of evidence for individual co-factors association with KSHV?	Systematic review

Co-factors may include the following: Host (age, genetic profile, previous diseases, immune status, occupation), Environment (socioeconomic, pollution) and Agent (Infectious disease, nutrition)

8.4 Prevention of Kaposi's sarcoma

8.4.1 The search for risk factors for HIV-associated Kaposi's sarcoma oncogenesis

The results from chapter 7 suggest that high antibody titres to two KSHV proteins are associated with risk of developing HIV-associated KS. Two key questions stem from this study: (1) what risk factors determine why some individuals get KS; and (2) what sort of test would be ideal for predicting risk? The answers to these may allow for KS preventative measures.

This thesis reported that some individuals with HIV-KSHV co-infection are more likely to progress to KS than matched individuals (also infected with HIV-KSHV). Current evidence suggests that in HIV-KSHV co-infected individuals, factors that cause additional immune modulations change the risk of KS and the factors include ART¹²⁴, steroids^{144, 145} and immune reconstitution inflammatory syndrome³⁹⁷. Additional risk factors for HIV-associated KS may be present and these could conceivably include parasites and immune modulating genes. There is a clear need to fully define risk factors for KS in addition to HIV infection because if identified, these risk factors may have the potential to be avoided or modified to reduce the risk of KS oncogenesis. Furthermore, in the era of ART, KS occurring at CD4 counts above 500 and KS not remitting with treatment may be rising^{413, 414}, increasing the need for risk factors to be fully understood. Information on a large number of co-factors could

be accrued through a case-control study and followed with a longitudinal cohort study (Table 8.3). Samples collected from such studies could be used to determine biological interaction between KSHV and any co-factor.

8.4.2 Malaria as a co-factor for Kaposi's sarcoma

The immunomodulatory effects of *P.falciparum* parasitaemia make it a potential co-factor³⁵⁴. So, in addition to influencing the transmission of KSHV, it is intriguing to speculate that malaria could also be implicated in the oncogenesis of KS in at least one of two ways: (1) malaria could induce the KSHV reactivation required for pathogenesis of KS; and (2) malaria could facilitate the dissemination of the virus to sites of disease. A point to further speculate is if malaria prophylaxis in HIV-KSHV co-infected individuals could reduce their risk of progression to KS. It may be possible in some parts of sub-Saharan Africa with establish malaria programmes and cancer registration to demonstrate an association between malaria transmission rates and KS incidence.

Table 8.3: Suggested studies to increase level of evidence for co-factors for KS

Research question	Proposed study design
Does malaria control reduce KS incidence?	Cancer registry
Are certain co-factors more common in individuals with KS?	Case control
Among a KSHV-HIV co-infected cohort what co-factors increase risk of progression to KS?	Cohort
What is the biological mechanism for the co-factors effect on KSHV reactivation and progression to disease?	Quantitative studies
What is the level of evidence for individual co-factors association with KS?	Systematic review

Co-factors may include the following: Host (age, genetic profile, previous diseases, immune status, occupation), Environment (socioeconomic, pollution) and Agent (Infectious disease, nutrition)

8.4.3 The development of a risk assessment tool for progression to Kaposi's sarcoma

Whether antibody levels could be used to determine clinical risk of KSHV-related disease remains undetermined. Following multiple KSHV antibodies may increase the clinical validity of serology as a risk assessment tool. It may be that compared to a defined "normal range"

both the absolute difference and dynamics of antibody titre may be important. It is likely that the patient clinical profile and/or other biomarkers, perhaps in combination with antibodies to KSHV, will be required. Indicators of risk of KS that are valid in a clinical setting are urgently needed to screen for and prevent KSHV-related pathology. At the current time, once KS is clinically evident the main aims of treatment are symptom palliation, prevention of disease progression, and reducing psychological stress. Targeting individuals in need of ART may be particularly important in resource-limited settings where drugs to treat HIV are lacking and clinicians may have to prioritize individuals to receive treatment. Furthermore, modalities to treat or palliate more bulky symptomatic tumours, including radiotherapy and chemotherapy, may not be available. Currently no ART guidelines for early intervention in preventing KS are in routine practice and in part this is due to the inability to predict those at greatest risk of KS.

Risk assessment tools that allow the calculation of 5-year absolute risk are currently available for cancers such as breast cancer and lung cancer^{415, 416}. A risk assessment tool for KS will likely require a combination of information on the background of the patient and parameters about the patient's immune status and KSHV viral protein or genetic profile. Patient information may include age, gender, residence and length of time with HIV-KSHV co-infection. Immune status parameters may include, nadir CD4 count, current HIV viral load and history of opportunistic infections. The KSHV profile may include KSHV antibody titre, and KSHV viral load. Monitoring of risk and data collection will likely need to be dynamic as parameters may alter over time due to environmental and treatment related changes. Future studies may identify and allow the inclusion of KSHV viral proteins or genes that are oncogenic markers.

8.4 Final conclusion

The results from this thesis suggest a potential biological interaction between *P.falciparum* and KSHV. If this paradigm is true, basic malaria control measures could reduce KSHV population prevalence. As HIV may impact vulnerability to KSHV infection, it is an intriguing possibility that primary infection with KSHV in certain risk groups may be further reduced with HIV prevention or ART. Transmission of KSHV is likely dependant on many factors; research is required to elucidate this further. Risk factors for progression to KS among HIV-KSHV co-infected individuals include immune modulators. However, those currently known only explain part of the risk and research is needed to inform prevention measures. A valid risk assessment tool to identify those at the highest risk of KS oncogenesis is urgently needed and the information that may be required for such a tool is proposed.

The impact of oncoviruses on the health of large numbers of people across the world remains huge and yet these cancers have the potential to be prevented. KSHV transmission and halting pathogenesis are attractive targets to prevent KS. It is hoped that the findings documented in this thesis go some small way to achieving that much needed goal.

Appendices

1. Table of papers rejected from meta-analysis
2. Wakeham K, Webb EL, Sebina I, Muhangi L, Miley W, Johnson WT, Ndibazza J, Elliott AM, Whitby D, Newton R. Parasites and Kaposi's sarcoma associated herpesvirus (KSHV) in Uganda. Infect Agent Cancer. 2011 Sep 30;6(1):15
3. Wakeham K, Webb EL, Sebina I, Muhangi L, Miley W, Johnson WT, Ndibazza J, Whitby D, Newton R, Elliott AM. Transmission of Kaposi's sarcoma associated herpesvirus (KSHV) in mother-child pairs in Uganda. J Acquir Immune Defic Syndr. 2013 June 1;63(2):228

Appendix 1: Articles rejected from meta-analysis

Title	Authors	Publication details	Rejection basis
High seroprevalence of antibodies to human herpesvirus-8 in Egyptian children: evidence of nonsexual transmission.	Andreoni M, El-Sawai G, Rezza G, Ensioli B, Nicastrì E, Ventura L, Ercoli L, Sarmati L, Rocchi G.	J Natl Cancer Inst. 1999 Mar; 31(9):465-9.	HIV not tested for
Seroprevalence of HHV-8 infection in the pediatric population of two university hospitals in Rio de Janeiro, Brazil.	Avelleira JC, Lupi O, Caterino-de-Araujo A, Santos-Fortuna Edle L.	Int J Dermatol. 2006 Apr; 45(4):381-3.	Not sub-Saharan Africa
Correlates of human herpesvirus 8 seropositivity among heterosexual men in Kenya.	Baeten JM, Chohan BH, Lavreys L, Rakwar JP, Ashley R, Richardson BA, Mandaliya K, Bwayo JJ, Kreiss JK.	AIDS. 2002 Oct 18; 16(15):2073-8.	Sex-worker or high risk sex cohort
Sex and geographic patterns of human herpesvirus 8 infection in a nationally representative population based sample in Uganda.	Bryahwaho B, Dollard SC, Pfeiffer RM, Shebl FM, Munuo S, Amin MM, Hladik W, Parsons R, Mbulaitaye SH.	J Infect Dis. 2010 Nov 1; 202(9):1347-53.	Adolescent
Serologic evidence for mother-to-child transmission of Kaposi sarcoma-associated herpesvirus infection.	Bourboulla D, Whitby D, Boshoff C, Newton R, Beral V, Carrara H, Lane A, Stas F.	JAMA. 1998 Jul 1; 280(1):31-2.	HIV not tested for
Distribution of Kaposi sarcoma-associated herpesvirus/human herpesvirus 8 in maternal saliva and breast milk in Zambia: implications for transmission.	Brayfield BP, Kankasa C, West JT, Muyanga J, Bhat G, Kiasakala W, Mitchell CD, Wood C.	J Infect Dis. 2004 Jun 15; 189(12):2260-70.	Duplicate dataset
Human herpesvirus 8 infection in children and adults in a population-based study in rural Uganda.	Butler LM, Wiers WA, Balinandi S, Downing R, Dollard S, Nellands TB, Gupta S, Rutherford GW, Hermin J.	J Infect Dis. 2011 Mar 1; 203(5):625-34.	HIV not tested for
A search for human herpesvirus 8 (HHV-8) in HIV-1 infected mothers and their infants does not suggest vertical transmission of HHV-8.	Calabra ML, Gasperini P, Barblerato M, Ometto L, Zanchetta M, De Rossi A, Chicco-Bianchi L.	Int J Cancer. 2000 Jan 15; 85(2):296-7.	Not sub-Saharan Africa
Human herpesvirus 8 infection among adolescents in the REACH cohort.	Casper C, Meier AS, Wald A, Morrow RA, Corey L, Moscicki AB.	Arch Pediatr Adolesc Med. 2006 Sep; 160(9):937-42.	Adolescent
Searching for antibodies to HHV-8 in children born to HIV-1 infected mothers from São Paulo, Brazil: relationship to maternal infection.	Caterino-de-Araujo A, Cibella SE.	J Trop Pediatr. 2003 Aug; 49(4):247-50.	Not sub-Saharan Africa
Human herpesvirus-8 (HHV-8) antibodies in women from São Paulo, Brazil. Association with behavioral factors and Kaposi's sarcoma.	Caterino-de-Araujo A, de los Santos-Fortuna E, Carbone PH, Cibella SE, Moreira AA.	Braz J Infect Dis. 2003 Dec; 7(6):395-401.	Not sub-Saharan Africa
Viral coinfections among African children infected with human immunodeficiency virus type 1.	Chakraborty R, Rees G, Bourboulla D, Cross AM, Dixon JR, D'Agostino A, Musoke R, Boshoff C, Rowland-Jones SL, Klennerman P.	Clin Infect Dis. 2003 Apr 1; 36(7):922-4. Epub 2003	All HIV infected
Mixed patterns of transmission of human herpesvirus-8 (Kaposi's sarcoma-associated herpesvirus) in Malawian families.	Cook RD, Hodgson TA, Vaughn AC, Molyneux EM, Borgstein E, Sherry A, Teo CG, Porter SK.	J Gen Virol. 2002 Jul; 83(Pt 7):1613-9.	HIV not tested for
Demographic and immune correlates of human herpesvirus 8 seropositivity in Malawi, Africa.	DeSantis SM, Pau CP, Archibald LK, Nwanyanwu OC, Kazembe PN, Dobbie H, Jarvis WR, Jason J.	Int J Infect Dis. 2002 Dec; 6(4):266-71.	Hospital cohort
Substantial regional differences in human herpesvirus 8 seroprevalence in sub-Saharan Africa: insights on the origin of the Kaposi's sarcoma belt."	Dollard SC, Bouter LM, Jones AM, Mermin JH, Chidzonga M, Chipato T, Shibuski CH, Brander C, Mosam A, Kiepiela P, Hladik W, Martin JN.	Int J Cancer. 2010 Nov 15; 127(10):2395-401.	HIV not tested for
Human herpesvirus 8 serological markers and viral load in patients with AIDS-associated Kaposi's sarcoma in Central African Republic.	Duprez R, Kassa-Kelembho E, Plancoulaine S, Briand J, Fossi M, Kobangue L, Hinzart P, Tuerre H, Gessain A.	J Clin Microbiol. 2005 Sep; 43(9):4840-3.	All HIV infected
Transmission of human herpesvirus 8 by sexual activity among adults in Lagos, Nigeria.	Eltom MA, Mbulaitaye SM, Daada AJ, Whitby D, Biggar RJ.	AIDS. 2002 Dec 6; 16(18):2473-8.	Sex-worker or high risk sex cohort
Seroprevalence of human herpes virus 8 in different Eritrean population groups.	Enbom M, Tolfevstam T, Ghebrekidan H, RudÅ©n U, Grandien M, Wahren B, Linde A.	J Clin Virol. 1999 Dec; 14(3):167-72.	HIV not tested for
HIV-associated Kaposi sarcoma in African children.	Feller L, Khammissa RA, Gugushe TS, Chikete UM, Wood NH, Meyerov R, Lemmer J.	SADJ. 2010 Feb; 65(1):20-2.	Participants have Kaposi sarcoma
High seroprevalence of human herpesvirus 8 (HHV-8) in HIV-1-infected pregnant women of Southeastern Italy: association with injection drug use and hepatitis C virus infection.	Fiore JR, Volpe A, Tosatti MA, De Valentin L, Favio A, Chironna M, Lisca A, Vimercati A, Angarano G, Chicco-Bianchi L, Calabraz ML.	J Med Virol. 2004 Apr; 72(4):656-60.	Not sub-Saharan Africa
Seroprevalence of Kaposi's sarcoma-associated herpesvirus and risk factors in Xinjiang, China.	Fu B, Sun F, Li B, Yang L, Zeng Y, Sun X, Xu F, Rayner S, Guadalupe M, Gao SJ, Wang L.	J Med Virol. 2009 Aug; 81(8):1422-31.	Not sub-Saharan Africa
Clinical presentation and outcome of epidemic Kaposi sarcoma in Ugandan children.	Gant S, Kakuru A, Wald A, Walusansa V, Corey L, Casper C, Orem J.	Pediatr Blood Cancer. 2010 May; 54(5):670-4.	Participants have Kaposi sarcoma
Human herpesvirus 8 primary infection occurs during childhood in Cameroon, Central Africa.	Gessain A, Maucia re P, van Beveren M, Plancoulaine S, Ayoub A, Essame-Oyono JL, Martin PM, de The G.	Int J Cancer. 1999 Apr 12; 81(2):189-92.	HIV not tested for
Antibodies to human herpesvirus 8 in women and infants born in Haiti and the USA.	Goedert JJ, Kedes DH, Genem D.	Lancet. 1997 May 10; 349(9062):1368.	Not sub-Saharan Africa

Appendix 1: Continuation					
HLA polymorphisms and detection of Kaposi sarcoma-associated herpesvirus DNA in saliva and peripheral blood among children and their mothers in the Uganda sickle cell anemia KSHV Study.	Guech-Ongey M, Verboom M, Pfeiffer RM, Schulz TF, Ndugwya CM, Owor AM, Bakaki PM, Bhatia K, Figueiredo C, Elz-Vesper B, Blaszyk R, Mbulaiteye SM.	Infect Agent Cancer. 2010 Nov;18(5):21.	HIV not tested for		
Seroprevalence of human herpesvirus 8 among Zambian women of childbearing age without Kaposi's sarcoma (KS) and mother-child pairs with KS.	He J, Bhat G, Kankasa C, Chintu C, Mitchell C, Duan W, Wood C.	J Infect Dis. 1998 Dec;178(6):1787-90.	Duplicate dataset		
Prevalence of human herpes virus-8 and hepatitis B virus among HIV seropositive pregnant women enrolled in the Mother-to-Child HIV Transmission Prevention Program at Saint Camille Medical Centre in Burkina Faso.	Iboudo D, Karou D, Nadembega WM, Savadogo A, Djeneba O, Pignatelli S, Pietra V, Bere A, Simpore J, Traore AS.	Pak J Biol Sci. 2007 Sep 1;10(17):2831-7.	All HIV infected		
Mother-to-child HIV and HHV-8 transmission in neonates at Saint Camille Medical Centre in Burkina Faso.	Iboudo D, Simpore J, Sanou DS, Karou D, Sia DJ, Ouermi D, Bisseye C, Sagna T, Odolimi S, Bueli F, Pietra V, Pignatelli S, Gnoula C, Nikiema JB, Castelli F.	Pak J Biol Sci. 2009 Jun 15;12(12):908-13.	All HIV infected		
Sequence analyses of human herpesvirus-8 strains from both African human immunodeficiency virus-negative and -positive childhood endemic Kaposi's sarcoma show a close relationship with strains identified in Lebanese children and high variation in the K1 glycoprotein.	Kasolo FC, Monze M, Obel N, Anderson RA, French C, Gompels UA.	J Gen Virol. 1998 Dec;79 (Pt 12):3055-65.	Participants have Kaposi sarcoma		
Infection with AIDS-related herpesviruses in human immunodeficiency virus-negative infants and endemic childhood Kaposi's sarcoma in Africa.	Kasolo FC, Mpaibwani E, Gompels UA.	J Gen Virol. 1997 Apr;78 (Pt 4):847-55.	Participants have Kaposi sarcoma		
Diverse genotypes of Kaposi's sarcoma associated herpesvirus (KSHV) identified in infant blood infections in African childhood-KS and HIV/AIDS endemic region.	Kasolo FC, Spinks J, Bina H, Bates M, Gompels UA.	J Med Virol. 2007 Oct;79(10):1555-61.	Participants have Kaposi sarcoma		
Human herpesvirus 8: seroprevalence and correlates in prostitutes in Mombasa, Kenya.	Laurys L, Chopan B, Ashley R, Richardson BA, Corey L, Mandaliya K, Ndiya Achola JO, Kreis JK.	J Infect Dis. 2003 Feb 1;187(3):359-63.	Sex-worker or high risk sex cohort		
Pregnancy and human herpesvirus 8 reactivation in human immunodeficiency virus type 1-infected women.	Lisco A, Barberato M, Fiore JR, Gasperini P, Favla A, Volpe A, Chiroma M, Pastore G, Chicco-Bianchi L, Calabrà M.	J Clin Microbiol. 2006 Nov;44(11):3863-71.	Not sub-Saharan Africa		
Evidence for horizontal and not vertical transmission of human herpesvirus 8 in children born to human immunodeficiency virus-infected mothers.	Lyall EG, Patton GS, Sheldon J, Stainsby C, Mullen J, O'Shea S, Smith NA, De Ruiter A, McClure MO, Schulz TF.	Pediatr Infect Dis J. 1999 Sep;18(9):795-9.	All HIV infected		
Seroprevalence of human herpesvirus 8 infection in children born to HIV-1-infected women in São Paulo, Brazil.	Machado DM, Sumita LM, Pannuti CS, Succi RC, Moraes-Pinto MJ, Souza VA.	Braz J Med Biol Res. 2005 Feb;38(2):237-40.	Not sub-Saharan Africa		
No evidence of sexual transmission of Kaposi's sarcoma herpes virus in a heterosexual South African population.	Malope BI, MacPhail P, Mbisa G, MacPhail C, Stein L, Raatsikhophla EM, Ndlovu L, Sitas F, Whitby D.	AIDS. 2008 Feb 19;22(4):519-26.	Sex-worker or high risk sex cohort		
Kaposi's sarcoma herpesvirus and HIV-1 seroprevalences in prostitutes in Djibouti.	Marcelin AG, Grandadam M, Flandre P, Nicand E, Milliancourt C, Koeck JL, Philippon M, Teyssou R, Agut H, Dupin N, Calvez V.	J Med Virol. 2002 Oct;68(2):164-7.	Sex-worker or high risk sex cohort		
Human herpesvirus 8 infection among various population groups in southern Israel.	Margalith M, Chatyenne LG, Fuchs E, Owen C, Lee CR, Yermiyahu T, Whitman JE, Abisahi DV.	J Acquir Immune Defic Syndr. 2003 Dec 15;34(5):S100-104.	Not sub-Saharan Africa		
Comparison of human herpesvirus 8 and Epstein-Barr virus seropositivity among children in areas endemic and non-endemic for Kaposi's sarcoma.	Marro E, Bultenys M, Stewart JA, Spira TJ, Cannon MJ, Thacher TD, Bruns R, Pelett PE, Dolard SC.	J Med Virol. 2004 Jan;72(1):126-31.	HIV not tested for		
Prevalence and transmission of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) in Ugandan children and adolescents.	Mayama S, Cuevas LE, Sheldon J, Omar OH, Smith DH, Okong P, Silvei B, Hart CA, Schulz TF.	Int J Cancer. 1998 Sep 11;77(6):817-20.	HIV not tested for		
Water, socioeconomic factors, and human herpesvirus 8 infection in Ugandan children and their mothers.	Mbulaiteye SM, Biggar RJ, Pfeiffer RM, Bakaki PM, Garmache C, Owor AM, Katongole-Mbidde E, Ndugwya CM, Goedert JJ, Whitby D, Engels EA.	J Acquir Immune Defic Syndr. 2005 Apr 1;38(4):471-4.	HIV not tested for		
Seroprevalence and risk factors for human herpesvirus 8 infection, rural Egypt.	Mbulaiteye SM, Pfeiffer RM, Dolan B, Tsang VC, Noh J, Mkhaili NN, Abdel-Hamid M, Hashem M, Whitby D, Thomas Strickland G, Goedert JJ.	Emerg Infect Dis. 2008 Apr;14(4):586-91.	HIV not tested for		
Detection of Kaposi sarcoma-associated herpesvirus DNA in saliva and buffy-coat samples from children with sickle cell disease in Uganda.	Mbulaiteye SM, Pfeiffer RM, Engels EA, Marshall V, Bakaki PM, Owor AM, Ndugwya CM, Katongole-Mbidde E, Goedert JJ, Biggar RJ, Whitby D.	J Infect Dis. 2004 Oct 15;190(6):1382-6.	HIV not tested for		
Human herpesvirus 8 infection within families in rural Tanzania.	Mbulaiteye SM, Pfeiffer RM, Whitby D, Brubaker GR, Shao J, Biggar RJ.	J Infect Dis. 2003 Jun 1;187(11):1780-5.	HIV not tested for		
High levels of Epstein-Barr virus DNA in saliva and peripheral blood from Ugandan mother-child pairs.	Mbulaiteye SM, Walters M, Engels EA, Bakaki PM, Ndugwya CM, Owor AM, Goedert JJ, Whitby D, Biggar RJ.	J Infect Dis. 2006 Feb 1;193(3):422-6.	HIV not tested for		
Early childhood infection by human herpesvirus 8 in Zambia and the role of human immunodeficiency virus type 1 coinfection in a highly endemic area.	Mimnas V, Crabtree KL, Chao A, M'Soka TJ, Kankasa C, Bultenys M, Mitchell CD, Wood C.	Am J Epidemiol. 2008 Aug 1;168(3):311-20.	Duplicate dataset		
The Zambia Children's KS-HHV8 Study: rationale, study design, and study methods.	Mimnas V, Crabtree KL, Chao A, M'Soka TJ, Kankasa C, Bultenys M, Mitchell CD, Wood C.	Am J Epidemiol. 2011 May 1;173(9):1085-92.	Duplicate dataset		
Early childhood infection of Kaposi's sarcoma-associated herpesvirus in Zambian households: a molecular analysis.	Olp LN, Shea DM, White MK, Gondwe C, Kankasa C, Wood C.	Int J Cancer. 2013 Mar 1;132(5):1182-90.	Duplicate dataset		

