BURDEN OF INFECTION AND GENETIC CHARACTERIZATION OF HUMAN HERPES VIRUS TYPE 8 IN HIV INFECTED INDIVIDUALS IN NORTHERN SOUTH AFRICA

By

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A Thesis Submitted in Fulfillment of the Requirements for the Award of the Degree of Doctor of Philosophy in Microbiology

To

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DECLARATION

I, Elizabeth Mashu Etta, hereby declare that this thesis for the award of a Doctor of Philosophy degree in Microbiology at the University of Venda has not been submitted before for any degree examination at this or any other University. It is my own work and design and execution, and all the reference materials contained therein have been duly acknowledged.

Signature …… **Date: January 2019**

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SUMMARY OF THE THESIS

Human herpes virus type 8 (HHV-8), also known as Kaposi's sarcoma associated herpes virus (KSHV), is the etiologic agent of Kaposi's sarcoma (KS), and AIDS related Kaposi's sarcoma (AIDS-KS). HHV-8 which is a member of the Herpesviridae family, exhibits extensive genetic diversity globally. In endemic regions, infection with HHV-8 occurs very early on in life, which is an indication of both environmental and vertical routes of transmission. The advent of HIV leads to the classification of an AIDS-KS defining condition in HIV infections. This suggests that in regions where HIV and HHV-8 are endemic, KS may become common in a mature HIV epidemic. Just like the prevalence of HIV in Northern South Africa is generally high as in most regions of the country, as the HIV epidemic matures in South Africa, it is important to understand the burden and distribution of HHV-8 infection, and the likely genotypes infecting the population. The main objective of the thesis was to establish the epidemiology and infecting genotypes of HHV-8 in Northern South Africa (Limpopo Province), where no data exists.

First, a systematic review of the literature was carried out for the entire African continent to determine the seroprevalence and genotype distribution of HHV-8 in all African countries (n=53). In this review, Sudan and South Sudan were considered as one country. Articles were searched using the PRISMA guideline and exported using an article grid. More than two-thirds (64%) of the studies reported on seroprevalence, 29.3% on genotypes; and 9.5% were on both seroprevalence and genotypes. About 45% (24/53) of the African countries had data on HHV-8 seroprevalence exclusively, and more than half (53%) had data on either seroprevalence or genotypes. Almost half (47%) of the countries had no data on HHV-8 infection. There was high heterogeneity in the types of tests and interpretation algorithms used in determining HHV-8 seropositivity across the different studies.

Generally, seroprevalence ranged from 2.0% in a group of young children in Eritrea to 100% in a small group of individuals with KS in the Central Africa Republic and a larger group of KS in individuals in Morocco. Approximately, 16% of all the studies reported on children. The difference in seroprevalence across the African region was not significant (95% CI, X^2 =0.86; p =0.35), although specifically, a relatively significant

level of infection was observed in HIV-infected children. About 38% of the countries had data on *K1* genotypes A, A5, B, C, F and Z which occurred at frequencies of 5.3%, 26.3%, 42.1%, 18.4%, 5.3% and 2.6% respectively. Twenty-three percent of the countries had data for *K15* genotypes, whereas genotypes P, M and N occurred at frequencies of 52.2%, 39.1% and 8.7% respectively. Data on HHV-8 inter-genotype recombinant is scanty. Our finding suggests that HHV-8 is endemic on the entire African continent, and in HIV endemic regions, but there is need for a harmonized testing protocol for better understanding of HHV-8 seropositivity. HHV-8 genotype A5 and B for *K1* gene and genotype P and M for *K15* gene are the most predominant genotypes in Africa. The review, for the first time, has provided information on HHV-8 burden on the entire African continent, and suggests that vaccine development efforts for Africa should focus on genotypes B and P.

The second component of the investigation focused on the burden of HHV-8 in an HIV population in Northern South Africa (Limpopo Province). Plasma from 3501 HIV infected individuals from 5 districts in Limpopo Province were assessed for antibodies to both the lytic antigen (ORFK8.1) and the latent antigen (ORF73). The distribution of infection was analyzed based on demographic, socioeconomic, and immunological parameters. Statistical inferences for significant differences were determined by Chi-square at a confidence interval of 95%. P-values less than 0.05 were considered significant. About 19.0% of the study population was positive for antibodies to either the lytic or latent antigens or both. Prevalence of antibodies to the lytic antigen was significantly higher than prevalence of antibodies to the latent antigen (17.3% vs 4.1%; p=0.0001). Significant differences were observed for age groups, racial population groups, districts and year of sample collection (p=<0.0001, p=<0.0001, p=<0.0001 and p=0.0385) respectively. Associations were found between both antigens in comparison to the different variables such as age group, racial population groups and districts (R^2 value ranging between 0.886 and 1.0). The burden of HHV-8 has now been established for the first time in Northern South Africa.

The third aspect of the investigation was a meta-analysis of HHV-8 seroprevalence in Southern Africa in order to understand the impact of geographical location (urban vs rural) on infection. The analysis revealed a significant association between urban settings and HHV-8 infection (p=0.0001).

The fourth component of the thesis examined the detection of HHV-8 antigen through polymerase chain reaction (PCR) in 534 participants in HIV infected and HIV non-infected populations. A selection of mouthwash DNA samples were subjected to Next Generation Sequencing (NGS) for subsequent genotype inference. Mouth wash samples were obtained from each consenting individual before eating or smoking, and their DNA was purified. A 233bp fragment of the ORF26 gene of HHV-8 was amplified by PCR. HHV-8 was detected in 150 of the 534 participants (28.1%). A significant difference in detection was observed for gender, HIV status, district and the level of education (p=0,0003; p=0.0094; p=0.0002 and p=0.0095) respectively. Consensus sequences were derived from NGS reads for 13 samples. The genotyping results revealed that genotype Q, B, E and N are the genotypes predominant in the study population. As such no mixed infections were detected.

Therefore, from the investigations foregoing have demonstrated for the first time the following: (1) HHV-8 is endemic in the entire African continent, which suggest a co-endemicity in regions already endemic for HIV; (2) HHV-8 is endemic in Northern South Africa; (3) Urban settings in Southern Africa are associated with high HHV-8 infection; (4) HHV-8 genotypes Q, B, E and N may be predominant in Northern South Africa, with B and P common on the entire African continent. Hence, studies should focus on the generation of full length HHV-8 genomes of the common genotypes to support the selection of genes for vaccine design and development.

SCIENTIFIC PRESENTATIONS

The following communications have emanated from this study:

Conference proceedings

Elizabeth M. Etta, Lufuno G. Mavhandu-Ramarumo, Denis M. Tebit, George Gachara, Miley Wendell, Vicky Marshall, Denise Whitby, Pascal O. Bessong. Kaposi's sarcoma associated herpes virus infection in an HIV infected cohort in northern rural South Africa. Limpopo DOH Research Day. Theme: Working together-setting the stage for evidence based planning.12 October 2018, Limpopo, Bolivia Lodge Polokwane (**Third prize oral presentation).**

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

^{0}C	Degree Celsius
%	Percentage
µl	Microlitre
AIDS	Acquired Immune Deficiency Syndrome
AIDS-KS sarcoma	Acquired Immune Deficiency Syndrome associated - Kaposi's
BLAST	Basic Local Alignment Search Tool
bp	base pairs
CMV	Cytomegalovirus
DNA	Deoxyribonucleic acid
EBV	Epstein-Barr Virus
ELISA	Enzyme-linked Immunosorbent Assay
FLIP	FLICE Inhibitory Protein
G-C	Guanine-Cytosine
GPCR	G-protein-coupled receptor
GSK	Glycogen synthase kinase
HAART	Highly active antiretroviral therapy
HHV	Human Herpes Virus
HHV-8	Human Herpes Virus type 8
HIV	Human immunodeficiency Virus
HSV	Herpes simplex Virus
HVS	Herpesvirus saimiri
ICTV	International Committee on Taxonomy of Viruses
IFA	Immunofluorescence Assay

IFN	Interferon
IgG	Immunoglobulin G
IL	Interleukin
IRF	Interferon regulator factor
ITAM	Immunoreceptor tyrosine-based activation Motif
KS	Kaposi's sarcoma
KSHV	Kaposi's sarcoma-associated Herpes Virus
LAMP	Latent antigen membrane protein
LANA	Latency-associated nuclear antigen
LUR	Long Unique Region
mA	Milliampere
mg	Milligram
ml	Millilitre
NCBI	National Center for Biotechnology Information
ng	nanogram
NJ	Neighbour Joining
nt	Nucleotide
MCD	Multicentric Castleman's Disease
MSM	Men who had sex with men
OR	Odd ratio
ORFs	Open reading frames
PBMCs	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PEL	Primary Effusion Lymphoma
PRISMA	Preferred Reporting Item for Systematic reviews and meta-analysis
PTEN	Phosphatase and tensin homolog

SNPs	Single Nucleotide Polymorphism
SPSS	Statistical package for the social sciences
TAT	Transcriptional transactivator
TNF	Tumour necrosis factor
TR	Terminal Repeats
UV	Ultra-violet
V	Volt
VOS	Viral Oncology Section
VZV	Varicella Zoster Virus

CHAPTER ONE

GENERAL INTRODUCTION

1.1 INTRODUCTION

Kaposi's sarcoma associated herpesvirus (KSHV) is also called human herpesvirus type 8 (HHV-8). However, in this thesis the acronym HHV-8 will be used to refer to the virus. It has been established that HHV-8 is the etiological agent of kaposi's sarcoma (KS), which is characterized by proliferating spindle cells of endothelial origin and formation of excessive uncontrollable blood vessels (Chang et al., 1994; Abere et al., 2017). Since its discovery, the virus is estimated to have infected millions of people. In immuno-competent individuals, the signs and symptoms may be mild or remain asymptomatic whereas it causes high rates of morbidity and mortality in immunocompromised individuals. A clear majority of these HHV-8 infections occur in low-income and middle-income countries (resource-limited countries). Sub-Saharan Africa, which is classified as a resource-limited region, is considered the region most severely hit by HHV-8 infection, with a prevalence range between 2% to 90% (Enbom et al., 1999; Whitby et al., 2004).

HHV-8 is a human gamma herpes virus and has been frequently detected in saliva, plama, peripheral blood mononuclear cells (PBMC), semen and biopsy from patients with all forms of Kaposi's sarcoma (KS) disease (Schalling et al., 1995), as well as in specific types of AIDS-associated lymphomas (Cesarman et al., 1995; Soulier et al., 1995). KS has been recognized for a long time as being associated with HIV infection and has been classified as an AIDS defining illness. HHV-8 has been established as the causative agent for Kaposi Sarcoma (KS); Primary Effusion Lymphoma (PEL) and Multicentric Castleman Disease (MCD); and malignancies occurring more frequently in AIDS patients (Cesarman et al., 1995; 1996; 2001). HHV-8 infection is endemic among adult populations in Africa. The prevailing view is that childhood transmission is primarily responsible for the high seroprevalence of HHV-8 among adults that is observed throughout the continent. In the AIDS population, HHV-8 was implicated as the etiological agent of AIDS-associated Kaposi's sarcoma (AIDS-KS) (Chang et al., 1994).

