HUMAN HERPES VIRUS TYPE 8 AND ITS COINFECTION WITH HUMAN IMMUNODEFICIENCY VIRUS AND *TREPONEMA PALLIDUM* AMONG FEMALE SEX WORKERS IN MALINDI SUB-COUNTY, KENYA

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Human Herpes Virus Type 8 and its co-infection with Human Immunodeficiency Virus and *Treponema pallidum* among female sex workers in Malindi Sub-County, Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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This thesis has been submitted for examination with our approval as supervisors.

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DEDICATION

I dedicate this work to my dear parents, Agnes Nzivo and Aquilas Nzivo, my loving brothers Falin Wambua, Andrew Muthiani and Brian Muli, and my lovely niece Victoria Wambua.

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LIST OF ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immunodeficiency Virus
AMURT	Ananda Marga Universal Relief Team
CSWS	Commercial sex workers
ELISA	Enzyme Linked Immunosorbent Assay
IDUS	Intravenous drug users
IFA	Immunoflorescence assay
FSWs	Female Sex Workers
HAART	Highly Active Antiretroviral Therapy
HHV-8	Human Herpes Virus type 8
HCMV	Human cytomegalovirus
HIV	Human Immunodeficiency Virus
HSV	Herpes simplex virus
KANCO	Kenya AIDS NGO Consortium
KEMRI	Kenya Medical Research Institute
KS	Kaposi's sarcoma
KSHV	Kaposi's Sarcoma Associated Virus
MCD	Multi-centric Castleman Disease
MSMS	Men having sex with men
NGO	Non-Governmental Organization
ORF	Open Reading Frame
PCR	Polymerase Chain Reaction
PEL	Primary Effusion Lymphoma

PBMCs	Peripheral Blood Mononuclear Cells
SCC	Squamous cell carcinoma
SPSS	Statistical package for Social Scientist
STIS	Sexually Transmitted Infections

ABSTRACT

The prevalence of Human Herpes Virus type 8 (HHV-8) is high in Sub-Saharan Africa (36-60%) in the general population. The risk factors associated with HHV-8 range from socio-demographic to geographic and behavioral characteristics. Documented studies on HHV-8 in Kenya are few and data on its co-infection with Human Immunodeficiency Virus (HIV) and syphilis is scanty. This study aimed at determining the prevalence of HHV-8, associated risk factors and co-infection with HIV and syphilis among Female Sex Workers (FSWs). A cross-sectional survey involving 268 participants was conducted and blood samples screened for antibodies against HHV-8 using enzyme linked Immunosorbent assay (ELISA). Infections by HIV and syphilis were tested using parallel algorithm and one step Anti-TP test respectively. Socio-demographic data was collected using a questionnaire administered by a health worker. The prevalence of HHV-8 was 25% (67/268), HIV 16.4% (44/268) whereas syphilis accounted for 2.24% (6/268). Of those infected with HHV-8, a co-infection of 12% (8/67) was recorded with HIV and 3% (2/67) with syphilis. FSWs in marriage posed a significant risk to HHV-8 infection (OR 2.90; p=0.043). There was no significant association between HHV-8 and HIV positivity (OR 0.62; p=0.257) or syphilis positivity (OR 1.52; p=0.636). HHV-8/HIV co-infection was associated with increased age (OR 11.21; p=0.027) and syphilis infection (OR 21.33; p=0.001). Lack of association between HHV-8 and HIV or syphilis in this study shows that sexual mode does not play a significant role in HHV-8 transmission in this population. Continuous monitoring of HHV-8 infection and its potential co-infections is recommended among FSWs and by extension the general population.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

HHV-8 also known as Kaposi's sarcoma associated Herpes virus (KSHV) was first identified in 1994, in tumor biopsy of an AIDS related Kaposi's sarcoma (KS). It is a double stranded DNA virus and most recently discovered human tumor virus. HHV-8 is associated with of all forms of Kaposi's sarcoma including; classic, endemic, AIDs associated KS and iatrogenic KS (Betsem *et al.,* 2014; Braz-Silva *et al.,* 2017).

The prevalence of HHV-8 has been shown to be high among FSWs, compared to men and women in defined sexual relationship. For example, the prevalence of HHV-8 among FSWs in Brazil was 5.6% compared to 1.3% among women in the general population and in Nigeria a seroprevalence of 31% was reported among commercial sex workers (CSWs) compared to 19% in healthy adults of general population (Eltom *et al*., 2002; Caterino de Araujo *et al.,* 2007). High prevalence of HHV-8/HIV co-infection among FSWS has been reported in Africa and in Latin America. While the prevalence of HIV ranges from 6-10% in sub-Saharan Africa that of HHV-8 ranges from 36-60% (Tumwine *et al.,* 2017). Co-infection of HHV-8/HIV should be of public health importance as it results in Kaposi's sarcoma which enhances progression of HIV to AIDS. Kaposi's sarcoma is an AIDS-defining illness and is the second most common malignancy present in HIV-1 infected patients worldwide and the most common in Kenya (Rogena *et al.,* 2015). Despite this fact, the prevalence of HHV-8 in the general population is unknown and scanty among key populations. HIV infections and syphilis, whose causative agent is *Treponema palladum* among adults, are markers of high risk sexual behavior. HIV and syphilis have been associated with HHV-8 by a number of studies while a lack of association been reported in others (Lavreys *et al.,* 2003; Zakari *et al.,* 2012; Zhang *et al.,* 2014). High prevalence of HHV-8, HIV and syphilis observed within the FSW renders this population unique as a bridge for transmission between this key population and the general population.

Understanding HHV-8 prevalence and its potential risk factors remains a prerequisite for development of strategies for prevention of HHV-8 transmission and subsequent diseases

associated with this oncogenic virus. Reliable data on HHV-8/HIV co-infection is also important as the presence of each virus determines the course of the other. This data would be important in defining policies for interventions among HIV patients who are in need of appropriate care, especially in the low resource countries.

1.2 Statement of the problem

Commercial sex is rampant in all major towns in Kenya (FIDA Kenya, 2009). Malindi is a tourist attraction area with people of diverse socio-economic status. The difference in social economic class between the residents and wealthy, mainly tourist has contributed to commercial sex. Young girls often drop out of school as a result of poverty and are lured to sex work at a tender age hoping to improve their lives and those of their families (Hope 2013). Due to lack of education, alcohol abuse and sexual violence, they engage in unsafe sexual activities. Sex work increases the risk of HHV-8, HIV and syphilis acquisition. Previous studies have shown that risky sexual behavior such as multiple sex partners, inconsistent condom use and increased sex acts enhances transmission of these infections. HHV-8 can also be transmitted via saliva through kissing (Minhas and Wood 2014). The prevalence of HHV-8 among HIV-1 negative FSWs and truck drivers in Mombasa Kenya was reported as 44% and 43% respectively (Baeten *et al.*, 2002; Lavrey *et al.,* 2003).

1.3 Justification

HHV-8 has been implicated in Kaposi's sarcoma, multicentric castlemans disease and Primary Effusion Lymphoma. For instance, KS is the most common AIDS defining malignancy in Kenya and the most common cancer in the general population in Sub-Saharan Africa (Rogena *et al.*, 2015). Kaposi's sarcoma enhances progression of HIV/AIDS and causes deaths in these patients. Despite high HHV-8 prevalence reported previously, a few have been carried out in the country on HHV-8 and data on co-infection with HIV and syphilis even scanty. Due to rampant sex tourism, international trade, migration a high rate of spread of HHV-8 from regions of high prevalence to those of low prevalence such as Europe and North America is anticipated, thus HHV-8 not only remains restricted but spread globally. FSWs act as a bridge for transmission between this population and the general population, hence completing the HHV-8 transmission

cycle. A study on HHV-8 will give insight on the prevalence of HHV-8 and co-infection HIV and syphilis which are of public health concern. The results of the study will contribute to monitoring and surveillance of HHV-8 in HIV infected populations.

1.4 Research questions

1. What is the prevalence of HHV-8, HIV and syphilis mono-infections and co-infections among female sex workers in Malindi Sub-County?
2. What are the risk factors associated with HHV-8, HIV and syphilis mono-infections and co-infections among female sex workers?
3. What is the genetic diversity of HHV-8 among female sex workers population?

1.5 Objectives

1.5.1 General objective

To determine the prevalence of HHV-8 and determine its co-infections with HIV and *Treponema pallidum* infections among female sex workers in Malindi Sub-County, Kenya

1.5.2 Specific objectives

1. To determine the prevalence of HHV-8, HIV and syphilis mono-infections and co-infections among female sex workers in Malindi Sub-County, Kenya.
2. To determine the risk factors associated with HHV-8, HIV and syphilis mono-infection and co-infections among female sex workers in Malindi Sub-County.
3. To determine genetic diversity of HHV-8 among female sex workers population in Malindi Sub-County.

CHAPTER TWO

LITERATURE REVIEW

2.1 Herpes viruses

Herpes viruses are divided into three subfamilies; *Alphaherpesvirinae, Betaherpesvirinae* and *Gammaherpesvirinae.* The subfamilies are based on biological properties and tissue tropism. Further subdivision is based on genomic sequence arrangement and relation to viral protein (Edelman, 2005; Malope, 2012; Speicher, 2012). *Alphaherpesvirinae* subfamily includes the genera *Simplex, Varicellovirus, Mardivirus and Iltovirus.* They have a broad host range, short replicative cycle in the epithelial cells and they rapidly grow in cell cultures. Viruses in this group include Herpes simplex virus 1 and 2 (HSV1 and 2) and Human herpes virus 3 (Edelman, 2005; Speicher, 2012). The subfamily *Betaherpesvirinae* is composed of genera *Cytomegalovirus, Muromegolovirus, Roseolovirus and Proboscivirus*. Beta herpes viruses have a restricted host range and a long replicative cycle in the epithelial cells. Infection by beta viruses progresses slowly in cell culture systems. The species in this subfamily are Human herpes virus 5, 6 and 7 (Edelman, 2005; Chandran and Hutt-fletcher, 2006). The genera in *Gammaherpesvirinae* subfamily are *Lymphocryptovirus, Rhadinovirus, Macavirus and Percavirus.* Gamma viruses have a restricted host range found within organisms that are part of family or order of natural host. They are specific for either B or T cells with latent virus. They replicate in haemopoietic cells, epithelial cells and fibroblasts. Gamma herpes viruses are the only known herpes viruses that cause tumors (Edelman, 2005; Chandran and Hutt-fletcher, 2006; Malope, 2012).

2.2 Genome characteristics

Herpes viruses are characterized based on size, base composition and structural arrangement in unique and repeated base composition. The length of the double stranded DNA ranges from 120-230kbs. Base composition is about 31% to 77% guanine and cytosine content and encode between 70-200genes. Herpes viruses also have terminal repeats resulting from their base sequence arrangement. Herpes viruses are further subdivided into six structurally distinct groups designated A-F based on pattern and recurrence of this repeat base sequence (Alagiozoglou, 1999; Malope, 2012; Speicher, 2012).

2.3 Biological characteristics of herpes viruses

Herpes viruses express a large number of enzymes involved in metabolism of nucleic acid, DNA synthesis and processing of proteins. DNA synthesis and capsid formation occurs in the nucleus of host cell and viral capsid is enveloped at nucleus membrane. Productive viral replication results in host cell destruction. Herpes viruses can remain latent in their host and are reactivated following cellular stress. They are commonly associated with horizontal person to person transmission due to the persistence of latent infection and asymptomatic shedding of the virus. HHV-8 is an exception as its prevalence varies by population and has uneven geographical distribution (Edelman, 2005; Chandran and Hutt-fletcher, 2006).

2.4 Human Herpes Virus type 8

2.4.1 Classification

HHV-8 is in the *Herpesviridae* family and *Gammaherpesvirinae* subfamily together with Epstein Barr virus (EBV) and Herpes virus Samiri. HHV-8 is the first known human member of genus *Rhadinovirus.* *Rhadinoviruses* share typical genome structure which contains numerous sequences that appear to be sequestered from host cellular DNA (Nicholas *et al.*, 1998; Edelman, 2005). Two new viruses with more homology to HHV-8 than any other herpes viruses have been described in monkeys (Edelman, 2005; Malope 2012).

2.4.2 Epidemiology of HHV-8

The epidemiologic patterns of HHV-8 differ by population and geography (Qiwen *et al.*, 2017; Tembo *et al.,* 2017). HHV-8 prevalence is high in areas where non-HIV associated forms of KS (classic or endemic forms) have been common like Africa and the Mediterranean (Minhas and Wood, 2014; Ramezani *et al.*, 2016; Tembo *et al.*, 2017). Sero-epidemiological surveys have reported HHV-8 prevalence in sub-Saharan Africa is up to 60% in general population where KS is endemic and less than 4% in Europe and United States (Rohner *et al.,* 2016; Tumwine *et al.,* 2017) . In the Mediterranean the prevalence ranges between 10-40% and is 0-20% in most parts of Asia (Guech-Ongey *et al.*, 2010; Leao *et al.*, 2013).

The prevalence of HHV-8 is unevenly distributed in Africa (El-Mallawany *et al.,* 2018). HHV-8 seropositivity and KS incidences have been reported to be low in Western,

Northern and Southern Africa compared to Eastern and Central Africa (Biryahwaho *et al.*, 2011). In sub-Saharan Africa, marked differences in the geographic distribution of HHV-8 and KS suggest that environmental cofactors influence HHV-8 transmission, control and progression to KS. Prevalence of HHV-8 in Nigeria, Zimbabwe and South Africa was reported as 19, 24.7 and 23.1% respectively (Eltom *et al.,* 2002; Dollard *et al.,* 2010). In East Africa HHV-8 prevalence of 47.1, 3.4-89 (infants to adults aged >45) and 43% were reported in Uganda, Tanzania and Kenya respectively (Baeten *et al*., 2002; Mbulaiteye *et al.*, 2003; Dollard *et al.,* 2010). In Cameroon a prevalence of 62% was recorded among HIV negative individuals (Mbondji-Wonje *et al.,* 2013). A prevalence of 34.9% was recorded among pregnant women and 31% in their cord blood after delivery in Gabon (Capan-Melser *et al.,* 2015)

2.4.3 Kaposi's sarcoma in Kenya

Kaposi's sarcoma was endemic in Africa even before the appearance of HIV in 1985 and experienced major growth as HIV infection spread (Senba *et al*., 2011; Rogena *et al*., 2015; El-Mallawany *et al.,* 2018). In Africa, 80,000 cases of cancers are diagnosed each year with KS accounting for 6.9% of the cases (Lupia *et al.,* 2015). The prevalence of HIV and HHV-8 has led to the marked prevalence of KS in sub-Saharan Africa. KS is second most common cancer affecting males (12.9% of all malignancies) and the fifth most common cancer of females (5.1% of all cancers) in sub-Saharan Africa (Senba *et al*., 2011; Bender Ignacio *et al.,* 2016). KS is the leading AIDs defining malignancy in Kenya (Rogena *et al.,* 2015). KS in Kenya is relatively low unlike in other regions (50% of all tumors in men in Uganda) in East Africa (Korir *et al*., 2015).