Appendix 1: Continuation			
Respective roles of serological status and blood specific antihuman herpesvirus 8 antibody levels in human herpesvirus 8 intrafamilial transmission in a highly endemic area.	Planoulaine S, Abel L, Tráčgouat D, Duprez R, van Beveren M, Tortevoye P, Froment A, Gessain A.	Cancer Res. 2004 Dec 1;64(23):8782-7.	HIV not tested for
Human herpesvirus 8 transmission from mother to child and between siblings in an endemic population.	Planoulaine S, Abel L, van Beveren M, Tráčgouat DA, Joubert M, Tortevoye P, de The G, Gessain A.	Lancet. 2000 Sep 23;356(9235):1062-5.	HIV not tested for
Prevalence and risk factors for human herpesvirus 8 infection in northern Cameroon.	Rezza G, Tchongmeu OB, Andreoni M, Bugarini R, Toma L, Bakary DK, Gilkoutou M, Sarmati L, Monini P, Pezzotti P, Ensoli B.	Sex Transm Dis. 2000 Mar;27(3):159-64.	Hospital cohort
Increased prevalence of Kaposi's sarcoma-associated herpesvirus in the Kaposi's sarcoma-endemic area of western Kenya in 1981-2000.	Senba M, Buziba N, Mori N, Morimoto K, Nakamura T.	Acta Virol. 2011;55(2):161-4.	Participants have Kaposi sarcoma
Human herpesvirus 8 seropositivity among sexually active adults in Uganda.	Shebi FM, Dollard SC, Pfeiffer RM, Biryahwaho B, Amin MM, Munuo SS, Hladik W, Parsons R, Graubard BI, Mbulateye SM.	PLoS One. 2011;6(6):e21286.	Sex-worker or high sexual risk
Prevalence of antibodies to human herpesvirus-8 in populations with and without risk for infection in São Paulo State.	Souza VA, Sumita LM, Freire W, Sato HK, Grandi JL, Pierrrotti LC, Nascimento RC, Pannud CS.	Braz J Med Biol Res. 2004 Jan;37(1):123-7.	Not sub-Saharan Africa
Seroprevalence of HHV 8 antibodies among the general population and HIV positive persons in the Czech Republic.	Suchánková I, Stanková M, Roubalová K, Vandasová J, Bráčeková M.	J Clin Virol. 2003 Sep;28(1):70-6.	Not sub-Saharan Africa
Human herpesvirus 8 seroprevalence among internationally adopted children coming from Eastern Europe.	Viviano E, Vitale F, Perna AM, Cataldo F, Firenze A, Romano N.	New Microbiol. 2009 Jan;32(1):1-5.	Not sub-Saharan Africa
Correlates of human herpes virus-8 and herpes simplex virus type 2 infections in Northern Cameroon.	Volpi A, Sarmati L, Suligoi B, Montano M, Rezza G, Andreoni M.	J Med Virol. 2004 Nov;74(3):467-72.	Sex-worker or high risk sex cohort
HIV - associated and non - HIV associated types of Kaposi's sarcoma in an African population in Tanzania. Status of immune suppression and HHV-8 seroprevalence.	Wamburu G, Masenga EJ, Mushi EZ, Schmid-Grendelmeier P, Kempf W, Orfanos CE.	Eur J Dermatol. 2006 Nov-Dec;16(6):677-82.	Participants have Kaposi sarcoma
Prevalence of Kaposi sarcoma-associated herpesvirus compared with selected sexually transmitted diseases in adolescents and young adults in rural Rakai District, Uganda.	Wawer MJ, Eng SM, Serwadda D, Sewankambo NK, Kiwanuka N, Li C, Gray RH.	Sex Transm Dis. 2001 Feb;28(2):77-81.	Sex-worker or high risk sex cohort
Prevalence of infection with human herpesvirus 8/Kaposi's sarcoma herpesvirus in rural South Africa.	Wilkinson D, Shaldon J, Gilks CF, Schulz TF.	S Afr Med J. 1999 May;89(5):554-7.	Hospital cohort
Mortality among HIV-1- and human herpesvirus type 8-affected mother-infant pairs in Zambia.	Wojcicki J, Mwanhamuntu M, Minhas V, Djokic B, Kankasa C, Klaskala W, Brayfield B, Phiri S, Wood C, Mitchell CD.	Cancer Epidemiol Biomarkers Prev. 2008 Sep;17(9)	Duplicate dataset
Prevalence of human herpesvirus 8 DNA sequences in human immunodeficiency virus-negative individuals without Kaposi's sarcoma in Greece.	Zavos G, Gazouli M, Papaconstantinou I, Lukas JC, Zografidis A, Kostakis A, Nasoulas G.	In Vivo. 2005 Jul-Aug;19(4):729-32.	Not sub-Saharan Africa
Kaposi's sarcoma in childhood: an analysis of 100 cases from Uganda and relationship to HIV infection.	Ziegler JL, Katongole-Mbidde E.	Int J Cancer. 1996 Jan 17;65(2):200-3.	Participants have Kaposi sarcoma

RESEARCH ARTICLE

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Parasite infection is associated with Kaposi's sarcoma associated herpesvirus (KSHV) in Ugandan women

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Abstract

Background: Immune modulation by parasites may influence susceptibility to bacteria and viruses. We examined the association between current parasite infections, HIV and syphilis (measured in blood or stool samples using standard methods) and antibodies against Kaposi's sarcoma herpesvirus (KSHV), measured by ELISA, in 1915 stored plasma samples from pregnant women in Entebbe, Uganda.

Results: Seroprevalence of KSHV was higher in women with malaria parasitaemia (73% vs 60% $p = 0.01$), hookworm (67% vs 56% $p = 0.001$) and *Mansonella perstans* (69% vs 59% $p = 0.05$); seroprevalence increased with increasing intensity of hookworm infection ($p < 0.001$ [trend]). No associations were found for HIV, five other parasites or active syphilis. These effects were not explained by socioeconomic status or education.

Conclusions: Specific parasite infections are associated with presence of antibodies against KSHV, perhaps mediated via their effect on immune function.

Background

Infection with KSHV is the underlying cause of Kaposi's sarcoma (KS), although it may not be sufficient [1]. Immune suppression, such as that caused by human immunodeficiency virus (HIV), significantly increases the risk of KS among KSHV infected people and is associated with increased viral load and viral shedding [2-8]. Among people without HIV infection or other forms of overt immune suppression, geographic and temporal variation in the incidence of KS and in the prevalence of KSHV suggest that cofactors may be important in facilitating both transmission and disease [9-18]. Whether cofactors act directly or via effects on the immune system is unclear [19].

Many environmental co-factors for KSHV transmission and disease have been suggested, including volcanic soils [20], limestone [21] and 'oncoweeds' - that is plants with

carcinogenic properties or the ability to reactivate KSHV *in vitro* - although epidemiologic evidence of a role for these agents remains scant [22]. Ecological studies in the Mediterranean area found that eradication of mosquitoes and other blood sucking arthropods was associated with declines both in the prevalence of KSHV and in the incidence of KS [9-14,18,23]. The 'promoter arthropod hypothesis' suggests that insect blood feeding increases KSHV transmission through viral reactivation and KS through inflammatory mechanisms associated with the bite [11,14].

Studies of KS in Africa have identified risk factors for KS that might be common to risk of certain parasites, such as exposure to water, high rainfall and walking barefoot [15,24-26]. Previously reported risk factors for KSHV, such as use of surface rather than piped water, may also be consistent with increased exposure to parasites [27]. Ecological associations between malaria and KSHV or KS in Africa are inconclusive [15]. Only one study has attempted to measure parasite burden among cases with KS and controls; KS patients had a higher carriage of certain intestinal helminths than did controls [28].

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Parasites impact on immune function [29,30] and could, therefore, modulate the host response to KSHV. The association between EBV (another gamma herpesvirus) and malaria is well documented [31-34]. Parasite-related immune modulation may increase susceptibility to KSHV infection and may also be associated with increased viral shedding and transmission, leading to an increased prevalence of KSHV infection and increased incidence of KS. We tested the hypothesis that parasites may be associated with KSHV by examining associations between current parasite infections and presence of antibodies against KSHV.

Methods

The investigation was conducted within an existing study in Uganda - the Entebbe Mother and Baby Study (EMaBS) - a large on-going double blind randomised placebo controlled trial designed to determine the impact of helminth infections and their treatment on vaccine responses and infectious disease outcomes. Detailed information about the study design has been reported elsewhere [35]. Briefly, consenting pregnant women resident in Entebbe and Katabi were recruited from the government funded antenatal clinic at Entebbe hospital, Uganda. Blood samples were obtained by venepuncture, and processed for syphilis, HIV serology, CD4 count and for examination for malaria parasites and *Mansonella perstans*. A stool sample was obtained for examination for intestinal helminths. Of note, women were apparently well on the day of enrollment, so infections identified were essentially asymptomatic. Information was collected on clinical and socio-demographic variables and socio-economic status was defined according to a composite variable comprising information on several relevant factors [35].

Maternal plasma samples from the enrolment visit stored at -80°C at the Uganda Virus Research Institute (UVRI), Entebbe, Uganda - were identified for 1915 women. KSHV serologic testing was based on ELISA for recombinant proteins to K8.1, a KSHV structural glycoprotein expressed during lytic infection, and for ORF 73, a nuclear antigen expressed during latency as previously described [36,37]. Each ELISA plate contained three positive and three negative controls for quality control and cut-off calculation. Both K8.1 and ORF 73 assay have high performance accuracy with a sensitivity of 98.78% and 89.02% respectively and specificity of 98.79% and 97.57% respectively [36]. The ELISAs were performed at the Uganda Virus Research Institute (UVRI) by the study lead and a technician, both of whom were blinded to patient details. The two assays were used to define evidence of KSHV - individuals were considered to be seropositive if they were positive to either assay and negative if both ELISA assays were negative. The assays were transferred

to UVRI from the Viral Oncology Section (VOS), National Cancer Institute (NCI), USA and analysis of the positive and negative controls showed comparable performance at NCI and UVRI. The geometric mean optical density (OD) of the ORF 73 positive and negative controls was 2.50 and 0.04 respectively at VOS, NCI and 2.62 and 0.06 respectively at UVRI. The K8.1 positive and negative controls were 2.23 and 0.10 at NCI and 2.22 and 0.06 at UVRI. Analysis of 375 samples tested in duplicate at both VOS, NCI and UVRI resulted in Kappa values of 0.89 for the K8.1 ELISA and 0.86 for the ORF 73 ELISA.

For parasite intensities, hookworm was measured by egg counts in stool and categorised as light (< 1,000 eggs per gram (epg) of stool), moderate (1,000 to 3,999 epg) and heavy infection ($\geq 4,000$ epg) [35,38]. The intensity of malaria infection was categorised as being below ("low") or above ("high") the median parasite count, per 200 white blood cells. For microfilariae, tertiles of the number of filaria per millilitre of blood were used to categorise into low, medium and high infection intensity.

Data were analysed using Stata11SE (StataCorp LP, College Station, Texas, USA). Potential associations between KSHV seropositivity and each potential risk factor were estimated using the Pearson chi-squared test or Fisher's exact test where expected numbers were small. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using logistic regression modeling controlling for age and other possible confounders. The possibility of multiple parasite infections increasing risk of KSHV seropositivity more or less than expected under a multiplicative model was assessed by fitting terms for interactions between parasites in the logistic regression model. The agreement between K8.1 and ORF73 ELISA assays was assessed formally by calculating a Kappa statistic. All p values were 2-sided and we considered $p < 0.05$ to be statistically significant.

Results and Discussion

The median age of women in the study was 23 years (IQR 19-27); most were in the third trimester of pregnancy (54% (1032/1915) with 46% (879/1915) in the second). The seroprevalence of HIV was 10% (193/1915) and the median CD4 count among those who were seropositive was 551 (IQR 368-796). The highest level of educational attainment reached by the majority of women was primary (50% [961/1911]), with 62% (1191/1915) describing themselves as unemployed or housewives and 82% (1561/1905) reporting a personal income of less than 30,000 Ugandan Shillings (approx. \$12USD) per month. The prevalence of antibodies to K8.1 was 41% and to ORF 73 was 52%. 32% of women were seropositive to both antigens and 61% had antibodies to either ORF 73 and/or K8.1. There was moderate concordance between latent KSHV ORF 73 and lytic

KSHV K8.1 assays in detecting KSHV seropositivity ($k = 0.43$), consistent with previous studies [37,39]. Prevalence of antibodies did not change significantly with age, although the age range of study participants was relatively narrow. Previous studies of women in a similarly narrow age range in Africa have showed little or no association with age [27,37,39,40]. As expected, prevalence decreased with increasing maternal education and household socioeconomic status [27]

Common infections among the participants were hookworm (44%), *Mansonella perstans* (21%), *Schistosoma mansoni* (18%), asymptomatic *Plasmodium falciparum* parasitaemia (10%) and HIV (10%). The prevalence of antibodies to K8.1, ORF 73, both antigens and either antigen was 41%, 52%, 32% and 61% respectively. Unadjusted ORs for the association of infections (including eight current parasitic infections), socio-demographic and behavioral factors with antibodies against KSHV are shown in Table 1. In Table 2, the variables found to be associated with KSHV serostatus have been examined again with adjustment for each other. Seropositivity to KSHV was significantly associated with malaria parasitaemia, hookworm

and *Mansonella perstans*. The prevalence of antibodies to KSHV increased with increasing intensity of hookworm infection ($p < 0.001$ [trend]; as measured by egg counts in stool), from 56% among those with no infection to 67% in those with light/moderate infection (12 - the limit of detection - to 3,999 eggs per gram (epg) of stool) to 72% in those with heavy infection ($\geq 4,000$ epg); no consistent trends were observed for malaria parasite density or *Mansonella perstans* intensity, but most infections were light (Table 3).

Mode of KSHV transmission is yet to be fully elucidated, but high acquisition rates during childhood imply a non-sexual route [7,27,37,40-49]. In studies of mother-child pairs [37,40,50], the impact of HIV on KSHV seropositivity is unclear with some studies reporting a positive impact [37] and others reporting borderline or null association [40,50]. We observed no statistically significant association between HIV and KSHV seropositivity. There was no association between KSHV seropositivity and CD4 count in HIV infected women ($p = 0.13$). The lack of association with syphilis is consistent with previous studies reporting no association with KSHV and markers of sexual behavior

Table 1 Crude associations with antibodies against KSHV and sociodemographic and clinical risk factors among Ugandan women

Factor	Prevalence of women KSHV seropositive*	OR (95% CI)	p**
Age			
14-19 years	65% (288/443)	1	
20-24 years	62% (446/717)	0.89 (0.70-1.14)	
25-29 years	56% (252/444)	0.71 (0.54-0.93)	
30-34 years	58% (125/216)	0.74 (0.53-1.03)	
> = 35 years	55% (52/94)	0.67 (0.43-1.05)	0.007 [trend]
Maternal education			
None	74% (50/68)	1	
Primary	64% (617/960)	0.65 (0.37-1.12)	
Senior	59% (418/713)	0.51 (0.29-0.89)	
Tertiary	45% (76/169)	0.29 (0.16-0.55)	<0.001 [trend]
Household SES***			
1 (lowest)	69% (75/109)	1	
2	71% (112/158)	1.10 (0.65-1.88)	
3	64% (369/581)	0.79 (0.51-1.22)	
4	58% (310/531)	0.64 (0.41-0.99)	
5	57% (222/389)	0.60 (0.38-0.95)	
6 (highest)	50% (55/111)	0.45 (0.26-0.77)	<0.001 [trend]
HIV			
Negative	60% (1033/1721)	1	
Positive	67% (130/193)	1.38 (1.00-1.89)	0.04
Malaria parasites			
No	60% (1010/1697)	1	
Yes	73% (135/185)	1.84 (1.31-2.58)	<0.001
Active syphilis			
No	61% (1110/1833)	1	
Yes	68% (52/76)	1.41 (0.86-2.31)	0.25

Table 1 Crude associations with antibodies against KSHV and sociodemographic and clinical risk factors among Ugandan women (Continued)

Hookworm			
No	56% (594/1070)	1	
Yes	67% (562/836)	1.64 (1.36-1.98)	<0.001
<i>Mansonella perstans</i>			
No	59% (884/1508)	1	
Yes	69% (277/402)	1.55 (1.23-1.96)	<0.001
<i>Schistosoma mansoni</i>			
No	61% (952/1568)	1	
Yes	60% (204/338)	1.0 (0.78-1.25)	0.90
<i>Strongyloides stercoralis</i>			
No	61% (1007/1665)	1	
Yes	63% (144/230)	1.10 (0.82-1.46)	0.54
<i>Trichuris trichiura</i>			
No	60% (1044/1739)	1	
Yes	67% (112/167)	1.36 (0.97-1.90)	0.07
<i>Ascaris lumbricoides</i>			
No	61% (1125/1859)	1	
Yes	66% (31/47)	1.27 (0.69-2.33)	0.45
<i>Trichostrongylus</i> species			
No	61% (1149/1887)	1	
Yes	37% (7/19)	0.38 (0.15-0.96)	0.03
Use of mosquito spray in the home			
No	62% (942/1510)	1	
Yes	55% (219/400)	0.73(0.58-0.90)	0.005
Use of bed net			
No	61% (574/952)	1	
Yes	60% (587/959)	1.04 (0.87-1.25)	0.68
Walk barefoot			
Yes	61% (925/1505)	1	
No	58% (236/407)	1.15 (0.92-1.44)	0.20

* Individuals were considered KSHV positive if they had a positive ORF 73 and/or K8.1 ELISA. Participants were considered negative if both ELISAs were negative.

** All estimated using Chi-squared test except for *Trichostrongylus* species which was estimated using a Fishers exact test. All tests of statistical significance two sided.

*** Socio-economic status

[37,47,51,52]. In unadjusted analyses, the use of insecticide in the home was associated with a lower prevalence of antibodies against KSHV ($p = 0.005$) although use of a bed net and walking barefoot (a risk factor for hookworm infection) was not. Effects of increasing numbers of infections on KSHV seropositivity combined multiplicatively; there were no interactions between the effects of HIV, malaria parasitaemia, hookworm or *Mansonella perstans* on KSHV infection (results not shown). KSHV seropositivity was not associated with trimester or pregnancy duration as measured in months. The results for ORF 73 and K8.1 separately, did not materially differ.

This study has a number of important limitations. It is possible that associations arose as a result of residual confounding by socio-economic status (SES), although adjustment for certain markers of SES had no effect on the findings. Furthermore, the work was cross sectional

and so associations identified should be confirmed in longitudinal studies. Also, the study participants were pregnant and pregnancy itself may modulate immune function. However, since all comparisons were internal within the study (i.e. comparing one group of pregnant women with another), it is difficult to see how this could have impacted on the results.

Conclusions

The findings reported here provide evidence of an association between specific parasites and presence of antibodies against KSHV. Specific parasite infections may increase KSHV replication or cause reactivation, thereby increasing the likelihood of detecting antibodies against KSHV. Alternatively, specific parasites may increase susceptibility to infection - we cannot, in this study, distinguish between these two possibilities. Co-factors for

Table 2 Adjusted associations with antibodies against KSHV and sociodemographic and clinical risk factors among Ugandan women.

Factor**	KSHV seropositive*	
	OR (95% CI)	P***
Age		
Trend OR	0.94 (0.86-1.02)	0.14
Maternal education		
Trend OR	0.79 (0.69-0.91)	0.001
Household SES****		
Trend OR	0.90 (0.83-0.98)	0.01
HIV status		
Negative	1	
Positive	1.35 (0.97-1.89)	0.08
Malaria parasites		
No	1	
Yes	1.60 (1.12-2.27)	0.01
Hookworm		
No	1	
Yes	1.40 (1.14-1.71)	0.001
Mansonella perstans		
No	1	
Yes	1.29 (1.00-1.65)	0.05

* Individuals were considered KSHV positive if they had a positive ORF 73 and/or K8.1 ELISA. Participants were considered negative if both ELISAs were negative.

**All factors are adjusted for each other

***All tests of statistical significance two sided.

**** Socio-economic status

KSHV transmission and disease have been sought to explain the elevated prevalence of KSHV and incidence of KS in sub-Saharan Africa. Data presented here suggest that parasites may constitute one such co-factor. Further epidemiological and laboratory studies are needed to fully understand the role of parasites as a risk factor for infection with KSHV.

Ethical approval

Ethical approval for this study was obtained from three bodies: Uganda Virus Research Institute Science and Ethics Committee, Entebbe, Uganda; Uganda National Council for Science and Technology; and the University of York, UK.

Acknowledgements

Wellcome Trust as a PhD Training Fellowship held by KW (grant number 090132), a Wellcome Trust Senior Fellowship held by AME (grant number 064693) and funded in part by the Intramural Program of the National Cancer Institute, National Institutes of Health, Department of Health and Human Services (contract HHSN261200800001E). Wellcome Trust is a UK registered charity No.210183. We thank all the participants and the staff of the Entebbe Mother and Baby Study and the staff of the laboratory and statistics departments of the Medical Research Council/Uganda Virus Research Institute Uganda Research Unit on AIDS who made this study possible.

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Table 3 Crude associations with antibodies against KSHV and intensity of parasite infection in Uganda women.

Infection	Prevalence of women	KSHV seropositive*	OR (95% CI)	P**
Hookworm				
Uninfected	56%	(594/1070)	1	
Light	67%	(478/714)	1.62 (1.33-1.98)	
Moderate	67%	(61/91)	1.62 (1.04-2.56)	
Heavy	72%	(23/32)	2.05 (0.94-4.47)	<0.001[trend]
Malaria parasites				
No	40%	(687/1697)	1	
Low	75%	(68/91)	2.01 (1.24-3.26)	
High	66%	(81/123)	1.31 (0.89-1.93)	0.02[trend]
Mansonella perstans				
Uninfected	59%	(884/1508)	1	
Light	69%	(98/142)	1.57 (1.09-2.28)	
Moderate	74%	(68/92)	2.00 (1.24-3.22)	
Heavy	66%	(111/169)	1.35 (0.97-1.89)	0.02[trend]

* Individuals were considered KSHV positive if they had a positive ORF 73 and/or K8.1 ELISA. Participants were considered negative if both ELISAs were negative.