What is worth noting is that equatorial Africa has the highest incidences of KS in the world, thus earning the name 'KS belt' (Dollard et al., 2010). The seroprevalence of HHV-8 varities are based on geographic and environmental conditions. That is 30-40% of individuals infected with HIV in North America to above 60% in Sub-Saharan

Africa (Dedicoat et al., 2003; Ablashi et al., 1999).

Furthermore, characterization of HHV-8 viral strains has been focused on tumor biopsy and saliva samples from KS patients. Tumor biopsies and mouth wash samples are convenient sources of viral DNA, as they have a high viral load. There is limited knowledge on the genetic characterization of the virus in the Northern province of South Africa. Hence, it is of great importance to characterize HHV-8 isolates. The most sensitive approach, Next Generation Sequencing(NGS) was used to characterize HHV-8, ORF 26 gene. Analyses obtained in this research have provided data on the infecting genotypes in Northern South Africa.

1.2 STUDY RATIONALE

The scope of infection of HHV-8, and its subsequent transmission at the population level is related to human and environmental risks factors (Biryahwaho et al., 2010). Transmission of HHV-8 through saliva, sexual secretions, and blood transfusions have been determined (Minhas and Wood, 2014). It is therefore plausible that HHV-8 is present in all African countries and even endemic across the continent. Data from this premise supports continent wide efforts in stemming the spread of HHV-8 infection. Studies from South Africa have demonstrated the presence of HHV-8 in the country (Bourboulia et al., 1998; Sitas et al., 1999; Wilkinson et al., 1999. More recent studies have shown a relatively high HHV-8 seroprevalence that is as high as 44% in some regions of the country. As most of these studies on seroprevalence were limited in scope and diversity of the study population, this restricted the interpretation even within the entire region of the country where the study was carried out.

And yet, one of the motivation for this study was to establish serological and molecular laboratory techniques that can routinely be used to unravel the biological, pathological and epidemiological properties of this virus as different aspects of this study was aimed at gaining a better understanding of the epidemiological patterns of HHV-8 in the Northern South African populations.

In addition, with the knowledge that HHV-8 causes KS, and that KS is an AIDS defining illness, it is important to establish the burden and distribution of HHV-8 in regions such as the Northern region of South Africa (Limpopo Province), where HIV is highly endemic, more especially since no data on HHV-8 exists in this part of the country. So

to get to grip with the stated point, all the districts in the Limpopo Province were brought into focus. This type of information would potentially allow the modeling of the future burden of KS in a country where HIV is endemic, and in which infected individuals are expected to live much longer than previously because of the universal access to antiretroviral therapy.

Nevertheless, data on the genetics of any pathogen is key to the selection of genes for vaccine design and development efforts. Therefore, a fundamental description of the infecting genotypes of HHV-8 in Northern South Africa should be a significant contribution to the scientific resource. In this regard, the application of genomic platforms, such as NGS that provide the opportunity for a detailed examination of viral sequences, could afford the possibility to identify mixed genotypic infections, as well as minority populations, which may otherwise be missed in a population-based sequencing approach (Arsenic et al., 2015).

1.3 HYPOTHESES

1. Since risk factors for HHV-8 acquisition exist in all societies, and data in Africa is scanty, it was therefore hypothesized that HHV-8 is generally prevalent in all African countries.

2. From the findings of relatively high HHV-8 prevalence from other parts of South Africa, it was hypothesized that there is a high prevalence of HHV-8 across Northern South Africa irrespective of location, gender, age, HIV status and ethnicity.

3. On the basis of high HHV-8 prevalence, it was hypothesized that many genetic variants are responsible for the infection across Northern South Africa.

1.4 RESEARCH QUESTIONS

1. What is the distribution and level of infection of HHV-8 in Africa?
2. What are the most common infecting genotypes of HHV-8 in Africa?

3. What is the burden, distribution and infecting genotypes of HHV-8 in Northern South Africa?

1.5 STUDY OBJECTIVES

The overall goal of the study was to describe the seroprevalence and genetic genotypes of HHV-8 in Africa, and to establish the burden of HHV-8 infection in Northern South Africa.

1.5.1 SPECIFIC OBJECTIVES

The specific objectives were to:

1. To systematically describe the epidemiology of HHV-8 in Africa from 1998 - 2018.
2. To determine the distribution of antibodies to latent (ORF73) and lytic (K8.1) antigens of HHV-8 in Northern South Africa.
3. To perform a meta-analysis on the association between HHV-8, HIV seropositivity and geographical settings in Southern Africa.
4. To determine the infecting genotypes of HHV-8 in Northern South Africa.

1.6 INVESTIGATIONS PRESENTED IN THE STUDY

The subsequent chapters presented in this study deal with different investigations on HHV-8.

Chapter 1 presents the general introduction of the study, study rationale, objectives, hypothesis, research questions and ideas of the investigations that are presented in the individual chapters of the study.

Chapter 2 reports on the background and biology of HHV-8.

Chapter 3 reports on the systematic review of HHV-8 in Africa. This comprises of all the countries on the continent (n=53).

Chapter 4 presents data on the seroprevalence of HHV-8 from all 5 districts of the Limpopo Province of South Africa (Capricorn, Waterberg, Vhembe, Mopani and Sekhukhune districts).

Chapter 5 presents the meta-analysis of the association between HHV-8 seropositivity and geographical settings within the Southern African countries, in contrast to data obtained in chapter 4 (Northern South Africa), focusing on rural and urban settings.

There is high variability within the HHV-8 genome. In this vein, **Chapter 6** presents data on the genotypes from Northern South Africa using a minor lytic gene, while applying a modern and advanced sequencing technology (Next Generation Sequencing).

Each chapter (3-6) consists of abstract, introduction, experimental procedures, results, discussions, conclusions and references. Major findings of the thesis, and recommendations for future investigations are presented in **Chapter 7**.

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CHAPTER TWO

BACKGROUND AND THE BIOLOGY OF HHV-8

2.1 Discovery of human herpes virus 8

This virus was named after the tumor it causes, Kaposi's sarcoma. HHV-8 was discovered by Dr. Moritz Kaposi, a Hungarian dermatologist. It was termed idiopathic multiple pigmented sarcoma of the skin. Later primary effusion lymphoma and multicentric castleman's disease were also described (Kaposi, 1872; Boshoff, and Weiss, 2002). In 1984, there was still uncertainity whether KS is caused by an infectious agent. However, this was later confirmed by the presence of herpes-type virus in tumor specimens (Walter et al., 1984). Globally, prior to the HIV/AIDS epidemic, KS was generally rare and even insignificant in various African regions including the sub-Saharan African region. This is because HHV-8 infection on its own can not develop into KS. The ability for HHV-8 developing into KS was thought to be influenced by the immunosuppression status in the host (Cesarman et al., 1995; Pellet and Roizman, 2007). Thus, the pursuit of the virus linked to KS led to the discovery of two small fragments of DNA (KS330Bam and KS631Bam) present in almost all AIDS-KS patient samples. These fragments had DNA sequences peculiar to KS biopsis from AIDS-patients as compared to individuals without AIDS. Figure 2.1 shows a representative phylogenetic relationship, illustrating the different classification of herpes viruses and their similarities.

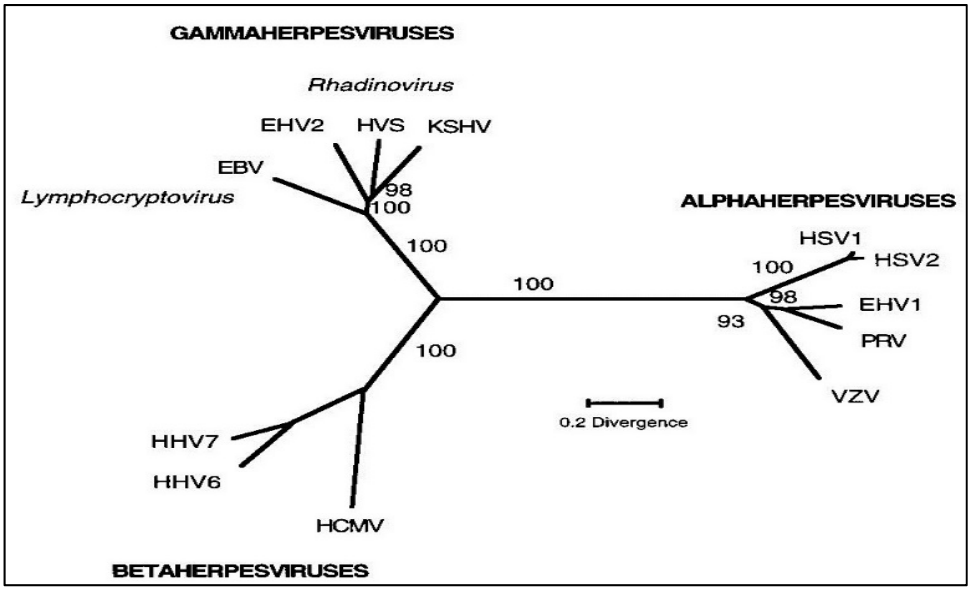

Figure 2.1: Classification and phylogenetic relationship of herpesviruses. The phylogenetic tree clearly shows those viruses which are alpha, beta and gamma herpes, constructed at 100 boot strap replicates (adapted from Moore et al., 1996a).

2.2 Classification of herpes virus

Herpesviruses are large families of enveloped, linear double-stranded DNA with relatively large complex genomes of about 100-300nm in size. Herpesviruses consist of a linear genome encoding with approximately 200 genes. Their sequences vary according to family and species type. They encode a variety of enzymes, which are involved in DNA and protein synthesis, as well as nucleic acid metabolism. Herpes was derived from a Greek word “Herpein” which means to creep. Herpesvirus infections are usually endemic, with sexual contact as a common mode of transmission. In fact, other transmission routes have been described. According to The International Committee on Taxonomy of Viruses (ICTV), herpes viruses are classified under *Herpesvirales* and family, which are further classified into 3 subfamilies: *Alphaherpesvirinae*; *Betaherpesvirinae*; and *Gammaherpesvirinae.* Eight herpes viruses have been discovered and these establish latent infections in both vertebrate and invertebrate species (see figure 2.2). These classifications are based on their physical and biological properties, genomic structure, genomic organization, sequence similarity, presence of glycoprotein B gene or from sequence analysis of the DNA polymerase gene (Davison et al., 2009).

The *Alphaherpesviruses* are a subfamily of *Herpesviridae*. They are primarily differentiated by their rapid reproductive capacity, with the ability of reproducing in a variety of host tissues. They also have an extremely short growth cycle, although they cause latent infection. Examples include: herpes simplex viruses 1 and 2 (HSV-1 and HSV-2) and varicella zoster virus (VZV).

The *Betaherpesviruses* are also a subfamily of *Herpesviridae*. They are primarily classified by their long reproductive life cycle, marked by a slow infection rate. They are restricted to a few host cells such as the kidney and the secretory gland. They cause latent infection. Examples include: cytomegalovirus or herpesvirus 5 (HCMV and HHV-5), human herpesvirus 6 and 7 (HHV-6 and HHV-7). An alternative name for HHV-7 is roseolavirus. This name was derived from the disease roseola infantum, which is common in children (Knipe and Howley, 2001).