KS can involve any site in HIV patients including bone marrow, pancreas, gastrointestinal tract, lungs, liver, heart, testis, oral cavity including the palate and larynx (Alagiozoglou, 1999; Senba *et al.*, 2011; Speicher, 2012; Machado *et al.*, 2016). HIV/AIDS positive patients have a high chance of developing KS compared to HIV negative patients (da Silva and de Oliveira, 2011). KS causes 60-70% deaths in persons untreated for HIV within 1 year of diagnosis. HIV patients with KS on treatment have a fourfold death rate compared to those without (Semeere *et al*., 2016). In Kenya, 1.6 million people were living with HIV in 2016 and approximately 36,000 deaths occurred due to AIDS (UNAIDS, 2017). High incidence and prevalence of HIV is often

accompanied with the burden of malignant lesions. KS and the squamous cell carcinoma (SCC) of the vulva and cervix dominated between the period 2000 and 2003 (Rogena *et al.,* 2015). KS is still the leading cancer among HIV positive patients across all ages but the incidence of SCC of the conjunctiva is surpassing that of cervix and vulva (Rogena *et al.,* 2015). Senba *et al.,* (2011) reported HHV-8 prevalence of 10.3% in KS specimen samples obtained from 1981-1985 and 50.1-63.5% in samples obtained from 1986-2000 from KS endemic area of Western Kenya. The possible explanation for the linear increase is that endemic KS changed to epidemic one.

In Kenya, KS has been associated with low CD4 count (≤350), imperfect adherence to HAART and HIV WHO stages 3 and 4 (Lupia *et al.,* 2015). Highly Active Antiretroviral Therapy (HAART) has been reported to be effective against KS and has led to significant decrease of AIDS related malignancies in developed countries over years (Korir *et al.*, 2015; Lupia *et al.*, 2015). In Kenya, HAART has led to decrease in KS mortality rate and increase in number of persons living with HIV infection and AIDs related malignancies (Lupia *et al.*, 2015; Rogena *et al.,* 2015). This could be as a result of increase in survival rate and endemicity of HHV-8 in East Africa (Rogena *et al.*, 2015). KS still develops in approximately 15% of AIDS patients (Lupia *et al*., 2015)

2.4.4 HHV-8 infection among Female Sex Workers

The prevalence of HHV-8 among FSWs varies considerably from one region to another. In Asia, studies in China among FSWs reported prevalence of 10-16.7% (Ju *et al.,* 2012; Zhang *et al.,* 2014). This was lower than HHV-8 prevalence of 22.2%, 31.2% and 32.7% among immune compromised patients, IDUs and homosexual men respectively in the same country (Zhang *et al*., 2014). In the Latin America a study among HIV positive FSWs in Honduras reported a HHV-8 prevalence of 36% compared to 9.9% among non FSWs (Sosa *et al.,* 1998). Caterino-de-Araujo *et al.* (2007), reported a HHV-8 prevalence of 6.7% among FSWs Imbituba, Santa Catarina Brazil.

In Mediterenean and South of Europe, HHV-8 prevalence of 42% and 11.5% was reported among FSWs and low risk women in the general population respectively in Sicily (Perna *et al.*, 2000). De Sanjose *et al.* (2002) in Spain reported HHV-8 prevalence of 16% and 8% within the same populations. Spanish prostitutes had a lower HHV-8 prevalence compared to foreign prostitutes, 12% and 17.3% respectively. Another study

in Spain aiming to look at potential of HHV-8 sexual transmission reported HHV-8 prevalence of 10%, 10% and 25% in general population women, HIV positive women and prostitutes respectively (Santón *et al.*, 2002). HHV-8 prevalence among FSWs in Italy was 32.7%. In Italy, foreign prostitutes had a higher prevalence of 42.9% compared to those of Western Europe origin with 10.3% (Smacchia *et al.*, 2004).

In Africa, a study in Cameroon, reported a high HHV-8 prevalence (51.3%) among FSWs compared to 36.6% among HIV positive pregnant women (Betsetti *et al*., 1998). A relatively low HHV-8 seroprevalence of 8% was documented among FSWs in Eritrea (Enbom *et al.,* 1999). Eltom *et al*. (2002) reported a HHV-8 prevalence of 31% among commercial sex workers compared to 19% among low risk men and women in the general population in Nigeria. In Djibouti, female prostitutes working in the streets and female prostitutes working in luxury bars recorded HHV-8 prevalence of 26% and 20% respectively (Marcelin *et al.*, 2002). HHV-8 prevalence was documented as 44% among HIV seronegative prostitutes in Kenya (Lavrey *et al.*, 2003). The prevalence of HHV-8 among FSWs, miners and male and female residents of a township reported prevalence of HHV-8 of 50.5%, 48.4%, 47.5% and 46% respectively (Malope *et al.,* 2008). HHV-8 prevalence was reported as 45% among FSWs in Jos, Nigeria (Zakari *et al.*, 2012).

2.4.5 HHV-8 Transmission

HHV-8 is transmitted via sexual and non-sexual route. In endemic regions like in Africa, the Mediterranean and among Brazilian Amerindians, non-sexual route of transmission has been reported as the main route through which HHV-8 is acquired (Mbulaiteye *et al.*, 2003; Dedicoat *et al.,* 2004; Borges *et al.,* 2012;) while in non-endemic countries of Europe and America, HHV-8 is believed to be transmitted via sexual route (Giuliani *et al*., 2007; Rohner *et al*., 2016). HHV-8 DNA has been isolated in major body fluids such as blood and blood products, saliva, oropharyngeal mucosa secretions, semen and cervico-vaginal secretions and rarely via organ transplantation which represent both vertical and horizontal transmission (Taylor *et al.,* 2004; Ogoina *et al.,* 2011; Phipps *et al.,* 2014; Minhas & Wood, 2014;). Transmission of HHV-8 via saliva is the main route through which HHV-8 is acquired (De Sanjose *et al.*, 2009; Crabtree *et al.,* 2013; Bender Ignacio *et al.*, 2016). There is a controversy of HHV-8 sexual transmission among heterosexuals. Some studies have reported HHV-8 sexual transmission among

heterosexuals (Eltom *et al.,* 2002; Lavreys *et al.,* 2003; Caterino-de-araujo *et al.,* 2007; Zakari *et al.,* 2012). These was not observed in other studies (Enbom *et al.,* 1999; Engels *et al*., 2007; Malope *et al.,* 2008; Shebl *et al.,* 2011).

2.4.5.1 Non-sexual transmission

HHV-8 is believed to be transmitted through non-sexual route due to its high prevalence in children especially in Africa, Mediterranean and among Brazilian Amerindians (Giuliani *et al.,* 2007; Malope *et al.,* 2008; Minhas and Wood, 2014; Rohner *et al.,* 2016). Children are believed to acquire HHV-8 from their mothers in the uterus or intrapartum but rarely does this occur. This is because HHV-8 DNA has been reported in the cervico vaginal secretions of HHV-8/HIV co-infected mothers (Minhas & Wood, 2014). Breast milk has been found to contain HHV-8 (Dedicoat *et al*., 2004) but there is inconsistency in reporting (Crabtree *et al.,* 2013; Minhas & Wood, 2014). HHV-8 is known to infect monocytes, macrophages and epithelial cells found in cellular components of mature milk suggesting HHV-8 may be found (Dedicoat *et al.,* 2004; Minhas & Wood, 2014). However recent research does not point this route as an important one (Crabtree *et al.,* 2013; Minhas & Wood, 2014). The other route children may acquire HHV-8 is through exposure to saliva of seropositive mothers, care givers or other siblings (Mbulaiteye *et al.,* 2003; Dedicoat *et al*., 2004; Mbulaiteye and Goedert, 2011; Tozetto-Mendoza *et al.,* 2018). Studies have reported high HHV-8 prevalence in children of HHV-8 seropositive mothers, with the prevalence increasing when both parents are positive, living in large families and siblings of close age (Borges *et al.,* 2012). Practices that expose children to saliva include; sharing sweets, food and sauce plate, pre-mastication of medicinal plants given to a child and soothing of insect bites using saliva among others (Mbulaiteye and Goedert, 2011). Reports also of childhood infection are also emerging in Europe and America where HHV-8 infection is low (Goedert *et al.,* 2003; Malope 2012) but there is paucity of data about when and how transmission occurs.

Among adults in sub-Saharan Africa, practices such as healing and medicinal plants practices, initiation or ritual practices and feeding practices have been reported as means through which HHV-8 is transmitted (Enbom *et al.,* 1999; Borges *et al.,* 2012; Mbulaiteye & Goedert, 2011). In Western and developed countries kissing is the main practice which enhances transmission among adults (Giuliani *et al.,* 2007; Crabtree *et a*l.,

2013). Studies investigating sexual practices among MSMs have reported salivary exposure as a possible route of transmission during oro-genital and oro-anal (Minhas & Wood, 2014). Saliva has been reported to shed more HHV-8 than other body fluids (Taylor *et al.*, 2004; Phipps *et al*., 2014; Braz-Silva *et al.*, 2017). The shedding rate of HHV-8 DNA in different oral and genital mucosa sites was reported as 32%, 28%, 4%, 2.3%, 9% and 18% in saliva, mouth swabs, cervical, vaginal mucosa, plasma and peripheral blood mononuclear cells respectively (Taylor *et al.*, 2004). However kissing (saliva) as route of HHV-8 transmission fails to explain why HHV-8 is common in homosexuals in Europe and America and not among heterosexuals considering it is a common practice. It is difficult to understand how kissing could spread the virus and keep it only among gay men (Martin, 2011).

2.4.5.2 Sexual route

In Europe, America and Australia, HHV-8 is believed to be transmitted mainly by sexual route (Rohner *et al.*, 2016). In these countries, HHV-8 is prevalent among homosexual men and migrants from endemic areas (Rohner *et al*., 2014). In Africa and the Mediterranean, though non-sexual transmission is predominant, HHV-8 has also been reported to be transmitted sexually with high HHV-8 prevalence being recorded among female and male sex workers and people with high risk sexual behavior (Perna *et al.*, 2000; Eltom *et al.*, 2002; Lavreys *et al*., 2003; Zakari *et al.*, 2012). Some of the risk factors associated with HHV-8 sexual transmission include; increased number of sexual partners, HIV seropositivity, history of STIs (Cannon *et al.*, 2001; Eltom *et al.*, 2002; Lavreys *et al.*, 2003; Corey *et al.*, 2006; Zakari *et al.*, 2012). Evidence conflicting sexual transmission of HHV-8 among heterosexuals continues to emerge from studies all over the world (Giuliani *et al.*, 2007; Malope *et al.*, 2008; Shebl *et al.*, 2011; Zhang *et al.*, 2014).

2.5 HHV-8 and HIV co-infection

HHV-8 and HIV interact and regulate infection with each other and in human carcinogenesis. HHV-8 infection is highly prevalent in HIV positive adults with up to 20000 fold increase of developing KS than in general population and 300 fold higher than in persons with any type of immunosuppression (da Silva and de Oliveira, 2011). Molecular interactions between the viruses play an important role in progression of

diseases. HHV-8 replication can be induced by cytokines such as interleukins 1, interferon –F, oncostatin M, hepatocyte growth factors/ Scatter factors which are produced due to HIV infected cells. HIV infection stimulates replication of HHV-8 in co-infected and adjacent cells. Trans-activator of transcription (Tat) protein of HIV enhances the lytic cycle replication of HHV-8 thus increasing spindle cells, viral load and expression of various viral genes with oncogenic potential and it stimulates the development of KS lesions (Pyakurel *et al.,* 2007: da Silva & de Oliveira, 2011).

Conversely, HHV8 promotes progression of HIV to AIDS. HHV-8 promotes transendothelial spread of HIV by inducing HIV replication as well as promoting infection of new HIV target cells (Ogoina *et al.*, 2011). HHV-8 Latency associated nuclear antigen (LANA) activates LTR of HIV and stimulates expression of HIV P24. In addition, immediate early gene ORF 50 (the major transactivator of HHV-8 lytic cycle) acts synergistically with HIV 1 Tat protein enhancing transactivation of HIV-1 LTR. This leads to potent stimulation of HIV-1 gene expression (Pyakurel *et al.*, 2007). Sex workers and women engaging in high-risk sexual behavior are therefore likely to be co-infected with HHV-8 and HIV than the general population.

In Western Sicily, a HHV-8 prevalence of 42% was reported among HIV-positive female prostitutes compared to 30% among HIV negative ones (Perna *et al.,* 2000). Greenblatt *et al*. (2001) reported a prevalence of HHV-8 of 15% among HIV-positive and 6.3% among HIV negative high-risk women. HHV-8 seroprevalence was also reported to be higher among HIV-positive FSWs with a pooled odds ratio of 4.1 compared to the HIV negative ones in Spain (Smacchia *et al.,* 2004). Zakari *et al*. (2012) also observed the prevalence of HHV-8 to be higher among HIV-positive FSWs than HIV negative ones ($p<0.05$) in Nigeria. However, a large study in South Africa among sex workers, miners and residents of a township reported non-significant difference in the prevalence of HHV-8 among HIV-positive and HIV negative individuals recording 48.5% and 46.8% respectively (Malope *et al.,* 2008). Mbondji-wonje *et al.,* (2013) recorded prevalence of 61% and 62% among HIV positive and HIV negative individuals in Cameroon respectively.

2.6 HHV-8 and syphilis

Treponema pallidum is the causative agent of syphilis and can be spread by a number of routes including kissing, birth placenta, close contact with active lesions with vast majority transmitted through sexual contact (vaginal, anal or oral). Syphilis infection is known to facilitate transmission and acquisition of HIV (Musyoki *et al.,* 2015). The prevalence of syphilis worldwide has reduced drastically in the past 3 decades with a pooled global mean prevalence of 1.11% in studies compiled between 1996-2016 and 0.5% in studies compiled between 2005-2012 reported (Newman *et al.,* 2015; Smolak *et al.,* 2018). In Kenya the prevalence of syphilis in the general population is 1.8% and estimated to be higher among HIV infected individuals and high risk groups such as FSWs and men having sex with men (MSM). A survey among FSWs in Kisumu by Vandenhoudt *et al.* (2013) showed that syphilis infection had decreased from 10.8% to 3.3% in duration of 5 years. Earlier a prevalence of 2.5% and 1.7% was reported among FSWS as compared to the general population. A syphilis prevalence of 0.9% was reported among FSWs in Nairobi (Musyoki *et al.,* 2015). Syphilis infection among FSWs has been associated with HHV-8 infection by a number of studies (Cannon *et al*., 2001; Greenblatt *et al* 2007) while others did not find any significant association (Corey *et al.,* 2006; Giuliani *et al.,* 2007; Malope *et al.,* 2008; Zhang *et al.*, 2014). A previous study in Kenya among HIV-1 seronegative FSWs also associated syphilis (p=0.004) with HHV-8 infection (Lavreys *et al.,* 2003). However due to the controversy surrounding sexual transmission of HHV-8, syphilis could be a marker of high risk sexual behavior rather than an indicator of HHV-8. Syphilis has been associated with HIV and HSV-2 which are too markers of high risk sexual behavior.