** All estimated using Chi-squared test. All tests of statistical significance two sided.

Authors' contributions

KW conceived and coordinated the study, carried out the KSHV ELISA assays, performed the statistical analysis and drafted the manuscript, ELW performed the statistical analysis and helped to draft the manuscript, IS carried out KSHV ELISA assays, LM managed the study database, WM set up, validated and carried out the KSHV ELISA assays and helped to draft the manuscript, WTM helped with statistical analysis and drafting the manuscript, JN was project leader for the EMaBS cohort, AME is principle investigator for the EMaBS cohort, conceived the study and helped with statistical analysis and drafting the manuscript, DW is head of VOS, NCI, conceived the study and drafted the manuscript and RN drafted the manuscript. All authors have read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 12 July 2011 Accepted: 30 September 2011

Published: 30 September 2011

References

1. Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, Benbrahim-Tallaa L, Guha N, Freeman C, Galichet L, Coglianò V: **A review of human carcinogens-Part B: biological agents.** *Lancet Oncol* 2009, **10**:321-322.
2. Engels EA, Biggar RJ, Marshall VA, Walters MA, Gamache CJ, Whitby D, Goedert JJ: **Detection and quantification of Kaposi's sarcoma-associated herpesvirus to predict AIDS-associated Kaposi's sarcoma.** *AIDS* 2003, **17**:1847-1851.
3. Laney AS, Dollard SC, Jaffe HW, Offermann MK, Spira TJ, Gunthel CJ, Pellett PE, Cannon ML: **Repeated measures study of human herpesvirus 8 (HHV-8) DNA and antibodies in men seropositive for both HHV-8 and HIV.** *AIDS* 2004, **18**:1819-1826.
4. Laney AS, Cannon ML, Jaffe HW, Offermann MK, Ou CY, Radford KW, Patel MM, Spira TJ, Gunthel CJ, Pellett PE, Dollard SC: **Human herpesvirus 8 presence and viral load are associated with the progression of AIDS-associated Kaposi's sarcoma.** *AIDS* 2007, **21**:1541-1545.
5. Merat R, Amara A, Lebbe C, de The H, Morel P, Saib A: **HIV-1 infection of primary effusion lymphoma cell line triggers Kaposi's sarcoma-associated herpesvirus (KSHV) reactivation.** *Int J Cancer* 2002, **97**:791-795.
6. Bagni R, Whitby D: **Kaposi's sarcoma-associated herpesvirus transmission and primary infection.** *Curr Opin HIV AIDS* 2009, **4**:22-26.
7. Sitas F, Carrara H, Beral V, Newton R, Reeves G, Bull D, Jentsch U, Pacella-Norman R, Bourboulla D, Whitby D, Boshoff C, Weiss R: **Antibodies against human herpesvirus 8 in black South African patients with cancer.** *N Engl J Med* 1999, **340**:1863-1871.
8. Grundhoff A, Ganem D: **Inefficient establishment of KSHV latency suggests an additional role for continued lytic replication in Kaposi sarcoma pathogenesis.** *J Clin Invest* 2004, **113**:124-136.
9. Ascoli V, Belli S, Benedetti M, Trinca S, Ricci P, Comba P: **High incidence of classic Kaposi's sarcoma in Mantua, Po Valley, Northern Italy (1989-1998).** *Br J Cancer* 2001, **85**:379-382.
10. Ascoli V, Facchinelli L, Valerio L, Zucchetto A, Dal Maso L, Coluzzi M: **Distribution of mosquito species in areas with high and low incidence of classic Kaposi's sarcoma and seroprevalence for HHV-8.** *Med Vet Entomol* 2006, **20**:198-208.
11. Ascoli V, Senis G, Zucchetto A, Valerio L, Facchinelli L, Budroni M, Dal Maso L, Coluzzi M: **Distribution of 'promoter' sandflies associated with incidence of classic Kaposi's sarcoma.** *Med Vet Entomol* 2009, **23**:217-225.
12. Ascoli V, Zambon P, Manno D, Guzzinati S, Zorzi M, Arca B, Costantini C, Coluzzi M: **Variability in the incidence of classic Kaposi's sarcoma in the Veneto region, Northern Italy.** *Tumori* 2003, **89**:122-124.
13. Coluzzi M, Calabro ML, Manno D, Chieco-Bianchi L, Schulz TF, Ascoli V: **Reduced seroprevalence of Kaposi's sarcoma-associated herpesvirus (KSHV), human herpesvirus 8 (HHV8), related to suppression of Anopheles density in Italy.** *Med Vet Entomol* 2003, **17**:461-464.
14. Coluzzi M, Manno D, Guzzinati S, Tognazzo S, Zambon P, Arca B, Costantini C, Ascoli V: **The bloodsucking arthropod bite as possible cofactor in the transmission of human herpesvirus-8 infection and in the expression of Kaposi's sarcoma disease.** *Parassitologia* 2002, **44**:123-129.
15. Cook-Mozaffari P, Newton R, Beral V, Burkitt DP: **The geographical distribution of Kaposi's sarcoma and of lymphomas in Africa before the AIDS epidemic.** *Br J Cancer* 1998, **78**:1521-1528.
16. Dediccoat M, Newton R: **Review of the distribution of Kaposi's sarcoma-associated herpesvirus (KSHV) in Africa in relation to the incidence of Kaposi's sarcoma.** *Br J Cancer* 2003, **88**:1-3.
17. Dollard SC, Butler LM, Graves Jones AM, Mermin JH, Chidzonga M, Chipato T, Shibuski CH, Brander C, Mosam A, Kiepiela P, Hladik W, Martin JN: **Substantial regional differences in human herpesvirus 8 seroprevalence in sub-Saharan Africa: Insights on the origin of the "KS Belt".** *Int J Cancer* 2010.
18. Tanzi E, Zappa A, Caramaschi F, Amendola A, Lassagna D, Gatti L, Ascoli V, Rezza G, Zanetti AR: **Human herpesvirus type 8 infection in an area of Northern Italy with high incidence of classic Kaposi's sarcoma.** *J Med Virol* 2005, **76**:571-575.
19. Brown EE, Whitby D, Vitale F, Marshall V, Mbisa G, Gamache C, Lauria C, Alberg AJ, Serraino D, Cordiali-Fei P, Messina A, Goedert JJ: **Virologic, hematologic, and immunologic risk factors for classic Kaposi sarcoma.** *Cancer* 2006, **107**:2282-2290.
20. Montesu MA, De Marco R, Cottoni F: **Soil silicates and Kaposi's sarcoma in Sardinia.** *Lancet* 1995, **346**:1436-1437.
21. Ziegler JL: **Endemic Kaposi's sarcoma in Africa and local volcanic soils.** *Lancet* 1993, **342**:1348-1351.
22. Whitby D, Marshall VA, Bagni R, Miley WJ, McCloud TG, Hines-Boykin R, Goedert JJ, Conde BA, Nagashima K, Mikovits J, Dittmer DP, Newman DJ: **Reactivation of Kaposi's sarcoma-associated herpesvirus by natural products from Kaposi's sarcoma endemic regions.** *Int J Cancer* 2007, **120**:321-328.
23. Ascoli V, Facchinelli L, Valerio L, Manno D, Coluzzi M: **Kaposi's sarcoma, human herpesvirus 8 infection and the potential role of promoter-arthropod bites in northern Sweden.** *J Med Virol* 2006, **78**:1452-1455.
24. Ziegler JL, Newton R, Katongole-Mbidde E, Mbulaitaye S, De Cock K, Wabinga H, Mugerwa J, Katubira E, Jaffe H, Parkin DM, Reeves G, Weiss R, Beral V: **Risk factors for Kaposi's sarcoma in HIV-positive subjects in Uganda.** *AIDS* 1997, **11**:1619-1626.
25. Ziegler J, Newton R, Bourboulla D, Casabonne D, Beral V, Mbidde E, Carpenter L, Reeves G, Parkin DM, Wabinga H, Mbulaitaye S, Jaffe H, Weiss R, Boshoff C: **Risk factors for Kaposi's sarcoma: a case-control study of HIV-seronegative people in Uganda.** *Int J Cancer* 2003, **103**:233-240.
26. Hutt MS: **The epidemiology of Kaposi's sarcoma.** *Antibiot Chemother* 1981, **29**:3-11.
27. Mbulaitaye SM, Biggar RJ, Pfeiffer RM, Bakaki PM, Gamache C, Owor AM, Katongole-Mbidde E, Nduwaga CM, Goedert JJ, Whitby D, Engels EA: **Water, socioeconomic factors, and human herpesvirus 8 infection in Ugandan children and their mothers.** *J Acquir Immune Defic Syndr* 2005, **38**:474-479.
28. Lin CJ, Katongole-Mbidde E, Byekwaso T, Orem J, Rabkin CS, Mbulaitaye SM: **Intestinal parasites in Kaposi sarcoma patients in Uganda: indication of shared risk factors or etiologic association.** *Am J Trop Med Hyg* 2008, **78**:409-412.
29. Maizels RM: **Parasite immunomodulation and polymorphisms of the immune system.** *J Biol* 2009, **8**:62.
30. Maizels RM, Pearce EJ, Artis D, Yazdanbakhsh M, Wynn TA: **Regulation of pathogenesis and immunity in helminth infections.** *J Exp Med* 2009, **206**:2059-2066.
31. Moormann AM, Chelimo K, Sumba OP, Lutze ML, Ploutz-Snyder R, Newton D, Kazura J, Rochford R: **Exposure to holoendemic malaria results in elevated Epstein-Barr virus loads in children.** *J Infect Dis* 2005, **191**:1233-1238.
32. Moormann AM, Chelimo K, Sumba PO, Tisch DJ, Rochford R, Kazura JW: **Exposure to holoendemic malaria results in suppression of Epstein-Barr virus-specific T cell immunosurveillance in Kenyan children.** *J Infect Dis* 2007, **195**:999-1008.
33. Priou E, Kimmel R, Chelimo K, Middeldorp JM, Odada PS, Ploutz-Snyder R, Moormann AM, Rochford R: **Serological evidence for long-term Epstein-Barr virus reactivation in children living in a holoendemic malaria region of Kenya.** *J Med Virol* 2009, **81**:1088-1093.
34. Rainey JJ, Mwanda WO, Wairimu P, Moormann AM, Wilson ML, Rochford R: **Spatial distribution of Burkitt's lymphoma in Kenya and association with malaria risk.** *Trop Med Int Health* 2007, **12**:936-943.
35. Elliott AM, Kizza M, Quigley MA, Ndibazza J, Nampijja M, Muhangi L, Morison L, Namujju PB, Muwanga M, Kabatereine N, Whitworth JA: **The impact of helminths on the response to immunization and on the incidence of infection and disease in childhood in Uganda: design of a randomized, double-blind, placebo-controlled, factorial trial of**

- deworming interventions delivered in pregnancy and early childhood [ISRCTN32849447]. *Clin Trials* 2007, **4**:42-57.
36. Mbisa GL, Miley W, Gamache CJ, Gillette WJ, Esposito D, Hopkins R, Busch MP, Schreiber GB, Little RF, Yarchoan R, Ortiz-Conde BA, Labo N, Whitby D: **Detection of antibodies to Kaposi's sarcoma-associated herpesvirus: a new approach using K8.1 ELISA and a newly developed recombinant LANA ELISA.** *J Immunol Methods* 2010, **356**:39-46.
37. Malope BI, Pfeiffer RM, Mbisa G, Stein L, Ratsikhopho EM, O'Connell DL, Sitas F, MacPhail P, Whitby D: **Transmission of Kaposi sarcoma-associated herpesvirus between mothers and children in a South African population.** *J Acquir Immune Defic Syndr* 2007, **44**:351-355.
38. Nalibazza J, Muhangi L, Akishule D, Kiggundu M, Ameke C, Owela J, Kizindo R, Duong T, Kleinschmidt I, Muwanga M, Elliott AM: **Effects of deworming during pregnancy on maternal and perinatal outcomes in Entebbe, Uganda: a randomized controlled trial.** *Clin Infect Dis* 2010, **50**:531-540.
39. de Sanjose S, Marshall V, Sola J, Palacio V, Almirall R, Goedert JJ, Bosch FX, Whitby D: **Prevalence of Kaposi's sarcoma-associated herpesvirus infection in sex workers and women from the general population in Spain.** *Int J Cancer* 2002, **98**:155-158.
40. Dedicato M, Newton R, Alkharshah KB, Sheldon J, Szabados I, Ndllovu B, Page T, Casabonne D, Gilks CF, Cassol SA, Whitby D, Schulz TF: **Mother-to-child transmission of human herpesvirus-8 in South Africa.** *J Infect Dis* 2004, **190**:1068-1075.
41. Wawer MJ, Eng SM, Serwadda D, Sewankambo NK, Kiwanuka N, Li C, Gray RH: **Prevalence of Kaposi sarcoma-associated herpesvirus compared with selected sexually transmitted diseases in adolescents and young adults in rural Rakai District, Uganda.** *Sex Transm Dis* 2001, **28**:77-81.
42. Olsen SJ, Chang Y, Moore PS, Biggar RJ, Melbye M: **Increasing Kaposi's sarcoma-associated herpesvirus seroprevalence with age in a highly Kaposi's sarcoma endemic region, Zambia in 1985.** *AIDS* 1998, **12**:1921-1925.
43. Minhas V, Crabtree KL, Chao A, M'Soka TJ, Kankasa C, Bulteyns M, Mitchell CD, Wood C: **Early childhood infection by human herpesvirus 8 in Zambia and the role of human immunodeficiency virus type 1 coinfection in a highly endemic area.** *Am J Epidemiol* 2008, **168**:311-320.
44. Mbulateye SM, Walters M, Engels EA, Bakaki PM, Ndugwa CM, Owor AM, Goedert JJ, Whitby D, Biggar RJ: **High levels of Epstein-Barr virus DNA in saliva and peripheral blood from Ugandan mother-child pairs.** *J Infect Dis* 2006, **193**:422-426.
45. Mbulateye SM, Pfeiffer RM, Engels EA, Marshall V, Bakaki PM, Owor AM, Ndugwa CM, Katongole-Mbidde E, Goedert JJ, Biggar RJ, Whitby D: **Detection of kaposi sarcoma-associated herpesvirus DNA in saliva and buffy-coat samples from children with sickle cell disease in Uganda.** *J Infect Dis* 2004, **190**:1382-1386.
46. Mbulateye S, Marshall V, Bagni RK, Wang CD, Mbisa G, Bakaki PM, Owor AM, Ndugwa CM, Engels EA, Katongole-Mbidde E, Biggar RJ, Whitby D: **Molecular evidence for mother-to-child transmission of Kaposi sarcoma-associated herpesvirus in Uganda and K1 gene evolution within the host.** *J Infect Dis* 2006, **193**:1250-1257.
47. Malope BI, MacPhail P, Mbisa G, MacPhail C, Stein L, Ratsikhopho EM, Ndllovu L, Sitas F, Whitby D: **No evidence of sexual transmission of Kaposi's sarcoma herpes virus in a heterosexual South African population.** *AIDS* 2008, **22**:519-526.
48. Klaskala W, Brayfield BP, Kankasa C, Bhat G, West JT, Mitchell CD, Wood C: **Epidemiological characteristics of human herpesvirus-8 infection in a large population of antenatal women in Zambia.** *J Med Virol* 2005, **75**:93-100.
49. Butler LM, Dorsey G, Hladik W, Rosenthal PJ, Brander C, Nelands TB, Mbisa G, Whitby D, Kiepiela P, Mosam A, Mzolo S, Dollard SC, Martin JN: **Kaposi sarcoma-associated herpesvirus (KSHV) seroprevalence in population-based samples of African children: evidence for at least 2 patterns of KSHV transmission.** *J Infect Dis* 2009, **200**:430-438.
50. Brayfield BP, Phiri S, Kankasa C, Muyanga J, Mantina H, Kwenda G, West JT, Bhat G, Marx DB, Klaskala W, Mitchell CD, Wood C: **Postnatal human herpesvirus 8 and human immunodeficiency virus type 1 infection in mothers and infants from Zambia.** *J Infect Dis* 2003, **187**:559-568.
51. de Sanjose S, Mbisa G, Perez-Alvarez S, Benavente Y, Sukvirach S, Hieu NT, Shin HR, Anh PT, Thomas J, Lazzano E, Matos E, Hennero R, Munoz N, Molano M, Franceschi S, Whitby D: **Geographic variation in the prevalence of Kaposi sarcoma-associated herpesvirus and risk factors for transmission.** *J Infect Dis* 2009, **199**:1449-1456.
52. Newton R, Ziegler J, Bourboulla D, Casabonne D, Beral V, Mbidde E, Carpenter L, Reeves G, Parkin DM, Wabinga H, Mbulateye S, Jaffe H, Weiss R, Boshoff C: **The sero-epidemiology of Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) in adults with cancer in Uganda.** *Int J Cancer* 2003, **103**:226-232.

doi:10.1186/1750-9378-6-15

Cite this article as: Wakeham *et al.*: Parasite infection is associated with Kaposi's sarcoma associated herpesvirus (KSHV) in Ugandan women. *Infectious Agents and Cancer* 2011 **6**:15.

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Risk Factors for Seropositivity to Kaposi Sarcoma–Associated Herpesvirus Among Children in Uganda

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Background: Determinants of Kaposi sarcoma associated herpesvirus (KSHV) seropositivity among children living in sub-Saharan African populations where infection is endemic are not well understood. Local environmental factors, including other infectious agents, may be key.

Methods: Within the context of a well-characterized birth cohort, we examined associations between various factors and antibodies against KSHV, measured in stored plasma samples from 1823 mother child pairs in Entebbe, Uganda.

Received for publication October 12, 2012; accepted January 18, 2013.
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Presented at the 14th International Workshop on Kaposi's Sarcoma Herpesvirus (KSHV) and Related Agents, August 2011, Helsinki; 13th International Workshop on Kaposi's Sarcoma Herpesvirus (KSHV) and Related Agents, August 2010, Los Angeles.

Funded by the Wellcome Trust as a PhD Training Fellowship held by K. Wakeham (grant no. 090132), a Wellcome Trust Senior Fellowships held by A. M. Elliott (grant nos. 064693 and 079110) and funded in part by the Intramural Program of the National Cancer Institute, National Institutes of Health, Department of Health and Human Services (contract HHSN261200800001E). Wellcome Trust is a UK registered charity no. 210183.

The authors have no conflicts of interest to disclose.

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Results: Seroprevalence increased with increasing age of the child ($P = 0.0003$) and was higher among those with KSHV seropositive mothers than in those without (12% vs 9%; odds ratio: 1.4, 95% confidence interval: 1.1 to 2.0). It was also higher among children with HIV infection (29% vs 10%; odds ratio: 3.1, 95% confidence interval: 1.2 to 8.3) or malaria parasitemia (30% vs 10%; odds ratio: 4.1, 95% confidence interval: 2.4 to 7.0) than in children without. These associations were not explained by socioeconomic status.

Conclusions: The finding that KSHV serostatus is associated with malaria parasitemia in children is novel. In a country endemic for KSHV, malaria may be a cofactor for KSHV infection or reactivation among children.

Key Words: Kaposi sarcoma-associated herpesvirus, Sub-Saharan Africa, children, HIV, Kaposi sarcoma

(*J Acquir Immune Defic Syndr* 2013;63:228–233)

BACKGROUND

Kaposi sarcoma–associated herpesvirus (KSHV) seroprevalence exhibits marked worldwide geographical variation,^{1,2} which may represent regional differences in modes of transmission, or point to the existence of cofactors for infection or reactivation. Studies from sub-Saharan Africa report high KSHV prevalence, with primary infection beginning in childhood and increasing with age.^{3,4} There is considerable evidence, too, that KSHV transmission occurs via saliva^{5,6} and in Africa transmission from mothers and siblings, it is likely to be an important route.^{4,7} Risk factors governing childhood vulnerability to infection with KSHV remain only partly elucidated. Exposures in childhood including other infectious diseases may be key. Understanding transmission dynamics is a prerequisite for the development of strategies to prevent spread and the subsequent diseases associated with this important oncogenic infection.

This study took place in Uganda, a country with a high endemic prevalence of KSHV⁸ and a relatively high incidence of the Kaposi sarcoma, both among HIV-infected people and among those without HIV.^{9,10} We hypothesize that this region has specific cofactors driving KSHV transmission. We have previously reported a high prevalence of antibodies to KSHV in pregnant women in Uganda, and risk factors for infection

among these women included certain parasites.⁸ Few studies have so far examined parasitic infections as cofactors for seropositivity in childhood. This analysis describes the association between antibodies to KSHV in Ugandan children aged 1–5 years and common local exposures including HIV, malaria, and helminths in addition to maternal and household factors.

METHODS

Study Population

The investigation was nested within an existing study in Uganda—the Entebbe Mother and Baby Study—a large double-blind randomized placebo-controlled trial designed to determine the impact of helminth infections and their treatment on vaccine responses and infectious disease outcomes (International Standard Randomised Controlled Trials No. 32849447). The details of this study have been reported elsewhere.¹¹ Briefly, consenting women in their second or third trimester of pregnancy, resident in Entebbe and Katabi, Uganda were recruited between April 2003 and November 2005 from the government funded antenatal clinic. The residential area for the women covers rural, urban, and fishing communities. At enrolment, sociodemographic data were collected, and blood samples were obtained by venepuncture and processed for HIV serology and CD4 count. Women found to be infected with HIV were offered single-dose nevirapine to prevent vertical HIV transmission.

Children were followed from birth and at the time of this study were between 4 and 5 years old. Children were also seen for vaccinations and if unwell, for medical treatment. At routine yearly visits, clinical data, blood for full blood count, blood slides for malaria and *Mansonella perstans*, and stool samples were collected from well children. Any helminth infections were treated. In addition, at approximately 6 weeks and 18 months of age, blood samples were obtained from children of HIV-infected mothers to ascertain the child's HIV status. Blood samples from mothers and their children were processed and plasma stored in the -80°C freezer archive facility at the Uganda Virus Research Institute (UVRI), Entebbe, Uganda.

Diagnosis of Infections

Stool was examined using the Kato-Katz^{12,13} method for identification of hookworm, *Schistosomiasis mansoni*, *Trichuris trichiura*, *Ascaris lumbricoides*, and *Trichostrongylus* species and by charcoal culture for *Strongyloides stercoralis*.¹³ Two slides from a single stool sample were examined for each individual within 30 minutes for hookworm and the next day for other ova and parasites. Blood was examined for *M. perstans* by a modified Knott method.¹⁴ *Plasmodium falciparum* was diagnosed by examination of thick blood films and asymptomatic malaria parasitemia defined as the presence of parasites without fever. In 6-week-old children, HIV was indicated by plasma viral load.¹⁵ In mothers and children more than 18 months of age, HIV was identified by serology using a triple rapid test serial testing algorithm.¹⁶

KSHV Serological Testing

The mother's enrolment plasma sample and the last available plasma sample for each child were selected for KSHV serological testing using enzyme-linked immunosorbent assays (ELISAs) for recombinant proteins K8.1 (a KSHV structural glycoprotein expressed during lytic infection) and ORF 73 (a nuclear antigen expressed during latency) as previously described.^{8,17,18} The ELISAs were performed at UVRI by the study lead and a technician, both of whom were blinded to patient details. The assays were transferred to UVRI from the Viral Oncology Section, Frederick National Laboratory for Cancer Research, United States, and have been used in more than 40 studies worldwide. Analysis of the positive and negative controls showed comparable performance at Viral Oncology Section, Frederick National Laboratory for Cancer Research, and at UVRI.⁸ Ten percent of samples were tested in duplicate and agreement formally tested by calculating a Kappa statistic.¹⁹ The Kappa score for agreement for the duplicate samples for K8.1 was 0.86 and for ORF 73 was 0.92. These scores represent almost perfect agreement.¹⁹

Statistical Analysis

Data were analyzed using Stata11SE (StataCorp LP, College Station, TX). As individual responses to KSHV antigens are complex and no gold standard assay exists to simplify the presentation of results, we combined the K8.1 and ORF 73 results to define evidence of KSHV infection as "positive" if either of the 2 assays were positive and "negative" if both assays were negative. The agreement between K8.1 and ORF 73 ELISA assays was assessed by calculating a Kappa statistic.¹⁹ A composite variable for household socioeconomic status was derived based on home building materials and number of rooms and items collectively owned at the mothers home.¹⁶ Children were categorized into 3 HIV status groups: HIV unexposed, HIV exposed (to maternal HIV) but not infected, and HIV exposed and infected. Because the prevalence of helminth infections in children was low, a variable for current infection with any helminth was created indicating infection with *Trichostrongylus* sp, *A. lumbricoides*, hookworm, *M. perstans*, *S. mansoni*, *S. stercoralis*, or *T. trichiura*. Other risk factors for childhood KSHV seropositivity considered were (1) childhood variables: gender, age, asymptomatic malaria parasitemia, and number of previous symptomatic malaria episodes; (2) maternal factors: KSHV serostatus, age at birth of child, educational attainment, marital status, number of babies born alive, number of children living in the home, personal monthly income, and trial treatment arm; and (3) risk factors for malaria and helminth infections: walking distance to Lake Victoria, type of toilet, use of mosquito nets, spraying home for mosquitoes, and water collection source (lake, well, bore hole, stand pipe, tap).