There is another subfamily known as *Gammaherpesviruses* of *Herpesviridae*, which are further subdivided into two genera: lymphocrytovirus (LCV) and Rhadinivirus

(RDV). They establish both latent and lytic infections. They utilize host range characteristics of the natural host and they infect cells such as B or T cells. HHV-8 is the only RDV virus. Examples include: EBV (Epstein Barr virus) and HHV-8 (human herpes virus 8).

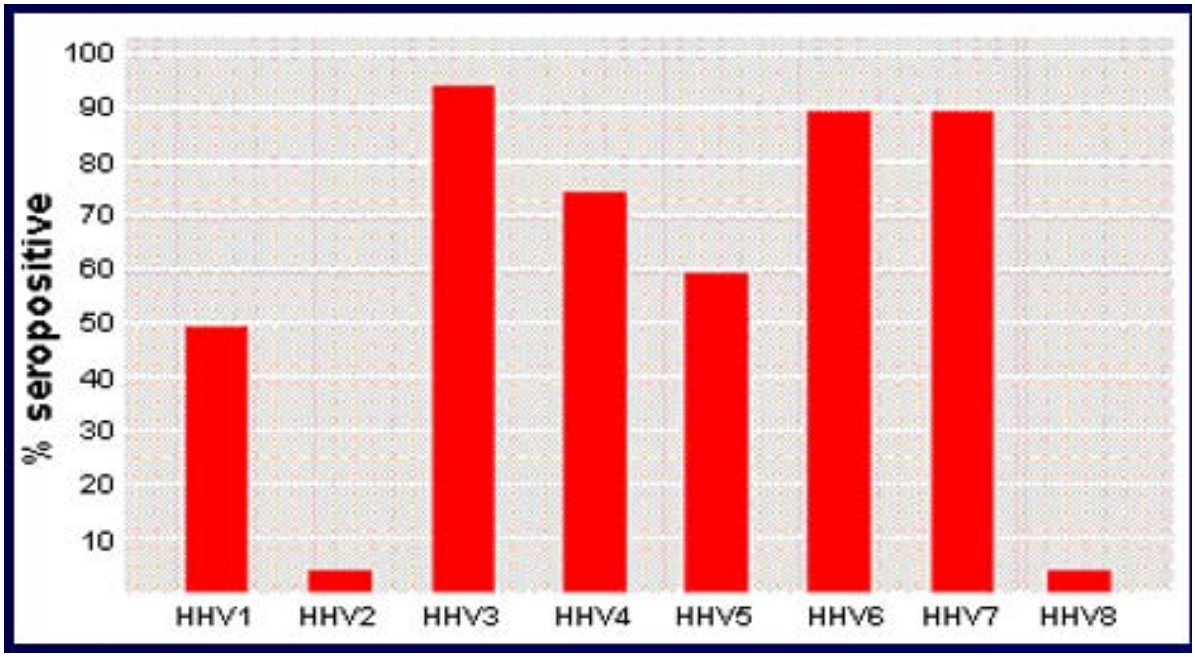

Figure 2.2: Seropositive percentages of herpesviruses (adapted from Julis Solomon, www. Brown.edu, 2018).

Kaposi's sarcoma has been classified into four different types (see figure 2.3):

(a) Classic KS, which affects elderly men in the Meriterranean.

(b) Endemic KS, which affects individuals mostly based within Western and Central Africa.

(c) Transplant-associated or iatrogenic KS, mostly common in drug users

(d) Epidemic KS also called AIDS-KS, is the most common AIDS defining cancer, present in men who have sex with men (MSM).

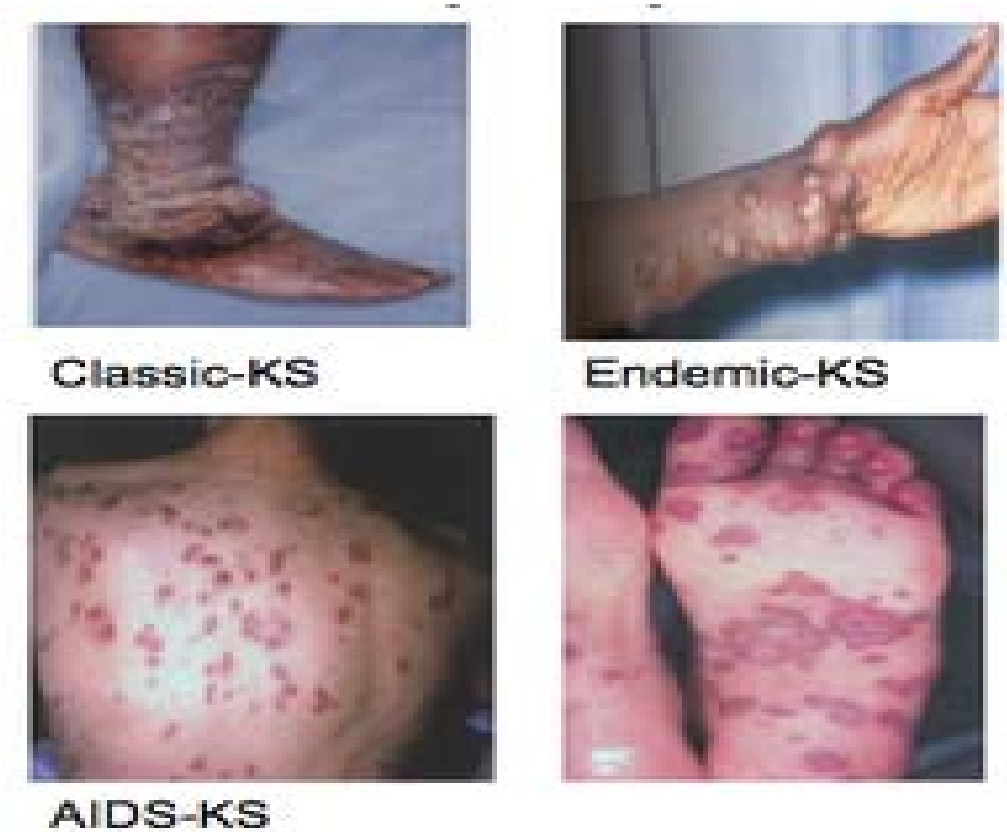

Figure 2.3: Different types of Kaposi's sarcoma (adapted from PI.wikidoc.org, 2018).

2.3 Virology of HHV-8

HHV-8 is an oncogenic gamma 2 herpes virus and its central core is embedded in a proteinaceous spindle (Sarid et al., 1998). HHV-8 has a common viral structure, consisting of the envelope, tegument, capsid and finally the core (Mettenleiter, 2002) (see figure 2.4A) Its viral tegument is protected by a lipid envelope membrane and the envelope is the outer layer of the virion containing many unique viral glycoproteins. During attachment and cell entry, these glycoproteins are used. The tegument is distributed asymmetrically between the envelope and the capsid and its thickness varies depending on the location of the virus within the infected cell. The DNA is enclosed by an icosahedral capsid of approximately 100nm in diameter with about 162 capsomeres. The capsid is surrounded by an amorphous protein coat called tegument, which contains approximately 30 viral proteins. The exterior of the tegument is surrounded by the lipid bilayer envelope (Sarid et al., 2001; Owen et al., 2015) (see figure 2.4B).

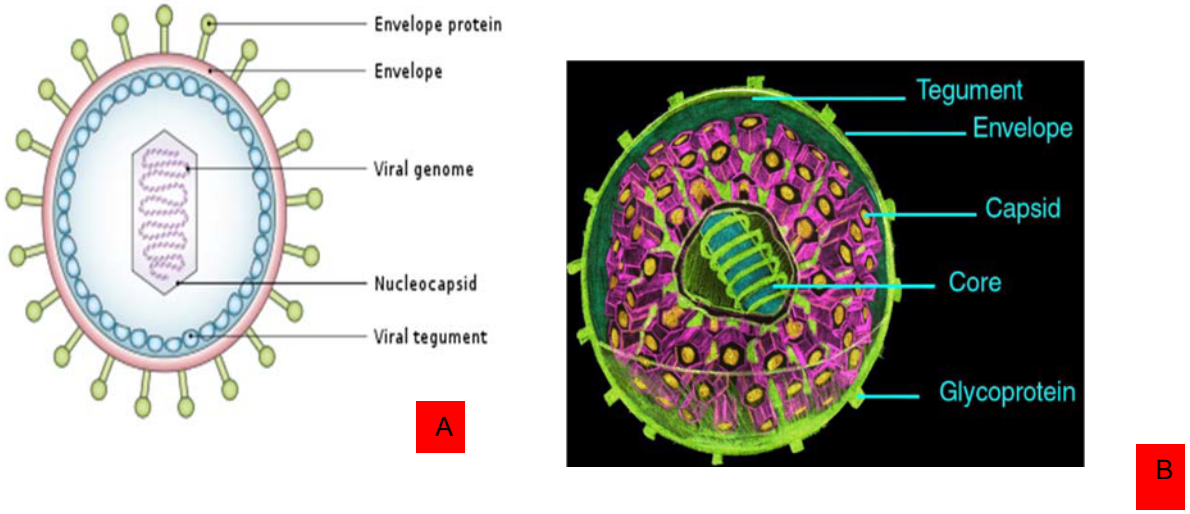

Figure 2.4: Structure of a herpesvirus particle. All herpesvirus family members show the same general structure. **A** shows the nucleocapsid core which protects the DNA, while **B** shows capsid which provides viral proteins for the virus (adapted from http://stdgen.northwestern.edu/stdgen/bacteria/hhv2/herpes.html).

The genome of herpesvirus is characterized according to the basis of the base composition, size, and structural arrangement. The length of the Herpesvirus dsDNA genome ranges from 140 to 160 kilobases (Kb), with over 140.5 kilobases in the central long unique region (LUR) which is flanked by multiple terminal repeat (TR) sequences (20-30kb sequences) at both ends of the genome (Moore and Chang, 2001). HHV-8 is closely related in gene content with other members of the genus (Ouyang et al., 2014), although there are variations within the genome. The variation in the genome sizes of isolates is as a result of the number of TRs the genome has and this ranges from 16 to 75 (Duprez et al., 2007). The overall Guanine-Cytosine (G-C) content in the LUR and TR is 53.5% and 84.5% respectively. There is also 59% G/C rich content base pair composition on average across the entire genome, with a variation of this composition at particular areas across the genome (Knipe et al., 2001). Within the LUR, there are 95 open reading frames (ORF), of which over 60 ORFs are homologous to other rhadinoviruses. ORF50 (Replication Transactivation Activator) is the primary viral protein responsible for the switching from the latent stage to the lytic stage (Kedes et al., 1996), and ORF25 encodes novel proteins not observed in other human herpesviruses.