2.7 HHV-8 Genome

HHV-8 has a double stranded DNA. The genome is approximately 165kbp in length including over 90 open reading frames designated ORFs 4 to 75 (Lacoste *et al*., 2000; Leao *et al.,* 2013). Its genome is smaller than that of HCMV (230kb) but larger than that of HSV-2 (152kb). One Hundred and forty (140kbp) of this DNA contains coding information (Chandran and Hutt-fletcher, 2006; Betsem *et al*., 2014). Its genome is flanked by long terminal repeats of 1.4kb which contain many GC non-coding sequences on either side. A large region of the HHV 8 genome is conserved among herpes viruses. Divergent regions in the HHV 8 genome contain more than 20 HHV-8 unique genes.

The genes are designated with prefix K. The regions are found in between the conserved gene (Edelman, 2005; Leao *et al*., 2013). HHV-8 genome is shown in Fig 2.1.

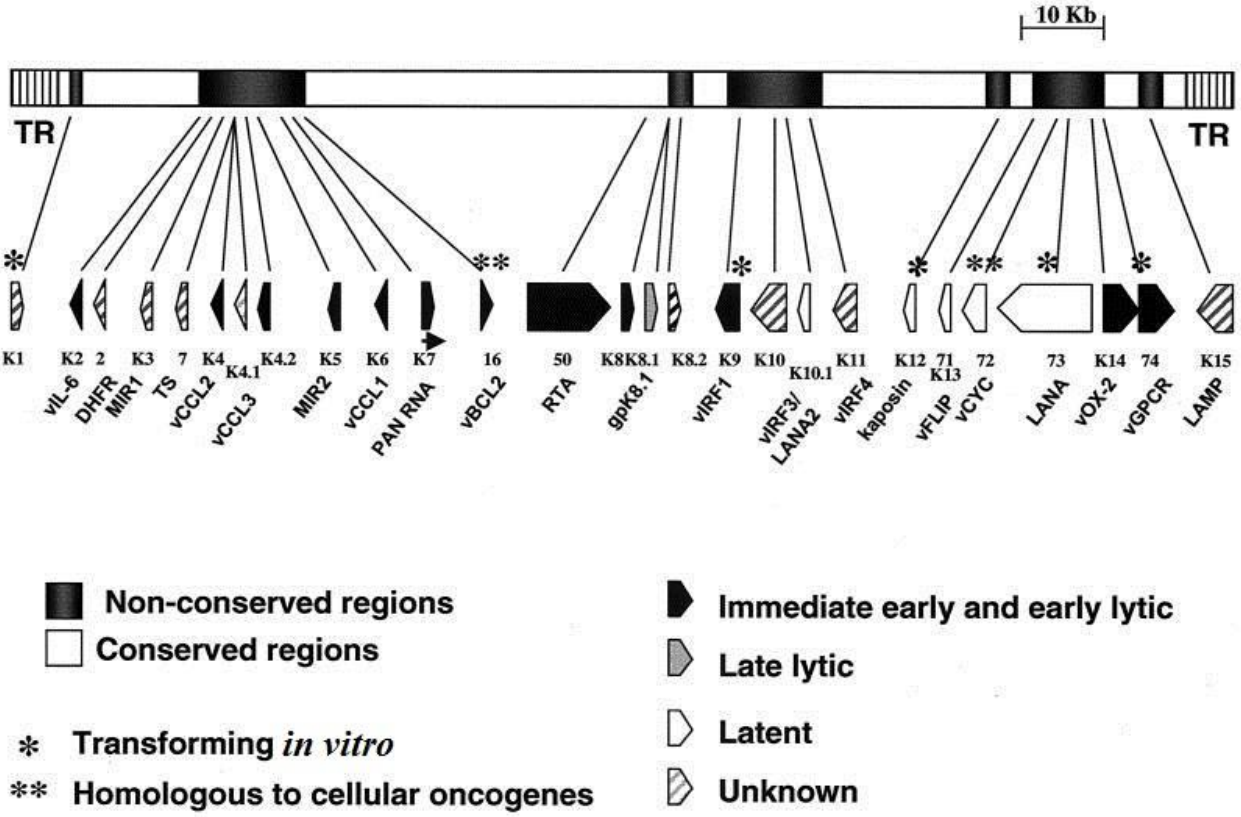

Figure 2.1: HHV-8 genome structure (Ablashi *et al.*, 2002).

K1 region of the HHV-8 genome on the extreme left is the most highly variable region. KI4 and K15 on the right side are variable and have also been used to categorize the virus.

2.8 HHV-8 subtypes and phylogenetic relationships

The 5' and 3' regions of HHV-8 are disproportionately variable compared to the central region. They have both been used to categorize HHV-8. Based on the highly variable K1 region that encodes transmembrane protein, phylogenetic analysis have allowed identification of subtypes A, B, C, D, E, F and Z whose distribution parallels that of ORF26 variants (Olp *et al.*, 2013; Betsem *et al.*, 2014; Tozetto-mendoza *et al.*, 2016). The subtypes are further subdivided into different variants. Subtype A has the variants A1 – A5, subtype B has variants B1 to B4 and subtype C is subdivided into variants C1

to C6 (Lacoste *et al*., 2000; Tornesello *et al*., 2010). Based on ORF 26 (a minor capsid gene), variants A/C, J, K/M, D/E, B, Q, R and N diversely distributed in different geographical regions have been identified. The other subtypes are P and M (for predominant and minor) alleles based on K15 gene on the 3'end of the herpes genome (Olp *et al.*, 2015; Tornesello *et al*., 2010)

Subtypes A, C, A/C, J and K are prevalent in Middle East, Europe, N. America and Northern Asia. Subtype A variants are widespread in Africa, Australia (A1 only) and the Mediterranean region. Subtype C variants (C1 – C4), are commonly distributed along the Middle East and Mediterranean regions. Subtypes C1 and C3 have been described in Northern America and Australia. Subtype C has been described in South America and Asia. B and A5 subtypes are prevalent in Africa while N, R and Q are exclusively in sub-Saharan Africa. Actually, Subtype B is mainly restricted to Africa and in those of African lineage. The D variant is found in Pacific Islands and Australia, E among Brazilian Amerindians, D/E indigenous South Asians and Polynesians, Z variant in Zambian children and F among a Bantu tribe in Uganda (Tornesello *et al*., 2010; Malope *et al*., 2012; Speicher, 2012).

HHV-8 subtypes A, B, C, D and E differ by 15-30% based on the heterogeneity of 289 amino acid sequence of K1 region and 8.5% at the nucleotide level (Nicholas *et al*., 1998; Ouyang *et al*., 2014). Subtypes A and C are more closely related while subtype A and B most distantly related. Subtype B is believed to have originated from Central Africa based on a correlation between human migration routes and phylogenetic analysis. Subtype A and C diverged from B following human migration to N. America and Europe. Subtype D and E are thought to have originated from subtype C as human migrated from Australasia to Oceania (Alagiozoglou, 1999; Lacoste *et al.*, 2000; Malope, 2012; Speicher, 2012). Subtypes P, M and N have a 70% inter allele divergence at amino acid level. There is little variation within allele groups but extreme divergence across alleles. Subtypes P and M are closely related with an identity of up to 30% while N allele is highly divergent from P and M (Leao *et al.*, 2013; Olp *et al.*, 2015). There is no relationship which has been established between subtypes based on ORF K1 and those based on K15 region (Nicholas *et al.*, 1998; Olp *et al.*, 2015; Tornesello *et al*., 2010).

2.9 HHV-8 Laboratory detection

HHV-8 can be detected by either serological assays or molecular methods. Serological assays commonly used include; Enzyme Linked Immunosorbent Assay (ELISA), Immunoflourescent assay (IFA), western blot and Immunohistochemistry. They are the main methods of choice for large epidemiological studies (Malope, 2012; Speicher, 2012; Machado *et al.*, 2016). HHV-8 DNA can be absent even in HHV-8 seropositive individuals and therefore serological methods have remained to be methods of choice (Minhas & Wood, 2014; Machado *et al.*, 2016). They detect HHV-8 antibodies to latent and lytic protein. IFA are used for determining HHV-8 serology in population with low probabilities of infection while ELISAs are used for analyzing large batches of samples as they are more sensitive easier and faster (Speicher 2012). Molecular detections methods are also used to mainly validate serological assays, analyze HHV-8 associated disease development, progression and ascertain effect of antiretroviral therapy (Malope 2012). Polymerase Chain Reaction (PCR) has been used in detection of HHV-8 DNA and in determining viral load. HHV-8 DNA has been isolated in Peripheral Blood mononuclear Cells (PBMCs), oral and nasal secretions, blood specimen among others. Only 40-60% patients have HHV-8 DNA in plasma and in PBMCs (Speicher 2012). HHV-8 DNA is detected in 100% of PEL cells Body Cavity B- Lymphoma cells (BCBL) and in approximately 95% in KS lesions of known HHV-8 positive patients (Chandran and Hutt-fletcher, 2006).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

Malindi sub-County is a tourist destination along the Kenyan coast. The area has for a longtime been associated with sex tourism coupled with drug abuse (Hope 2013). Economically the region relies on tourism. Although predominantly Muslim, the region has attracted people from different parts of Kenya thus becoming cosmopolitan with different religions, cultures and races. Tourists frequenting Malindi are mainly from Europe, with tourists from Italy and Germany accounting for the highest proportion. In the sub-County, the sample collection points were in Ananda Marga Universal Relief Team (AMURT) Health Centre and Kenya AIDS NGO Consortium (KANCO) drop-in Centre. They were chosen because they work directly with FSWs and therefore, would facilitate easy recruitment of study subjects from different parts of Malindi sub-County. The clinics in the two centers facilitated safe collection of blood samples. They both recruit sex workers from all around Malindi sub-County to provide services to them. The map of Kenya and Malindi sub-county showing AMURT health Centre and KANCO drop-in Centre are shown in Figure 3.1.

3.2 Study design

A cross sectional study was carried out to assess HHV-8 and its co-infection with HIV and *T. pallidum* in the population of FSWs.

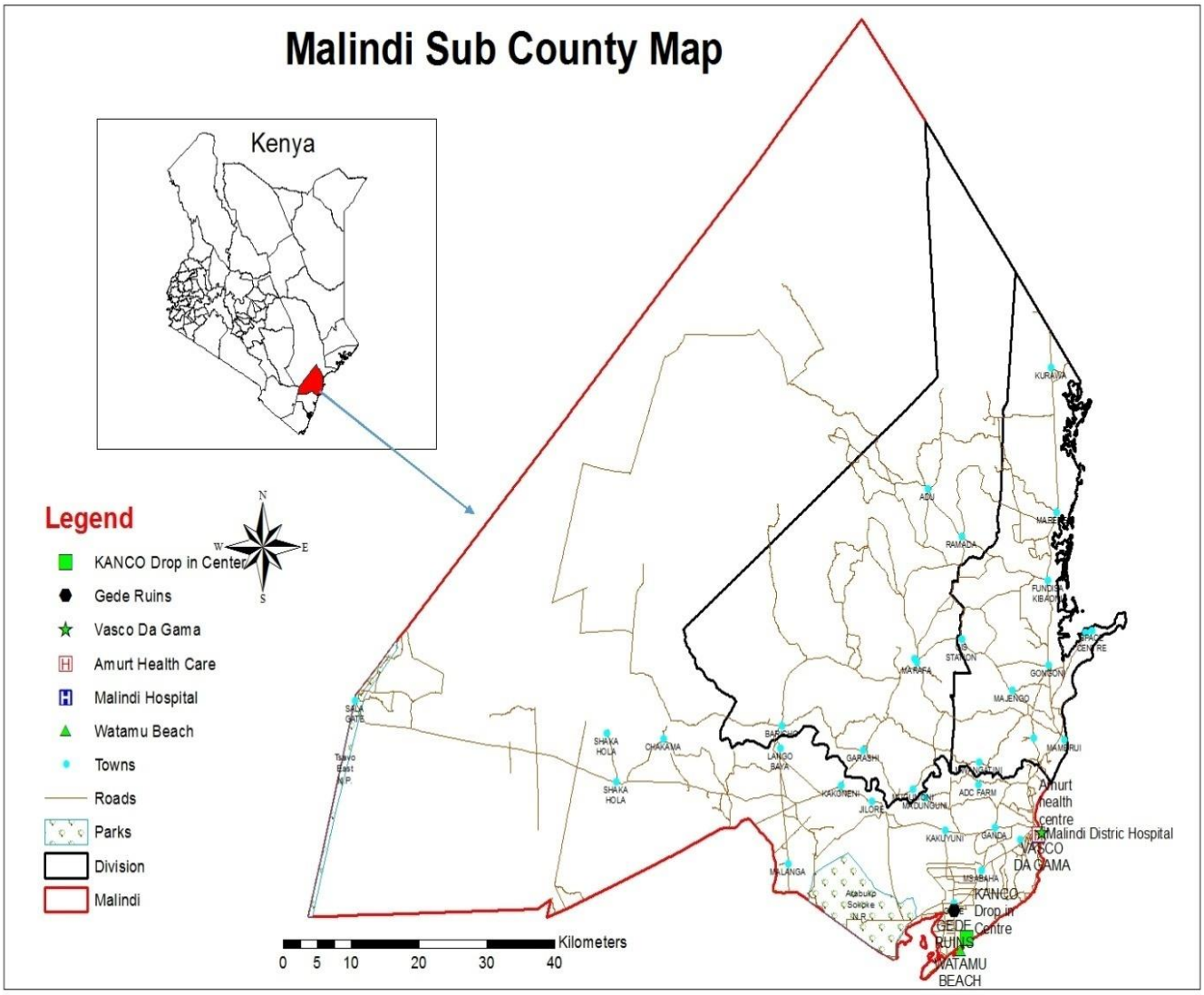

Figure 3.1: A map of Kenya and Malindi sub-County showing sample collection points in 2015-2016.

3.3 Study population

The study recruited FSWs in the years 2015-2016 from Malindi Sub-County. Sex work is the practice of providing sexual services in exchange for money and other material compensation (FIDA Kenya, 2008). The prevalence of HHV-8, HIV and syphilis has been reported to be high among FSWS due to high risk behavior. There is however a controversy surrounding sexual transmission of HHV-8. This population provided a good base for study of HHV-8 and co-infection with HIV and syphilis in Kenya.

3.3.1 Inclusion criteria

FSWs above 18 years of age living in Malindi sub-County were included.

3.3.2 Exclusion criteria

FSWs who did not consent to be tested for HHV-8, HIV or syphilis.

Females visiting the Centre who were not FSWs

Female visiting the Centre who were non-Malindi residents

3.4 Sampling procedure

Purposive random sampling was carried out. Females visiting the Centre were approached by the PI, enquired their purpose for visit and if they were FSWs as the Centre gives services to mainly FSWs. Upon confirming that they were sex workers, they were consented and recruited. FSWs visited AMURT Health Centre for either treatment, family planning procedures, to collect condoms or to attend routine meetings among others. In KANCO drop-in Centre, FSWs visited to collect injecting needles, for treatment, counseling meetings or for other services. New recruits to the Centre who were brought in the course of the study by peer educators were also included.