Potential bias in the sample of mother-child pairs was investigated by comparing the covariate distributions of mother-child pairs included in this study to the distributions of those in the cohort who had no samples available for inclusion. Further to this, mother-child pairs where the child was lost to follow-up at an early age were compared with pairs with longer child follow-up.

The outcome of interest for all analyses was KSHV serostatus of the child as defined above. The initial analysis was based on the a priori hypothesis that the age of the child would be a major determinant of risk of infection. Therefore, all odds ratios in the initial analysis (model 1) are adjusted for age of child. A multivariable logistic model (model 2) was then constructed including all risk factors that were associated with the outcome at the 5% level in model 1. Statistical significance was assessed using the likelihood ratio test. Departure from linear trend was considered for all ordered categorical exposure variables by calculation of a likelihood ratio test. To assess factors that potentially modify the risk of a KSHV seropositive mother having a KSHV seropositive child, interactions between maternal KSHV serostatus and HIV status or asymptomatic malaria parasitemia in the child were added to the regression model. No adjustment was made for multiple comparisons. All *P* values were 2-sided and we considered *P* < 0.05 to be statistically significant.

Ethical Approval

Written informed consent was obtained from each woman, for her own and her child's participation. Ethical approval for this study was obtained from the UVRI, Science and Ethics Committee, Entebbe, Uganda, and the Uganda National Council for Science and Technology, Kampala, Uganda.

RESULTS

Plasma samples were available for KSHV serological testing from 1823 (86%) mother-child pairs. Twenty sets of twins were excluded from these analyses. Maternal age at sampling ranged from 14 to 43 years (median 23 years). The majority of women (74%) were multigravida and most were married (85%). The majority of women (85%) reported a monthly income below the World Bank poverty line of 1.25 USD per day and 25% reported that they could not read. Antenatal HIV seroprevalence among mothers was 10% with a median CD4 count for HIV seropositive women of 553 (interquartile range: 368–813) and 4% of women had active syphilis. Among children, the prevalence of asymptomatic malaria parasitemia was 5% and HIV seroprevalence was 1% overall. Helminth infections were rare among children: 3% prevalence of *Trichostrongylus* sp and 1% prevalence of *A. lumbricoides* infection; hookworm, *M. perstans*, *S. mansoni*, *S. stercoralis*, and *T. trichiura* were all detected in fewer than 1% of children.

Mother-child pairs with no samples available for inclusion were more likely to include a HIV seropositive mother with less education. Mothers of children whose last available sample was at 1 year were generally younger and more likely to be HIV seropositive compared with mothers of children followed up for a longer period.

Among mothers, the prevalence of antibodies to K8.1 was 41% and to ORF 73 was 53%; 32% were seropositive to both antigens and 61% had antibodies to either K8.1 or ORF 73. The seroprevalence of KSHV among children was 9% to K8.1, 6% to ORF 73, 4% to both antigens, and 11% to either ORF 73 or K8.1. There was moderate concordance between lytic K8.1 and

latent ORF 73 assays in detecting KSHV seropositivity in the mothers (*K* = 0.43) and the children (*K* = 0.46).

Factors significantly associated with KSHV seropositivity among children are presented in Table 1. Associations for age and maternal education were consistent with linear trends. Of childhood factors, increasing age of the child was strongly associated with KSHV seropositivity in crude and adjusted analyses (model 2). Childhood infections with malaria and HIV were both associated with increased risk of being KSHV seropositive. Of maternal factors, KSHV seropositive status and low educational attainment were associated with KSHV seropositivity in the child; the effect of maternal KSHV serostatus was reduced by adjusting for other potential confounders (model 2) and the association with maternal education was not statistically significant. In an analysis restricted to HIV seropositive mothers, being a HIV seropositive child was associated with a 2-fold increase in the risk of being KSHV seropositive compared with being a HIV-negative child (odds ratio: 1.9, 95% confidence interval: 0.6 to 5.4). Use of a mosquito net in the home was marginally associated with a decreased risk of childhood KSHV seropositivity in model 1, but this association was not retained after adjusting for other potential confounders (model 2).

No modifiers of the relationship between KSHV serostatus of the mother and child were detected.

DISCUSSION

In this study of children aged 1–5 years in Uganda, we have demonstrated for the first time an association between KSHV seropositivity and the presence of asymptomatic malaria parasitemia. We have also confirmed associations between KSHV serostatus in Ugandan children and the following cofactors: age of the child, HIV infection in the child, and maternal KSHV serostatus.

Parasites have been hypothesized as potential cofactors for KSHV transmission. KSHV is an immune-sensitive virus,²⁰ and the survival of parasites is dependent on their ability to interfere with host immune function.²¹ Ecological studies from Italy report substantial declines in KSHV seroprevalence and Kaposi sarcoma incidence in association with eradication of mosquitoes.²² It is notable that malaria is known to affect immune control of another human gamma herpesvirus, the Epstein-Barr virus. In addition, malaria interacts with the Epstein-Barr virus thereby increasing the risk of Burkitt lymphoma, the most common cancer reported in children in East Africa.^{23,24} Malaria may impair immune defence and therefore increase susceptibility to primary KSHV infection. In addition, malaria infection may cause KSHV reactivation increasing viral replication and shedding in saliva. Repeated malaria infections have been shown to suppress T-cell immunity,²⁵ and this may have a detrimental effect on the control of KSHV infection. We have previously reported an association between KSHV seropositivity and malaria in women in Uganda.⁸ Longitudinal studies will be required to examine the nature of this association further.

HIV is an important candidate for influencing KSHV transmission rates between KSHV-infected mothers and their

TABLE 1. Factors Associated With KSHV Seropositivity in Ugandan Children Aged 1–5 Years

Factor	Prevalence*	Age adjusted OR (95% CI)†	P‡	Adjusted OR (95% CI)§	P‡
Child factors at time of sampling					
Age of child (yrs)					
1	4% (5/129)	1		1	
2	7% (13/182)	1.9 (0.7–5.5)		2.1 (0.7–6.9)	
3	10% (70/677)	2.9 (1.1–7.2)		3.6 (1.3–10.3)	
4	13% (76/585)	3.7 (1.5–9.3)		5.0 (1.8–14.4)	
5	14% (36/250)	4.2 (1.6–10.9)		6.0 (2.0–17.7)	
<i>P</i> for trend			<0.0001		0.0003
HIV status if the child (18 missing values)					
Not exposed	10% (169/1635)	1		1	
Exposed but uninfected	15% (22/143)	1.5 (1.0–2.5)		1.6 (1.0–2.7)	
Infected	29% (8/27)	4.3 (1.8–10.2)		3.1 (1.2–8.3)	
<i>P</i> for heterogeneity			0.004		0.03
Asymptomatic malaria parasitemia (128 missing values)					
No	10% (164/1617)	1		1	
Yes	30% (23/78)	3.9 (2.4–6.7)		4.1 (2.4–7.0)	
<i>P</i> for heterogeneity			<0.0001		<0.0001
Maternal and household factors at time of sampling					
Maternal KSHV serostatus					
Negative	9% (61/708)	1		1	
Positive	12% (139/1115)	1.6 (1.1–2.2)		1.4 (1.0–2.0)	
<i>P</i> for heterogeneity			<0.0001		0.04
Maternal education (4 missing values)					
None	16% (11/67)	1		1	
Primary	12% (110/920)	0.6 (0.3–1.2)		0.5 (0.3–1.1)	
Senior	10% (68/670)	0.5 (0.2–1.0)		0.5 (0.2–1.0)	
Tertiary	7% (11/162)	0.3 (0.1–0.8)		0.3 (0.1–0.8)	
<i>P</i> for trend			0.04		0.08
Use of mosquito net in the home (3 missing values)					
No	12% (112/904)	1		1	
Yes	10% (97/916)	0.7 (0.6–1.0)		0.8 (0.6–1.1)	
<i>P</i> for heterogeneity			0.04		0.3

*KSHV seropositive to either K8.1 or ORF 73.

†All adjusted for age of child (model 1).

‡*P* value for association of exposure with outcome of KSHV seropositivity.

§All adjusted for age of child, HIV exposure status, asymptomatic malaria parasitemia, maternal KSHV serostatus, maternal education, and use of mosquito net in the home (model 2).

||Antenatal enrolment visit.

OR, odds ratio; CI, confidence interval.

children. HIV-infected subjects may be more likely to shed KSHV in saliva and HIV-associated immune deficiency may increase susceptibility to KSHV infection. We found some evidence that having an HIV-infected mother was a risk factor for KSHV seropositivity if the child was HIV negative, and strong evidence that if the child was HIV positive, the odds of KSHV seropositivity were increased compared with HIV unexposed uninfected children and with HIV-exposed uninfected children. This study confirms the findings of others^{4,18,26} that HIV infection is associated with an increased seroprevalence of KSHV in children; only 1 study did not find such an association.²⁷ Whether HIV is acting to increase vulnerability to infection or causing reactivation in children infected with KSHV in the past will require further longitudinal studies of KSHV infection.

In sub-Saharan Africa, KSHV is endemic and infection prevalence in children increases with age, suggesting nonsexual horizontal transmission.²⁸ There is little evidence of vertical KSHV transmission, or that the virus can be passed through breast milk.^{4,29} KSHV is likely to be transmitted through saliva.^{5,6} The seroprevalence of KSHV in this study increased from 4% in children aged 1 year to 15% in 5-year-old children. In keeping with other studies, KSHV infection status of the mother was positively associated with infection risk in the child.^{4,18} However, nearly a third of KSHV seropositive children had a KSHV antibody negative mother. It is possible that these mothers became infected with KSHV after the antenatal enrolment sample or that the child was infected from another source. KSHV transmission within families in sub-Saharan Africa has been documented with infection risk for the child

showing associations in decreasing order of magnitude with infection in the mother, father, and next oldest sibling.⁷ Childhood KSHV seropositivity was not associated with maternal age, in line with other studies in which women had a narrow age range.^{30,31}

In this study, markers of socioeconomic status, namely household socioeconomic status, maternal education, maternal monthly income, and type of toilet, were not associated with antibodies to KSHV. The absence of an association between childhood KSHV seropositivity and maternal educational attainment is in keeping with other studies from sub-Saharan Africa.^{26,30} In a recent extensive report of socioeconomic factors in mother-child pairs in Uganda, the risk of being KSHV seropositive was low among mother-child pairs in the high-income group, although the trend in risk with increasing income was not linear.³⁰ Other factors including maternal and paternal occupation, household electricity, and household density were not found to be associated. Contact with water or collecting water from ground, stream, river, lake, or wetland sources attracted particular attention in early reports of risk factors for Kaposi sarcoma. Ground water was not thought to be a marker of socioeconomic status, but rather that it may contain a potential cofactor such as a parasite.^{30,32,33} In Uganda, previous studies have investigated type of water supply and antibodies to KSHV. In children and their mothers, use of surface water for drinking was associated with an increased risk of KSHV seropositivity in the crude but not adjusted analysis.³⁰ We found no association with water source nor did 2 other studies of children and adolescents attending an urban hospital in Uganda.^{5,34}

Our study of a large mother-child population benefited from the use of robust data, collected through a well-conducted randomized controlled trial, the initial findings of which have been published elsewhere.¹⁵ The moderate concordance between lytic K8.1 and latent ORF 73 assays in detecting KSHV seropositivity is consistent with previous studies.^{4,18} However, there were several limitations. The mothers had relatively high CD4 counts and were receiving HIV care that may have impacted on our ability to assess the role of HIV alone. The mother's plasma sample was collected remotely in time from the child's sample and the overall number of HIV-infected children was small. Power to detect potential associations with childhood helminth infections was small because of the low prevalence of these infections. Selection biases may have underestimated the association with HIV and antibodies to KSHV. Finally, the cross-sectional design makes it difficult to differentiate between risk factors for primary infection and reactivation (leading to a boost in antibody titers). Further longitudinal studies are needed that collect suitable material to study KSHV viral load and KSHV-specific T-cell function.

Cofactors for KSHV transmission are incompletely understood. Fully elucidating the factors impacting on infectiousness and vulnerability will be the key to reducing KSHV transmission and thereby the incidence of HIV-associated and -unassociated Kaposi sarcoma in sub-Saharan Africa. This study raises new and interesting questions surrounding the relationship between KSHV and the malaria parasite while confirming child's age, HIV infection, and maternal KSHV status as key cofactors.

ACKNOWLEDGMENTS

The authors thank the participants and staff of the Entebbe Mother and Baby Study and the staff of the laboratory and statistics departments of the Medical Research Council/Uganda Research Unit on AIDS, who made this study possible.

REFERENCES

1. Dedicoat M, Newton R. Review of the distribution of Kaposi's sarcoma-associated herpesvirus (KSHV) in Africa in relation to the incidence of Kaposi's sarcoma. *Br J Cancer*. 2003;88:1-3.
2. Dollard SC, Butler LM, Graves Jones AM, et al. Substantial regional differences in human herpesvirus 8 seroprevalence in sub-Saharan Africa: insights on the origin of the "KS Belt." *Int J Cancer*. 2010;127:2395-2401.
3. Mbulateye SM, Biggar RJ, Bakaki PM, et al. Human herpesvirus 8 infection and transfusion history in children with sickle-cell disease in Uganda. *J Natl Cancer Inst*. 2003;95:1330-1335.
4. Dedicoat M, Newton R, Alkharsh KR, et al. Mother-to-child transmission of human herpesvirus-8 in South Africa. *J Infect Dis*. 2004;190:1068-1075.
5. Mbulateye SM, Pfeiffer RM, Engels EA, et al. Detection of Kaposi sarcoma-associated herpesvirus DNA in saliva and buffy-coat samples from children with sickle cell disease in Uganda. *J Infect Dis*. 2004;190:1382-1386.
6. Butler LM, Dollard SC, Amin M, et al. Relationship between salivary shedding and seropositivity for Kaposi's sarcoma-associated herpesvirus (KSHV) and Epstein-Barr virus (EBV) among South African children and adults: insights on why EBV infection is ubiquitous and KSHV is not. Paper presented at: 12th International Conference on Malignancies in AIDS and Other Acquired Immunodeficiencies (ICMAOD); April 26-27 2010; Bethesda, MD.
7. Mbulateye SM, Pfeiffer RM, Whitby D, et al. Human herpesvirus 8 infection within families in rural Tanzania. *J Infect Dis*. 2003;187:1780-1785.
8. Wakeham K, Webb EL, Sebina I, et al. Parasite infection is associated with Kaposi's sarcoma associated herpesvirus (KSHV) in Ugandan women. *Infect Agents Cancer*. 2011;6:15.
9. Wabinga HR, Parkin DM, Wabwire-Mangen F, et al. Trends in cancer incidence in Kyadondo County, Uganda, 1960-1997. *Br J Cancer*. 2000;82:1585-1592.
10. Taylor JF, Templeton AC, Vogel CL, et al. Kaposi's sarcoma in Uganda: a clinico-pathological study. *Int J Cancer*. 1971;8:122-135.
11. Elliott AM, Kizza M, Quigley MA, et al. The impact of helminths on the response to immunization and on the incidence of infection and disease in childhood in Uganda: design of a randomized, double-blind, placebo-controlled, factorial trial of deworming interventions delivered in pregnancy and early childhood [ISRCTN32849447]. *Clin Trials*. 2007;4:42-57.
12. Katz N, Claves A, Pellegrino J. A simple device for quantitative stool thick-smear technique in Schistosomiasis mansoni. *Rev Inst Med Trop Sao Paulo*. 1972;14:397-400.
13. Bukusuba JW, Hughes P, Kizza M, et al. Screening for intestinal helminth infection in a semi-urban cohort of pregnant women in Uganda. *Trop Doct*. 2004;34:27-28.
14. Melrose WD, Turner PF, Pisters P, Turner B. An improved Knott's concentration test for the detection of microfilariae. *Trans R Soc Trop Med Hyg*. 2000;94:176.
15. Webb EL, Mawa PA, Ndiranza J, et al. Effect of single-dose anthelmintic treatment during pregnancy on an infant's response to immunisation and on susceptibility to infectious diseases in infancy: a randomised, double-blind, placebo-controlled trial. *Lancet*. 2011;377:52-62.
16. Musingi L, Woodburn P, Omara M, et al. Associations between mild-to-moderate anaemia in pregnancy and helminth, malaria and HIV infection in Entebbe, Uganda. *Trans R Soc Trop Med Hyg*. 2007;101:899-907.
17. Mbisa GL, Miley W, Gamache CJ, et al. Detection of antibodies to Kaposi's sarcoma-associated herpesvirus: a new approach using K8.1 ELISA and a newly developed recombinant LANA ELISA. *J Immunol Methods*. 2010;356:39-46.
18. Malope BI, Pfeiffer RM, Mbisa G, et al. Transmission of Kaposi sarcoma-associated herpesvirus between mothers and children in a South African population. *J Acquir Immune Defic Syndr*. 2007;44:351-355.

19. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics*. 1977;33:159–174.
20. Areste C, Blackbourn DJ. Modulation of the immune system by Kaposi's sarcoma-associated herpesvirus. *Trends Microbiol*. 2009;17:119–129.
21. Maizels RM. Parasite immunomodulation and polymorphisms of the immune system. *J Biol*. 2009;8(7):62.
22. Ascoti V, Facchinelli L, Valerio L, et al. Distribution of mosquito species in areas with high and low incidence of classic Kaposi's sarcoma and seroprevalence for HHV-8. *Med Vet Entomol*. 2006;20:198–208.
23. Moomann AM, Chelimo K, Sumba OP, et al. Exposure to holoendemic malaria results in elevated Epstein-Barr virus loads in children. *J Infect Dis*. 2005;191:1233–1238.
24. Moomann AM, Chelimo K, Sumba PO, et al. Exposure to holoendemic malaria results in suppression of Epstein-Barr virus-specific T cell immunosurveillance in Kenyan children. *J Infect Dis*. 2007;195:799–808.
25. Moomann AM, Heller KN, Chelimo K, et al. Children with endemic Burkitt lymphoma are deficient in EBNA1-specific IFN-gamma T cell responses. *Int J Cancer*. 2009;124:1721–1726.
26. Brayfield BP, Phiri S, Kankasa C, et al. Postnatal human herpesvirus 8 and human immunodeficiency virus type 1 infection in mothers and infants from Zambia. *J Infect Dis*. 2003;187:559–568.
27. Butler LM, Dorsey G, Hladik W, et al. Kaposi sarcoma-associated herpesvirus (KSHV) seroprevalence in population-based samples of African children: evidence for at least 2 patterns of KSHV transmission. *J Infect Dis*. 2009;200:430–438.
28. Malope BI, MacPhail P, Mbisa G, et al. No evidence of sexual transmission of Kaposi's sarcoma herpes virus in a heterosexual South African population. *AIDS*. 2008;22:519–526.
29. Brayfield BP, Kankasa C, West JT, et al. Distribution of Kaposi sarcoma-associated herpesvirus/human herpesvirus 8 in maternal saliva and breast milk in Zambia: implications for transmission. *J Infect Dis*. 2004;189:2260–2270.
30. Mbulaiteye SM, Biggar RJ, Pfeiffer RM, et al. Water, socioeconomic factors, and human herpesvirus 8 infection in Ugandan children and their mothers. *J Acquir Immune Defic Syndr*. 2005;38:474–479.
31. de Sanjose S, Mbisa G, Perez-Alvarez S, et al. Geographic variation in the prevalence of Kaposi sarcoma-associated herpesvirus and risk factors for transmission. *J Infect Dis*. 2009;199:1449–1456.
32. Ziegler J, Newton R, Bourboullia D, et al. Risk factors for Kaposi's sarcoma: a case-control study of HIV-seronegative people in Uganda. *Int J Cancer*. 2003;103:233–240.
33. McHardy J, Williams EH, Geser A, et al. Endemic Kaposi's sarcoma: incidence and risk factors in the West Nile District of Uganda. *Int J Cancer*. 1984;33:203–212.
34. Mayama S, Cuevas LE, Sheldon J, et al. Prevalence and transmission of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) in Ugandan children and adolescents. *Int J Cancer*. 1998;77:817–820.

References

1. Mesri EA, Cesarman E, Boshoff C. Kaposi's sarcoma and its associated herpesvirus. *Nature reviews Cancer* 2010; **10**(10): 707-19.
2. Ziegler JL, Templeton AC, Vogel CL. Kaposi's sarcoma: a comparison of classical, endemic, and epidemic forms. *Semin Oncol* 1984; **11**(1): 47-52.
3. Chang Y, Moore PS. Kaposi's Sarcoma (KS)-associated herpesvirus and its role in KS. *Infectious agents and disease* 1996; **5**(4): 215-22.
4. Moore PS, Chang Y. Detection of herpesvirus-like DNA sequences in Kaposi's sarcoma in patients with and without HIV infection. *N Engl J Med* 1995; **332**(18): 1181-5.
5. Dedicoat M, Newton R. Review of the distribution of Kaposi's sarcoma-associated herpesvirus (KSHV) in Africa in relation to the incidence of Kaposi's sarcoma. *Br J Cancer* 2003; **88**(1): 1-3.
6. Wojcicki J, Mwanahamuntu M, Minhas V, et al. Mortality among HIV-1- and human herpesvirus type 8-affected mother-infant pairs in Zambia. *Cancer Epidemiol Biomarkers Prev* 2008; **17**(9): 2238-43.
7. Wakeham K, Webb EL, Sebina I, et al. Risk factors for seropositivity to Kaposi's sarcoma associated herpesvirus (KSHV) among children in Uganda. *J Acquir Immune Defic Syndr* 2013.
8. Wakeham K, Webb EL, Sebina I, et al. Parasite infection is associated with Kaposi's sarcoma associated herpesvirus (KSHV) in Ugandan women. *Infect Agent Cancer* 2011; **6**(1): 15.
9. Minhas V, Brayfield BP, Crabtree KL, Kankasa C, Mitchell CD, Wood C. Primary gamma-herpesviral infection in Zambian children. *BMC Infect Dis* 2010; **10**: 115.
10. Mbulaiteye SM, Pfeiffer RM, Whitby D, Brubaker GR, Shao J, Biggar RJ. Human herpesvirus 8 infection within families in rural Tanzania. *The Journal of infectious diseases* 2003; **187**(11): 1780-5.
11. Mbulaiteye SM, Pfeiffer RM, Dolan B, et al. Seroprevalence and risk factors for human herpesvirus 8 infection, rural Egypt. *Emerg Infect Dis* 2008; **14**(4): 586-91.
12. Mantina H, Kankasa C, Klaskala W, et al. Vertical transmission of Kaposi's sarcoma-associated herpesvirus. *Int J Cancer* 2001; **94**(5): 749-52.
13. Malope BI, Pfeiffer RM, Mbisa G, et al. Transmission of Kaposi sarcoma-associated herpesvirus between mothers and children in a South African population. *J Acquir Immune Defic Syndr* 2007; **44**(3): 351-5.
14. Malope BI, MacPhail P, Mbisa G, et al. No evidence of sexual transmission of Kaposi's sarcoma herpes virus in a heterosexual South African population. *AIDS* 2008; **22**(4): 519-26.
15. Klaskala W, Brayfield BP, Kankasa C, et al. Epidemiological characteristics of human herpesvirus-8 infection in a large population of antenatal women in Zambia. *J Med Virol* 2005; **75**(1): 93-100.
16. He J, Bhat G, Kankasa C, et al. Seroprevalence of human herpesvirus 8 among Zambian women of childbearing age without Kaposi's sarcoma (KS) and mother-child pairs with KS. *The Journal of infectious diseases* 1998; **178**(6): 1787-90.