Genes of HHV-8 can be grouped into three namely; common genes (ORF26, ORF73), cellular-homologous genes (ORF16, ORF71); and unique genes (K genes). Open reading frames are found in long unique regions of the genome (Neipel et al., 1997). *K1* genes are the most hypervariable glycoprotein gene within the genome, with a size of approximately 15kb.Transcription experiments with alternative splicing reveal new genes of HHV-8 with similar functions (Pfeiffer et al., 2004; Gottwein, 2012). The major difference between HHV-8 and the other herpesviruses is that, it encrypts macrophage, cytokines such as interleukin-6, inflammatory proteins, interferon regulatory factors and the regulatory genes such as cyclin D and G-protein coupled receptor which are used during the life cycle.

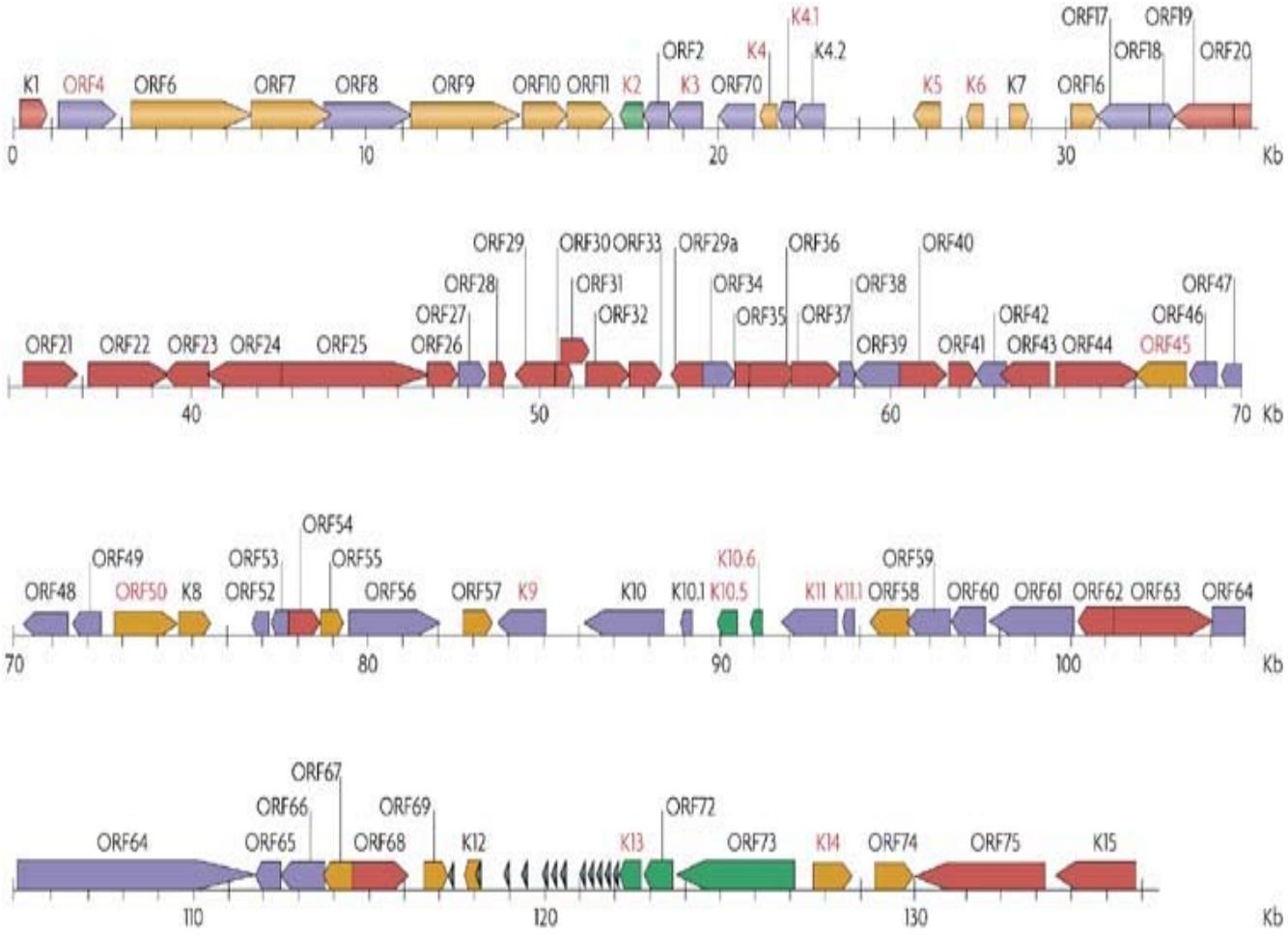

Figure 2.5: Genetic organization of HHV-8 genome. (adapted from Coscoy, 2007).

2.4 Biological properties of HHV-8

There are four well defined biological properties identified for HHV-8. Firstly, HHV-8 has been shown to express a large number of enzymes involved in nucleic acid metabolism, DNA synthesis and processing of proteins (Pellet and Roizman, 2007). Secondly, replication of HHV-8 occurs within the nucleus. Therefore, HHV-8 takes advantage of the host's transcription machinery and DNA repair enzymes to support a large genome with complex arrays of genes. The genes are characterized as either essential or dispensable for growth in cell culture. The essential genes regulate transcription and therefore, the assemble of the virion while the dispensable genes enhance the cellular environment for virus production that is necessary for viral defense from the host immune system and to promote cell-to-cell distribution (Pellet and Roizman, 2007).

The third property of HHV-8 is that it produces viral infections which lead to host cell destruction. Fourthly, HHV-8 can remain in a latent state within the host cell and reactivates following cellular stress such as co-infection with other viruses. Herpesvirus latency involves stable maintenance of the viral genome in the nucleus with limited expression of a small subset of viral genes. Due to persistence of the latent infections and asymptomatic shedding of the virus, HHV-8 prevalence varies by population groups and has an uneven geographical distribution (Ablashi et al., 2002; Pellet and Roizman, 2007). HHV-8 is also shed asymptomatically in saliva and can be transmitted horizontally.

2.5 Molecular epidemiology of HHV-8 genotypes and variants

The genome of HHV-8 displays a high level of genetic diversity and the clustering of genotypes at certain loci which is similar to other human herpes viruses. Moreso, the diversity observed in HHV-8 is higher than what is found in other human herpesviruses. With this great diversity, there is a need to better understand this phenomenon by providing explicit information on the evolution of the genome over a short period as well as the biology, and pathogenesis of diseases associated with HHV-8 (Hayward and Zong, 2007). HHV-8 encodes broad genetically diverse K-genes (*K1-K15*). *K1* alleles are more diverse and unique in relation to geographic distribution. All the genes of HHV-8 have been studied, but the most studied are ORF-*K1* and ORF-*K15*. These two genes are the most diverse and are located at the 3’ and 5’ termini of

the genome respectively. *K1* has been better studied and has shown to have more variation than *K15*.

The HHV-8 genome is classified into different strains through sequencing the ORF-*K1*, *K15* and ORF26 genes (diverse and conserved genes of the genome) (Zong et al.,1999; Tornesollo et al., 2010). HHV-8 strains labelled genotypes A – E, M, N, P, Q and Z have been identified world wide (Cassar et al., 2007; Zhu and Paul 2008). Genotype A is common in North America and the Western part of Europe, wherea Genotype A5 is common in Africa (Kouri et al., 2005). Genotype B is common in Africa and in those who do not live in Africa but are of African origin (Kajumbula et al., 2006). Genotype C has also been described in South America and Asia. Genotype D is the least described and has been identified in the Brazilian Amerindians of different tribes (Ishak et al., 2007). Genotype E is common in the South American population (Boshoff and Weiss, 2001). Genotype N is a novel genotype which has been described in South Africa (Isaac et al., 2015). Genotype Z has been described in Zambia for the first time in child population (Kasolo et al., 1998).

The genotypes are further divided into different variants. Genotype A has the variants A1 to A5; genotype B has B1 to B4 variants; and genotype C is subdivided into variants C1 to C6. All these variants are globally distributed. But the other genotypes, variants have not been described as yet.

Furthermore, preliminary studies have shown that the most common circulating genotypes for *K1* are A, A5, B, C, D, E, F and Z with a variability of about 30%. *K1* has two variable regions of VR1 and VR2 (Biggar et al., 2000). Studies have been carried out in some provinces in South Africa. A recent study in Cape Town, South Africa by Isaac and colleagues (2015), showed that the most dominant genotypes for *K1* were A5 and B2. Among all the genes of HHV-8, ORF 26 gene is one of the most conserved gene, with polymorphisms being detected within this region of the genome (Zong et al., 1997). In 2010, Tornesello and colleagues reported the diversity of ORF26, with 9 genotypes detected; (A/C, B1, B2, D/E, J, K/M, N, Q, R). Most of these genotypes cluster within geographic origins of samples and also show strong linkage to *K1* genotypes. For example, B2 genotype of ORF 26 is linked to B3 and A5 of *K1* genotype.

2.6 Life Cycle of HHV-8

HHV-8 has two main phases in its life cycle which are: latent and lytic phases. The virus establishes a lifetime latent infection in particular cells within the host with intermediate lytic replication. In 2013, Pellet and Roizman reported on latency of HHV-8 for the 3 herpes viruses, occurring in different cells within the host. The production and development of HHV-8 infection is established by the interaction between the virus, host cells and host immune system. Viral glycoproteins, such as gb, K8.1, ORF4, and attachment receptor, such as heparin sulfate are used by the virus to attach and gain entry into the host cell (Birkmann et al., 2001). When the virus enters the host cell it is transported to the nucleus where episome formed through viral DNA circularizes and is later transported to lymphocytes.

Furthermore, endothelial cells, monocyte-derived dendritic cells (MDDC) and monocytes, acting as antigens presenting cells (APC) serve as conduits for the entry of HHV-8 into the host (Kerur et al., 2010; Akula et al., 2002). Hahn et al (2012), demonstrated that ephrin receptor tyrosine kinase A2, which interacts with viral glycoprotein L and H dimer on heparin sulfate-negative is a receptor for HHV-8. The reason for the broad range of cellular targets of HHV-8 might be due to the ubiquitous nature of heparan sulfate expression on host cells (Campbell et al., 2014). No viral particle is produced in lymphocytes and a viral antigen called latency-associated nuclear antigen (LANA), which expresses different proteins such as vFIP, vCYC, Kaposins, helps the virus to resist any host immune pressure, providing an environment for lifetime latency. During the latent stage, HHV-8 genome exists as an episome in the nucleus of the infected cells and not as a provirus (Cesarman et al., 1995). The episome is maintained by the latent gene, latency-associated nuclear antigen (LANA).