3.5 Sample size calculation

Sample size was calculated using the prevalence of HHV-8 and HIV co-infection among FSWs in Nigeria reported as 20% as there was no similar study carried out in Kenya (Zakari *et al.,* 2012). This study in Nigeria was selected because it was similar to the study been carried out. Previous studies in Kenya did not test co-infection. The formula

by Chadha (2006) was used for calculating the appropriate sample size with 95% confidence level and ±5% precision.

$$n = \frac{z^2 pq}{e^2}$$

Where,

n is the sample size

z is the X axis value of the normal curve that cuts off an area α at the tails of the normal curve. Where 1-α is equal to the desired confidence level (1.96).

e is the desired level of precision (0.05)

p is proportion of FSWs who are at risk of acquiring HHV-8 infection(0.20)

q is equal to 1-p

Where e=0.05, p=0.20, q=0.80, Z=1.96

$$245 = \frac{1.96^2 \times 0.20 \times 0.80}{0.05^2}$$

Hence 268 FSWs were recruited for this study

3.6 Data and Sample collection

3.6.1 Socio-demographic data

A structured interviewer administered questionnaire was used to collect data from the study participants. This included socio-demographic information on age, level of education and marital status and medical history questions such as HIV status, STI history. Sexual behavioral data such as; number of years as a sex worker, age one became sexually active, intravenous drug use, number of clients per day and use of condom was also included.

3.6.2 Blood collection

Venous blood (5 ml) was drawn from each study participant by a well-trained phlebotomist at the study sites and transferred to Malindi sub-County Hospital

Laboratory. The blood was then separated into plasma and cells at the Hospital Laboratory biosafety level 2 (BSL-2) and transported to KEMRI HIV Laboratory (BSL-2) under cold chain and where it was stored at -20°C.

3.7 Laboratory testing

3.7.1 Separation of whole blood into plasma and PBMCs

Five milliliters (5mls) of blood samples in EDTA tubes was centrifuged at 1500 rpm for 1 min. The upper layer (plasma) was drawn off using a clean pipette, added into a cryo vial and labeled. The PBMCs were drawn into another cryo vial and labeled and was used for DNA extraction experiment.

3.8.2 Serological tests

3.8.2.1 HHV-8 test

The plasma samples were tested using HHV-8 Enzyme Linked Immunosorbent Assay (ELISA) from Sunlong Biotechnologies Ltd, China following manufacturer's instructions (Appendix I).

3.7.2.2 HIV testing

For HIV screening, a parallel algorithm for plasma samples was done, with Determine^{TM} (Alere Medical Co. Ltd) and First Response® HIV 1-2-0 (Premier Medical Co. Ltd) being used in parallel following manufacturer's instructions (Appendix II).

3.8.2.3 Syphilis testing

The test for *Treponema pallidum*, the causative agent for syphilis was done using one step Anti-TP test using plasma samples following manufacturer's instructions (Appendix III).

3.8 HHV-8 DNA extraction

DNA was extracted from PBMCs using Qiagen kit following manufacturer's instructions (Appendix IV).

3.9 PCR Amplification

3.9.1 PCR Primers

Amplification of HHV-8 DNA was done using HHV-8 K1-1/K1-2 primers in the 1st round PCR and the DNA was re-amplified by the nested Klinn5/Klinn6 primers that

target K1 region of HHV-8 genome (Leao *et al.*, 2013). Other primers used were K1a-f /K1a-r and K1b-f/ K1b-r (Whitby *et al.*, 2004) and K1F/K1R (not published). The sequences of the primers are shown in Table 3.1.

Table 3.1: HHV-8 primers used to amplify HHV-8 DNA

	Primers	**Sequence (5' to 3')**	**Product size**
1 set of Primers	K1-1	CCTTTCTCTGCTATCGTCTC	450bp
	K1-2	GGTAGATGCCAAACGGTAAC	
	K1inn5	CCCTGGATGATTTCAACGC	
	K1inn6	ACATGCTGACCACAAGTGAC	
2nd set of primer	K1a-f	ATGTTCCTGTATGTTGTCTGC	750bp
	K1a-r	AGTACCAATCCACTGGTTGCG	
	K1b-f	GTCTGCAGTCTGGCGGTTTGC	
	K1b-r	CTGGTTGCGTATAGTCTTCCG	
3rd set of Primers	K1F	GTTCTGCCAGGCATAGTC	1050bp
	K1R	AATAAGTATCCGACCTCAT	

3.9.2 Nested PCR using 1st set of primers

In the first round PCR, 2.5 µl of the extracted product, 2.5 µl 10x buffer, 7.5 µl of $MgSO_4$, 0.5 µl of each primer (K1-1/K1-2), 10.0 µl of nuclease free water, 0.5 µl Taq polymerase and 1.0 µl of dNTPs were used. An automatic thermocycler was programmed to perform one cycle of 5 minutes at 94°C followed by 34 cycles of 94°C for 1minute (denaturation temperature), 60°C for 1 min (annealing) and extension at 72°C for 1 minute. The final extension was at 72°C for 7 minutes. For the second round, 2 µl of the 1st ran PCR product, 3 µl of 10x buffer and primers Klinn5 and Klinn6 were

used. The same amount of the other reagents and cycling conditions as in the first round were used.

3.9.3 Nested PCR using 2nd set of primers

In the first round PCR, 1 µl of the extracted product, 2.5 µl 10x buffer, 2 µl of $MgCl_2$, 0.3 µl of each primer (K1a-f/K1a-r), 18.6 µl of nuclease free water, 0.5 µl Taq polymerase and 0.1 µl of dNTPs were used. An automatic thermocycler was programmed to perform one cycle of 10 minutes at 94°C followed by 34 cycles of 94°C for 30seconds (denaturation temperature), 72°C for 2 min (annealing) and extension at 72°C for 7 minutes. For the second round, the same amount of reagents using primers K1b-f and K1b-r were used. Same cycling conditions were maintained.

3.9.4 Direct PCR using 3rd set of primers

The PCR master mix included 4 µl of the extracted product, 5 µl 10x buffer, 2 µl of $MgSO_4$, 0.5 µl of each primer (K1F/K1R), 37.3 µl of nuclease free water, 0.2 µl Taq polymerase and 0.5 µl of dNTPs were used. An automatic thermocycler was programmed to perform one cycle of 10 minutes at 94°C followed by 34 cycles of 94°C for 30 seconds (denaturation temperature), 72°C for 2 min (annealing) and extension at 72°C for 7 minutes.

3. 10 Gel Electrophoresis

The nested PCR amplicons were then electrophoresed in a 2% agarose gel with Tris/Borate/EDTA (TBE) (electrophoresis buffer) already stained with ethidium bromide Examination was done in an alpha imager (CA, USA). Visualization of an approximately 450 bp (1nd set of primers), 750 bp (2nd set of primers) and 1050 bp product (3rd set of primers) was considered positive for HHV-8 DNA.

3.11 Gel Extraction

Gel extraction was done using QIAquick Gel Extraction Kit (Appendix V).

3.12 DNA purification

The PCR products were purified using QIAquick DNA purification kit from QIAGEN following manufacturer's instructions. To 4 µl of Exonuclease 1, shrimp-alkaline

phosphatase (Exosap), 10 µl of each sample was added in a PCR tube. The mixture was then incubated in a thermocycler for 37°C for 15 min followed by 80°C for 15 min. It was then stored at -20°C and later sent to Macrogen, Europe for sequencing.

3.13 Data Management and Analysis

All socio-demographic data and behavioral characteristics data obtained using questionnaire and medical information obtained from the laboratory was entered into an excel spread sheet. It was then imported to SPSS version 22. It was also converted to binary data in excel and exported to Stata version 10.

The number of women who tested positive for HHV-8, HIV and syphilis was recorded and data subjected to SPSS where percentages were calculated to obtain the prevalence. The number of those co-infected with HHV-8 and HIV, HHV-8 and syphilis and HIV and syphilis was also recorded. The percentages were calculated to obtain the prevalence of co-infection.

To determine the basic characteristics of all study subjects, socio-demographic and behavioral characteristics was subjected to SPSS version 22 where descriptive statistics (frequencies) were determined for the categorical variables; Age groups, marital status, level of education, duration as a sex worker (≤6 versus (vs.) ≥6), age at 1st sexual intercourse (≤18 vs. ≥18) and number of clients per day (1-3 vs. ≥4). Mean, median and standard deviation were determined for the continuous variable (age).

To determine association of HHV-8, HIV and syphilis mono-infection and HHV-8/HIV and HHV-8/syphilis co-infection with socio-demographic and behavioral characteristics, data was subjected to Stata version 10. In an excel spread sheet, data was converted to binary data and exported to Stata version 10 where odds ratio (OD), 95% confidence intervals (95%CI) and p value of infection were determined for all the categorical variables.

A sample was considered to contain HHV-8 DNA if it showed a band at the 450bp mark using K1-1, K1-2, K1inn5, and K1inn6, 750bp mark using K1a-f / K1a-r and K1b-f / K1b-r or at the 1050bp mark using K1F/K1R by comparing with the 1kb marker on gel images.

3.14 Ethical approval

This study was approved by KEMRI Scientific and Ethics Review Unit (SCC 2915) and Kilifi County Department of Health (Appendix V1, VII and VIII respectively). Participants were given information about the nature of the study and informed that participation was voluntary. The study involved an invasive procedure (drawing venous blood) and therefore a well-trained phlebotomist carried out the procedure. Those willing to take part in the study were required to sign an informed consent (Appendix IX). All ethical considerations regarding the use of human subjects were followed. To ensure confidentiality, identifying unique numbers were used on the questionnaires (Appendix X) and in labeling cryovials tubes containing the blood samples collected. Data was entered into excel sheets and were password protected only allowing the principal investigator's access. The PI took the results to AMURT Health Centre and KANCO drop-in Centre to avoid any breach of confidentiality. The participants benefitted from this study by knowing their HIV and syphilis status. FSWs positive for HIV were referred to Malindi Sub-County Hospital for care and management. Results were important to Malindi Sub-County as they reported the prevalence of HHV-8, HIV and syphilis prevalence and their co-infection among the high risk group in the region.

CHAPTER FOUR

RESULTS

4.1 Socio demographic and behavioral characteristics of study participants

The study recruited 268 FSWs who were between ages 18-57 with a mean of age 29.15 years, median age of 28 years and a standard deviation of 7.3. Majority 87/268 (32.5%) of FSWs were between ages 25-29 followed those between18-24 years 74 (27.6%). FSWs at ages 30-34 were 55 (20.5%) and those 35 years of age and above were 52 (19.4%). Single FSWs 180 (67.2%) were the majority followed by the widowed and separated were (62) 23.1% while married FSWs were the fewest 26 (9.7%). Majority of the sex workers had attended primary school 151 (67.2%) while two (0.7%) had attended tertiary education. Eighty five 85 (31.7 %) had attended secondary school while (30) 11.2% had never been to school. Intravenous drug users were 26 (9.7%). A majority of FSWs had 1-3 clients per day 139 (51.9%) while 129 (48.1%) had more than 4 clients per day. Majority 195 (72.8%) of the sex workers used condom consistently while 73 (27.2%) used condom occasionally. Sixty eight (25.4) sex workers had been in the business for more than six years while 200 (74.6%) had been in the business for 1-5 years. Majority 237 (88.4%), of the FSWs started engaging in sexual intercourse while they were less than 18 years of age while the rest 31 (11.6%) were above 18 years during 1st sexual encounter (Table 4.1).

Table 4.1: Socio demographic and behavioral characteristics of female sex workers in Malindi Sub-County in 2015-2016

Risk factor	Number	Percentage (%)
Age		
35 and above	52	19.4
30-34	55	20.5
25-29	87	32.5
18-24	74	27.6
marital status		
Single	180	67.2
Married	26	9.7
Widowed/separated	62	23.1
Education Level		
Primary	151	56.4
Secondary	85	31.7
Tertiary**	2**	0.7
NonFormal	30	11.2
IDU		
Yes	26	9.7
No	242	90.3
Clients per day		
4 -7	129	48.1
1 - 3	139	51.8
Condom use		
Occasionally	73	27.2
Consistently	195	72.8
Duration as a sex worker		
≥6	68	25.4
1-5	200	74.6
sex debut		
< 18	237	88.4
≥18	31	11.6

**Number too small for statistical inference, IDU- intravenous drug use

4.2 Prevalence of mono-infections and co-infections tested in the study subjects

Sixty seven (67) out of 268 FSWs tested positive for HHV-8. Forty four (44) FSWs tested positive for HIV and 6 tested positive for syphilis. The FSWs who tested positive for both HHV-8 and HIV were 8, 2 tested positive for HHV-8 and syphilis and 2 tested positive for HIV and syphilis (Figure 4.1). Co-infection between different infections was recorded in the study population. The prevalence of HHV-8/HIV co-infection was 12% (8/67). The prevalence of HHV-8/syphilis co-infection was 3% (2/67) slightly lower than that of HIV/syphilis recorded as 4.5% (2/44). There was one (1/268) female sex worker who was triple infected 0.37% (Figure 4.2).

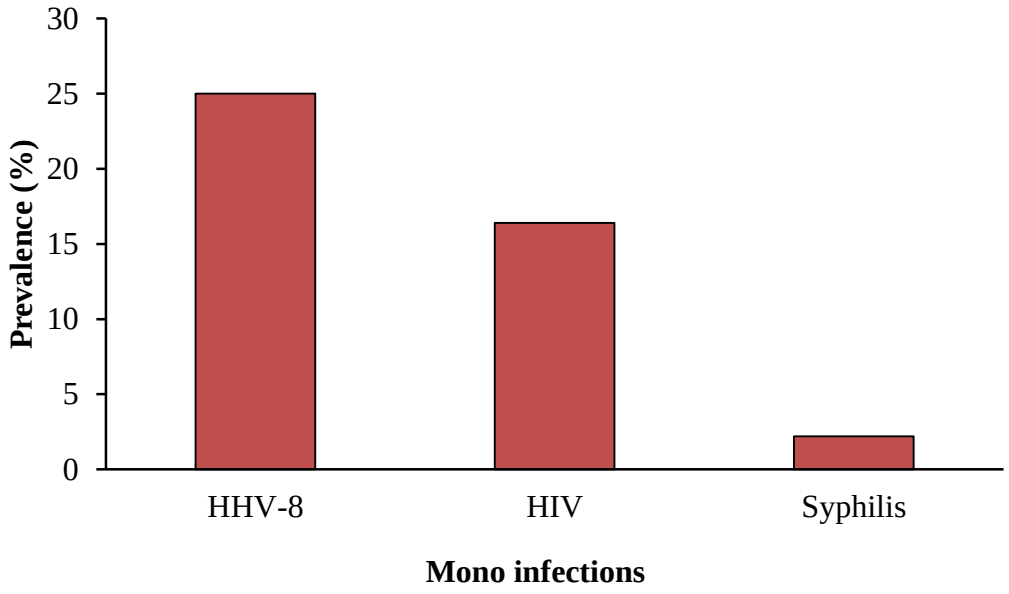

Figure 4.1: The prevalence of HHV-8, HIV and syphilis among FSWs in Malindi Sub-County

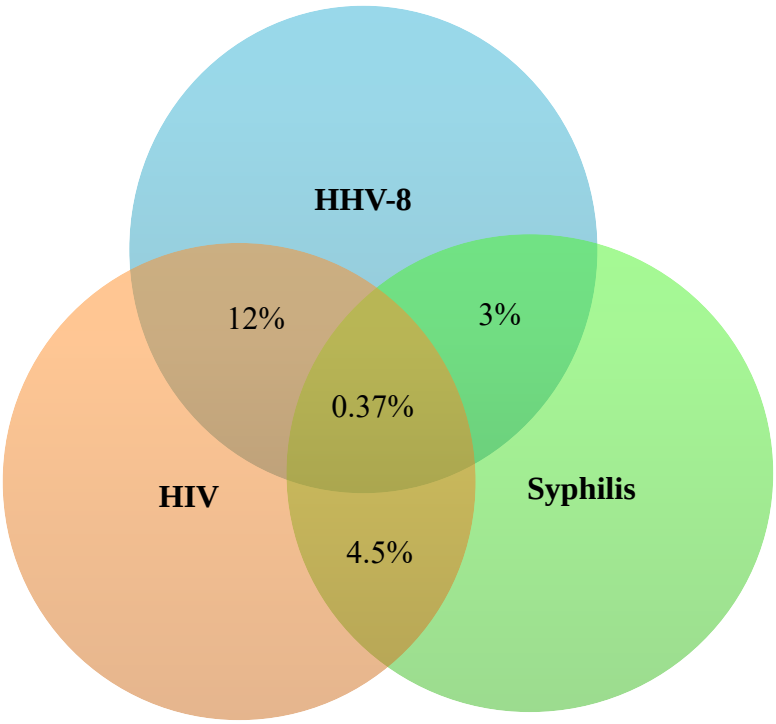

Figure 4.2: The prevalence of co-infections and triple infection by HHV-8, HIV and syphilis among FSWs.