17. Gaye-Diallo A, Toure AT, Gessain A, et al. [Preliminary study of human Herpesvirus type 8 infection in pregnant women in Dakar (Senegal)]. *Bull Soc Pathol Exot* 2001; **94**(3): 231-4.
18. Eltom MA, Mbulaiteye SM, Dada AJ, Whitby D, Biggar RJ. Transmission of human herpesvirus 8 by sexual activity among adults in Lagos, Nigeria. *AIDS* 2002; **16**(18): 2473-8.
19. de-The G, Bestetti G, van Beveren M, Gessain A. Prevalence of human herpesvirus 8 infection before the acquired immunodeficiency disease syndrome-related epidemic of Kaposi's sarcoma in East Africa. *J Natl Cancer Inst* 1999; **91**(21): 1888-9.
20. de Sanjose S, Mbisa G, Perez-Alvarez S, et al. Geographic variation in the prevalence of Kaposi sarcoma-associated herpesvirus and risk factors for transmission. *The Journal of infectious diseases* 2009; **199**(10): 1449-56.
21. Ceffa S, Buonomo E, Altan AM, et al. Seroprevalence of HHV8 in a cohort of HIV-negative and HIV-positive patients in Mozambique. *Annali di igiene : medicina preventiva e di comunita* 2007; **19**(6): 519-23.
22. Brayfield BP, Phiri S, Kankasa C, et al. Postnatal human herpesvirus 8 and human immunodeficiency virus type 1 infection in mothers and infants from Zambia. *The Journal of infectious diseases* 2003; **187**(4): 559-68.
23. Brayfield BP, Kankasa C, West JT, et al. Distribution of Kaposi sarcoma-associated herpesvirus/human herpesvirus 8 in maternal saliva and breast milk in Zambia: implications for transmission. *The Journal of infectious diseases* 2004; **189**(12): 2260-70.
24. Whitby D. Seroprevalence and risk factors for human herpesvirus 8 infection in Cameroon. *Personal communication* 2013.
25. Dedicoat M, Newton R, Alkharsah KR, et al. Mother-to-child transmission of human herpesvirus-8 in South Africa. *The Journal of infectious diseases* 2004; **190**(6): 1068-75.
26. Butler L, Dollard SC, Amin M, Mosam A, Martin J. Relationship between salivary shedding and seropositivity for Kaposi's sarcoma-associated herpesvirus (KSHV) and Epstein-Barr virus (EBV) among South African children and adults: insights on why EBV infection is ubiquitous and KSHV is not. 12th International Conference on Malignancies in AIDS and Other Acquired Immunodeficiencies (ICMAOI); 2010; Bethesda, USA; 2010.
27. Mbulaiteye SM, Pfeiffer RM, Engels EA, et al. Detection of kaposi sarcoma-associated herpesvirus DNA in saliva and buffy-coat samples from children with sickle cell disease in Uganda. *The Journal of infectious diseases* 2004; **190**(8): 1382-6.
28. Vitale F, Briffa DV, Whitby D, et al. Kaposi's sarcoma herpes virus and Kaposi's sarcoma in the elderly populations of 3 Mediterranean islands. *Int J Cancer* 2001; **91**(4): 588-91.
29. Beral V, Peterman TA, Berkelman RL, Jaffe HW. Kaposi's sarcoma among persons with AIDS: a sexually transmitted infection? *Lancet* 1990; **335**(8682): 123-8.
30. Parkin DM, Namboozee S, Wabwire-Mangen F, Wabinga HR. Changing cancer incidence in Kampala, Uganda, 1991-2006. *Int J Cancer* 2010; **126**(5): 1187-95.

31. Wabinga HR, Parkin DM, Wabwire-Mangen F, Mugerwa JW. Cancer in Kampala, Uganda, in 1989-91: changes in incidence in the era of AIDS. *Int J Cancer* 1993; **54**(1): 26-36.
32. Wabinga HR, Parkin DM, Wabwire-Mangen F, Namboozee S. Trends in cancer incidence in Kyadondo County, Uganda, 1960-1997. *Br J Cancer* 2000; **82**(9): 1585-92.
33. Quinlivan EB, Wang RX, Stewart PW, et al. Longitudinal sero-reactivity to human herpesvirus 8 (KSHV) in the Swiss HIV Cohort 4.7 years before KS. *J Med Virol* 2001; **64**(2): 157-66.
34. Ziegler JL, Simonart T, Snoeck R. Kaposi's sarcoma, oncogenic viruses, and iron. *J Clin Virol* 2001; **20**(3): 127-30.
35. Elliott AM, Kizza M, Quigley MA, et al. The impact of helminths on the response to immunization and on the incidence of infection and disease in childhood in Uganda: design of a randomized, double-blind, placebo-controlled, factorial trial of deworming interventions delivered in pregnancy and early childhood [ISRCTN32849447]. *Clin Trials* 2007; **4**(1): 42-57.
36. Kamali A, Carpenter LM, Whitworth JA, Pool R, Ruberantwari A, Ojwiya A. Seven-year trends in HIV-1 infection rates, and changes in sexual behaviour, among adults in rural Uganda. *AIDS* 2000; **14**(4): 427-34.
37. Kaposi M. Idiopathisches multiples pigmentsarkom der Haut. *Archiv fur Dermatologie und Syphilis* 1872; (4): 265-73 (translated CA 1982;32:342-7).
38. Oriel JD. Moritz Kaposi (1837-1902). *International journal of STD & AIDS* 1997; **8**(11): 715-7.
39. Oettle AG. Geographical and racial differences in the frequency of Kaposi's sarcoma as evidence of environmental or genetic causes. *Acta - Unio Internationalis Contra Cancrum* 1962; **18**: 330-63.
40. Cook-Mozaffari P, Newton R, Beral V, Burkitt DP. The geographical distribution of Kaposi's sarcoma and of lymphomas in Africa before the AIDS epidemic. *Br J Cancer* 1998; **78**(11): 1521-8.
41. Hutt MS. The epidemiology of Kaposi's sarcoma. *Antibiot Chemother* 1981; **29**: 3-11.
42. Hutt MS. Classical and endemic form of Kaposi's sarcoma. A review. *Antibiot Chemother* 1983; **32**: 12-7.
43. Hutt MS. Kaposi's sarcoma. *Br Med Bull* 1984; **40**(4): 355-8.
44. Master SP, Taylor JF, Kyalwazi SK, Ziegler JL. Immunological studies in Kaposi's sarcoma in Uganda. *Br Med J* 1970; **1**(5696): 600-2.
45. Oettle AG. Cancer in the South African Bantu. *Cancer progress* 1960: 232-40.
46. Taylor JF, Templeton AC, Vogel CL, Ziegler JL, Kyalwazi SK. Kaposi's sarcoma in Uganda: a clinico-pathological study. *Int J Cancer* 1971; **8**(1): 122-35.
47. Williams EH, Williams PH. A note on an apparent similarity in distribution of onchocerciasis, femoral hernia and Kaposi's sarcoma in the West Nile district of Uganda. *East African Medical Journal* 1966; **43**(6).
48. Moosa MR. Racial and ethnic variations in incidence and pattern of malignancies after kidney transplantation. *Medicine* 2005; **84**(1): 12-22.
49. CDC. Kaposi's Sarcoma and Pneumocystis Pneumonia among homosexual men - New York City and California. *MMWR* 1981; (30): 306-8.

50. Uldrick TS, Whitby D. Update on KSHV epidemiology, Kaposi Sarcoma pathogenesis, and treatment of Kaposi Sarcoma. *Cancer Lett* 2011; **305**(2): 150-62.
51. Parkin DM, Sitas F, Chirenje M, Stein L, Abratt R, Wabinga H. Part I: Cancer in Indigenous Africans--burden, distribution, and trends. *Lancet Oncol* 2008; **9**(7): 683-92.
52. Bouvard V, Baan R, Straif K, et al. A review of human carcinogens--Part B: biological agents. *Lancet Oncol* 2009; **10**(4): 321-2.
53. Wang HW, Trotter MW, Lagos D, et al. Kaposi sarcoma herpesvirus-induced cellular reprogramming contributes to the lymphatic endothelial gene expression in Kaposi sarcoma. *Nature genetics* 2004; **36**(7): 687-93.
54. Iscovich J, Boffetta P, Franceschi S, Azizi E, Sarid R. Classic kaposi sarcoma: epidemiology and risk factors. *Cancer* 2000; **88**(3): 500-17.
55. Iscovich J, Boffetta P, Winkelmann R, Brennan P, Azizi E. Classic Kaposi's sarcoma in Jews living in Israel, 1961-1989: a population-based incidence study. *AIDS* 1998; **12**(15): 2067-72.
56. Barozzi P, Luppi M, Facchetti F, et al. Post-transplant Kaposi sarcoma originates from the seeding of donor-derived progenitors. *Nat Med* 2003; **9**(5): 554-61.
57. Ziegler JL. Kaposi's sarcoma: introduction and overview. *J Acquir Immune Defic Syndr* 1990; **3 Suppl 1**: S1-3.
58. Gallant JE, Moore RD, Richman DD, Keruly J, Chaisson RE. Risk factors for Kaposi's sarcoma in patients with advanced human immunodeficiency virus disease treated with zidovudine. Zidovudine Epidemiology Study Group. *Arch Intern Med* 1994; **154**(5): 566-72.
59. Sitas F, Newton R. Kaposi's sarcoma in South Africa. *J Natl Cancer Inst Monogr* 2001; (28): 1-4.
60. Parkin DM, Wabinga H, Nambooze S, Wabwire-Mangen F. AIDS-related cancers in Africa: maturation of the epidemic in Uganda. *AIDS* 1999; **13**(18): 2563-70.
61. Athale UH, Patil PS, Chintu C, Elem B. Influence of HIV epidemic on the incidence of Kaposi's sarcoma in Zambian children. *J Acquir Immune Defic Syndr Hum Retrovirol* 1995; **8**(1): 96-100.
62. Cattani P, Cerimele F, Porta D, et al. Age-specific seroprevalence of Human Herpesvirus 8 in Mediterranean regions. *Clin Microbiol Infect* 2003; **9**(4): 274-9.
63. Sivayathorn A, Srihira B, Leesanguankul W. Prevalence of skin disease in patients infected with human immunodeficiency virus in Bangkok, Thailand. *Annals of the Academy of Medicine, Singapore* 1995; **24**(4): 528-33.
64. Eltom MA, Jemal A, Mbulaiteye SM, Devesa SS, Biggar RJ. Trends in Kaposi's sarcoma and non-Hodgkin's lymphoma incidence in the United States from 1973 through 1998. *J Natl Cancer Inst* 2002; **94**(16): 1204-10.
65. Engels EA, Pfeiffer RM, Goedert JJ, et al. Trends in cancer risk among people with AIDS in the United States 1980-2002. *AIDS* 2006; **20**(12): 1645-54.
66. Armstrong AW, Lam KH, Chase EP. Epidemiology of classic and AIDS-related Kaposi's sarcoma in the USA: incidence, survival, and geographical distribution from 1975 to 2005. *Epidemiol Infect* 2013; **141**(1): 200-6.

67. Einollahi B, Rostami Z, Nourbala MH, et al. Incidence of malignancy after living kidney transplantation: a multicenter study from iran. *Journal of Cancer* 2012; **3**: 246-56.
68. Shmueli D, Shapira Z, Yussim A, Nakache R, Ram Z, Shaharabani E. The incidence of Kaposi sarcoma in renal transplant patients and its relation to immunosuppression. *Transplantation proceedings* 1989; **21**(1 Pt 3): 3209-10.
69. Piselli P, Busnach G, Citterio F, et al. Risk of Kaposi sarcoma after solid-organ transplantation: multicenter study in 4,767 recipients in Italy, 1970-2006. *Transplantation proceedings* 2009; **41**(4): 1227-30.
70. Zavos G, Bokos J, Papaconstantinou J, et al. Study of "de novo" malignancies among greek renal transplant recipients. *Transplantation proceedings* 2003; **35**(4): 1399-403.
71. Grossman Z, Iscovich J, Schwartz F, et al. Absence of Kaposi sarcoma among Ethiopian immigrants to Israel despite high seroprevalence of human herpesvirus 8. *Mayo Clinic proceedings Mayo Clinic* 2002; **77**(9): 905-9.
72. Iscovich J, Boffetta P, Brennan P. Classic Kaposi's sarcoma in Arabs living in Israel, 1970-1993: a population-based incidence study. *Int J Cancer* 1998; **77**(3): 319-21.
73. Touloumi G, Kaklamanis L, Potouridou I, et al. The epidemiologic profile of Kaposi's sarcoma in Greece prior to and during the AIDS era. *Int J Cancer* 1997; **70**(5): 538-41.
74. Hjalgrim H, Frisch M, Melbye M. Incidence rates of classical Kaposi's sarcoma and multiple myeloma do not correlate. *Br J Cancer* 1998; **78**(3): 419-20.
75. Hjalgrim H, Melbye M, Lecker S, Frisch M, Thomsen HK, Larsen SO. Epidemiology of classic Kaposi's sarcoma in Denmark between 1970 and 1992. *Cancer* 1996; **77**(7): 1373-8.
76. Hjalgrim H, Melbye M, Pukkala E, et al. Epidemiology of Kaposi's sarcoma in the Nordic countries before the AIDS epidemic. *Br J Cancer* 1996; **74**(9): 1499-502.
77. Hjalgrim H, Tulinius H, Dalberg J, Hardarson S, Frisch M, Melbye M. High incidence of classical Kaposi's sarcoma in Iceland and the Faroe Islands. *Br J Cancer* 1998; **77**(7): 1190-3.
78. Dal Maso L, Polesel J, Ascoli V, et al. Classic Kaposi's sarcoma in Italy, 1985-1998. *Br J Cancer* 2005; **92**(1): 188-93.
79. Cottoni F, Masala MV, Pattaro C, et al. Classic Kaposi sarcoma in northern Sardinia: a prospective epidemiologic overview (1977-2003) correlated with malaria prevalence (1934). *Journal of the American Academy of Dermatology* 2006; **55**(6): 990-5.
80. Cottoni F, De Marco R, Montesu MA. Classical Kaposi's sarcoma in north-east Sardinia: an overview from 1977 to 1991. *Br J Cancer* 1996; **73**(9): 1132-3.
81. Kaldor JM, Coates M, Vettom L, Taylor R. Epidemiological characteristics of Kaposi's sarcoma prior to the AIDS epidemic. *Br J Cancer* 1994; **70**(4): 674-6.
82. SEER. Surveillance Epidemiology and End Results (SEER) <http://seer.cancer.gov>, accessed October 12th 2012.
83. Registry Scotland. <http://www.isdscotland.org/Health-Topics/Cancer/Scottish-Cancer-Registry/> Incidence and mortality by ICD10 code, accessed 12th January 2013.

84. ONS. Cancer incidence and mortality in the United Kingdom, 2008-2010, Office for National Statistics (ONS) <http://ons.gov.uk/ons/datasets-and-tables/index.html?pageSize=50&sortBy=none&sortDirection=none&newquery=cancer&content-type=Reference+table&content-type=Dataset>.
85. Bah E, Parkin DM, Hall AJ, Jack AD, Whittle H. Cancer in the Gambia: 1988-97. *Br J Cancer* 2001; **84**(9): 1207-14.
86. Banda LT, Parkin DM, Dzamalala CP, Liomba NG. Cancer incidence in Blantyre, Malawi 1994-1998. *Tropical medicine & international health : TM & IH* 2001; **6**(4): 296-304.
87. Koulibaly M, Kabba IS, Cisse A, et al. Cancer incidence in Conakry, Guinea: first results from the Cancer Registry 1992-1995. *Int J Cancer* 1997; **70**(1): 39-45.
88. Echimane AK, Ahnoux AA, Adoubi I, et al. Cancer incidence in Abidjan, Ivory Coast: first results from the cancer registry, 1995-1997. *Cancer* 2000; **89**(3): 653-63.
89. Nontuthuzelo I, Bradshaw D, Gelderblom WC, Parkin D. Cancer incidence in a rural population of South Africa, 1998-2002. *International Journal of Cancer* 2010; **127**(10): 2420 - 9.
90. Chokunonga E, Levy LM, Bassett MT, Mauchaza BG, Thomas DB, Parkin DM. Cancer incidence in the African population of Harare, Zimbabwe: second results from the cancer registry 1993-1995. *Int J Cancer* 2000; **85**(1): 54-9.
91. Mohanna S, Ferrufino JC, Sanchez J, Bravo F, Gotuzzo E. Epidemiological and clinical characteristics of classic Kaposi's sarcoma in Peru. *Journal of the American Academy of Dermatology* 2005; **53**(3): 435-41.
92. Mohanna S, Maco V, Bravo F, Gotuzzo E. Epidemiology and clinical characteristics of classic Kaposi's sarcoma, seroprevalence, and variants of human herpesvirus 8 in South America: a critical review of an old disease. *International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases* 2005; **9**(5): 239-50.
93. Mohanna S, Maco V, Gown A, Morales D, Bravo F, Gotuzzo E. Is classic Kaposi's sarcoma endemic in Peru?: report of a case in an indigenous patient. *Am J Trop Med Hyg* 2006; **75**(2): 324-6.
94. Biggar RJ, Whitby D, Marshall V, Linhares AC, Black F. Human herpesvirus 8 in Brazilian Amerindians: a hyperendemic population with a new subtype. *The Journal of infectious diseases* 2000; **181**(5): 1562-8.
95. Bouchardy C, Mirra AP, Khlat M, Parkin DM, de Souza JM, Gotlieb SL. Ethnicity and cancer risk in Sao Paulo, Brazil. *Cancer Epidemiol Biomarkers Prev* 1991; **1**(1): 21-7.
96. Jang HS, Cha JH, Oh CK, Kwon KS. A case of classic Kaposi's sarcoma with multiple organ involvement. *J Dermatol* 2000; **27**(11): 740-4.
97. Ross RK, Casagrande JT, Dworsky RL, Levine A, Mack T. Kaposi's sarcoma in Los Angeles, California. *J Natl Cancer Inst* 1985; **75**(6): 1011-5.
98. Robey RC, Lagos D, Gratrix F, et al. The CD8 and CD4 T-cell response against Kaposi's sarcoma-associated herpesvirus is skewed towards early and late lytic antigens. *PloS one* 2009; **4**(6): e5890.
99. Woodberry T, Suscovich TJ, Henry LM, et al. Impact of Kaposi sarcoma-associated herpesvirus (KSHV) burden and HIV coinfection on the detection of T cell responses to KSHV ORF73 and ORF65 proteins. *The Journal of infectious diseases* 2005; **192**(4): 622-9.

100. Lambert M, Gannage M, Karras A, et al. Differences in the frequency and function of HHV8-specific CD8 T cells between asymptomatic HHV8 infection and Kaposi sarcoma. *Blood* 2006; **108**(12): 3871-80.
101. Pellet C, Chevret S, Blum L, et al. Virologic and immunologic parameters that predict clinical response of AIDS-associated Kaposi's sarcoma to highly active antiretroviral therapy. *The Journal of investigative dermatology* 2001; **117**(4): 858-63.
102. Robey RC, Mletzko S, Gotch FM. The T-Cell Immune Response against Kaposi's Sarcoma-Associated Herpesvirus. *Advances in virology* 2010; **2010**: 340356.
103. Wang QJ, Jenkins FJ, Jacobson LP, et al. Primary human herpesvirus 8 infection generates a broadly specific CD8(+) T-cell response to viral lytic cycle proteins. *Blood* 2001; **97**(8): 2366-73.
104. Bourboulia D, Aldam D, Lagos D, et al. Short- and long-term effects of highly active antiretroviral therapy on Kaposi sarcoma-associated herpesvirus immune responses and viraemia. *AIDS* 2004; **18**(3): 485-93.
105. Barozzi P, Bonini C, Potenza L, et al. Changes in the immune responses against human herpesvirus-8 in the disease course of posttransplant Kaposi sarcoma. *Transplantation* 2008; **86**(5): 738-44.
106. Cattani P, Capuano M, Graffeo R, et al. Kaposi's sarcoma associated with previous human herpesvirus 8 infection in kidney transplant recipients. *Journal of clinical microbiology* 2001; **39**(2): 506-8.
107. Andreoni M, Goletti D, Pezzotti P, et al. Prevalence, incidence and correlates of HHV-8/KSHV infection and Kaposi's sarcoma in renal and liver transplant recipients. *J Infect* 2001; **43**(3): 195-9.
108. Brown EE, Whitby D, Vitale F, et al. Virologic, hematologic, and immunologic risk factors for classic Kaposi sarcoma. *Cancer* 2006; **107**(9): 2282-90.
109. Goedert JJ, Vitale F, Lauria C, et al. Risk factors for classical Kaposi's sarcoma. *J Natl Cancer Inst* 2002; **94**(22): 1712-8.
110. Anderson LA, Lauria C, Romano N, et al. Risk factors for classical Kaposi sarcoma in a population-based case-control study in Sicily. *Cancer Epidemiol Biomarkers Prev* 2008; **17**(12): 3435-43.
111. Vasto S, Malavolta M, Pawelec G. Age and immunity. *Immunity & ageing : I & A* 2006; **3**: 2.
112. Campbell TB, Borok M, Gwanzura L, et al. Relationship of human herpesvirus 8 peripheral blood virus load and Kaposi's sarcoma clinical stage. *AIDS* 2000; **14**(14): 2109-16.
113. Engels EA, Biggar RJ, Marshall VA, et al. Detection and quantification of Kaposi's sarcoma-associated herpesvirus to predict AIDS-associated Kaposi's sarcoma. *AIDS* 2003; **17**(12): 1847-51.
114. Whitby D, Howard MR, Tenant-Flowers M, et al. Detection of Kaposi sarcoma associated herpesvirus in peripheral blood of HIV-infected individuals and progression to Kaposi's sarcoma. *Lancet* 1995; **346**(8978): 799-802.
115. Jacobson LP, Jenkins FJ, Springer G, et al. Interaction of human immunodeficiency virus type 1 and human herpesvirus type 8 infections on the incidence of Kaposi's sarcoma. *The Journal of infectious diseases* 2000; **181**(6): 1940-9.