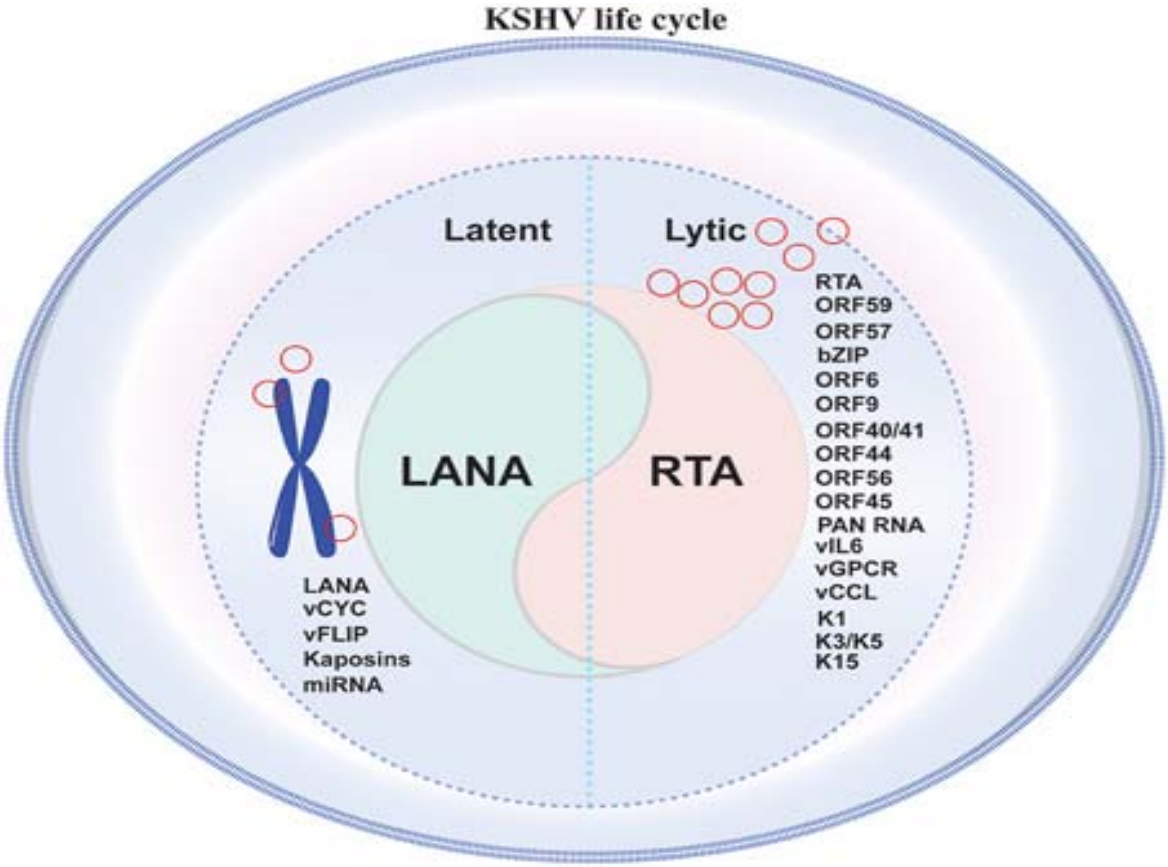

Figure 2.6A: Life cycle of HHV-8, illustrating the genes needed for long life infection (latent and lytic stages) (adapted from Birkmann et al., 2001).

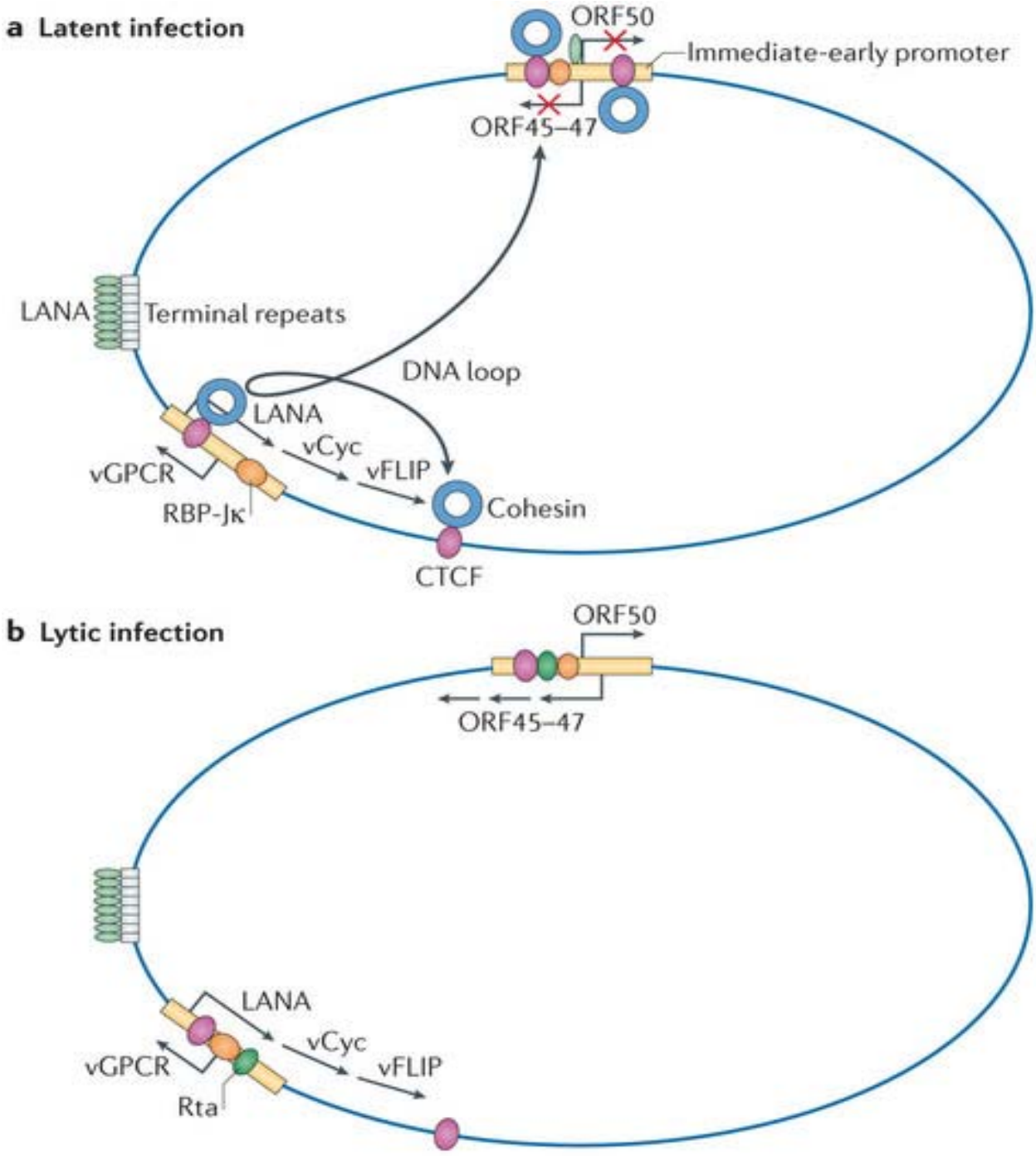

Figure 2.6B: Illustration of latent and lytic cycle (adapted from Birkmann et al., 2001).

2.7 HHV-8 Epidemiology

Globally, there is great difference of HHV-8 prevalence among countries. The lowest prevalence of less than 3% has been observed in America, European population and the Northern part of Africa. Most of the population infected are men who later develop KS. In this population children are not infected.

In Africa and the Mediterranean where the infection is ubiquitous, children and adults across all age groups are highly infected (Malope et al., 2007). HHV-8 is very common in African regions were HIV-AIDS associated with KS is very common (Olsen et al., 1998).

Furthermore, in African countries, the seroprevalence of HHV-8 has been described as being increased from child birth to adulthood (Malope et al., 2007; Newton et al., 2017). This suggests transmission of HHV-8 between mothers and their children and other close members of the family.

2.7.1 HHV-8 Epidemiology in Non-African countries

In the American, European and Asian populations, HHV-8 appears to be limited, as it ranges from 3-7% in Hungary, Switzerland, United Kingdom, France, Spain and Germany. But in HIV-infected individuals, it ranges from 16% in women in Germany to 31% in UK's homosexuals. In Greece and Italy, prevalence from blood donors was 35% (Preiser et al., 2001; Juhasz et al., 2001; Koelle et al., 1997). In contrast, moderate prevalence of approximately 15 - 25% is observed in Mediterranean regions where classic KS is prevalent (Chironna et al*.,* 2006; Cattani et al*.,* 2003). HHV-8 seroprevalence ranges from 13% to 48% amongst four regions in the North-Western part of China (Dilnur et al., 2001). A prevalence of less than 5% has been reported in Saudi Arabia (Almuneef et al., 2001).

2.7.2 HHV-8 Epidemiology in African countries

As such in this section, just a highlight of HHV-8 epidemiology will be reported. Details of HHV-8 in Africa in the form of a systematic review is fully decribed in chapter 3. Africa is greatly affected by HIV/AIDS with a high prevalence reported in Southern African countries. Sub-Saharan Africa is one of the regions in the world with a high prevalence of

HHV-8, with adults and children most infected (Mbondji Wonje et al., 2013; Malope et al., 2007; Dedicoat and Newton, 2003). Higher HHV-8 seroprevalences of up to 90% have been reported in Southern African countries: South Africa; Malawi; Mozambique; Zambia; Botswana and Zimbabwe (Olsen et al., 1998; Wojcicki et al., 2004; Malope et al., 2007; Campbell et al., 2009; Dollard et al., 2010). In South Africa, it has been estimated that approximately 5 per 1000 population are at risk every year in association with the AIDS epidemic (Cook et al., 1999). There are very few studies describing the seroepidemiology of HHV-8 in Central African populations. However, in Cameroon, a high seroprevalence has been reported of HHV-8 (Jacky et al., 2015).

Varying HHV-8 seroprevalences have also been described in North African countries. With high HHV-8 seroprevalence described in Egyptian children (Andreoni et al., 2001). Furthermore, a lower HHV-8 seroprevalence was reported in Tunisian children, pregnant women and blood donors respectively (Hannachi et al., 2012).

Varying HHV-8 seroprevalences has also been described in East and West African countries. High HHV-8 seroprevalence have been reported in Kenyan men and Ethiopian immigrants (Margalith et al., 2003; Lemma et al., 2009). In West Africa, high HHV-8 seroprevalences have been reported in pregnant women from Burkina Faso (Collenberg et al., 2006). HHV-8 seroprevalence was 23% in Ghananian HIV negative blood donors, but was higher (66%) in HIV infected individuals (Adjei et al., 2008).

2.8 Transmission of HHV-8

The transmission patterns of HHV-8 remain unclear and are part of a continuous argument world wide. There exists a general consensus that HHV-8 transmission is mainly transmitted through saliva among all age groups and populations. However, the manner in which this virus is acquired is still not clear. Saliva has also been reported as the possible route by which men who have sex with men acquire the virus (MSM).

In countries where HHV-8 infection and KS are common, sexual ransmission route has been defined amongst bisexual and homosexual populations. To support this fact, numerous studies globally have showed a strong association between HHV-8 and sexual

behaviour (Martin et al., 1998; Engels et al., 2007).

On the other hand, reports have also showed that the virus is not sexually transmitted. Non sexual transmission route can be the only explanation for an increase of HHV-8 infections noted in children and adolescents (Whitby et al., 2000; Mbulaiteye et al., 2008). Some possible non-sexual routes of HHV-8 transmission in children include: horizontal and vertical transmission from an infected mother to her child, water sources, intra and extra familial person-to- person transmission and oral transmission (Mbulaiteye et al., 2005). In 2004, Brayfield reported on the transmission route through oral body fluids such as saliva, as well as through colostrum and breastmilk, though the detection rate was not significant.