4.3 Likelihood of infections

The prevalence of HHV-8 (25%) was significantly different from HIV prevalence (16.4%) p=0.0143 and syphilis prevalence (2.24%) p=0.0001. HIV prevalence was significantly different from syphilis prevalence p=0.0001. The prevalence of co-infections was not significantly different. The prevalence of HIV/syphilis co-infection was not significantly different from HHV-8/HIV p=0.19 and HHV-8/syphilis prevalence p=0.7 (Table 4.2)

Table 4.2: Likelihood of infections

Condition	n(N)	Prevalence	OR	p value
Mono infections				
HHV-8	67(268)	25	14.6	0.0001
HIV	44(268)	16	8.6	0.0001
Syphilis	6(268)	2.2	Reference	
Co-infections				
HHV-8/HIV	8(67)	11.9	2.8	0.19
HHV-8/Syphilis	2(67)	3	0.6	0.7
HIV/Syphilis	2(44)	4.5	Reference	

4.4 Infections among HIV negative and HIV positive FSWs

Out of 44 HIV positive FSWs, 8 were positive for HHV-8 (18%) 8/44 compared to 26.3% (59/224) among HIV negative FSWs. Syphilis infection was recorded in two out of 44 (4.5%) HIV positive FSWs and in 4 out of 224 (1.8%) HIV negative FSWs (Figure 4.3).

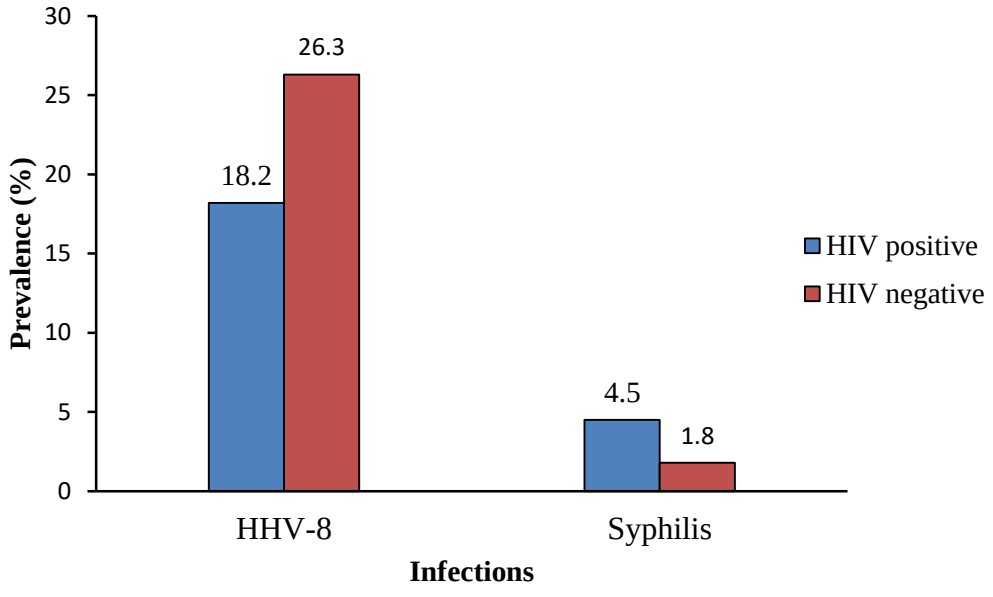

Figure 4.3: Prevalence of HHV-8 and syphilis among HIV positive and HIV negative FSWs

4.5 Risk factors associated with HHV-8 infection among female sex workers

Assessment of the risk factors for infection revealed that there was an increased risk of infection among the married FSWs (OR 2.90; p=0.042) compared to the single (OR 1.59; p=0.214) or the widowed/separated (referent). HHV-8 was not significantly associated with level of education; primary (0.89; p=0.802), secondary (OR 0.90 p=0.832) and tertiary (OR 2.75; p=0.492). In addition HHV-8 infection was not associated with age. The referent group (18-24) was more likely to be infected than the other age groups; 35 and above (0.92; p=0.858), 30-34 (0.70 p=0.399) and 25-29 (0.75-0.435).

In this study, no significant association between HHV-8 and intravenous drug use (OR 0.89; p=0.812) was observed. On sexual behavioral characteristics, there was no significant association of HHV-8 with number of clients (0.9 p=0.724), condom use (OR 0.98 P=0.937), number of years as a sex worker (OR 0.639; p=0.197) or engaging in sexual intercourse at early age (<18) (OR 0.95; p=0.912). The table 4.3 summarizes prevalence and OR of HHV-8 infection across all variables.

Table 4.3 Socio-demographic and sexual behavioral characteristics associated with HHV-8 among FSWs

		HHV-8 infection		
Risk factors		**n (%)**	**OR (95% CI)**	**p-value**
Age				
35 and above	52	21 (31.3)	0.92 (0.42-2.06)	0.858
30-34	55	20 (29.9)	0.70 (0.31-1.59)	0.399
25-29	87	12 (17.9)	0.75 (0.37-1.53)	0.435
18-24	74	14(20.9)	1	
marital status				
Single	180	46 (68.7)	1.59 (0.76-3.31)	0.214
Married	26	10 (14.9)	2.90 (1.04-8.07)	**0.042**
Widowed/separated	62	11 (16.4)	1	

Education Level				
Primary	151	37 (55.2)	0.89 (0.37-2.17)	0.802
Secondary	85	21 (31.3)	0.90 (0.35-2.33)	0.832
Tertiary**	2**	1(1.5)	2.75 (0.15-49.36)	0.492
NonFormal	30	8 (11.9)	1	
IDU				
Yes	26	6 (9.0)	0.89 (0.34 -2.31)	0.812
No	242	61 (91.0)	1	
Clients per day				
4 and above	129	31(46.3)	0.9 (0.52-1.57)	0.724
1 to 3	139	36 (53.7)	1	
Condom use				
Occasionally	73	18 (26.9)	0.98 (0.52-1.82)	0.937
Consistently	195	49 (73.1)	1	
Duration as a sex worker				
≥6	68	13 (19.4)	0.63 (0.32-1.26)	0.197
1-5	200	54 (80.6)	1	
sex debut				
< 18	237	59 (88.1)	0.95 (0.40-2.24)	0.912
≥18	31	8 (11.9)	1	

*** Too few to achieve statistical significance. HHV-8 was associated with being married.

4.6 Risk factors associated with HIV infection

Evaluation of socio-demographic characteristics showed an inverse association between HIV infection and being single OR 0.46 (CI 0.23-0.94) as opposed to been married (OR 0.38; p=0.149) or widowed/ separated (referent). HIV was not significantly associated with level of education; primary (OR 0.99; p=0.99), secondary (OR 1.87, p=0.294) and tertiary (OR 6.5; p=0.216). Those who had attended tertiary education among the study participants were two. HIV infection was significantly associated with increased age; 35

years and above (OR14.79; P=0.000) 30-34 years (OR 4.63; p=0.027) and lastly age group 25-29 (OR 3.78; p=0.046). On assessing behavioral characteristics, HIV infection was significantly associated with intravenous drug use (OR 2.5; p=0.043), increased number of sexual partners (OR 4.0; p=0.0002), inconsistent condom use (OR 2.69; p=0.004), increased number of years as FSW (OR 3.0; p=0.002) and weakly associated with sexual intercourse encounter at an early age <18 (OR 0.42; p=0.049). Table 4.4 summarizes prevalence and OR of HIV infection across all variables.

Table 4.4: Socio-demographic and behavioral risk factors associated with HIV infection among FSWs

		HIV infection		
Risk factors	**N**	**n (%)**	**OR (95% CI)**	**p-value**
Age				
35 and above	52	20 (45.4)	14.79 (4.10-53.37)	**0**
30-34	55	9 (20.5)	4.63 (1.19-18.01)	**0.027**
25-29	87	12 (27.3)	3.78 (1.02-13.97)	**0.046**
18-24	74	3 (6.8)	1	
marital status				
Single	180	25 (56.8)	0.46(0.23-0.94)	**0.034**
Married	26	3 (6.8)	0.38 (0.099-1.41)	0.149
Widowed/separated	62	16 (36.4)	1	
Education Level				
Primary	151	20 (45.5)	0.99 (0.31-3.14)	0.99
Secondary	85	19 (43.2)	1.87 (0.58-6.03)	0.294
Tertiary**	2**	1 (2.3)	6.5(0.34- 126.06)	0.216
NonFormal	30	4 (9.1)	1	
IDU				
Yes	26	8 (18.2)	2.5 (1.03-6.29)	**0.043**
No	242	36 (81.8)	1	
Clients per day				
4 and above	129	33(75)	4.0 (1.92-8.32)	**0.0002**
1 to 3	139	11(25)	1	

Condom use				
Occasionally	73	20 (45.5)	2.69 (1.38-5.25)	**0.004**
Consistently	195	24 (54.5)	1	
Duration as a sex worker				
≥6	68	18 (40.9)	3.0 (1.51-6.02)	**0.002**
1-5	200	26 (59.1)	1	
sex debut				
< 18	237	35 (79.5)	0.42(0.18-1.00)	0.05
≥18	31	9 (20.5)	1	

The bolded value show where significant association was observed. HIV was associated with increased age and all high risk sexual behavior; intravenous drug use, occasional condom use, increased clients per day and increased duration as a sex worker.

4.7 Risk factors associated with Syphilis infection among female sex workers

Syphilis infection was not associated with any of the evaluated socio-demographic or sexual behavior characteristics. However, FSWs aged 35 years and above were at an increased rate of syphilis infection (OR 1.7; p=0.519) compared to the other age groups but there was no significant association. Neither level of education nor Marital status was significantly associated with syphilis infection in this study (Table 4.5). None of the married women or those who were intravenous drug users were infected with syphilis. FSWs who had more than 4 clients in a day (OR 1.08 p=0.926), those who inconsistently used condom (OR 1.3; p=0.556) and who were sex workers for a longer period (OR 1.48; p=0.596) were all at an increased rate of infection than their counter parts but there was no significant association (Table 4.5).

Table 4.5: Socio-demographic and behavioral risk factors associated with syphilis infection among FSWs

Syphilis infection				
Risk factors	**N**	**n (%)**	**OR (95% CI)**	**p- value**
Age				
35 and above	52	3 (50)	1.7 (0.33-8.23)	0.519
30-34	55	0	1	
25-29	87	3(50)	1	
18-24	74	0	1	
marital status				
Single	180	4 (60)	0.68 (0.12-3.82)	0.663
Married	26	0	1	
Widowed/separated	62	2 (40)	1	
Education Level				
Primary	151	3 (50)	0.58(0.05-5.85)	0.65
Secondary	85	2 (33.3)	0.70 (0.06-8.00)	0.773
Tertiary**	2**	0	1	
NonFormal	30	1 (16.7)	1	
Clients per day				
4 and above	129	3 (50)	1.08 (0.21-5.45)	0.926
1 to 3	139	3(50)		
Condom use				
Occasionally	73	5 (83.3)	1.3 (0.31-5.74)	0.556
Consistently	195	1 (16.7)	1	
Duration as a sex worker				
≥6	68	2 (66.7)	1.48 (0.34-6.34)	0.596
1-5	200	4 (33.3)	1	
sex debut				
<18	237	5 (83.3)	0.44 (0.08-2.27)	0.329
≥18	31	1 (16.7)	1	

None of the variables tested were significantly associated with syphilis. Syphilis infection was minimal among FSWs and only occurred in two age groups; 25-29 and ≥35.

4.8 HHV-8/HIV co-infection among female sex workers in Malindi sub-County

Co-infection of HHV-8 with HIV among FSWs in Malindi was associated with increased age. FSWs above 35 years were more likely to be co-infected (OR 11.21; p=0.027) as compared to those in age group 30-34 (OR 1.59; p=0.7444) and 25-29 (referent). HHV-8/HIV co-infection was not recorded among FSWs in the age group 18-24. Marital status and level of education were both not significantly associated with HHV-8/HIV co-infection. However those who had attended primary school (OR1.9; p=0.449) were more likely to be infected than the other groups within the cluster. Other intra cluster analyses showed that FSWs who were Intravenous drug users (OR 3.2; p=0.16), who had more than 4 clients in a day (OR 3.3 p=0.144) and those who inconsistently used condom (OR 1.63; p=0.512) were all at an increased rate of co-infection than their counter parts but there was no significant association. Increased duration as a sex worker and early involvement in sexual intercourse showed no significant association (OR 0.98 p=0.98) and (OR 0.91 p=0.933) respectively (Table 4.6).

Table 4.6: Risk factors of HHV-8/HIV co-infection among FSWs

		HHV-8/HIV Co-infection		
Risk factor	**N**	**n (%)**	**OR (95% CI)**	**p- value**
Age				
35 and above	52	6 (75)	11.21(1.31-96.02)	**0.027**
30-34	55	1 (12.5)	1.59 (0.097-25.99)	0.744
25-29	87	1 (12.5)	1	
18-24	74	0	1	
marital status				
Single	180	4 (50)	0.45 (0.97-2.06)	0.301
Married	26	1 (12.5)	0.79 (0.08-7.93)	0.839
Widowed/separated	62	3(37.5)	1	
Education Level				
Primary	151	5 (62.5)	1.9 (0.35-10.83)	0.449
Secondary	85	2 (25.5)	1	

Tertiary**	2**	1(12.5)	1	
NonFormal	30	0	1	
IDU				
Yes	26	2 (25)	3.27 (0.63-17.44)	0.16
No	242	6 (75)	1	
Clients per day				
4 and above	129	6 (75)	3.3 (0.66-16.86)	0.1441
1 to 3	139	2 (25)	1	
Condom use				
Occasionally	73	3 (37.5)	1.63 (0.38-6.99)	0.512
Consistently	195	5 (62.5)	1	
Duration as a sex worker				
≥6	68	2 (25)	0.98 (0.19-4.97)	0.98
1-5	200	6 (75)	1	
sex debut				
< 18	237	7 (87.5)	0.91(0.11-7.68)	0.933
≥18	31	1(12.5)	1	

The only socio-demographic characteristic associated with HHV-8/HIV co-infection was increased age (above 35).