116. Biggar RJ, Engels EA, Whitby D, Kedes DH, Goedert JJ. Antibody reactivity to latent and lytic antigens to human herpesvirus-8 in longitudinally followed homosexual men. *The Journal of infectious diseases* 2003; **187**(1): 12-8.
117. Newton R, Carpenter L, Casabonne D, et al. A prospective study of Kaposi's sarcoma-associated herpesvirus and Epstein-Barr virus in adults with human immunodeficiency virus-1. *Br J Cancer* 2006; **94**(10): 1504-9.
118. Moore PS, Kingsley LA, Holmberg SD, et al. Kaposi's sarcoma-associated herpesvirus infection prior to onset of Kaposi's sarcoma. *AIDS* 1996; **10**(2): 175-80.
119. Cannon MJ, Dollard SC, Black JB, et al. Risk factors for Kaposi's sarcoma in men seropositive for both human herpesvirus 8 and human immunodeficiency virus. *AIDS* 2003; **17**(2): 215-22.
120. Laney AS, Dollard SC, Jaffe HW, et al. Repeated measures study of human herpesvirus 8 (HHV-8) DNA and antibodies in men seropositive for both HHV-8 and HIV. *AIDS* 2004; **18**(13): 1819-26.
121. Alagiozoglou L, Morris L, Bredell H, Martin DJ, Sitas F. Human herpesvirus-8 antibodies and DNA in HIV-1 infected patients in South Africa. *Epidemiol Infect* 2003; **131**(3): 1125-9.
122. Campbell TB, Borok M, White IE, et al. Relationship of Kaposi sarcoma (KS)-associated herpesvirus viremia and KS disease in Zimbabwe. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2003; **36**(9): 1144-51.
123. Nsubuga MM, Biggar RJ, Combs S, et al. Human herpesvirus 8 load and progression of AIDS-related Kaposi sarcoma lesions. *Cancer Lett* 2008; **263**(2): 182-8.
124. Gill J, Bourboulia D, Wilkinson J, et al. Prospective study of the effects of antiretroviral therapy on Kaposi sarcoma--associated herpesvirus infection in patients with and without Kaposi sarcoma. *J Acquir Immune Defic Syndr* 2002; **31**(4): 384-90.
125. Spira TJ, Lam L, Dollard SC, et al. Comparison of serologic assays and PCR for diagnosis of human herpesvirus 8 infection. *Journal of clinical microbiology* 2000; **38**(6): 2174-80.
126. IARC. <http://monographs.iarc.fr/ENG/Monographs/vol100B/index.php>, accessed 6th January 2012.
127. Gao SJ, Kingsley L, Li M, et al. KSHV antibodies among Americans, Italians and Ugandans with and without Kaposi's sarcoma. *Nat Med* 1996; **2**(8): 925-8.
128. Newton R, Ziegler J, Bourboulia D, et al. Infection with Kaposi's sarcoma-associated herpesvirus (KSHV) and human immunodeficiency virus (HIV) in relation to the risk and clinical presentation of Kaposi's sarcoma in Uganda. *Br J Cancer* 2003; **89**(3): 502-4.
129. Simpson GR, Schulz TF, Whitby D, et al. Prevalence of Kaposi's sarcoma associated herpesvirus infection measured by antibodies to recombinant capsid protein and latent immunofluorescence antigen. *Lancet* 1996; **348**(9035): 1133-8.
130. Caterino-de-Araujo A, Magri MC, Manuel RC. Patterns of antibodies against latent and lytic antigens of human herpesvirus 8 in an endemic population and patients with Kaposi's sarcoma in Mozambique. *J Med Virol* 2010; **82**(9): 1576-81.

131. Sitas F, Carrara H, Beral V, et al. Antibodies against human herpesvirus 8 in black South African patients with cancer. *N Engl J Med* 1999; **340**(24): 1863-71.
132. Chatlynne LG, Lapps W, Handy M, et al. Detection and titration of human herpesvirus-8-specific antibodies in sera from blood donors, acquired immunodeficiency syndrome patients, and Kaposi's sarcoma patients using a whole virus enzyme-linked immunosorbent assay. *Blood* 1998; **92**(1): 53-8.
133. Melbye M, Cook PM, Hjalgrim H, et al. Risk factors for Kaposi's-sarcoma-associated herpesvirus (KSHV/HHV-8) seropositivity in a cohort of homosexual men, 1981-1996. *Int J Cancer* 1998; **77**(4): 543-8.
134. O'Brien TR, Kedes D, Ganem D, et al. Evidence for concurrent epidemics of human herpesvirus 8 and human immunodeficiency virus type 1 in US homosexual men: rates, risk factors, and relationship to Kaposi's sarcoma. *The Journal of infectious diseases* 1999; **180**(4): 1010-7.
135. Renwick N, Halaby T, Weverling GJ, et al. Seroconversion for human herpesvirus 8 during HIV infection is highly predictive of Kaposi's sarcoma. *AIDS* 1998; **12**(18): 2481-8.
136. Rezza G, Andreoni M, Dorrucchi M, et al. Human herpesvirus 8 seropositivity and risk of Kaposi's sarcoma and other acquired immunodeficiency syndrome-related diseases. *J Natl Cancer Inst* 1999; **91**(17): 1468-74.
137. Grulich AE, Olsen SJ, Luo K, et al. Kaposi's sarcoma-associated herpesvirus: a sexually transmissible infection? *J Acquir Immune Defic Syndr Hum Retrovirol* 1999; **20**(4): 387-93.
138. Franceschi S, Maso LD, Rickenbach M, et al. Kaposi sarcoma incidence in the Swiss HIV Cohort Study before and after highly active antiretroviral therapy. *Br J Cancer* 2008; **99**(5): 800-4.
139. Lodi S, Guiguet M, Costagliola D, et al. Kaposi sarcoma incidence and survival among HIV-infected homosexual men after HIV seroconversion. *J Natl Cancer Inst* 2010; **102**(11): 784-92.
140. Gaya A, Esteve A, Casabona J, et al. Amino acid residue at position 13 in HLA-DR beta chain plays a critical role in the development of Kaposi's sarcoma in AIDS patients. *AIDS* 2004; **18**(2): 199-204.
141. Foster CB, Lehrnbecher T, Samuels S, et al. An IL6 promoter polymorphism is associated with a lifetime risk of development of Kaposi sarcoma in men infected with human immunodeficiency virus. *Blood* 2000; **96**(7): 2562-7.
142. Maskew M, Macphail AP, Whitby D, Egger M, Wallis CL, Fox MP. Prevalence and predictors of kaposi sarcoma herpes virus seropositivity: a cross-sectional analysis of HIV-infected adults initiating ART in Johannesburg, South Africa. *Infect Agent Cancer* 2011; **6**: 22.
143. Polizzotto MN, Uldrick TS, Wang V, et al. KSHV activation, human and viral IL-6 production, and other cytokine dysregulation: Association with the symptomatology of KSHV-associated multicentric Castleman's disease. 12th International Conference on Malignancies in AIDS and Other Acquired Immunodeficiencies (ICMAOI) ; 2011; Bethesda, USA; 2011.
144. Trattner A, Hodak E, David M, Neeman A, Sandbank M. Kaposi's sarcoma with visceral involvement after intraarticular and epidural injections of

corticosteroids. *Journal of the American Academy of Dermatology* 1993; **29**(5 Pt 2): 890-4.

145. Trattner A, Hodak E, David M, Sandbank M. The appearance of Kaposi sarcoma during corticosteroid therapy. *Cancer* 1993; **72**(5): 1779-83.

146. Zong JC, Ciufu DM, Alcendor DJ, et al. High-level variability in the ORF-K1 membrane protein gene at the left end of the Kaposi's sarcoma-associated herpesvirus genome defines four major virus subtypes and multiple variants or clades in different human populations. *J Virol* 1999; **73**(5): 4156-70.

147. Kasolo FC, Monze M, Obel N, Anderson RA, French C, Gompels UA. Sequence analyses of human herpesvirus-8 strains from both African human immunodeficiency virus-negative and -positive childhood endemic Kaposi's sarcoma show a close relationship with strains identified in febrile children and high variation in the K1 glycoprotein. *J Gen Virol* 1998; **79** (Pt 12): 3055-65.

148. Whitby D, Marshall VA, Bagni RK, et al. Genotypic characterization of Kaposi's sarcoma-associated herpesvirus in asymptomatic infected subjects from isolated populations. *J Gen Virol* 2004; **85**(Pt 1): 155-63.

149. Cook PM, Whitby D, Calabro ML, et al. Variability and evolution of Kaposi's sarcoma-associated herpesvirus in Europe and Africa. International Collaborative Group. *AIDS* 1999; **13**(10): 1165-76.

150. Marshall V, Parks T, Bagni R, et al. Conservation of virally encoded microRNAs in Kaposi sarcoma--associated herpesvirus in primary effusion lymphoma cell lines and in patients with Kaposi sarcoma or multicentric Castleman disease. *The Journal of infectious diseases* 2007; **195**(5): 645-59.

151. Marshall V, Martro E, Labo N, et al. Kaposi sarcoma (KS)-associated herpesvirus microRNA sequence analysis and KS risk in a European AIDS-KS case control study. *The Journal of infectious diseases* 2010; **202**(7): 1126-35.

152. Ray A, Marshall V, Uldrick T, et al. Sequence analysis of Kaposi sarcoma-associated herpesvirus (KSHV) microRNAs in patients with multicentric Castleman disease and KSHV-associated inflammatory cytokine syndrome. *The Journal of infectious diseases* 2012; **205**(11): 1665-76.

153. Olweny CL, Kaddumukasa A, Atine I, Owor R, Magrath I, Ziegler JL. Childhood Kaposi's sarcoma: clinical features and therapy. *Br J Cancer* 1976; **33**(5): 555-60.

154. Christeff N, Winter C, Gharakhanian S, et al. Differences in androgens of HIV positive patients with and without Kaposi sarcoma. *J Clin Pathol* 1995; **48**(6): 513-8.

155. Kaloterakis A, Papasteriades C, Filiotou A, Economidou J, Hadjiyannis S, Stratigos J. HLA in familial and nonfamilial Mediterranean Kaposi's sarcoma in Greece. *Tissue Antigens* 1995; **45**(2): 117-9.

156. Strichman-Almashanu L, Weltfriend S, Gideoni O, Friedman-Birnbaum R, Pollack S. No significant association between HLA antigens and classic Kaposi sarcoma: molecular analysis of 49 Jewish patients. *Journal of clinical immunology* 1995; **15**(4): 205-9.

157. Guttman-Yassky E, Cohen A, Kra-Oz Z, et al. Familial clustering of classic Kaposi sarcoma. *The Journal of infectious diseases* 2004; **189**(11): 2023-6.

158. Tornesello ML, Biryahwaho B, Downing R, et al. Human herpesvirus type 8 variants circulating in Europe, Africa and North America in classic, endemic and epidemic Kaposi's sarcoma lesions during pre-AIDS and AIDS era. *Virology* 2010; **398**(2): 280-9.

159. Brown EE, Fallin D, Ruczinski I, et al. Associations of classic Kaposi sarcoma with common variants in genes that modulate host immunity. *Cancer Epidemiol Biomarkers Prev* 2006; **15**(5): 926-34.
160. Brown EE, Fallin MD, Goedert JJ, et al. A common genetic variant in FCGR3A-V158F and risk of Kaposi sarcoma herpesvirus infection and classic Kaposi sarcoma. *Cancer Epidemiol Biomarkers Prev* 2005; **14**(3): 633-7.
161. Webster-Cyriaque J. Development of Kaposi's sarcoma in a surgical wound. *N Engl J Med* 2002; **346**(16): 1207-10.
162. Davis DA, Rinderknecht AS, Zoetewij JP, et al. Hypoxia induces lytic replication of Kaposi sarcoma-associated herpesvirus. *Blood* 2001; **97**(10): 3244-50.
163. Semenza GL, Wang GL. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. *Mol Cell Biol* 1992; **12**(12): 5447-54.
164. Haque M, Davis DA, Wang V, Widmer I, Yarchoan R. Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) contains hypoxia response elements: relevance to lytic induction by hypoxia. *J Virol* 2003; **77**(12): 6761-8.
165. Ye F, Lei X, Gao SJ. Mechanisms of Kaposi's Sarcoma-Associated Herpesvirus Latency and Reactivation. *Advances in virology* 2011; **2011**.
166. Ye F, Zhou F, Bedolla RG, et al. Reactive oxygen species hydrogen peroxide mediates Kaposi's sarcoma-associated herpesvirus reactivation from latency. *PLoS Pathog* 2011; **7**(5): e1002054.
167. Li X, Feng J, Sun R. Oxidative stress induces reactivation of Kaposi's sarcoma-associated herpesvirus and death of primary effusion lymphoma cells. Proceedings of the 13th International Workshop on Kaposi's Sarcoma-Associated Herpesvirus and Related Agents; 2010; Los Angeles, USA; 2010.
168. Ye F, Zhou F, Bedolla R. The reactive oxygen species (ROS) hydrogen peroxide (H₂O₂) mediates Kaposi's sarcoma-associated herpesvirus (KSHV) reactivation. Proceedings of the 13th International Workshop on Kaposi's Sarcoma-Associated Herpesvirus and Related Agents; 2010; Los Angeles, USA; 2010.
169. Simonart T. Role of environmental factors in the pathogenesis of classic and African-endemic Kaposi sarcoma. *Cancer Lett* 2006; **244**(1): 1-7.
170. Pelsler C, Dazzi C, Graubard BI, Lauria C, Vitale F, Goedert JJ. Risk of classic Kaposi sarcoma with residential exposure to volcanic and related soils in Sicily. *Ann Epidemiol* 2009; **19**(8): 597-601.
171. Nascimento MC, de Souza VA, Sumita LM, et al. Comparative study of Kaposi's sarcoma-associated herpesvirus serological assays using clinically and serologically defined reference standards and latent class analysis. *Journal of clinical microbiology* 2007; **45**(3): 715-20.
172. Rabkin CS, Schulz TF, Whitby D, et al. Interassay correlation of human herpesvirus 8 serologic tests. HHV-8 Interlaboratory Collaborative Group. *The Journal of infectious diseases* 1998; **178**(2): 304-9.
173. Pellett PE, Wright DJ, Engels EA, et al. Multicenter comparison of serologic assays and estimation of human herpesvirus 8 seroprevalence among US blood donors. *Transfusion* 2003; **43**(9): 1260-8.

174. Schatz O, Monini P, Bugarini R, et al. Kaposi's sarcoma-associated herpesvirus serology in Europe and Uganda: multicentre study with multiple and novel assays. *J Med Virol* 2001; **65**(1): 123-32.
175. Mbisa GL, Miley W, Gamache CJ, et al. Detection of antibodies to Kaposi's sarcoma-associated herpesvirus: a new approach using K8.1 ELISA and a newly developed recombinant LANA ELISA. *J Immunol Methods* 2010; **356**(1-2): 39-46.
176. Tedeschi R, Dillner J, De Paoli P. Laboratory diagnosis of human herpesvirus 8 infection in humans. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology* 2002; **21**(12): 831-44.
177. Ablashi DV, Handy M, Bernbaum J, et al. Propagation and characterization of human herpesvirus-7 (HHV-7) isolates in a continuous T-lymphoblastoid cell line (SupT1). *Journal of virological methods* 1998; **73**(2): 123-40.
178. Davidovici B, Karakis I, Bourboulia D, et al. Seroepidemiology and molecular epidemiology of Kaposi's sarcoma-associated herpesvirus among Jewish population groups in Israel. *J Natl Cancer Inst* 2001; **93**(3): 194-202.
179. Engels EA, Atkinson JO, Graubard BI, et al. Risk factors for human herpesvirus 8 infection among adults in the United States and evidence for sexual transmission. *The Journal of infectious diseases* 2007; **196**(2): 199-207.
180. Hjalgrim H, Lind I, Rostgaard K, et al. Prevalence of human herpesvirus 8 antibodies in young adults in Denmark (1976-1977). *J Natl Cancer Inst* 2001; **93**(20): 1569-71.
181. Kouri V, Eng SM, Rodriguez ME, et al. Seroprevalence of Kaposi's sarcoma-associated herpesvirus in various populations in Cuba. *Revista panamericana de salud publica = Pan American journal of public health* 2004; **15**(5): 320-5.
182. Nascimento MC, de Souza VA, Sumita LM, et al. Prevalence of, and risk factors for Kaposi's sarcoma-associated herpesvirus infection among blood donors in Brazil: a multi-center serosurvey. *J Med Virol* 2008; **80**(7): 1202-10.
183. Nuvor SV, Katano H, Ampofo WK, Barnor JS, Sata T. Higher prevalence of antibodies to human herpesvirus 8 in HIV-infected individuals than in the general population in Ghana, West Africa. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology* 2001; **20**(5): 362-4.
184. Vitale F, Viviano E, Perna AM, et al. Serological and virological evidence of non-sexual transmission of human herpesvirus type 8 (HHV8). *Epidemiol Infect* 2000; **125**(3): 671-5.
185. Zavitsanou A, Sypsa V, Petrodaskalaki M, Kalapothaki V, Whitby D, Hatzakis A. Human herpesvirus 8 (HHV-8) infection in healthy urban employees from Greece: seroprevalence and associated factors. *J Med Virol* 2007; **79**(5): 591-6.
186. Pelsler C, Vitale F, Whitby D, et al. Socio-economic and other correlates of Kaposi sarcoma-associated herpesvirus seroprevalence among older adults in Sicily. *J Med Virol* 2009; **81**(11): 1938-44.
187. DeSantis SM, Pau CP, Archibald LK, et al. Demographic and immune correlates of human herpesvirus 8 seropositivity in Malawi, Africa.

International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases 2002; **6**(4): 266-71.

188. Wang H, Liu J, Dilimulati, et al. Seroprevalence and risk factors of Kaposi's sarcoma-associated herpesvirus infection among the general Uygur population from south and north region of Xinjiang, China. *Virology journal* 2011; **8**: 539.

189. Fujii T, Taguchi H, Katano H, et al. Seroprevalence of human herpesvirus 8 in human immunodeficiency virus 1-positive and human immunodeficiency virus 1-negative populations in Japan. *J Med Virol* 1999; **57**(2): 159-62.

190. Ablashi D, Chatlynne L, Cooper H, et al. Seroprevalence of human herpesvirus-8 (HHV-8) in countries of Southeast Asia compared to the USA, the Caribbean and Africa. *Br J Cancer* 1999; **81**(5): 893-7.

191. Malope-Kgokong BI, Macphail P, Mbisa G, et al. Kaposi's Sarcoma Associated-Herpes Virus (KSHV) Seroprevalence in Pregnant Women in South Africa. *Infect Agent Cancer* 2010; **5**: 14.

192. Kedes DH, Operskalski E, Busch M, Kohn R, Flood J, Ganem D. The seroepidemiology of human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus): distribution of infection in KS risk groups and evidence for sexual transmission. *Nat Med* 1996; **2**(8): 918-24.

193. Martin JN, Ganem DE, Osmond DH, Page-Shafer KA, Macrae D, Kedes DH. Sexual transmission and the natural history of human herpesvirus 8 infection. *N Engl J Med* 1998; **338**(14): 948-54.

194. Casper C, Redman M, Huang ML, et al. HIV infection and human herpesvirus-8 oral shedding among men who have sex with men. *J Acquir Immune Defic Syndr* 2004; **35**(3): 233-8.

195. Casper C, Wald A, Pauk J, Tabet SR, Corey L, Celum CL. Correlates of prevalent and incident Kaposi's sarcoma-associated herpesvirus infection in men who have sex with men. *The Journal of infectious diseases* 2002; **185**(7): 990-3.

196. Osmond DH, Buchbinder S, Cheng A, et al. Prevalence of Kaposi sarcoma-associated herpesvirus infection in homosexual men at beginning of and during the HIV epidemic. *JAMA* 2002; **287**(2): 221-5.

197. Kedes DH, Ganem D, Ameli N, Bacchetti P, Greenblatt R. The prevalence of serum antibody to human herpesvirus 8 (Kaposi sarcoma-associated herpesvirus) among HIV-seropositive and high-risk HIV-seronegative women. *JAMA* 1997; **277**(6): 478-81.

198. Cannon MJ, Dollard SC, Smith DK, et al. Blood-borne and sexual transmission of human herpesvirus 8 in women with or at risk for human immunodeficiency virus infection. *N Engl J Med* 2001; **344**(9): 637-43.

199. Casper C, Meier AS, Wald A, Morrow RA, Corey L, Moscicki AB. Human herpesvirus 8 infection among adolescents in the REACH cohort. *Archives of pediatrics & adolescent medicine* 2006; **160**(9): 937-42.

200. Martro E, Bulterys M, Stewart JA, et al. Comparison of human herpesvirus 8 and Epstein-Barr virus seropositivity among children in areas endemic and non-endemic for Kaposi's sarcoma. *J Med Virol* 2004; **72**(1): 126-31.

201. Blackbourn DJ, Levy JA. Human herpesvirus 8 in semen and prostate. *AIDS* 1997; **11**(2): 249-50.

202. Blackbourn DJ, Lennette ET, Ambroziak J, Mourich DV, Levy JA. Human herpesvirus 8 detection in nasal secretions and saliva. *The Journal of infectious diseases* 1998; **177**(1): 213-6.
203. Tasaka T, Said JW, Morosetti R, et al. Is Kaposi's sarcoma-associated herpesvirus ubiquitous in urogenital and prostate tissues? *Blood* 1997; **89**(5): 1686-9.
204. Pellett PE, Spira TJ, Bagasra O, et al. Multicenter comparison of PCR assays for detection of human herpesvirus 8 DNA in semen. *Journal of clinical microbiology* 1999; **37**(5): 1298-301.
205. Diamond C, Huang ML, Kedes DH, et al. Absence of detectable human herpesvirus 8 in the semen of human immunodeficiency virus-infected men without Kaposi's sarcoma. *The Journal of infectious diseases* 1997; **176**(3): 775-7.
206. Rubin MA, Parry JP, Singh B. Kaposi's sarcoma associated herpesvirus deoxyribonucleic acid sequences: lack of detection in prostatic tissue of human immunodeficiency virus-negative immunocompetent adults. *J Urol* 1998; **159**(1): 146-8.
207. Calabro ML, Fiore JR, Favero A, et al. Detection of human herpesvirus 8 in cervicovaginal secretions and seroprevalence in human immunodeficiency virus type 1-seropositive and -seronegative women. *The Journal of infectious diseases* 1999; **179**(6): 1534-7.
208. Pauk J, Huang ML, Brodie SJ, et al. Mucosal shedding of human herpesvirus 8 in men. *N Engl J Med* 2000; **343**(19): 1369-77.
209. Casper C, Carrell D, Miller KG, et al. HIV serodiscordant sex partners and the prevalence of human herpesvirus 8 infection among HIV negative men who have sex with men: baseline data from the EXPLORE Study. *Sexually transmitted infections* 2006; **82**(3): 229-35.
210. Goedert JJ, Neuland CY, Wallen WC, et al. Amyl nitrite may alter T lymphocytes in homosexual men. *Lancet* 1982; **1**(8269): 412-6.
211. Lucchini A, Dal Conte I, Di Perri G. Mucosal shedding of human herpesvirus 8. *N Engl J Med* 2001; **344**(9): 691; author reply -2.
212. Luppi M, Torelli G. The new lymphotropic herpesviruses (HHV-6, HHV-7, HHV-8) and hepatitis C virus (HCV) in human lymphoproliferative diseases: an overview. *Haematologica* 1996; **81**(3): 265-81.
213. Guttman-Yassky E, Kra-Oz Z, Dubnov J, et al. Infection with Kaposi's sarcoma-associated herpesvirus among families of patients with classic Kaposi's sarcoma. *Arch Dermatol* 2005; **141**(11): 1429-34.
214. Perna AM, Bonura F, Vitale F, et al. Antibodies to human herpes virus type 8 (HHV8) in general population and in individuals at risk for sexually transmitted diseases in Western Sicily. *International journal of epidemiology* 2000; **29**(1): 175-9.
215. Angeloni A, Heston L, Uccini S, et al. High prevalence of antibodies to human herpesvirus 8 in relatives of patients with classic Kaposi's sarcoma from Sardinia. *The Journal of infectious diseases* 1998; **177**(6): 1715-8.
216. Chen N, Nelson KE, Jenkins FJ, et al. Seroprevalence of human herpesvirus 8 infection in Northern Thailand. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2004; **39**(7): 1052-8.