Speaking of non-sexual route of transmission, it is worth noting that studies focused on sexual practices amongst MSM. A study by Bagni and Whitby (2009), proposed that salivary exposure during oro-anal or oro-genital sex may be the possible route of HHV-8 transmission in this group of individuals. Additionally, a study by Bulter et al (2009) reported an 87% HHV-8 infection from a population of men who were applying saliva as a lubricant during anal intercourse.

2.9 Laboratory Detection of HHV-8

A number of serological and molecular assays for the detection of HHV-8 in blood, body secretions and body tissues have been grooved. The techniques can be used for the detection of antibodies in selected proteins, as well as the entire HHV-8 genome. Seroepidemiological studies used Immuno Fluorescence Assay (IFA) (Kedes et al., 1996; Inoue et al., 2000), immunoblotts (Zhu et al., 1999) or Enzyme Linked Immuno-Sorbent Assay (ELISA) (Mbisa et al., 2010) for the detection of HHV-8 antibodies to lytic K8.1 and latent ORF73 proteins (Lang et al., 1999). Western blot and Immunohistochemistry (IHC) (Mbondji-wonje et al., 2014; Pereira et al., 2013). Using more than one serological assay is considered as most appropriate as they increase sensitivity and specificity in the detection of HHV-8 infection (Topino et al., 2001). The primary method for the molecular detection of HHV-8 is through Polymerase Chain Reaction (PCR), be it conventional or real time PCR. Furthermore, detection of HHV-8 in the population at high risk of KS

requires a more sensitive and less expensive method to enable rapid diagnosis.

2.10 Prevention and treatment of HHV-8

Treatment for HHV-8 or KS includes combined chemotherapy such as Vincristine, Bleomycin and Doxorubicin, radiation therapy, surgery and biological therapy which will effectively dissolve lesions be it localized or widespread, compared to a single HAART drug (Dedicoat et al., 2003; Holfmann et al., 2017; National Cancer Institute, 2017).

There are five approved agents used to inhibit HHV-8 replication. They include: alitretinoin gel, liposomal doxorubicin, glanciclovir, valganciclovir and HAART. Treatment is the only means of prevention. To date, there is no vaccine nor cure for HHV-8, but there are ongoing trials for vaccine design. Furthermore, a study by Casper et al., 2008 reported that, valganciclovir when administered orally effectively inhibits mucosal HHV-8 replication.

2.11 Risk factors and transmission of HHV-8 infection

Although Kaposi's sarcoma (KS) was described in 1872, there was no association between the disease and HHV-8 until 1994 (Chang et al., 1994). There is still no conclusive report on risk factors for HHV-8 infections in the healthy population. However, associated risk factors could be of genetic and/or environmental origin (in relation to behavioral, cultural, or social conditions). Kaposi's sarcoma is a slow progressive cancer of endothelial origin which is dependent on immune modulation, replication of virus and inflammatory cytokines produced by the infected endothelial as well as the immune cells (Knowlton et al., 2014). HHV-8/KS endemic regions such as Africa and Mediterranean regions, have more KS partners who are male. Reports have shown that this virus is transmitted through close contacts (deep kissing) with an HHV-8 infected person (Sheblet al., 2013; Pedergnana et al., 2012). Risks of KS are influenced by high HHV-8 viral loads, high HHV-8 titres, high HIV RNA load and other host factors not well understood yet (Quinlivan et al., 2002). Individuals at high risk of HHV-8 infection are: HIV/AIDS patients; infected caregivers; procedures involving organ transplantation; infected mothers; household family relatives; as well as men having sex with men (Begré et al., 2016; Wang

et al., 2016; Shebl et al., 2013). This is so because these individuals are immunosuppressed.

In 1996, Whitby and colleagues reported on a high risk of KS when HIV seroconversion precedes HHV-8 seroconversion or when the HIV/HHV-8 co-infected individual becomes more immunosuppressed. Furthermore, in an HHV-8 infected individual, there are some co-factors which can lead to the development of KS. These factors include: biting flies e.g. malaria parasite; volcanic soil; age; ethnicity; parasitic infections; phorbol ester plants; household behaviours; high socio-economic status and environmental factors (Wakeham et al., 2011; Whitby et al., 2007; Mbulaiteye et al., 2005).

Primary effusion lymphoma (PEL), also called body cavity-based lymphoma is a fast growing aggressive non-Hodgkin B-cell tumor, mostly found in individuals with weakened immune systems such HIV positive patients, as well as patients who undergo organ transplantation (Canadian Cancer Society, 2018). In 2005, Deloose and colleagues reported on the dangerous mass radiation emitted by PEL. Its diagnosis is based on clinical and morphological features (Antar et al., 2014).

Multicentric Castleman's disease (MCD) as its name implies, is a type of condition whereby multiple cells start growing around lymph nodes. It is a rare tumor condition which can also affect tissues around the chest and the stomach. In 2011, Bower described the manifestation of HHV-8 in HIV infected patients as systemic because organs such as splenomegaly, cytopenia and lymphadenopathy were involved.

2.12 HHV-8 epidemiology and HIV-1 co-infection in AIDS-KS

It has been known since the early days of the AIDS pandemic that HIV-1 is one of the most common co-pathogen known to worsen HHV-8 induced pathologies in humans (Bhutani et al., 2015; Engels et al., 2008). HIV is a sexually transmitted infection and is associated with other sexually transmitted diseases. This is so because HIV acts as the driving force, hence causes the immune system to be suppressed leading to co-infection with other viruses such as HHV-8. The ability for HIV-1 to induce tumors in humans is

associated with chronic infection in HHV-8 infected individuals (Okada et al., 2014; Fuime et al., 2015). Reports have shown that there is a greater risk of developing KS in AIDS positive patients as opposed to AIDS negative individuals (Zeng et al., 2007; Bonnet et al., 2004).

HHV-8 infection has been reported to be higher in HIV positive individuals than in HIV negative individuals (Larocca, 2005). HHV-8 infection is common in HIV/AIDS patients for the following reasons: (1) HIV-Tat gene, which helps in enhancing HHV-8 infectivity; (2) promotes angiogenesis by induction of some cytokines; and also (3) providing signal pathways when interacting with HHV-8 proteins (Krause et al., 2014; Aoki, 2007). HIV-1 infection has also been reported to increase the risk of Kaposi's sarcoma (Merat et al., 2002). Occurrence of MCD and PEL tumors are also higher in HIV co-infected patients as compared to HHV-8 infected HIV-1 negative patients (Powles et al., 2009).

2.13 HHV-8 host innate immune evasion

Since HHV-8 has developed several molecular mechanisms to evade host immunity, the host uses two levels of defense to counter microbial infection; the innate immune system (initial recognition of the incoming pathogen) and the adaptive immune system (systemic recognition and memory response to the pathogen). The innate immune response initiates the adaptive response, which may eventually lead to pathogen clearance. HHV-8 is recognized by the innate immune system upon viral infection of newly uninfected cells.

2.14 Mechanism of HHV-8 immune evasion

HHV-8 has no counter reactant to counter-react the host immune response to viral infection and replication. HHV-8 encodes various viral proteins which are used to destabilize the host immune response, hence blunting the innate immune signaling pathway from fighting infection. HHV-8 also blocks the IFN signaling. The host innate immune signaling is the cGas – sting DNA sensing pathway is blocked by LANA, ORF52 and VIF13, which further leads to DNA damage (Figure 2.7).

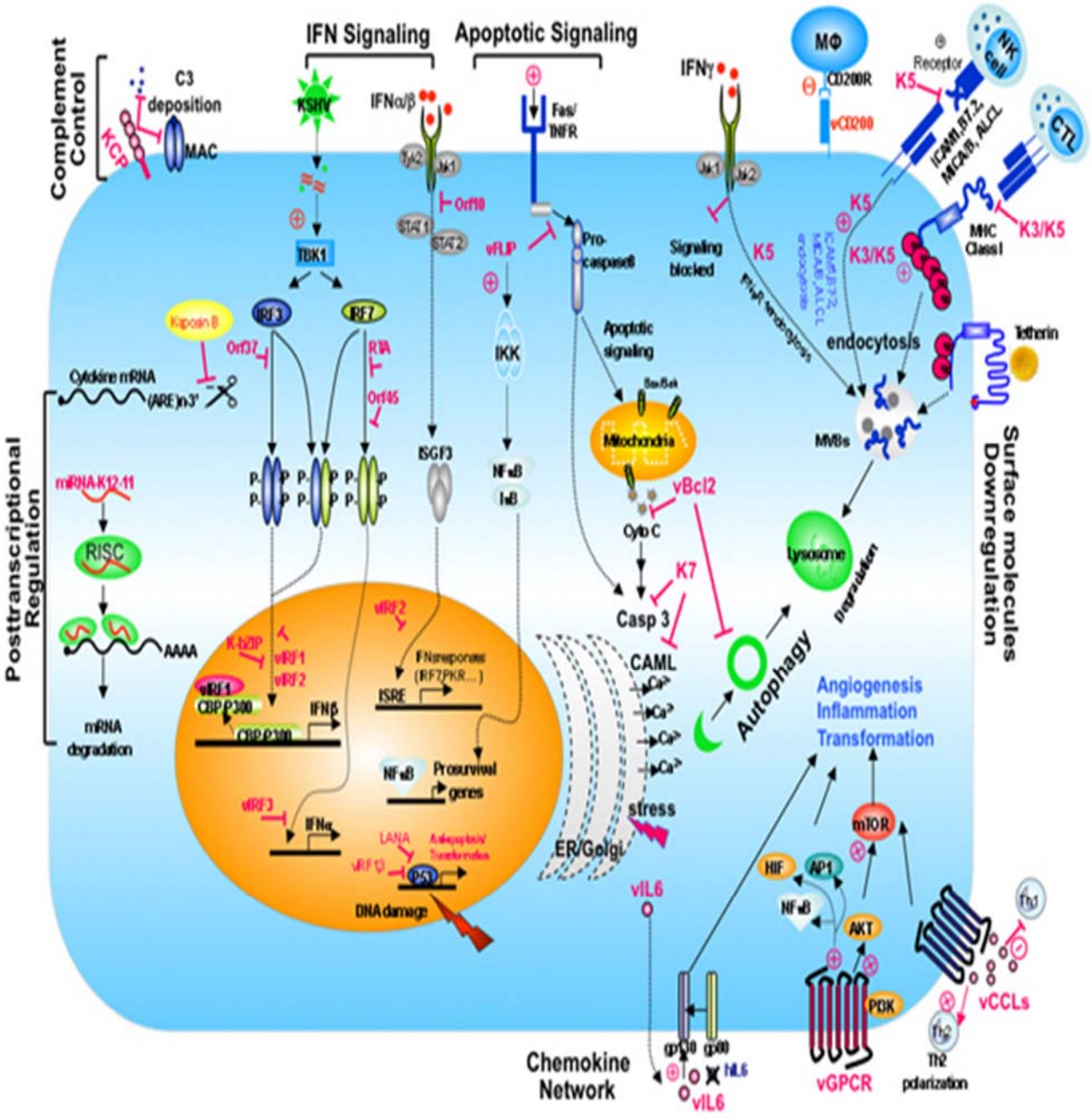

Figure 2.7: Illustration of the host innate immune evasion by HHV-8 (adapted from http//www. Medical journal, 2018).