4.9 Risk factors associated with HHV-8/syphilis infection among female sex workers

There was an increased risk of HHV-8/syphilis co-infection among FSWs aged 35 years and above but no significant association was observed (OR 1.68; p=0.714). HHV-8/syphilis co-infection was only recorded among FSWs with primary education but there was no significant association (OR 0.56; p=0.683). FSWs who used condom occasionally were at an increased risk of HHV-8/syphilis co-infection (OR 2.69; p=0.485). Having more than 6 clients per day (OR 1.08; p=0.96), increased duration as a sex worker (OR 0.58; p=0.726) and early sex debut (OR 0.67; p=0.797) were all not significantly associated with HHV-8/syphilis co-infection as summarized in Table 4.7.

Table 4.7: HHV-8/syphilis co-infection among female sex workers

	HHV-8/syphilis co-infection			
Risk factors	**N**	**n (%)**	**OR (95% CI)**	**p-value**
Age				
35 and above	52	1 (50)	1.68 (0.10-27.55)	0.714
30-34	55	0	1	
25-29	87	1 (50)	1	
18-24	74	0	1	
marital status				
Single	180	1 (50)	Omitted	
Married	26			
Widowed/separated	62	1 (50)		
Education Level				
Primary	151	2 (100)	0.56 (0.04-9.07)	0.683
Secondary	85	0		
Tertiary**	2**	0		
NonFormal	30	0		
Clients per day				
4 and above	129	1 (50)	1.08 (0.07-17.42)	0.96
1 to 3	139	1 (50)		
Condom use				
Occasionally	73	1 (50)	2.69 (0.17-43.65)	0.485
Consistently	195	1 (50)		
Duration as a sex worker				
≥6	68	0	0.58 (0.03-12.22)	0.726
1-5	200	2 (100)		
sex debut				
< 18	237	2 (100)	0.67 (0.03-14.25)	0.797
≥18	31	0		

None of the variables tested were significantly associated with HHV-8/syphilis co-infection. Out of the 67 FSWs infected with HHV-8, only 2 were co-infected with syphilis.

4.10 HHV-8, HIV and syphilis mono-infections and co-infections across all ages

Evaluation of prevalence of infections across all ages showed that HHV-8 infection was highest (31%) at age group 18-24 followed closely (30%) by age group 25-29. FSWs in the age group ≥35 recorded 21% with the lowest prevalence (18%) recorded in age group 30-34. HHV-8 infection was not significantly associated with age (p=0.792) unlike HIV which showed a significant association (p=0.000) with increased age. The highest HIV prevalence of 46% was observed among FSWs aged ≥35 years and lowest of 7% among FSWs 18-24 years of age. HIV was higher in age group 25-29 (26.5%) compared to 30-34 where the prevalence was 20.5%. Syphilis infection was observed in the age groups 25-29 and ≥35 only, which also recorded the highest HIV prevalence. HHV-8/HIV co-infection increased from 0% from age group 18-24 to 75% among FSWs aged 35 years and above with a p value of 0.001. HIV/syphilis co-infection was only recorded at age group 25-29. HHV-8/syphilis co-infection was recorded at age group 25-29 (50%) and ≥35 (50%) years. HIV/syphilis co-infection was observed only at the age group 25-29. HHV-8 infection was highest among FSWs aged 18-24 while HIV and HHV-8/HIV co-infection was highest among FSWs ≥35 years of age (Figure 4.4).

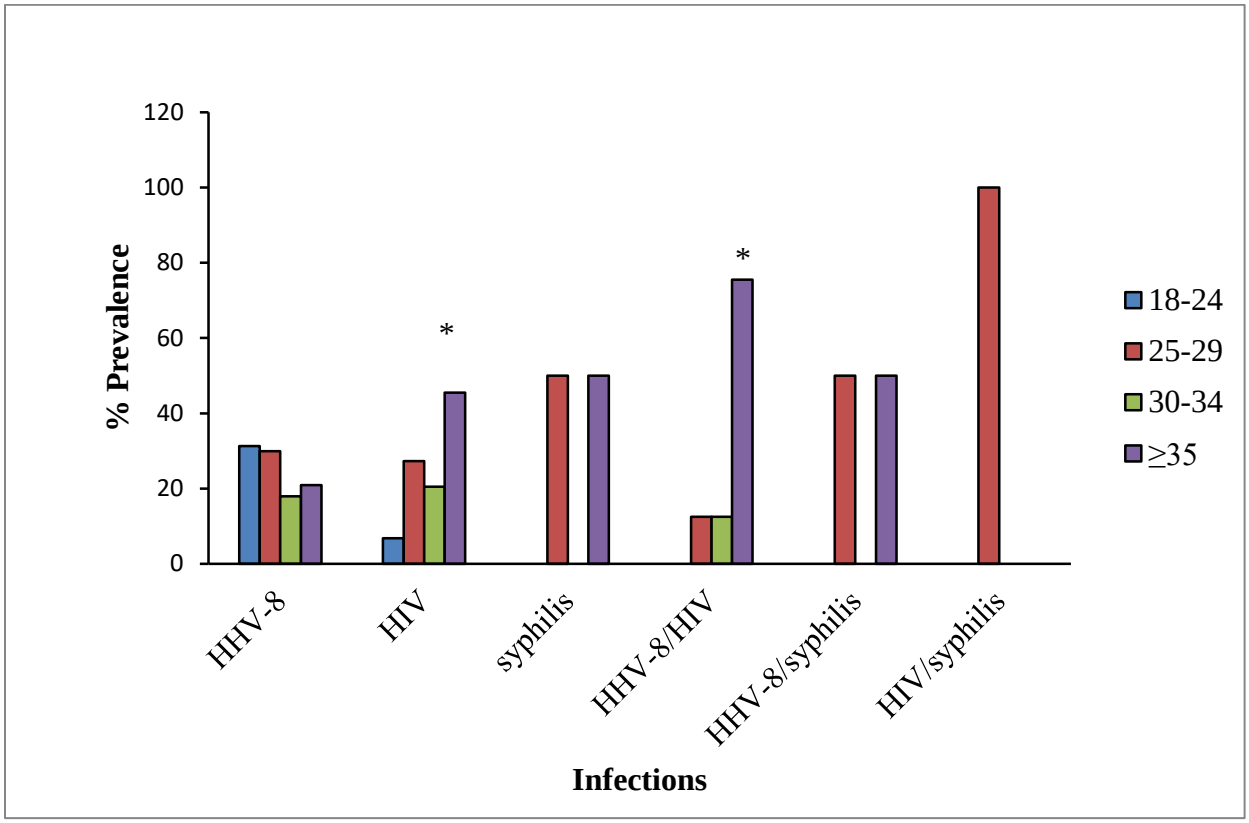

Figure 4.4: The prevalence of mono-infections and co-infections across all age groups. (*) Show infections that had significant association with age

4.11 HHV-8 DNA

Out of the 67 samples subjected to nested PCR using the first set of primers (K1-1, K1-2, K1inn, K1inn6) and second set of primers (K1a-f, K1a-r, k1b-f,K1b-r) HHV-8 DNA was not amplified. Using the third set of primers (K1F, K1R), the 13 samples that were amplified via direct PCR showed multiple bands. The First, second and the third set of primers used in the study targeted a 450 bp, 750 bp and 1050 bp product respectively. These were then visualized in gel (Plate 4.1). The gels were extracted and samples send for sequencing at Macrogen, Europe. No sequences for HHV-8 were detected (Appendix XI).

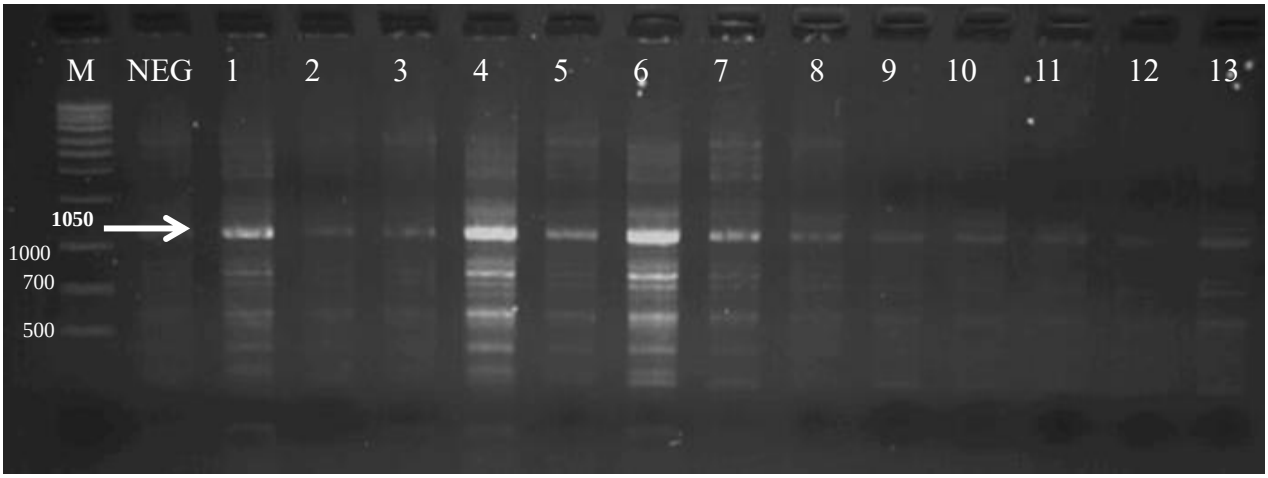

Plate 1: A target product of 1050 bp was detected in 13 samples. M: 1kb marker NEG: Negative control.

CHAPTER FIVE

DISCUSSION, LIMITATIONS, CONCLUSIONS AND RECOMMENDATIONS

This study determined the prevalence of HHV-8 to be 25% within a cohort of FSW population in Kenya. Although data on the prevalence in general population is scanty, findings from this study was lower than those previously reported in Kenya among HIV-1-seronegative FSW and truck drivers who had recorded 44% and 43% respectively (Beaten *et al.,* 2002; Lavreys *et al.,* 2003). This could be attributed to several factors such as improvement of socio-economic status over years hence improvement of living standards, campaigns and education on risky sexual behaviors by Ministry of Health and NGOs and access to HAART among others.

HIV prevalence of 16.4% among FSWs in this study was higher than the prevalence of HIV infection in the general population (5.9%) in Kenya. However the prevalence in this study was lower than the recorded overall seroprevalence of HIV among FSWs (29.3%) in Kenya (MOH, 2016). It suffices to note that low prevalence rates in this study could be attributed to structural interventions aimed at curbing HIV and its co-infections including supplying condoms, access to antiretroviral drugs, elimination of sexual violence, provision of needles and syringes to avoid needle sharing among IDUs and providing sex education to this high risk group within the study population that was obtained mainly from the drop-in Centre.

Syphilis infection in FSWs in Malindi sub-County was low at 2.24%. This could be as a result of clear symptoms of syphilis which allow for its early detection and treatment. In addition, availability of health care services through targeted programs for FSWs ensures consistent testing of STIs and treatment. However, detection of syphilis among the co-infected is still a significant finding since failure to detect or asymptomatic presence of syphilis in the patients would affect the treatment duration and success in the HIV infected. The detection of syphilis was also a clear sign that preventive measures such as condom use need to be pursued. There is an extensive network of service delivery for FSWS which provide HIV testing and counseling, however targeted and routine HIV and STI combination prevention strategies need to be scaled up or established to meet the needs of FSWS.

The prevalence of HHV-8/HIV co-infection was 12% among FSWs in Malindi sub-County. Data on HHV-8/HIV co-infection is neither available in the general population nor among FSWs in Kenya. The prevalence of HHV-8/HIV co-infection recorded in this study was lower than prevalence of co-infection among prostitutes in Jos, Nigeria and Imbituba, Brazil reported as 20% and 16.4% respectively (Caterino-de-Araujo *et al.,* 2007; Zakari *et al*., 2012). This could be as a result of decrease of HIV prevalence over years hence reducing co-infection with HHV-8. Improved immunity among HIV positive FSWs taking HAART could prevent acquisition of opportunistic infections resulting in low co-infection prevalence. Co-infection of HHV-8 in HIV infected patients has been associated with increased inflammation and immune activation and could lead to KS. Co-infection between HHV-8 and HIV is an indicator of a potential increase in progression to AIDS within this community.

HHV-8/syphilis co-infection observed in the study was minimal (3%). As mentioned earlier syphilis is a marker of high risk behavior. HIV/syphilis co-infection recorded of 4.5% and HHV-8/HIV/ syphilis triple infection although low is an indicator to unprotected sexual behavior within this group. Condom use being one of effective methods to control HIV and syphilis, in this study it was not associated with HHV-8 transmission hence its use to control HHV-8 will be of minimal effect. The lack of HHV-8 association with condom use has also been observed by (Mcdonald *et al.,* 2009; Zakari *et al.,* 2012; Zhang *et al.,* 2014).

HHV-8 infection was significantly associated with marriage (OR 2.90; p=0.042). The reason for this could not be explained within this study. However this was also observed in other studies. In South Africa increased infection among married people in a Carletonville village (OR 1.3, CI 1.1-1.7) was recorded (Malope *et al.,* 2008). Two studies in Uganda among a subset of participants from a population-based HIV/AIDS serobehavioral survey of adults aged 15–59 years recorded (OR 1.52 CI 1.17-1.97) and a p value of 0.001 among the married participants as compared to their unmarried counter parts (Biryahwaho *et al.,* 2011; Shebl *et al.,* 2011). Shebl *et al.* (2011) reported significant association of HHV-8 with marriage as an evidence of sexual transmission. However in this study, married FSWs were least likely to be infected by sexually transmitted infections: HIV (OR 0.38; 95% CI 0.099-1.41; p=0.149) and none were infected with syphilis indicating that HHV-8 may have not been transmitted sexually. A

study in Kenya (Baeten *et al.,* (2002), although among heterosexual men drivers of a trucking company observed HHV-8 significant association with marriage (OR 1.4; p=0.02). Lack of significant association of HHV-8 with marriage was recorded by (McDonald *et al.,* 2009; Ogoina *et al,* 2011; Zhang *et al*., 2014).

There was no significant association of HHV-8 infection with HIV infection (p=0.257) among FSWs in this study. This was consistent with studies which recorded p=0.660, p=0.57, p=0.47, OR 1.2 (CI 0.6-2.5) in Uganda, South Africa, Nigeria and USA respectively (Eltom *et al.,* 2002; Corey *et al.,* 2006; Malope *et al.,* 2008; Shebl *et al.,* 2011). However, it contrasted with other studies which reported significant association (Cannon *et al.,* 2001; Ogoina *et al*., 2011; Zakari *et al.,* 2012). HHV-8 and HIV presented with different modes of transmission in the present study, that is; sexual and non-sexual. HHV-8 infection among FSWs in Malindi sub-County did not present with characteristics of a sexually transmitted infection. HHV-8 infection unlike HIV infection was not associated with high risk sexual behavior such as inconsistent condom use, increased number of clients, increased duration as a sex worker or intravenous drug use, all of which were significantly associated with HIV as expected. This suggests sexual transmission of HHV-8 rarely occurred among the study participants. However, transmission of HHV-8 earlier in life could have masked sexual transmission of HHV-8 if it did occur.