217. Xie Y, Ruan B, Chen Y, Wu N, Hu M, Zhu B. Kaposi's sarcoma-associated herpesvirus infection in Chinese patients with chronic hepatitis B. *J Med Virol* 2011; **83**(5): 879-83.
218. Plancoulaine S, Abel L, van Beveren M, et al. Human herpesvirus 8 transmission from mother to child and between siblings in an endemic population. *Lancet* 2000; **356**(9235): 1062-5.
219. Smith NA, Sabin CA, Gopal R, et al. Serologic evidence of human herpesvirus 8 transmission by homosexual but not heterosexual sex. *The Journal of infectious diseases* 1999; **180**(3): 600-6.
220. Cunha AM, Caterino-de-Araujo A, Costa SC, et al. Increasing seroprevalence of human herpesvirus 8 (HHV-8) with age confirms HHV-8 endemicity in Amazon Amerindians from Brazil. *J Gen Virol* 2005; **86**(Pt 9): 2433-7.
221. Hladik W, Dollard SC, Downing RG, et al. Kaposi's sarcoma in Uganda: risk factors for human herpesvirus 8 infection among blood donors. *J Acquir Immune Defic Syndr* 2003; **33**(2): 206-10.
222. Biryahwaho B, Dollard SC, Pfeiffer RM, et al. Sex and geographic patterns of human herpesvirus 8 infection in a nationally representative population-based sample in Uganda. *The Journal of infectious diseases* 2010; **202**(9): 1347-53.
223. Enbom M, Tolfvenstam T, Ghebrekidan H, et al. Seroprevalence of human herpes virus 8 in different Eritrean population groups. *J Clin Virol* 1999; **14**(3): 167-72.
224. Kakoola DN, Sheldon J, Byabazaire N, et al. Recombination in human herpesvirus-8 strains from Uganda and evolution of the K15 gene. *J Gen Virol* 2001; **82**(Pt 10): 2393-404.
225. Newton R, Ziegler J, Bourboulia D, et al. The sero-epidemiology of Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) in adults with cancer in Uganda. *Int J Cancer* 2003; **103**(2): 226-32.
226. Olsen SJ, Chang Y, Moore PS, Biggar RJ, Melbye M. Increasing Kaposi's sarcoma-associated herpesvirus seroprevalence with age in a highly Kaposi's sarcoma endemic region, Zambia in 1985. *AIDS* 1998; **12**(14): 1921-5.
227. Pfeiffer RM, Wheeler WA, Mbisa G, et al. Geographic heterogeneity of prevalence of the human herpesvirus 8 in sub-Saharan Africa: clues about etiology. *Ann Epidemiol* 2010; **20**(12): 958-63.
228. Wawer MJ, Eng SM, Serwadda D, et al. Prevalence of Kaposi sarcoma-associated herpesvirus compared with selected sexually transmitted diseases in adolescents and young adults in rural Rakai District, Uganda. *Sex Transm Dis* 2001; **28**(2): 77-81.
229. Adjei AA, Armah HB, Gbagbo F, Boamah I, Adu-Gyamfi C, Asare I. Seroprevalence of HHV-8, CMV, and EBV among the general population in Ghana, West Africa. *BMC Infect Dis* 2008; **8**: 111.
230. Bourboulia D, Whitby D, Boshoff C, et al. Serologic evidence for mother-to-child transmission of Kaposi sarcoma-associated herpesvirus infection. *JAMA* 1998; **280**(1): 31-2.
231. Engels EA, Sinclair MD, Biggar RJ, et al. Latent class analysis of human herpesvirus 8 assay performance and infection prevalence in sub-saharan Africa and Malta. *Int J Cancer* 2000; **88**(6): 1003-8.

232. Engels EA, Whitby D, Goebel PB, et al. Identifying human herpesvirus 8 infection: performance characteristics of serologic assays. *J Acquir Immune Defic Syndr* 2000; **23**(4): 346-54.
233. Dollard SC, Butler LM, Graves Jones AM, et al. Substantial regional differences in human herpesvirus 8 seroprevalence in sub-saharan Africa: Insights on the origin of the "KS Belt". *Int J Cancer* 2010.
234. Lagunoff M, Ganem D. The structure and coding organization of the genomic termini of Kaposi's sarcoma-associated herpesvirus. *Virology* 1997; **236**(1): 147-54.
235. Lee H, Veazey R, Williams K, et al. Deregulation of cell growth by the K1 gene of Kaposi's sarcoma-associated herpesvirus. *Nat Med* 1998; **4**(4): 435-40.
236. Zong JC, Metroka C, Reitz MS, Nicholas J, Hayward GS. Strain variability among Kaposi sarcoma-associated herpesvirus (human herpesvirus 8) genomes: evidence that a large cohort of United States AIDS patients may have been infected by a single common isolate. *J Virol* 1997; **71**(3): 2505-11.
237. Poole LJ, Zong JC, Ciuffo DM, et al. Comparison of genetic variability at multiple loci across the genomes of the major subtypes of Kaposi's sarcoma-associated herpesvirus reveals evidence for recombination and for two distinct types of open reading frame K15 alleles at the right-hand end. *J Virol* 1999; **73**(8): 6646-60.
238. Stebbing J, Wilder N, Ariad S, Abu-Shakra M. Lack of intra-patient strain variability during infection with Kaposi's sarcoma-associated herpesvirus. *American journal of hematology* 2001; **68**(2): 133-4.
239. Hayward GS. KSHV strains: the origins and global spread of the virus. *Seminars in cancer biology* 1999; **9**(3): 187-99.
240. Qin Z, Jakymiw A, Findlay V, Parsons C. KSHV-Encoded MicroRNAs: Lessons for Viral Cancer Pathogenesis and Emerging Concepts. *International journal of cell biology* 2012; **2012**: 603961.
241. Liu H, Prugnolle F, Manica A, Balloux F. A geographically explicit genetic model of worldwide human-settlement history. *American journal of human genetics* 2006; **79**(2): 230-7.
242. Sabeti PC, Varilly P, Fry B, et al. Genome-wide detection and characterization of positive selection in human populations. *Nature* 2007; **449**(7164): 913-8.
243. Sirugo G, Hennig BJ, Adeyemo AA, et al. Genetic studies of African populations: an overview on disease susceptibility and response to vaccines and therapeutics. *Human genetics* 2008; **123**(6): 557-98.
244. Alkharsah KR, Dediccoat M, Blasczyk R, Newton R, Schulz TF. Influence of HLA alleles on shedding of Kaposi sarcoma-associated herpesvirus in saliva in an African population. *The Journal of infectious diseases* 2007; **195**(6): 809-16.
245. Guech-Ongey M, Verboom M, Pfeiffer RM, et al. HLA polymorphisms and detection of kaposi sarcoma-associated herpesvirus DNA in saliva and peripheral blood among children and their mothers in the uganda sickle cell anemia KSHV Study. *Infect Agent Cancer* 2010; **5**: 21.
246. Cottoni F, Masala MV, Santarelli R, et al. Susceptibility to human herpesvirus-8 infection in a healthy population from Sardinia is not directly correlated with the expression of HLA-DR alleles. *Br J Dermatol* 2004; **151**(1): 247-9.

247. Guerini FR, Mancuso R, Agostini S, et al. Activating KIR/HLA complexes in classic Kaposi's Sarcoma. *Infect Agent Cancer* 2012; **7**: 9.
248. O'Rourke DH, Suarez BK. Patterns and correlates of genetic variation in South Amerindians. *Annals of human biology* 1986; **13**(1): 13-31.
249. Long JC, Williams RC, McAuley JE, et al. Genetic variation in Arizona Mexican Americans: estimation and interpretation of admixture proportions. *American journal of physical anthropology* 1991; **84**(2): 141-57.
250. Sanchez E, Webb RD, Rasmussen A, et al. Genetically determined Amerindian ancestry correlates with increased frequency of risk alleles for systemic lupus erythematosus. *Arthritis and rheumatism* 2010; **62**(12): 3722-9.
251. de Souza VA, Sumita LM, Nascimento MC, et al. Human herpesvirus-8 infection and oral shedding in Amerindian and non-Amerindian populations in the Brazilian Amazon region. *The Journal of infectious diseases* 2007; **196**(6): 844-52.
252. Whitby D, Marshall VA, Bagni RK, et al. Reactivation of Kaposi's sarcoma-associated herpesvirus by natural products from Kaposi's sarcoma endemic regions. *Int J Cancer* 2007; **120**(2): 321-8.
253. Moormann AM, Snider CJ, Chelimo K. The company malaria keeps: how co-infection with Epstein-Barr virus leads to endemic Burkitt lymphoma. *Curr Opin Infect Dis* 2011; **24**(5): 435-41.
254. Piriou E, Asito AS, Sumba PO, et al. Early age at time of primary Epstein-Barr virus infection results in poorly controlled viral infection in infants from Western Kenya: clues to the etiology of endemic Burkitt lymphoma. *The Journal of infectious diseases* 2012; **205**(6): 906-13.
255. Andreoni M, El-Sawaf G, Rezza G, et al. High seroprevalence of antibodies to human herpesvirus-8 in Egyptian children: evidence of nonsexual transmission. *J Natl Cancer Inst* 1999; **91**(5): 465-9.
256. Butler LM, Dorsey G, Hladik W, et al. Kaposi sarcoma-associated herpesvirus (KSHV) seroprevalence in population-based samples of African children: evidence for at least 2 patterns of KSHV transmission. *The Journal of infectious diseases* 2009; **200**(3): 430-8.
257. Gessain A, Mauclere P, van Beveren M, et al. Human herpesvirus 8 primary infection occurs during childhood in Cameroon, Central Africa. *Int J Cancer* 1999; **81**(2): 189-92.
258. Mbulaiteye SM, Biggar RJ, Bakaki PM, et al. Human herpesvirus 8 infection and transfusion history in children with sickle-cell disease in Uganda. *J Natl Cancer Inst* 2003; **95**(17): 1330-5.
259. Mbulaiteye SM, Biggar RJ, Pfeiffer RM, et al. Water, socioeconomic factors, and human herpesvirus 8 infection in Ugandan children and their mothers. *J Acquir Immune Defic Syndr* 2005; **38**(4): 474-9.
260. Plancoulaine S, Abel L, Tregouet D, et al. Respective roles of serological status and blood specific antihuman herpesvirus 8 antibody levels in human herpesvirus 8 intrafamilial transmission in a highly endemic area. *Cancer research* 2004; **64**(23): 8782-7.
261. Sitas F, Newton R, Boshoff C. Increasing probability of mother-to-child transmission of HHV-8 with increasing maternal antibody titer for HHV-8. *N Engl J Med* 1999; **340**(24): 1923.
262. Butler LM, Dollard SC, Amin M, Mosam A, Martin JN. Relationship between salivary shedding and seropositivity for Kaposi's sarcoma-associated

- herpesvirus (KSHV) and Epstein-Barr virus (EBV) among South African children and adults: insights on why EBV infection is ubiquitous and KSHV is not. . 12th International Conference on Malignancies in AIDS and Other Acquired Immunodeficiencies (ICMAOI); 2010; Bethesda: Infectious Agents and Cancer; 2010.
263. Baeten JM, Chohan BH, Lavreys L, et al. Correlates of human herpesvirus 8 seropositivity among heterosexual men in Kenya. *AIDS* 2002; **16**(15): 2073-8.
264. Lavreys L, Chohan B, Ashley R, et al. Human herpesvirus 8: seroprevalence and correlates in prostitutes in Mombasa, Kenya. *The Journal of infectious diseases* 2003; **187**(3): 359-63.
265. Marcelin AG, Grandadam M, Flandre P, et al. Kaposi's sarcoma herpesvirus and HIV-1 seroprevalences in prostitutes in Djibouti. *J Med Virol* 2002; **68**(2): 164-7.
266. Shebl FM, Dollard SC, Pfeiffer RM, et al. Human herpesvirus 8 seropositivity among sexually active adults in Uganda. *PloS one* 2011; **6**(6): e21286.
267. Volpi A, Sarmati L, Suligoi B, Montano M, Rezza G, Andreoni M. Correlates of human herpes virus-8 and herpes simplex virus type 2 infections in Northern Cameroon. *J Med Virol* 2004; **74**(3): 467-72.
268. Taylor MM, Chohan B, Lavreys L, et al. Shedding of human herpesvirus 8 in oral and genital secretions from HIV-1-seropositive and -seronegative Kenyan women. *The Journal of infectious diseases* 2004; **190**(3): 484-8.
269. WHO. <http://www.afro.who.int/en/uganda/who-country-office-uganda.html>. accessed 8th April 2012.
270. UBOS. Uganda Bureau of Statistics (UBOS) and Macro International Inc. Uganda Demographic and Health Survey 2006, 2006.
271. IMF. <http://www.imf.org/external/data.htm>, accessed 16th May 2012.
272. Tann CJ, Kizza M, Morison L, et al. Use of antenatal services and delivery care in Entebbe, Uganda: a community survey. *BMC pregnancy and childbirth* 2007; **7**: 23.
273. Katz N, Chaves A, Pellegrino J. A simple device for quantitative stool thick-smear technique in Schistosomiasis mansoni. *Revista do Instituto de Medicina Tropical de Sao Paulo* 1972; **14**(6): 397-400.
274. Bukusuba JW, Hughes P, Kizza M, et al. Screening for intestinal helminth infection in a semi-urban cohort of pregnant women in Uganda. *Tropical doctor* 2004; **34**(1): 27-8.
275. Melrose WD, Turner PF, Pisters P, Turner B. An improved Knott's concentration test for the detection of microfilariae. *Trans R Soc Trop Med Hyg* 2000; **94**(2): 176.
276. Muhangi L, Woodburn P, Omara M, et al. Associations between mild-to-moderate anaemia in pregnancy and helminth, malaria and HIV infection in Entebbe, Uganda. *Trans R Soc Trop Med Hyg* 2007; **101**(9): 899-907.
277. Morgan D, Maude GH, Malamba SS, et al. HIV-1 disease progression and AIDS-defining disorders in rural Uganda. *Lancet* 1997; **350**(9073): 245-50.
278. Morgan D, Ross A, Mayanja B, Malamba S, Whitworth J. Early manifestations (pre-AIDS) of HIV-1 infection in Uganda. *AIDS* 1998; **12**(6): 591-6.
279. Mayanja BN, Shafer LA, Van der Paal L, et al. Effect of pregnancy on immunological and virological outcomes of women on ART: a prospective

cohort study in rural Uganda, 2004-2009. *Tropical medicine & international health : TM & IH* 2011; **12**(8): 920-8.

280. Mulder DW, Nunn AJ, Kamali A, Nakiyingi J, Wagner HU, Kengeya-Kayondo JF. Two-year HIV-1-associated mortality in a Ugandan rural population. *Lancet* 1994; **343**(8904): 1021-3.

281. Mulder DW, Nunn AJ, Wagner HU, Kamali A, Kengeya-Kayondo JF. HIV-1 incidence and HIV-1-associated mortality in a rural Ugandan population cohort. *AIDS* 1994; **8**(1): 87-92.

282. Nunn AJ, Mulder DW, Kamali A, Ruberantwari A, Kengeya-Kayondo JF, Whitworth J. Mortality associated with HIV-1 infection over five years in a rural Ugandan population: cohort study. *BMJ* 1997; **315**(7111): 767-71.

283. Kirungi WL, Musinguzi J, Madraa E, et al. Trends in antenatal HIV prevalence in urban Uganda associated with uptake of preventive sexual behaviour. *Sexually transmitted infections* 2006; **82 Suppl 1**: i36-41.

284. Hallett TB, Aberle-Grasse J, Bello G, et al. Declines in HIV prevalence can be associated with changing sexual behaviour in Uganda, urban Kenya, Zimbabwe, and urban Haiti. *Sexually transmitted infections* 2006; **82 Suppl 1**: i1-8.

285. Shafer LA, Biraro S, Nakiyingi-Miiró J, et al. HIV prevalence and incidence are no longer falling in southwest Uganda: evidence from a rural population cohort 1989-2005. *AIDS* 2008; **22**(13): 1641-9.

286. Shafer LA, Maher D, Weiss HA, Levin J, Biraro S, Grosskurth H. Contribution of population factors to estimation of human immunodeficiency virus prevalence trends: a cohort study in rural Uganda, 1989-2007. *American journal of epidemiology* 2011; **174**(10): 1175-82.

287. Shafer LA, White RG, Nsubuga RN, Chapman R, Hayes R, Grosskurth H. The role of the natural epidemic dynamics and migration in explaining the course of the HIV epidemic in rural Uganda: a modelling study. *International journal of epidemiology* 2011; **40**(2): 397-404.

288. Wolff B, Nyanzi B, Katongole G, Ssesanga D, Ruberantwari A, Whitworth J. Evaluation of a home-based voluntary counselling and testing intervention in rural Uganda. *Health policy and planning* 2005; **20**(2): 109-16.

289. Kellam P, Boshoff C, Whitby D, Matthews S, Weiss RA, Talbot SJ. Identification of a major latent nuclear antigen, LNA-1, in the human herpesvirus 8 genome. *Journal of human virology* 1997; **1**(1): 19-29.

290. Butler L, Dollard SC, Amin M, Mosam A, Martin J. Relationship between salivary shedding and seropositivity for Kaposi's sarcoma-associated herpesvirus (KSHV) and Epstein-Barr virus (EBV) among South African children and adults: insights on why EBV infection is ubiquitous and KSHV is not. 12th International Conference on Malignancies in AIDS and Other Acquired Immunodeficiencies (ICMAOI); 2010; Bethesda, USA; 2010.

291. Chatlynne LG, Ablashi DV. Seroepidemiology of Kaposi's sarcoma-associated herpesvirus (KSHV). *Seminars in cancer biology* 1999; **9**(3): 175-85.

292. Corchero JL, Mar EC, Spira TJ, Pellett PE, Inoue N. Comparison of serologic assays for detection of antibodies against human herpesvirus 8. *Clinical and diagnostic laboratory immunology* 2001; **8**(5): 913-21.

293. Lam LL, Pau CP, Dollard SC, Pellett PE, Spira TJ. Highly sensitive assay for human herpesvirus 8 antibodies that uses a multiple antigenic peptide derived

- from open reading frame K8.1. *Journal of clinical microbiology* 2002; **40**(2): 325-9.
294. Landis JR, Koch GG. An application of hierarchical kappa-type statistics in the assessment of majority agreement among multiple observers. *Biometrics* 1977; **33**(2): 363-74.
295. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977; **33**(1): 159-74.
296. International Agency for Research on Cancer L, France GLOBOCAN 2008 v2.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10 [Internet] Available from: <http://globocan.iarc.fr> Accessed 12th March 2013.
297. Chang Y, Ziegler J, Wabinga H, et al. Kaposi's sarcoma-associated herpesvirus and Kaposi's sarcoma in Africa. Uganda Kaposi's Sarcoma Study Group. *Arch Intern Med* 1996; **156**(2): 202-4.
298. Buonaguro FM, Tornesello ML, Beth-Giraldo E, et al. Herpesvirus-like DNA sequences detected in endemic, classic, iatrogenic and epidemic Kaposi's sarcoma (KS) biopsies. *Int J Cancer* 1996; **65**(1): 25-8.
299. Marchioli CC, Love JL, Abbott LZ, et al. Prevalence of human herpesvirus 8 DNA sequences in several patient populations. *Journal of clinical microbiology* 1996; **34**(10): 2635-8.
300. Lebbe C, Pellet C, Flageul B, et al. Sequences of human herpesvirus 8 are not detected in various non-Kaposi sarcoma vascular lesions. *Arch Dermatol* 1997; **133**(7): 919-20.
301. Ziegler J, Newton R, Bourboulia D, et al. Risk factors for Kaposi's sarcoma: a case-control study of HIV-seronegative people in Uganda. *Int J Cancer* 2003; **103**(2): 233-40.
302. Lennette ET, Blackbourn DJ, Levy JA. Antibodies to human herpesvirus type 8 in the general population and in Kaposi's sarcoma patients. *Lancet* 1996; **348**(9031): 858-61.
303. Rabkin CS, Shepherd FA, Wade JA. Human herpesvirus 8 and renal transplantation. *N Engl J Med* 1999; **340**(13): 1045-6.
304. Regamey N, Tamm M, Wernli M, et al. Transmission of human herpesvirus 8 infection from renal-transplant donors to recipients. *N Engl J Med* 1998; **339**(19): 1358-63.
305. Parravicini C, Olsen SJ, Capra M, et al. Risk of Kaposi's sarcoma-associated herpes virus transmission from donor allografts among Italian posttransplant Kaposi's sarcoma patients. *Blood* 1997; **90**(7): 2826-9.
306. Marcelin AG, Roque-Afonso AM, Hurtova M, et al. Fatal disseminated Kaposi's sarcoma following human herpesvirus 8 primary infections in liver-transplant recipients. *Liver transplantation : official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society* 2004; **10**(2): 295-300.
307. Frances C, Mouquet C, Marcelin AG, et al. Outcome of kidney transplant recipients with previous human herpesvirus-8 infection. *Transplantation* 2000; **69**(9): 1776-9.
308. Frances C, Mouquet C, Calvez V. Human herpesvirus 8 and renal transplantation. *N Engl J Med* 1999; **340**(13): 1045; author reply 6.
309. Emond JP, Marcelin AG, Dorent R, et al. Kaposi's sarcoma associated with previous human herpesvirus 8 infection in heart transplant recipients. *Journal of clinical microbiology* 2002; **40**(6): 2217-9.