2.15 Issues on HHV-8 vaccine development

Although vaccines have been the greatest success story in the prevention and control of infectious diseases, there are numerous obstacles in the design of an HHV-8 vaccine. These challenges include the HHV-8 hyper variability within the genome and infected individuals, and HHV-8 co-infection with other viruses such as HIV. However, there are several ongoing and therapeutic development efforts against HHV-8 innfection (Wu et al., 2012; Coen et al., 2014).

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CHAPTER THREE

HHV-8 SEROPREVALENCE AND GENOTYPE DISTRIBUTION IN AFRICA, 1998-2017: A SYSTEMATIC REVIEW

Appendix I (Review Article)

Abstract

Background: Human herpes virus type 8 (HHV-8) is the causative agent of Kaposi sarcoma (KS), a frequently seen malignancy in HIV/AIDS patients. Infection with HHV-8 occurs in humans, regardless of the immune status of the population, although HIV infection has been demonstrated to be an important risk factor for the development of KS. There is need for updated information on the trend of HHV-8 infection in Africa, moreso in the face of a mature HIV endemicity.

Objective: This review was aimed at describing the distribution of HHV-8 seroprevalence and viral genotypes in Africa and to identify gaps in our understanding of HHV-8 across the continent.

Methodology: MEDLINE, EMBASE, SCOPUS, and WEB OF SCIENCE databases were searched for observational studies reporting data between 1998 – 2017 on seroprevalence and genotypes of of HHV-8 in all 53 African countries (with Sudan and South Sudan considered as one country). Outcomes of the search were subjected to a systematic review process. Differences in the seroprevalence of HHV-8 across Africa regions were accessed for significance.

Results: One hundred and twenty-six studies (126) on HHV-8 sero-epidemiology or on HHV-8 genotypes, published from 1998 – 2017 met the inclusion criteria for analysis. The 126 studies were obtained from 81 articles. More than two-thirds (64%) of studies reported on seroprevlenceand 29.3% on genotypes; 9.5% were on both seroprevalence and genotypes. About 45% of African countries had data on HHV-8 seroprevalence exclusively, and more than (53%) had data on either seroprevalence or genotypes. Almost half (47%) of the countries had no data on HHV-8 infection. There was high heterogeneity in the types of tests and interpretation algorithms used in determing HHV-8 seropositivity across the different studies.Generally, seroprevalence ranged from 2.0% in a group of young children in Eritrea to 100% in a small group of individuals with KS in Central African Republic, and in a larger group of individuals with KS in Morocco. Approximately 16% of the studies reported on children. Difference in seroprevalence across the African regions was not significant (95% CI, x^2; p=0.35). About 38% of the

countries had data on *K1* genotypes. *K1* genotypes A, A5, B, C, F and Z occurred at frequencies of 5.3%, 26.3%, 42.1%, 18.45, 5.3% and 2.6% respectively. Twenty-three percent of the countries had data on *K15* genotypes P, M and N occurred at frequencies of 52.2%, 39.1% and 8.7%, respectively.

Conclusion: Our findings suggest that HHV-8 maybe endemic in the entire African continent especially in HIV endemic regions, but there is need for a harmonized testing protocol for a better understanding of HHV-8 seropositivity. Data on HHV-8 inter-genotype recombinants in Africa is scanty. *K1* genotypes A5 and B, and B, *K15* genotypes P and M, from Africa, should be considered in vaccince design efforts. Future research should consider approaches for preventing HHV-8 infection, investigate the impact of antiretroviral therapy on the pathogenesis of HHV-8, and characterize full-length HHV-8 genomes of genotype B and P from Africa to contribute to the selection of genes for vaccine development.

Keywords: HHV-8; seroprevalence; genotypes; systematic review; Africa.

3.1 INTRODUCTION

Human herpes virus type 8 (HHV-8) is the causative agent of four classes of Kaposi's sarcoma (KS), (William et al., 2005). These include: endemic, classic, iatrogenic and AIDS-associated KS. Of these, endemic-KS and AIDS-KS are the most aggressive. AIDS –KS has been highlighted in young homosexual men (CDC, 1981), while AIDS-KS and classic-KS are most common in elderly Mediterranean and individuals of Eastern Europe Boshoff and Weiss (2001). Furthermore, Cook (1998) reported on endemic-KS also called African endemic-KS as it was common in children and young adults in sub-Saharan Africa. Iatrogenic–KS has been observed in immunosuppressed patients who had undergone a solid organ transplant (Doutrelepont et al., 1996; Nagy et al., 2000).

Globally, the epidemiologic pattern of HHV-8 is uneven but follows that of KS and countries at high risk of KS report high prevalence of HHV-8. In a healthy population, there is great variability in the seroprevalence of HHV-8 as opposed to groups at increased risk of developing KS. Age and birth cohort effects are very important cofactors which can be used in measuring the number of HHV-8 viral copies in an individual. Birth cohort effect is defined as the reflecting periods of elevated HHV-8 transmission risk over time. HHV-8 undergoes both latent and lytic phases in its life cycle, whereby the virus remains within the host at a dormant stage until other cofactors triggers it to start replicating, leading to the lytic phase (De Franca et al., 2011; Stolka et al., 2014; Minhas and Wood, 2014). The mode of transmission of HHV-8 can either be vertical or horizontal, including mother to child transmission and from members of family units (Whitby et al., 2000; Mbulaiteye et al., 2004; Plancoulaine et al., 2004).

HHV-8 infection is ubiquitous in African regions with increased incidences of endemic Kaposi's sarcoma in the general population and AIDS-associated Kaposi's sarcoma in HIV/AIDS population (Mbondji Wonje et al., 2013; Sitas et al., 1999; *Gao* et al., 1996). An increase in KS has been observed due to HIV infection in Africa. However, with the scale up and improved access to combination antiretroviral therapy, it is expected that the incidence of KS may be mitigated as has been observed in the developed world (Dedicoat et al., 2003; Facciola et al., 2017). In contrast to observationin US, studies have

showed that HHV-8 seroprevalence increases from childhood to adulthood in African regions (Andreoni et al., 1999; Gessain et al., 1999; Wilkinson et al., 1999). Treatment for HHV-8 or KS includes combined chemotherapy such as Vincristine, Bleomycin and Doxorubicin, radiation therapy, surgery and biological therapy which will effectively dissolve lesions be it localized or widespread, compared to a single HAART drug (Dedicoat et al., 2003; Holfmann et al., 2017; National Cancer Institute, 2017).

HHV-8 is characterized by high genetic variability across the entire genome, with the highest level of genetic variation observed at the 5' and 3' ends of the genome. HHV-8 has been classified into genotypes A, A5, B, C, D, E, F and Z based on the hypervariable regions (VR1 and VR2) of the *K1* gene; genotypes P, M and N based on *K15* gene. Generally, these genotypes have been identified globally (Kasolo et al., 1998; Zong et al., 1999; Cook et al., 1999; Biggar et al., 2000; Meng et al., 2001; Whitby et al., 2004; Cassar et al., 2007; Hayward and Zong, 2007; White et al., 2008; Tornesello et al., 2010; Isaacs et al., 2016). There are 9 known genotypes for HHV-8 ORF26, however, genotypes B, Q, R and N are the most prevalent genotypes for HHV-8 ORF26 of which genotype B and N overlap with genotypes based on *K1* and *K15* genes.

There are several ongoing vaccines and therapeutic development efforts against HHV-8 (Coen et al., 2014; Wu et al., 2012). In this backdrop, it is important to understand the burden of HHV-8 in Africa where the infection appears to be relatively common. The current systematic review examined and analyzed data on the prevalence and molecular epidemiology of HHV-8 in all Africa countries from 1998 – 2017.

3.1.1 Objectives of the chapter

The main objective of the study was to systematically describe the epidemiology of HHV-8 in Africa from 1998 – 2017.

The specific objectives were:

1. To report on the seroprevalence of HHV-8 in Africa from 1998 – 2017.
2. To report on the circulating genotypes of HHV-8 in Africa from 1998 – 2017.

3.2 METHODOLOGY

3.2.1 Inclusion criteria for study analysis:

We conducted a systematic review of fpublished full text articles on HHV-8 seroprevalence and genotypes from 53 African countries according to the PRISMA guidelines, except for meta-analysis, Sudan and South Sudan were considered as one country. Cross-sectional, case report, retrospective, prospective and observational studies on HHV-8 seroprevalence and or genotypes were included for analysis. Full texts in French language were interpreted, analyzed and included in the analysis. However, there was no full text articles in other languages that met the inclusion criteria. African countries were assessed and categorized into Central, East, North, Southern and West Africa.

3.2.2 Relevant literature searches:

MEDLINE, EMBASE, SCOPUS, WEB OF SCIENCE databases and conference proceedings, were searched for published data from 1998–2017. Using electronic search, reference lists were screened for relevant and additional data.

3.2.2.1 MEDLINE search strategy using PubMed.

The search terms were: ((((((prevalence* OR epidemiology* OR incidence OR seroprevalence* OR seroepidemiology* OR sero-epidemiology* OR seropositivity* OR sero-positivity*)) OR ((((((seropidemiologic studies [MeSH Terms] OR prevalence [MeSH Terms] OR incidence [MeSH Terms])))))) AND ((((((herpesvirus 8, human [Term]) OR “human herpesrvirus 8”) OR “hhv-8”) OR “hhv-8” OR “kshv” OR “kaposi sarcoma associated herpesvirus”) OR “kaposi’s sarcoma associated herpes- virus”) OR “kaposi sarcoma-associated herpesvirus”) OR “kaposi’s sarcoma-associated herpesvirus”) OR “kaposi sar- coma herpesvirus”) OR kaposi virus))))) NOT ((animals[mh] NOT

humans[mh])). In addition, each search strategy by Rohner was concluded with our own search criteria as follows: reviews of hhv-8/kshv OR hhv-8 review and epidemiology OR genetic characterization of hhv-8/kshv, genetic OR characterization of KSHV and genotype distribution of hhv-8 *K1/K15*. All these search options were accompanied with an African country (name). This was done repeatedly for all 53 African countries.

3.2.2.2 EMBASE search strategy using science direct

The search terms were: #1.1 “human herpesvirus 8”/exp OR “human herpesvirus 8” OR “hhv-8” OR “hhv8” OR “kshv” OR “kaposi sarcoma associated herpesvirus”/exp OR “kaposi sarcoma associated herpesvirus” OR “kaposi sarcoma-associated herpesvirus”/exp OR “kaposi sarcoma-associated herpesvirus” OR kaposi NEXT/3 herpesvirus OR kaposi NEXT/3 virus AND [embase]/lim #1.2 “prevalence”/exp OR prevalence OR “seroprevalence”/exp OR seroprevalence OR “incidence”/exp OR incidence OR “seroepidemiology”/ exp OR seroepidemiology AND [embase]/lim #1.3 prevalen* OR inciden* OR epidemiolog* OR sero*epidemiolog* OR sero*prevalen* OR sero*positiv* AND [embase]/lim #1.4 #1.2 OR #1.3 #1.5 #1.1 AND #1.4 #1.6 “animals”/exp NOT “humans”/exp #1.7 #1.5 NOT #1.6.])). In addition, each search strategy was concluded with additional options as thus: reviews of hhv-8/kshv OR hhv-8 review and epidemiology OR genetic characterization of hhv-8/kshv, genetic OR characterization of KSHV and genotype distribution of hhv-8 *K1/K15*. All these search options were accompanied with an African country (name). This was done repeatedly for all 53 African countries.