Syphilis was not significantly associated with HHV-8 infection OR 1.52 (027-8.47). This was also observed by other studies OR 1.2 (0.9-1.6), 0.51 (0.18-1.51), OR 1.0 (0.1-8.9) and OR 2.98 (0.92-9.37) in South Africa, China, Italy and USA respectively (Smacchia *et al.*, 2004; Giuliani *et al.*, 2007; Malope *et al.*, 2008; Zhang *et al.*, 2014).

HIV infection and HHV-8/HIV co-infection were associated with increased age. This could be as result of increased HIV infections due to continuous exposure over years and reactivation of HHV-8 hence sero conversion due to a weakened immune system or new infections. However, a seemingly declining rate of HHV-8 infection by age could potentially mean that infection in a majority of our study participants may have been acquired earlier in life and a non-sexual route of transmission could be responsible. Lack of significant association with age has been recorded by other studies (Bestetti *et al*.,

1998; Smacchia *et al.*, 2004; Giuliani *et al.*, 2007; Malope *et al*., 2008; Zakari *et al.*, 2012) but was inconsistent with others which recorded a significant association (p=0.011, p=0.02 and p=0.0001) in Nigeria, Kenya and Uganda respectively (Eltom *et al.*, 2002; Lavreys *et al.*, 2003; Shebl *et al.*, 2011). HHV-8 infection among participants in the mentioned studies increased with age, while in this study HHV-8 infection was already at peak in women between 18-24 years of age. Studies have reported evidence of HHV-8 infection among children in sub-Saharan Africa indicating transmission of HHV-8 earlier in life mainly occurred via saliva (Mbulaiteye *et al.*, 2003; Malope *et al*., 2007; Crabtree *et al.*, 2013; Minhas & Wood, 2014; Rohner *et al.*, 2016). Two studies examined the shedding rate of HHV-8 in different mucosal sites reported there was more HHV-8 DNA in the saliva compared to other body secretions (Taylor *et al*., 2004; Phipps *et al.*, 2014). HHV-8/HIV co-infection was significantly associated with syphilis infection. FSWs in this study who were positive for syphilis were at an increased risk of HHV-8 and HIV co-infection. HHV-8/HIV co-infection was high among FSWs ≥35 who also recorded syphilis infection. This shows risky behavior in this age group.

HHV-8 was not associated with intravenous drug use a pattern consistent with other studies (Perna *et al.*, 2000; Greenblatt *et al*., 2001; Zhang *et al.*, 2014). However, other studies observed significant association between HHV-8 and intravenous drug use (Zavitsanou *et al.*, 2010 ; Kakavand-ghalehnoei *et al.*, 2016; Qiwen *et al.*, 2017). This could be due to the fact that KANCO drop-in Centre from which FSWs who are IDUs were recruited provides needles and syringes to avoid sharing hence reducing exposure. In addition HHV-8, transmission via blood has been reported to be rare. Zavitsanou *et al* (2009) reported that needle sharing was not significantly associated with HHV-8 and this could be explained by a study by Cannon *et al* (2001) that reported that HHV-8 transmission by blood was either inefficient or did not occur frequently. This was due to the observation that HHV-8 prevalence was low among women IDUs who had cases of needle sharing compared to HIV positive homosexual men. HHV-8 was weakly linked to intravenous drug use compared to other blood borne viruses such as HBV and HCV which showed high prevalence among IDUs (Cannon *et al*., 2001; Chandran & Hutt-fletcher, 2006).

Phylogenetic analysis was not conducted to determine the HHV-8 subtypes in the region due to technical and logistical challenges within the study period. HHV-8 DNA has been reported to be rare in peripheral blood samples even among HHV-8 seropositive individuals hence serological assays recommended as method of choice to test for HHV-8 (Speicher 2012; Minhas & Wood, 2014; Braz-Silva *et al.,* 2017). HHV-8 DNA has been isolated mainly in lymph nodes and saliva of healthy individuals and in KS lesions (Speicher, 2012; Minhas & Wood, 2014; Tembo *et al.*, 2017). However, a study in Brazil did not detect HHV-8 DNA in saliva samples indicating low levels of HHV-8 DNA in saliva in areas where KS is not endemic. In the study, 944 saliva samples were tested but HHV-8 DNA was not detected in 100% of the samples (Tozetto-Mendoza *et al.,* 2018).

Some studies have isolated HHV-8 DNA in blood samples. For instance, HHV-8 DNA was isolated in 9% (5/56) of PBMCs samples, 16.5% PBMCs samples from 156 HHV-8 seropositive healthy adults and in one (1) out of 12 HHV-8 seropositive individuals (Bigoni *et al.,* 1996; Brown *et al.,* 2005). HHV-8 DNA was isolated in 4.5% and 22.5% PBMCs samples of HHV-8 seropositive individuals in Tanzania and Centre Africa republic respectively (Belec *et al*, 1998; Enbom *et al.,* 2002). Taylor *et al* (2004) recorded HHV-8 shedding rate of 9% and 18% in plasma and PBMCs samples respectively. A study in Yaoundé, Cameroon detected HHV-8 DNA in 8.7% (15/88) blood of HHV-8 seropositive samples (Lontchi-yimagou *et al.*, 2018).

There are several studies that did not detect HHV-8 DNA in HHV-8 seropositive blood samples. Braz-Silva *et al* (2017) did not detect HHV-8 DNA in 738 blood samples including those from 18 oral shedders which were positive for HHV-8 DNA. HHV-8 DNA was not also isolated in any of PBMCs samples of both healthy and HIV infected individuals (Ramezani *et al.*, 2016). HHV-8 DNA was not isolated in whole blood samples from 803 HHV-8 seropositive blood donors and in any of cultured and uncultured PBMCs (Levi *et al.*, 2011). HHV-8 DNA was not detected in 684 blood donor samples including 40 HHV-8 seropositive ones (Qu *et al.*, 2011). HHV-8 DNA was not detected in blood samples using highly sensitive nested PCR and real-time PCR capable of detecting a single copy respectively (Hudnall *et al.* 2003: 2008). HHV-8 DNA was not detected in any of 138 blood samples including 33 that were seropositive.

The lack of detection of HHV-8 DNA in this study could be due to number of reasons. HHV-8 DNA was not present in the samples hence reason it was missing in the sequenced products. Korir *et al* (2015) reported KS to be rare in Kenya compared to other East African countries. Detection of HHV-8 DNA in blood samples has been associated with KS. It could also be the bands visualized on gel after direct PCR using K1-F/K1-R were false positives. Sanger sequencing results showed multiple templates (Appendix XI). To confirm if HHV-8 DNA was present in the samples it required the use of the highly sensitive next generation sequencing but was hindered by cost. Speicher (2012) reported that even with high sensitive PCR, most HHV-8 asymptomatic patients are negative for HHV-8 DNA in the blood.

Generally, there are a number of reasons to explain lack HHV-8 DNA in samples serologically positive for HHV-8. To begin with, HHV-8 shedding is not common contrary to other herpes viruses which are excreted and acquired through well-defined routes with horizontal transmission (Chandran & Hutt-fletcher, 2006; Bender Ignacio *et al.,* 2016; Tozetto-Mendoza *et al.,* 2018). Secondly, HHV-8 is a circulating virus not integrated into the human genome and exists as an episome rather than a provirus with low viral loads among healthy individuals (Alagiozoglou, 1999; Hulaniuk *et al.,* 2017). Peripheral blood B cells may not be the HHV-8 latency site in healthy individuals. Detection of HHV-8 DNA in PBMCs or plasma is associated with KS or future development of KS (Tedeschi *et al.,* 2001; Machado *et al.,* 2016; Ramezani *et al.*, 2016). HHV-8 viraemia is low in peripheral blood. HHV-8 DNA has been detected mainly in lymph nodes compared to blood in healthy individuals (Speicher, 2012). The other reason could be low concentration of DNA in the PCR mix hence limiting the possibility of viral DNA detection. Shedding of HHV-8 has been reported to be high among HIV positive than in healthy individuals (Ribeiro & Leao, 2011; Ramezani *et al.*, 2016). The lack of detection of HHV-8 DNA in this study among the HIV positive FSWs could be due to majority of the HIV positive FSWs present in the study were immunologically well controlled on HAART which has been reported to reduce HHV-8 DNA loads (Ramezani *et al.*, 2016).

5.1 Study Limitations

The design of this study was cross-sectional in nature hence a number of gaps were not filled. It was not possible to pair the participants with their spouses so as to determine with finality the role of sexual activity in HHV-8 transmission. Other activities out of the scope of this research which could be determined during the follow up were not assessed, hence the results are only subjective as at the time of sample collection. Further, this study was conducted within a group that has been enlightened on the dangers of sex work and is trained to live positively, hence may not give a true picture of FSWs in Kenya.

There is limited amount of HHV-DNA in blood samples as compared to saliva and KS biopsies.

5.2 Conclusions

This study concludes the following:

1. The prevalence of HHV-8 was 25% and significantly higher than the prevalence of HIV (16.4%) p=0.014and syphilis (2.24%) (p=0001).
2. Low prevalence of co-infection; HHV-8/HIV 12%, HHV-8/syphilis 2% and HIV/syphilis 4.5% was recorded.
3. HHV-8 was not significantly associated with HIV (p=0.257) or syphilis (p=0.636).
4. HHV-8 was associated with being married (p=0.042).
5. Prevalence of HHV-8/HIV co-infection increases with increasing age (p=0.027).
6. Unlike HIV, HHV-8 was not significantly associated with increased age, intravenous drug use, increased clients per day, occasional condom use and increased duration as a sex worker. This shows sexual route of HHV-8 transmission is rare within the FSW population in Kenya.

5.3 Recommendations

1. Create awareness to the FSWs on HHV-8 and enhance measures to curb the virus in this population and general population at large.
2. Enhance measures to curb HIV in FSWs population so as to avoid co-infection with HHV-8 as may lead to KS and progression to AIDs.

3. Longitudinal epidemiological studies should be undertaken to determine modes of transmission and risk factors for HHV-8 in different populations in Kenya.
4. Further research to be done on the availability of HHV-8 DNA in seropositive blood samples of healthy blood donors individuals, HIV positive and KS patients.

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APPENDICES

Appendix I: HHV-8 test

The plasma samples were tested using HHV-8 Enzyme Linked Immunosorbent Assay (ELISA) from Sunlong Biotechnologies Ltd, China following manufacturer's instructions. In a micro Elisa strip plate, 50 µl of both negative control and positive control were added in two wells each and one well was left empty as a blank control. In each sample well, 40 µl of sample dilution and 10 µl of each sample was added at the bottom. The contents were mixed by gently shaking. The micro ELISA plate was then sealed with a closure plate membrane and incubated for 30 min at 37°C. After incubation the seal was removed and each well washed 5 times with wash buffer using soak cycle for at least 30 seconds. In all wells except the blank, 50 ul of HRP-Conjugate reagent was added. The plate was incubated and samples washed again as described earlier. Fifty microliters (50 µl) of chromogen solution A and 50 µl of chromogen solution B were added to each well and incubated at 37°C for 15 minutes. A stop solution (50µl) was then added to each well to terminate the reaction. There was a color change from blue to yellow in some wells. The absorbance optical density (O.D) was then read at 450 nm using a micro titer plate reader. A cutoff value was calculated by getting the average value of two negative controls plus (+) the value 0.15 as directed by the manufacturer. Positive samples had an O.D above the cut off value.

Appendix II: HIV testing

For HIV screening, a parallel algorithm for plasma samples was done, with Determine™ (Alere Medical Co. Ltd) and First Response® HIV 1-2-0 (Premier Medical Co. Ltd) being used in parallel following manufacturer's instructions. To each of the two tests strip 50 µl of a sample was applied on the sample pad at the shown mark. Positive results showed two bands (test and control band) on each test strip while negative results showed only a single band on the control region of test strip.

Appendix III: Syphilis testing-

The test for *Treponemma pallidum*, the causative agent for syphilis was done using one step Anti-TP test using plasma samples following manufacturer's instructions. To each

strip, 60 µl of the sample was applied on the sample pad at the required mark on the bottom of the test strip. Results were interpreted within 15 minutes but not later than 20 minutes. Positive results showed purplish red colored test band and purplish red control on the membrane of the test strip. Negative results showed only one purplish red colored band on the control region.

Appendix IV: **DNA Extraction**

DNA was extracted from PBMCs using Qiagen kit following manufacturer's instructions. PBMCs samples initially stored at -20 ºC were allowed to thaw at room temperature. Into each of the sixty seven 1.5ml microcentrifuge tubes, 20 µl of proteinase K was added followed by 200 µl of the sample. Two hundred (200 µl) of AL buffer was added into each of the contents in microcentrifuge tubes and mixed by pulse vortexing for 15 seconds to yield a homogenous solution and ensure efficient lysis. The contents in the tubes were then incubated at 56°C for 10 minutes in an incubator and later centrifuged briefly to remove drops from the inside of the tubes. Absolute ethanol (200 µl) was added to each of the tubes contents and mixed by pulse vortexing for 15 seconds. They were again centrifuged briefly to remove drops from the inside of the tube.

The mixture was then carefully applied onto the QIAmp spin column in a collection tube without wetting the rim and spun at 8000 revolutions per minute (rpm) for 1 minute to allow DNA to bind and the tube with filtrate was discarded. The spin column was then placed in a clean 2ml collection tube and 500 µl of buffer AW1 (Wash buffer 1) added and centrifuged at 8000rpm for 1 minute. The collection tube containing filtrate was discarded and the spin column placed in another clean 2ml collection tube. Five hundred (500 µl) of buffer AW2 (wash buffer 2) was added and centrifuged at 14000 rpm for 5 minutes and the collection tube with filtrate was discarded. The QIAmp spin column was placed in a clean 2 ml collection tube and centrifuged at full speed for 1 minute to eliminate any chance of possible buffer AW2 carry over and tube with the filtrate was discarded.

The QIAmp spin column was then placed in a clean 1.5 ml microcentrifuge tube and 60 µl elution buffer (Buffer AVE). It was then incubated at room temperature for 1min and centrifuged at 8000rpm for 1 min. The eluted DNA was then stored at -80^{O}C awaiting PCR.

Appendix V: Gel Extraction

Gel extraction was done using QIAquick Gel Extraction Kit. DNA fragment was excised from the agarose gel with a sterile scalpel. The gel slice was weighed in a colorless tube. In each 100 mg of gel, 300 ul of Buffer QG was added. The tubes were then incubated at 50°C for 10 min with frequent vortexing after every 3 minutes until the gel slice had completely dissolved. After the gel slice had dissolved completely the color of the mixture turned yellow. Into the sample mix, 100 ul of isopropanol was added. The spin column was placed in 2 ml collection tube and the sample applied. It was then centrifuged at 13000 rpm for 1min. The flow-through was discarded and the spin column placed back in the same collection tube. Into the spin column, 0.5 ml of Buffer QG was added then centrifuged at 13000 rpm for 1 min. This was then washed by adding 0.75 ml of Buffer PE to spin column and allowed to stand for 5 min before it was centrifuged at 13000 rpm for 1 min. The flow-through was discarded, the spin column placed back in collection tube and centrifuged for an additional 1 min at 13,000 rpm. The column was then placed into a clean 1.5 ml microcentrifuge tube. To elute DNA, 30 µl of Buffer EB was added at the center of the membrane; the column was left to stand for 1 min and centrifuged at 13000 rpm for 1 min at maximum speed. Sample was then stored at -20°C and later sent to Macrogen, Europe for sequencing.