310. Garcia-Astudillo LA, Leyva-Cobian F. Human herpesvirus-8 infection and Kaposi's sarcoma after liver and kidney transplantation in different geographical areas of Spain. *Transplant immunology* 2006; **17**(1): 65-9.
311. Barozzi P, Luppi M, Masini L, et al. Lymphotropic herpes virus (EBV, HHV-6, HHV-8) DNA sequences in HIV negative Castleman's disease. *Clinical molecular pathology* 1996; **49**(4): M232-5.
312. Parravicini C, Chandran B, Corbellino M, et al. Differential viral protein expression in Kaposi's sarcoma-associated herpesvirus-infected diseases: Kaposi's sarcoma, primary effusion lymphoma, and multicentric Castleman's disease. *Am J Pathol* 2000; **156**(3): 743-9.
313. Asou H, Tasaka T, Said JW, Daibata M, Kamada N, Koeffler HP. Co-infection of HHV-6 and HHV-8 is rare in primary effusion lymphoma. *Leukemia research* 2000; **24**(1): 59-61.
314. Soulier J, Grollet L, Oksenhendler E, et al. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in multicentric Castleman's disease. *Blood* 1995; **86**(4): 1276-80.
315. Belec L, Mohamed AS, Authier FJ, et al. Human herpesvirus 8 infection in patients with POEMS syndrome-associated multicentric Castleman's disease. *Blood* 1999; **93**(11): 3643-53.
316. Maizels RM. Parasite immunomodulation and polymorphisms of the immune system. *J Biol* 2009; **8**(7): 62.
317. Brooker S, Hotez PJ, Bundy DA. The global atlas of helminth infection: mapping the way forward in neglected tropical disease control. *PLoS Negl Trop Dis* 2010; **4**(7): e779.
318. Brooker S, Kabatereine NB, Tukahebwa EM, Kazibwe F. Spatial analysis of the distribution of intestinal nematode infections in Uganda. *Epidemiol Infect* 2004; **132**(6): 1065-71.
319. Lin CJ, Katongole-Mbidde E, Byekwaso T, Orem J, Rabkin CS, Mbulaiteye SM. Intestinal parasites in Kaposi sarcoma patients in Uganda: indication of shared risk factors or etiologic association. *Am J Trop Med Hyg* 2008; **78**(3): 409-12.
320. Ziegler JL, Newton R, Katongole-Mbidde E, et al. Risk factors for Kaposi's sarcoma in HIV-positive subjects in Uganda. *AIDS* 1997; **11**(13): 1619-26.
321. WHO. Worldwide prevalence of anaemia 1993–2005: World Health Organisation (WHO) global database on anaemia http://whqlibdoc.who.int/publications/2008/9789241596657_eng.pdf, 2008.
322. Ndibazza J, Muhangi L, Akishule D, et al. Effects of deworming during pregnancy on maternal and perinatal outcomes in Entebbe, Uganda: a randomized controlled trial. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2010; **50**(4): 531-40.
323. WHO. Report of the World Health Organisation (WHO) informal consultation on hookworm infection and anaemia in girls and women. <https://apps.who.int/ctd/intpara/96-1.pdf>, 1994.
324. de Sanjose S, Marshall V, Sola J, et al. Prevalence of Kaposi's sarcoma-associated herpesvirus infection in sex workers and women from the general population in Spain. *Int J Cancer* 2002; **98**(1): 155-8.
325. Minhas V, Crabtree KL, Chao A, et al. Early childhood infection by human herpesvirus 8 in Zambia and the role of human immunodeficiency virus type 1

- coinfection in a highly endemic area. *American journal of epidemiology* 2008; **168**(3): 311-20.
326. Mbulaiteye SM, Walters M, Engels EA, et al. High levels of Epstein-Barr virus DNA in saliva and peripheral blood from Ugandan mother-child pairs. *The Journal of infectious diseases* 2006; **193**(3): 422-6.
327. Mbulaiteye S, Marshall V, Bagni RK, et al. Molecular evidence for mother-to-child transmission of Kaposi sarcoma-associated herpesvirus in Uganda and K1 gene evolution within the host. *The Journal of infectious diseases* 2006; **193**(9): 1250-7.
328. Hladik W, Dollard SC, Mermin J, et al. Transmission of human herpesvirus 8 by blood transfusion. *N Engl J Med* 2006; **355**(13): 1331-8.
329. Maizels RM, Pearce EJ, Artis D, Yazdanbakhsh M, Wynn TA. Regulation of pathogenesis and immunity in helminth infections. *J Exp Med* 2009; **206**(10): 2059-66.
330. McHardy J, Williams EH, Geser A, de-The G, Beth E, Giraldo G. Endemic Kaposi's sarcoma: incidence and risk factors in the West Nile District of Uganda. *Int J Cancer* 1984; **33**(2): 203-12.
331. Geddes M, Franceschi S, Barchielli A, et al. Kaposi's sarcoma in Italy before and after the AIDS epidemic. *Br J Cancer* 1994; **69**(2): 333-6.
332. Geddes M, Franceschi S, Balzi D, Arniani S, Gafa L, Zanetti R. Birthplace and classic Kaposi's sarcoma in Italy. *Associazione Italiana Registri Tumori. J Natl Cancer Inst* 1995; **87**(13): 1015-7.
333. Cottoni F, Masala MV, Budroni M, et al. The role of occupation and a past history of malaria in the etiology of classic Kaposi's sarcoma: a case-control study in north-east Sardinia. *Br J Cancer* 1997; **76**(11): 1518-20.
334. Coluzzi M, Calabro ML, Manno D, Chieco-Bianchi L, Schulz TF, Ascoli V. Reduced seroprevalence of Kaposi's sarcoma-associated herpesvirus (KSHV), human herpesvirus 8 (HHV8), related to suppression of Anopheles density in Italy. *Med Vet Entomol* 2003; **17**(4): 461-4.
335. Ascoli V, Facchinelli L, Valerio L, Manno D, Coluzzi M. Kaposi's sarcoma, human herpesvirus 8 infection and the potential role of promoter-arthropod bites in northern Sweden. *J Med Virol* 2006; **78**(11): 1452-5.
336. Coluzzi M, Calabro ML, Manno D, Chieco-Bianchi L, Schulz TF, Ascoli V. HHV-8 transmission via saliva to soothe blood-sucking arthropod bites. *Br J Cancer* 2004; **91**(5): 998-9; author reply 9.
337. Coluzzi M, Calabro ML, Manno D, Chieco-Bianchi L, Schulz TF, Ascoli V. Saliva and the transmission of human herpesvirus 8: potential role of promoter-arthropod bites. *The Journal of infectious diseases* 2004; **190**(1): 199-200; author reply -1.
338. Coluzzi M, Manno D, Guzzinati S, et al. The bloodsucking arthropod bite as possible cofactor in the transmission of human herpesvirus-8 infection and in the expression of Kaposi's sarcoma disease. *Parassitologia* 2002; **44**(1-2): 123-9.
339. Ascoli V, Facchinelli L, Valerio L, Zucchetto A, Dal Maso L, Coluzzi M. Distribution of mosquito species in areas with high and low incidence of classic Kaposi's sarcoma and seroprevalence for HHV-8. *Med Vet Entomol* 2006; **20**(2): 198-208.

340. Ascoli V, Senis G, Zucchetto A, et al. Distribution of 'promoter' sandflies associated with incidence of classic Kaposi's sarcoma. *Med Vet Entomol* 2009; **23**(3): 217-25.
341. Abrams DI. The relationship between Kaposi's sarcoma and intestinal parasites among homosexual males in the United States. *J Acquir Immune Defic Syndr* 1990; **3 Suppl 1**: S44-6.
342. Ascoli V, Belli S, Benedetti M, Trinca S, Ricci P, Comba P. High incidence of classic Kaposi's sarcoma in Mantua, Po Valley, Northern Italy (1989-1998). *Br J Cancer* 2001; **85**(3): 379-82.
343. Tanzi E, Zappa A, Caramaschi F, et al. Human herpesvirus type 8 infection in an area of Northern Italy with high incidence of classical Kaposi's sarcoma. *J Med Virol* 2005; **76**(4): 571-5.
344. Bettini S, Melis P. Leishmaniasis in Sardinia. III. Soil analysis of a breeding site of three species of sandflies. *Med Vet Entomol* 1988; **2**(1): 67-71.
345. Ziegler JL. Endemic Kaposi's sarcoma in Africa and local volcanic soils. *Lancet* 1993; **342**(8883): 1348-51.
346. van Riet E, Hartgers FC, Yazdanbakhsh M. Chronic helminth infections induce immunomodulation: consequences and mechanisms. *Immunobiology* 2007; **212**(6): 475-90.
347. Borkow G, Bentwich Z. Chronic immune activation associated with chronic helminthic and human immunodeficiency virus infections: role of hyporesponsiveness and anergy. *Clinical microbiology reviews* 2004; **17**(4): 1012-30, table of contents.
348. Kamal SM, El Sayed Khalifa K. Immune modulation by helminthic infections: worms and viral infections. *Parasite Immunol* 2006; **28**(10): 483-96.
349. Brown M, Mawa PA, Kaleebu P, Elliott AM. Helminths and HIV infection: epidemiological observations on immunological hypotheses. *Parasite Immunol* 2006; **28**(11): 613-23.
350. Loukas A, Prociv P. Immune responses in hookworm infections. *Clinical microbiology reviews* 2001; **14**(4): 689-703, table of contents.
351. Cooper PJ. Intestinal worms and human allergy. *Parasite Immunol* 2004; **26**(11-12): 455-67.
352. Valenti RM, Amodio E, Nam JM, et al. Delayed-type hypersensitivity in classic Kaposi sarcoma patients and controls. *Br J Cancer* 2011; **104**(3): 433-6.
353. Ziegler JL, Katongole-Mbidde E. Diminished delayed hypersensitivity responses in the legs and feet of patients with endemic Kaposi's sarcoma. *Trans R Soc Trop Med Hyg* 1996; **90**(2): 173-4.
354. Artavanis-Tsakonas K, Tongren JE, Riley EM. The war between the malaria parasite and the immune system: immunity, immunoregulation and immunopathology. *Clinical and experimental immunology* 2003; **133**(2): 145-52.
355. Stevenson MM, Riley EM. Innate immunity to malaria. *Nature reviews Immunology* 2004; **4**(3): 169-80.
356. Thorley-Lawson DA, Gross A. Persistence of the Epstein-Barr virus and the origins of associated lymphomas. *N Engl J Med* 2004; **350**(13): 1328-37.
357. Magrath I. Epidemiology: clues to the pathogenesis of Burkitt lymphoma. *British journal of haematology* 2012; **156**(6): 744-56.
358. Piriou E, Kimmel R, Chelimo K, et al. Serological evidence for long-term Epstein-Barr virus reactivation in children living in a holoendemic malaria region of Kenya. *J Med Virol* 2009; **81**(6): 1088-93.

359. Mancuso R, Brambilla L, Agostini S, et al. Intrafamilial transmission of Kaposi's sarcoma-associated herpesvirus and seronegative infection in family members of classic Kaposi's sarcoma patients. *J Gen Virol* 2011; **92**(Pt 4): 744-51.
360. Sowunmi A, Gbotosho GO, Adedeji AA, et al. Herpes simplex labialis in children with acute falciparum malaria. *Acta Trop* 2008; **106**(1): 68-71.
361. Kriesel JD, Gebhardt BM, Hill JM, et al. Anti-interleukin-6 antibodies inhibit herpes simplex virus reactivation. *The Journal of infectious diseases* 1997; **175**(4): 821-7.
362. Kriesel JD, Ricigliano J, Spruance SL, Garza HH, Jr., Hill JM. Neuronal reactivation of herpes simplex virus may involve interleukin-6. *Journal of neurovirology* 1997; **3**(6): 441-8.
363. Polizzotto MN, Uldrick TS, Hu D, Yarchoan R. Clinical Manifestations of Kaposi Sarcoma Herpesvirus Lytic Activation: Multicentric Castleman Disease (KSHV-MCD) and the KSHV Inflammatory Cytokine Syndrome. *Frontiers in microbiology* 2012; **3**: 73.
364. Webb EL, Mawa PA, Ndibazza J, et al. Effect of single-dose anthelmintic treatment during pregnancy on an infant's response to immunisation and on susceptibility to infectious diseases in infancy: a randomised, double-blind, placebo-controlled trial. *Lancet* 2011; **377**(9759): 52-62.
365. Okell LC, Ghani AC, Lyons E, Drakeley CJ. Submicroscopic infection in Plasmodium falciparum-endemic populations: a systematic review and meta-analysis. *The Journal of infectious diseases* 2009; **200**(10): 1509-17.
366. Woodburn PW, Muhangi L, Hillier S, et al. Risk factors for helminth, malaria, and HIV infection in pregnancy in Entebbe, Uganda. *PLoS Negl Trop Dis* 2009; **3**(6): e473.
367. Lisco A, Barbierato M, Fiore JR, et al. Pregnancy and human herpesvirus 8 reactivation in human immunodeficiency virus type 1-infected women. *Journal of clinical microbiology* 2006; **44**(11): 3863-71.
368. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *BMJ* 2009; **339**: b2535.
369. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; **327**(7414): 557-60.
370. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; **315**(7109): 629-34.
371. Ades AE, Lu G, Higgins JP. The interpretation of random-effects meta-analysis in decision models. *Medical decision making : an international journal of the Society for Medical Decision Making* 2005; **25**(6): 646-54.
372. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* 1994; **50**(4): 1088-101.
373. Gandhi M, Koelle DM, Ameli N, et al. Prevalence of human herpesvirus-8 salivary shedding in HIV increases with CD4 count. *Journal of dental research* 2004; **83**(8): 639-43.
374. Thurman AR, Doncel GF. Herpes simplex virus and HIV: genital infection synergy and novel approaches to dual prevention. *International journal of STD & AIDS* 2012; **23**(9): 613-9.
375. Miller CS, Berger JR, Mootoor Y, Avdiushko SA, Zhu H, Kryscio RJ. High prevalence of multiple human herpesviruses in saliva from human

- immunodeficiency virus-infected persons in the era of highly active antiretroviral therapy. *Journal of clinical microbiology* 2006; **44**(7): 2409-15.
376. Griffin E, Krantz E, Selke S, Huang ML, Wald A. Oral mucosal reactivation rates of herpesviruses among HIV-1 seropositive persons. *J Med Virol* 2008; **80**(7): 1153-9.
377. Morgan D, Malamba SS, Maude GH, et al. An HIV-1 natural history cohort and survival times in rural Uganda. *AIDS* 1997; **11**(5): 633-40.
378. Goudsmit J, Renwick N, Dukers NH, et al. Human herpesvirus 8 infections in the Amsterdam Cohort Studies (1984-1997): analysis of seroconversions to ORF65 and ORF73. *Proc Natl Acad Sci U S A* 2000; **97**(9): 4838-43.
379. Li M, MacKey J, Czajak SC, Desrosiers RC, Lackner AA, Jung JU. Identification and characterization of Kaposi's sarcoma-associated herpesvirus K8.1 virion glycoprotein. *J Virol* 1999; **73**(2): 1341-9.
380. Speck SH, Ganem D. Viral latency and its regulation: lessons from the gamma-herpesviruses. *Cell Host Microbe* 2010; **8**(1): 100-15.
381. Ganem D. KSHV and the pathogenesis of Kaposi sarcoma: listening to human biology and medicine. *J Clin Invest* 2010; **120**(4): 939-49.
382. Wen KW, Damania B. Kaposi sarcoma-associated herpesvirus (KSHV): molecular biology and oncogenesis. *Cancer Lett* 2010; **289**(2): 140-50.
383. Gallafent JH, Buskin SE, De Turk PB, Aboulaflia DM. Profile of patients with Kaposi's sarcoma in the era of highly active antiretroviral therapy. *J Clin Oncol* 2005; **23**(6): 1253-60.
384. Thompson MA, Aberg JA, Cahn P, et al. Antiretroviral treatment of adult HIV infection: 2010 recommendations of the International AIDS Society-USA panel. *JAMA* 2010; **304**(3): 321-33.
385. Hammer SM, Eron JJ, Jr., Reiss P, et al. Antiretroviral treatment of adult HIV infection: 2008 recommendations of the International AIDS Society-USA panel. *JAMA* 2008; **300**(5): 555-70.
386. Brau N, Fox RK, Xiao P, et al. Presentation and outcome of hepatocellular carcinoma in HIV-infected patients: a U.S.-Canadian multicenter study. *J Hepatol* 2007; **47**(4): 527-37.
387. Casper C, Krantz EM, Corey L, et al. Valganciclovir for suppression of human herpesvirus-8 replication: a randomized, double-blind, placebo-controlled, crossover trial. *The Journal of infectious diseases* 2008; **198**(1): 23-30.
388. Mocroft A, Youle M, Gazzard B, Morcinek J, Halai R, Phillips AN. Anti-herpesvirus treatment and risk of Kaposi's sarcoma in HIV infection. Royal Free/Chelsea and Westminster Hospitals Collaborative Group. *AIDS* 1996; **10**(10): 1101-5.
389. Costagliola D, Mary-Krause M. Can antiviral agents decrease the occurrence of Kaposi's sarcoma? Clinical Epidemiology Group from Centres d'Information et de Soins de l'Immunodeficiency Humaine. *Lancet* 1995; **346**(8974): 578.
390. Glesby MJ, Hoover DR, Weng S, et al. Use of antiherpes drugs and the risk of Kaposi's sarcoma: data from the Multicenter AIDS Cohort Study. *The Journal of infectious diseases* 1996; **173**(6): 1477-80.
391. Humphrey RW, O'Brien TR, Newcomb FM, et al. Kaposi's sarcoma (KS)-associated herpesvirus-like DNA sequences in peripheral blood mononuclear

cells: association with KS and persistence in patients receiving anti-herpesvirus drugs. *Blood* 1996; **88**(1): 297-301.

392. Giorgi JV, Lyles RH, Matud JL, et al. Predictive value of immunologic and virologic markers after long or short duration of HIV-1 infection. *J Acquir Immune Defic Syndr* 2002; **29**(4): 346-55.

393. Gottlieb GS, Sow PS, Hawes SE, et al. Equal plasma viral loads predict a similar rate of CD4+ T cell decline in human immunodeficiency virus (HIV) type 1- and HIV-2-infected individuals from Senegal, West Africa. *The Journal of infectious diseases* 2002; **185**(7): 905-14.

394. Braitstein P, Brinkhof MW, Dabis F, et al. Mortality of HIV-1-infected patients in the first year of antiretroviral therapy: comparison between low-income and high-income countries. *Lancet* 2006; **367**(9513): 817-24.

395. Nichols CM, Flaitz CM, Hicks MJ. Treating Kaposi's lesions in the HIV-infected patient. *Journal of the American Dental Association* 1993; **124**(11): 78-84.

396. Danzig JB, Brandt LJ, Reinus JF, Klein RS. Gastrointestinal malignancy in patients with AIDS. *Am J Gastroenterol* 1991; **86**(6): 715-8.

397. Bower M, Nelson M, Young AM, et al. Immune reconstitution inflammatory syndrome associated with Kaposi's sarcoma. *J Clin Oncol* 2005; **23**(22): 5224-8.

398. El Amari EB, Toutous-Trellu L, Gayet-Ageron A, et al. Predicting the evolution of Kaposi sarcoma, in the highly active antiretroviral therapy era. *AIDS* 2008; **22**(9): 1019-28.

399. Gill PS, Wernz J, Scadden DT, et al. Randomized phase III trial of liposomal daunorubicin versus doxorubicin, bleomycin, and vincristine in AIDS-related Kaposi's sarcoma. *J Clin Oncol* 1996; **14**(8): 2353-64.

400. Krown SE, Metroka C, Wernz JC. Kaposi's sarcoma in the acquired immune deficiency syndrome: a proposal for uniform evaluation, response, and staging criteria. AIDS Clinical Trials Group Oncology Committee. *J Clin Oncol* 1989; **7**(9): 1201-7.

401. Northfelt DW, Dezube BJ, Thommes JA, et al. Pegylated-liposomal doxorubicin versus doxorubicin, bleomycin, and vincristine in the treatment of AIDS-related Kaposi's sarcoma: results of a randomized phase III clinical trial. *J Clin Oncol* 1998; **16**(7): 2445-51.

402. Borok M, Fiorillo S, Gudza I, et al. Evaluation of plasma human herpesvirus 8 DNA as a marker of clinical outcomes during antiretroviral therapy for AIDS-related Kaposi sarcoma in Zimbabwe. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2010; **51**(3): 342-9.

403. Sorrell MF, Belongia EA, Costa J, et al. National Institutes of Health Consensus Development Conference Statement: management of hepatitis B. *Ann Intern Med* 2009; **150**(2): 104-10.

404. West JT, Wood C. The role of Kaposi's sarcoma-associated herpesvirus/human herpesvirus-8 regulator of transcription activation (RTA) in control of gene expression. *Oncogene* 2003; **22**(33): 5150-63.

405. Morris VA, Punjabi AS, Wells RC, Wittkopp CJ, Vart R, Lagunoff M. The KSHV viral IL-6 homolog is sufficient to induce blood to lymphatic endothelial cell differentiation. *Virology* 2012; **428**(2): 112-20.

406. Ramos JC, Sin SH, Staudt MR, et al. Nuclear factor kappa B pathway associated biomarkers in AIDS defining malignancies. *Int J Cancer* 2012; **130**(11): 2728-33.
407. Suthaus J, Stuhlmann-Laeisz C, Tompkins VS, et al. HHV-8-encoded viral IL-6 collaborates with mouse IL-6 in the development of multicentric Castleman disease in mice. *Blood* 2012; **119**(22): 5173-81.
408. Dean M, Jacobson LP, McFarlane G, et al. Reduced risk of AIDS lymphoma in individuals heterozygous for the CCR5-delta32 mutation. *Cancer research* 1999; **59**(15): 3561-4.
409. WHO. (<http://www.who.int/cancer/prevention/en/>).. Accessed 6th May 2013.
410. Parkin DM. The global health burden of infection-associated cancers in the year 2002. *Int J Cancer* 2006; **118**(12): 3030-44.
411. Cai Q, Verma SC, Lu J, Robertson ES. Molecular biology of Kaposi's sarcoma-associated herpesvirus and related oncogenesis. *Adv Virus Res* 2010; **78**: 87-142.
412. Munyagwa M, Baisley K, Levin J, Brian M, Grosskurth H, Maher D. Mortality of HIV-infected and uninfected children in a longitudinal cohort in rural south-west Uganda during 8 years of follow-up. *Tropical medicine & international health : TM & IH* 2012; **17**(7): 836-43.
413. Crum-Cianflone NF, Hullsiek KH, Ganesan A, et al. Is Kaposi's sarcoma occurring at higher CD4 cell counts over the course of the HIV epidemic? *AIDS* 2010; **24**(18): 2881-3.
414. Maurer T, Ponte M, Leslie K. HIV-associated Kaposi's sarcoma with a high CD4 count and a low viral load. *N Engl J Med* 2007; **357**(13): 1352-3.
415. National Lung Screening Trial Research T, Aberle DR, Adams AM, et al. Reduced lung-cancer mortality with low-dose computed tomographic screening. *N Engl J Med* 2011; **365**(5): 395-409.
416. Gail MH, Brinton LA, Byar DP, et al. Projecting individualized probabilities of developing breast cancer for white females who are being examined annually. *J Natl Cancer Inst* 1989; **81**(24): 1879-86.