Appendix VI: Approval from KEMRI Scientific and Ethics Review Unit

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1 **March 24, 2015**

TO: **MIRRIAM NZIVO,**
PRINCIPAL INVESTIGATOR

THROUGH: **DR. GEORGE NAKITARE,**
THE DIRECTOR, CVR,
NAIROBI

DIRECTOR
CENTRE FOR VIRUS RESEARCH
P.O. BOX 54840 2015
NAIROBI.

Dear Madam,

RE: **SSC PROTOCOL NO. 2915 - (*RESUBMISSION 2 OF INITIAL SUBMISSION*): CHARACTERIZATION OF HUMAN HERPES VIRUS TYPE 8 AMONG FEMALE COMMERCIAL SEX WORKERS IN MALINDI, KENYA-(*VERSION 2.0 DATED 10TH MARCH 2015*)**

Reference is made to your letter dated 9th March, 2015 and the revised documents received at the KEMRI/Scientific and Ethics Review Unit (SERU) on 11th March, 2015.

This is to inform you that the Committee notes that the issues raised at the 234th meeting of the KEMRI/Ethics and Review Committee held on 16th December, 2014 have been adequately addressed.

Consequently, the study is granted approval for implementation effective this **24th March, 2015** for a period of one year. Please note that authorization to conduct this study will automatically expire on **March 23, 2016.** If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **February 9, 2016**.

You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advise SERU when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

PROF. ELIZABETH BUKUSI,
ACTING HEAD,
KEMRI/SCIENTIFIC AND ETHICS REVIEW UNIT

CENTRE FOR VIRUS RESEARCH
25 MAR 2015
P.O. BOX 54840

In Search of Better Health

Appendix VII: Scientific and Ethic Review Unit approval to change study site

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1 **July 23, 2015**

TO: **MIRIAM NZIVO,**
PRINCIPAL INVESTIGATOR

THROUGH: **THE DIRECTOR, CVR,**
NAIROBI

Forwarded
Raphael Mihan
27th July 2015

FOR DIRECTOR
CENTRE FOR VIRUS RESEARCH
P.O. BOX 54628
NAIROBI.

Dear Madam,

RE: **SSC PROTOCOL NO. 2915 - (*RESUBMISSION-REQUEST FOR 1ST AMENDMENT*): CHARACTERIZATION OF HUMAN HERPES VIRUS TYPE 8 AMONG FEMALE COMMERCIAL SEX WORKERS IN MALINDI, KENYA.**

Reference is made to your letter dated 9th July 2015. The Scientific and Ethics Review Unit (SERU) acknowledges receipt of the revised document on 10th July, 2015.

This is to inform you that the Committee reviewed the documents submitted and is satisfied that the issues raised at the 240th C meeting of the KEMRI/SERU on 25th June 2015 have been adequately addressed. You are therefore **authorized** to implement the following Amendments accordingly:

- Change in study site from Malindi Sub-county hospital to Ananda Marga Universal Relief Team (AMURT) Health Center and Kenya AIDS NGO Consortium (KANCO) drop in center

Please note that you are responsible for submitting any further changes to the approved version of the study protocol to the SERU for review and the changes should not be initiated until written approval from the SERU is received.

Yours faithfully,

EAB

PROF. ELIZABETH BUKUSI,
ACTING HEAD,
KEMRI/ SCIENCE AND ETHICS REVIEW UNIT (SERU)

CENTRE FOR VIRUS RESEARCH
27 JUL 2015
P.O. Box 54628 - NAIROBI

Appendix VIII: Kilifi County Department of Health Approval

THE COUNTY GOVERNMENT OF KILIFI
RESEARCH COORDINATING UNIT, DEPARTMENT OF THE HEALTH SERVICES

Telephone: 0721627306
0721843015
0721359983
Email: langat.eva@gmail.com
aceobonyo@gmail.com
kazunguwilfred@hotmail.com

P.O. BOX 519-10808
KILIFI, KENYA

Date 31 July 2015

When Replying/Telephoning quote
REF: DOH/KLF/RESCH/VOL.I/38

Mirriam Mbithe Nzivo

Dear Madam,

RE: AUTHORIZATION TO CARRY OUT A STUDY IN KILIFI COUNTY

The research unit has received your amended request to conduct a study **"Characterization of Human Herpes Virus Type 8 among Female Sex Workers in Malindi, Kenya."**
We are pleased to grant you institutional authorization to proceed with your research. Upon completion of you research, you are required to submit a written report to the Research Unit detailing the findings, conclusion and recommendations of your study.

We wish you the very best as you conduct your research.

Regards,

Evaline Langat
Research Coordinator
KILIFI COUNTY HEALTH RESEARCH COORDINATING UNIT

NDTEA
10/8/2015
ATTN:
SCMO
DISTRICT MEDICAL OFFICER OF HEALTH
MALINDI DISTRICT HOSPITAL
MALINDI

Appendix IX: Consent to participate in research – English version

HUMAN HERPES VIRUS TYPE 8 AND ITS COINFECTION WITH HUMAN IMMUNODEFICIENCY VIRUS AND *TREPONEMA PALLIDUM* AMONG FEMALE SEX WORKERS IN MALINDI, KENYA

Principal Investigator: Mirriam Mbithe Nzivo

Co- investigators

Names	Title	Institution	Contact
Nancy Budambula	Associate Professor	University of Embu	0722524051
Raphael Lwembe	Doctor	KEMRI	0718876976
Eddy Odari	Doctor	JKUAT	0725259296

My name is Mirriam Mbithe Nzivo…and I will be conducting this research.

There is a type of Herpes virus known as Human herpes virus type 8 (HHV-8). Human herpes virus type 8 is transmitted through several routes such as sex, contact with contaminated saliva and blood or blood products. HHV-8 causes several diseases, including Kaposi's sarcoma (a cancer that causes a form of skin disease); the most frequent cancer among HIV positive patients and a major cause of deaths among these patients. Sex workers are a high risk group because they have sex with multiple partners and some SWs contact HHV-8 in saliva through kissing. Once infected, the virus persists in the body for life and may result in disease when activated. It is therefore important to be tested for the virus as one may transmit the virus to others unaware.

You will read the informed consent and a nurse will go through it again to ensure you understand the purpose of the study, procedures involved and possible risk/ benefits. If anything is not clear please ask questions. The data will be handled confidentially such that no unauthorized person will have to access it. If you married however we encourage that you share your findings with your spouse.

Purpose of study

The purpose of the study is to determine prevalence of HHV-8, HIV and syphilis mono-infections and HHV-8/HIV and HHV-8/ syphilis co-infection among FSWs in Malindi sub- County, Kenya. This information will be useful in constant monitoring and surveillance of HHV-8 in HIV infected populations and also give an insight on what to expect in similar groups in other parts of the country.

Procedure followed

To conduct this study, we need your permission to collect some of your personal information. We will only collect information needed for the study. The study requires 245 FSWs. All FSWs who will consent to the study and accept to have their blood drawn and tested for HHV-8 and HIV will be recruited to participate in the study. If you agree to participate a phlebotomist will take 5ml of blood sample from vein of your arm once. This will be taken to HIV laboratory in KEMRI for analysis. HHV-8 will be tested using specialized test known as ELISA and PCR while HIV will be tested using rapid tests as recommended. You will also be requested to fill a questionnaire with some of your personal and health information.

Benefits

There is direct benefit as you will get your results on HIV status and syphilis status which will be sent to the appropriate Centres from which the sex workers were recruited. You will also not incur any cost on the activities of the study. If tested positive, you will be referred to a hospital for control and management of the infection. Results will be useful as will give an insight on what to expect among similar group in other parts of country and also improve medical attention offered to those who are HHV-8 positive.

Risks

This study has minimal risk as no health risks are associated with blood taking except minimum discomfort associated with puncturing of your skin which will last only for few minutes. Blood sample will be taken by an experienced phlebotomist. Those tested positive for HIV will be counseled to avoid/ reduce psychological trauma associated in knowing infection status.

Voluntariness

Your participation in this study is voluntary. You can choose to decline to participate by failing to sign the consent form. At any point of the study you can withdraw and this will not affect your case at the health facility.

Confidentiality

The data collected using questionnaires will be handled and kept confidentially and will only be accessible to authorized persons. You will only be given an identifying unique number so your name is not required and you will be delinked from results. The findings of the study will be presented in conferences or written in articles but no information will be written that identifies you.

Project contacts

In case of any questions about this study, you may contact us through mobile number 0722-271077 or Email mirriamaquilas@yahoo.com or KEMRI ethical research committee:

The Secretary

KEMRI Ethics and Research committee

P.O. BOX 54840-00200 Nairobi
Tel. 020- 2722541 or 0722 205901 or 0733 400003.
Email address: **ercadmin@kemri.org**

Participant consent

1. I have read the consent document and understood.
2. The study's purpose, procedures, risks and possible benefits have been explained to me.
3. All my questions have been answered to satisfaction.
4. I voluntarily agree to participate in this study.

Name of participant……

Signature/Thumb mark……Date……

Consent agreement form

I participated in consent process and acknowledge enrollment of this participant into the study.

Name of principal investigator……

Signature……..Date……

Appendix X: Questionnaire

Answer all questions

Indicate a tick (√) where appropriate.

Social demographic data

1. Participant unique Identifying No.……..……
2. County of residence…….
3. Age …… ……...
4. Marital status ☐ Married ☐ Single ☐ Widowed/separated
5. Level of education ☐ None ☐ Primary ☐ Secondary ☐ Tertiary

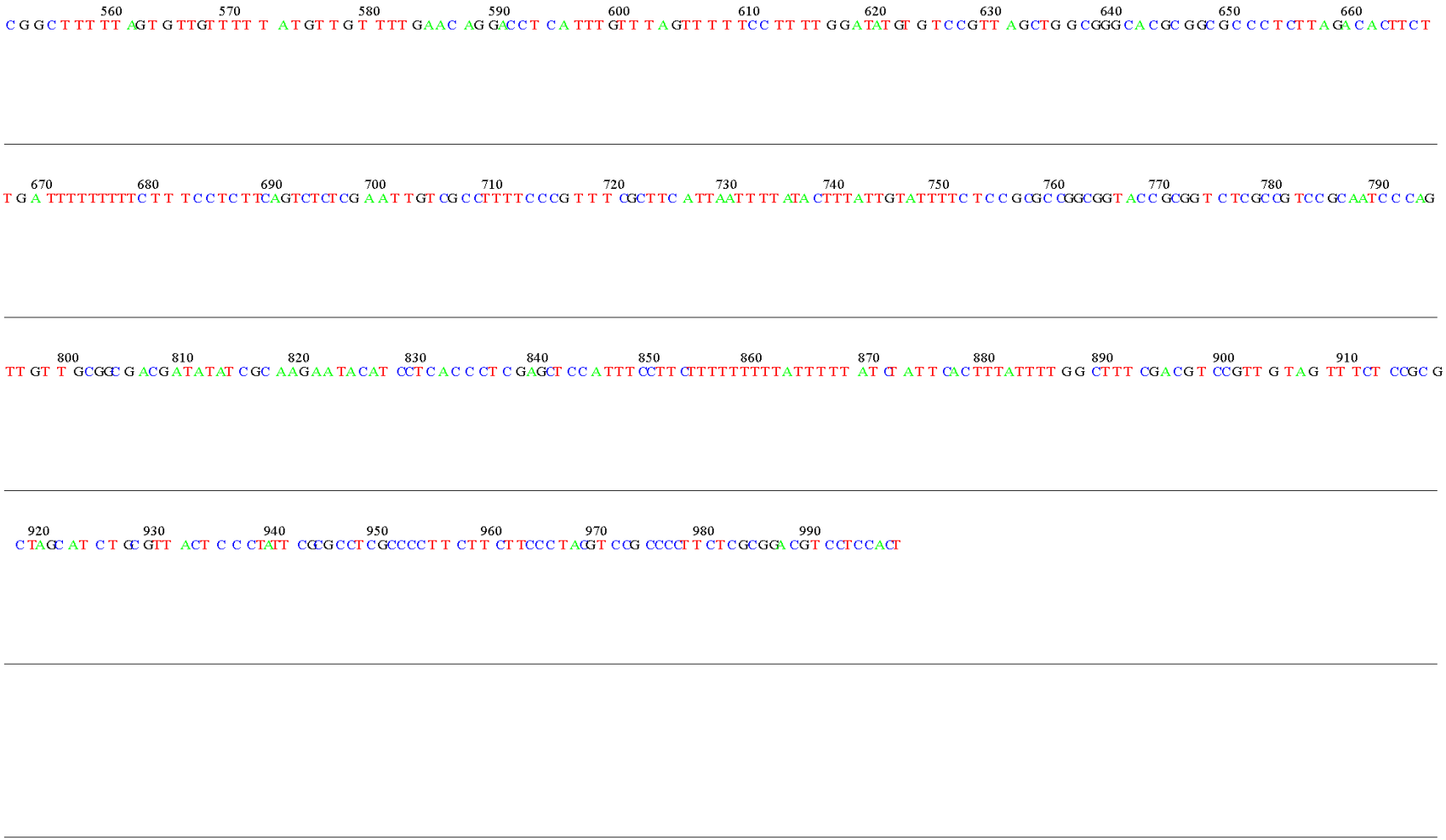
Medical History

6. Are you currently on hormonal contraception (pills)? ☐ Yes ☐ No
7. Do you have any intra-uterine device? ☐ Yes ☐ No
8. Have you ever been tested for HIV? ☐ Yes ☐ No
9. If YES, What is your HIV status? ☐ Positive ☐ Negative
10. Have you had any Sexually Transmitted Diseases in the past 5 years ☐ Yes ☐ No

Risk behavior

10. Do you use alcohol? ☐ Yes ☐ No
11. Are you using intravenous drugs? ☐ Yes ☐ No
12. At what age did you first engage in sex? ☐ <18 ☐ ≥ 18
13. How many clients do you have in a DAY? ☐ 1-3 ☐ 4 and above
14. How often do you use protection (female condoms) ☐ Consistently ☐ Occasionally ☐ Never
15. How often do your male partners use condoms (male condoms) ☐ Consistently ☐ Occasionally ☐ Never
16. How many years have you been a sex worker ☐ 1-5 ☐ 6 and above
17. Why are you a sex worker? .....

Appendix XI: Sanger sequencing results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800 810 820 830 840 850 860 870 880 890 900 910
T T G T T G C G G C C G A C G A T A T A T C G C A A G A A T A C A T C C T C A C C C T C G A G C T C C A T T T C C T T C T T T T T T T T T A T T T T T A T C T A T T C A C T T T A T T T T G G C T T T C G A C G T C C G T T G T A G T T T C T C C G C G
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C T A G C A T C T G C G T T A C T C C C T A T T C G C G C C T C G C C C C T T C T T C T T C C C T A C G T C C G C C C C T T C T C G C G G A C G T C C T C C A C T