3.2.2.3 SCOPUS search strategy using Elsevier

Search strategy are as follows: seroprevalence OR seroepidermiology OR sero-epidemiology OR seropositivity OR sero-positivity OR seropidemiologic studies OR prevalence OR incidence AND herpesvirus 8, human OR human herpesrvirus 8 OR hhv-8 OR hhv-8 OR kshv OR kaposi sarcoma associated herpesvirus OR kaposi’s sarcoma associated herpes- virus OR kaposi sarcoma-associated herpesvirus OR kaposi’s sarcoma-associated herpesvirus OR kaposi sarcoma herpesvirus/kaposi virus OR

reviews of hhv-8/kshv OR hhv-8 review and epidemiology OR genetic characterization of hhv-8/kshv OR genetic characterization of KSHV and genotype distribution of hhv-8 *K1/K15*. All these search options were accompanied with an African country (name). This was done repeatedly for all 53 African countries.

3.2.2.4 WEB OF SCIENCE search strategy using Analytic database

Search strategy were as follows: Seroprevalence OR seroepidemiology OR sero-epidemiology OR seropositicity OR sero-positivity OR seroepidemiologic studies OR prevalence OR incidence and herpesvirus 8, OR human herpesvirus 8 OR hhv-8 OR kshv OR kaposi sarcoma associated herpesvirus OR kaposi's sarcoma associated herpesvirus OR kaposi associated herpes virus OR kaposi's sarcoma associated herpes virus OR kaposi sarcoma herpesvirus OR kaposi sarcom virus, reviews on epidemiology of hhv-8/kshv, genetic characterization of hhv-8/kshv genotype distribution of hhv-8 *k1*/*k15*. All these search options were accompanied with an African country (name). This was repeatedly done for all 53 African countries.

3.2.2.5 Searching other sources

Additional searches were for additional data and focus was on African countries. We screened reference lists of relevant papers and searched the following conferences: Annual Meeting of the American Society of Clinical Oncology (ASCO) (http://www.asco.org/ASCOv2/ Meetings); International Conference on Malignancies in AIDS and Other Acquired Immuno-deficiencies (http://www.capconcorp.com/meeting/2013/14thICMAOI/ index.asp); Conference on Retroviruses and Opportunistic Infections (CROI) (www.croi2017.org).

3.2.3. Data extraction.

Two reviewers downloaded relevant articles from the above search options and classified each article using an article grid. These classifications were based on: the study objectives, design, sampling size, results, discussion and conclusion. For all included studies, information was extracted on: author(s), study type, country of study, country of participants, study population, sample size, sample type, number of males, females,

children, type of assay, seroprevalence, source of DNA, *K1* genotypes and *K15* genotypes. We used 'X' for parameters with no information. The various tests used for the determination of HHV-8 seropositivity were also considered. These tests were immunofluorescence (IFA) and enzyme immunoassay (EIA), immune-peroxidase (IMP), western blots and antigen ELISA tests (latent ORF73) and lytic K8.1). In addition, relevant articleson the genetic characterization of HHV-8 for *K1* and *K15* genes were analyzed. Figure 1 shows a PRISMA flow chart on the selection included for analysis.

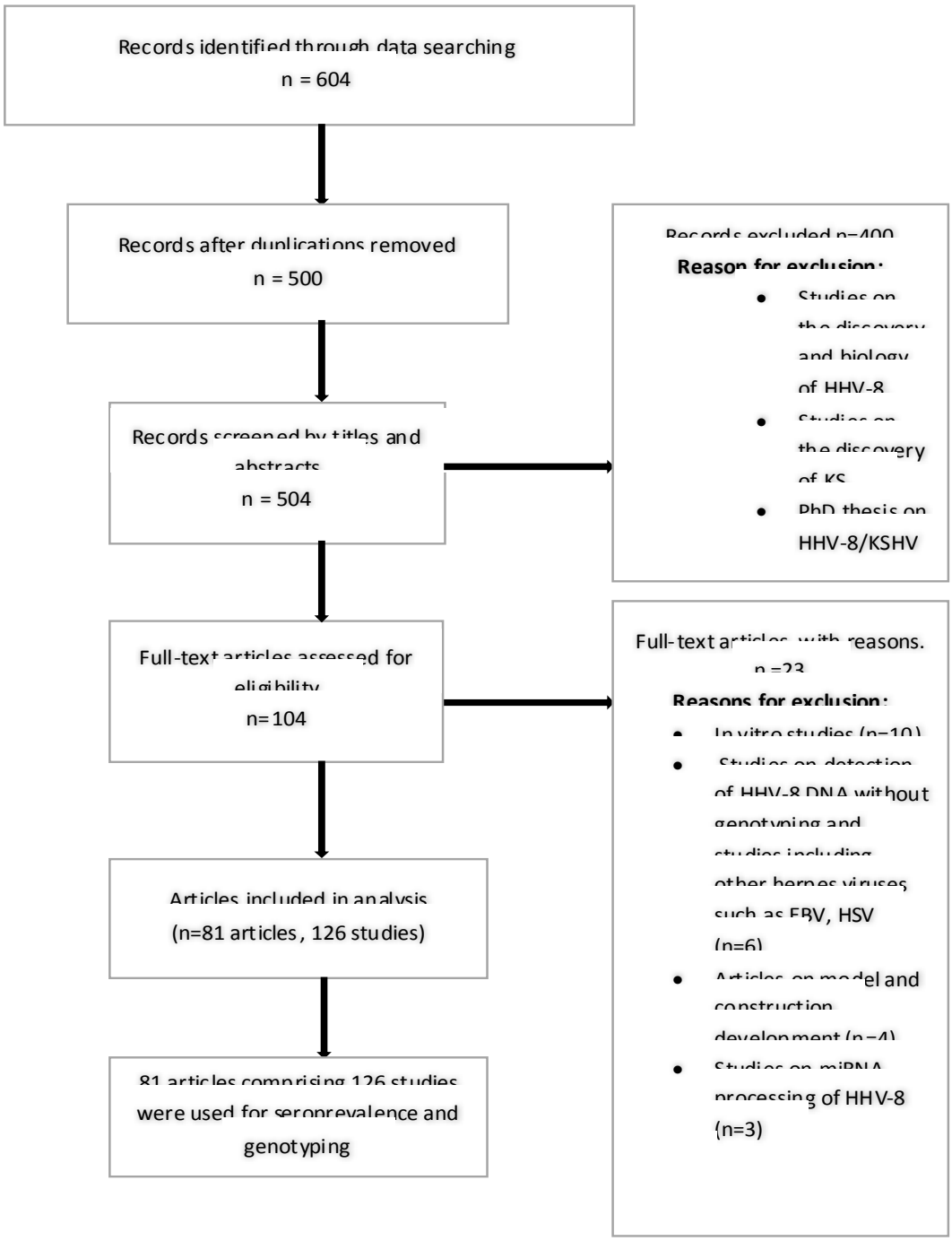

Figure 3.1: Flow chart on the selection of studies included for analysis.

3.3 RESULTS

The use of different approaches and algorithms to determine the sero-epidemiology of HHV-8 across different populations presents challenges for comparisons. Examples of these challenges include the use of different dilution factors in plasma or serum preparation for antibody detection, the use of ELISA with different principles and interpretation guideline, different immunofluorescence techniques, or a combination of these approaches. In addition to these, the inclusion of different populations (low and high risk), even in the same study community, increases the degree of complexity in deciphering an accurate picture of the distribution of HHV-8 infection among populations. Consequently, herein, we firstly present a descriptive analysis of the systematic literature search on studies on HHV-8 sero-epidemiology in Africa. Countries were categorized as follows: **Southern Africa**- Botswana, Zambia, Mozambique, South Africa, Zimbabwe, Namibia, Swaziland, Lesotho, Malawi; **Central Africa**- Angola, Cameroon, Central Africa Republic, Chad, Congo, Gabon, Democratic Republic of Congo, Equatorial Guinea, Sao Tome & Principle; **West Africa**- Nigeria, Burkina Faso, Guinea, Gambia, Ghana, Mali, Senegal, Sierra Leonne, Niger, Liberia, Benin, Cote d'Ivoire, Togo, Cape Verde, Mauritania; **East Africa**- Tanzania, Kenya, Uganda, Ethiopia, Rwanda, Burundi, Djibouti, Eritrea, Somalia, Madagascar, Comoros; and **North Africa**- Algeria, Egypt, Tunisia, Sudan (including South Sudan), Morocco, Libya, Western Sahara

3.3.1 Characteristics of studies included in the analysis:

We identified a total of 604 articles from database searches, which were reduced to 500 after the removal of duplicates. Observational studies included for analysis were: cross-sectional, prospective, retrospective, case report, which occurred at different settings such as rural, urban or hospital based. Out of the 500 articles, 81 articles met the inclusion criteria and were further analysed (Figure 3.1). Some studies reported on multi-country and multi-site studies. One hundred and twenty-six studies on HHV-8 sero-epidemiology or genotypes, published from 1998-2017, were obtained from the 81 articles and met the inclusion criteria for analysis. About 64% (81/12) of the studies were available on HHV-8 seroprevalence; 29.3% (37/126) were available on HHV-8 genotypes; and 9.55 (12/126) of the studies were available for both seroprevalence and genotypes. Overall, 52.8% (28/53) of the African countries had data on either seroprevalence or genotypes of HHV-8.

3.3.2 HHV-8 Seroprevalence Distribution in Africa

About 45% (24/53) of African countries had data on HHV-8 seroprevalence only. Bearing in mind that different test strategies were applied in determining seropositivity in different populations, seropositivity reported ranges from 0.0% in a group of blood donors in Morocco by indirect immunofluorescence (El Kassimi et al., 2003), through 2.0% in a group of young children in Eritrea by the detection of antibodies to ORF73 (Enbom et al., 1999), to 100% in a small group of individuals with KS in the Central Africa Republic using immunofluorescence and immune-peroxidase assays (Fouchard et al., 2000), although in a large group of individuals with KS in Morocco the prevalence was 92% by indirect immunofluorescence (El Kassimi et al., 2003). Out of the 126 studies, 20 (15.8%) reported on children, in which seroprevalence ranged from 2% to 69%. Regionally, the seroprevalence across the continent ranged as follows: Southern Africa (14.0 – 90.0%), Central Africa (17.4 – 100.0%), West Africa (14.0 – 83.1%), East Africa (2.0 – 93.0%) and North Africa (0.0 – 92.0%). Considering the highest reported seroprevalence from the different African regions, there was no significant difference among the African regions (95% CI, x^2 =0.35). Figure 2 represents the relative serprevalence of HHV-8 across Africa.

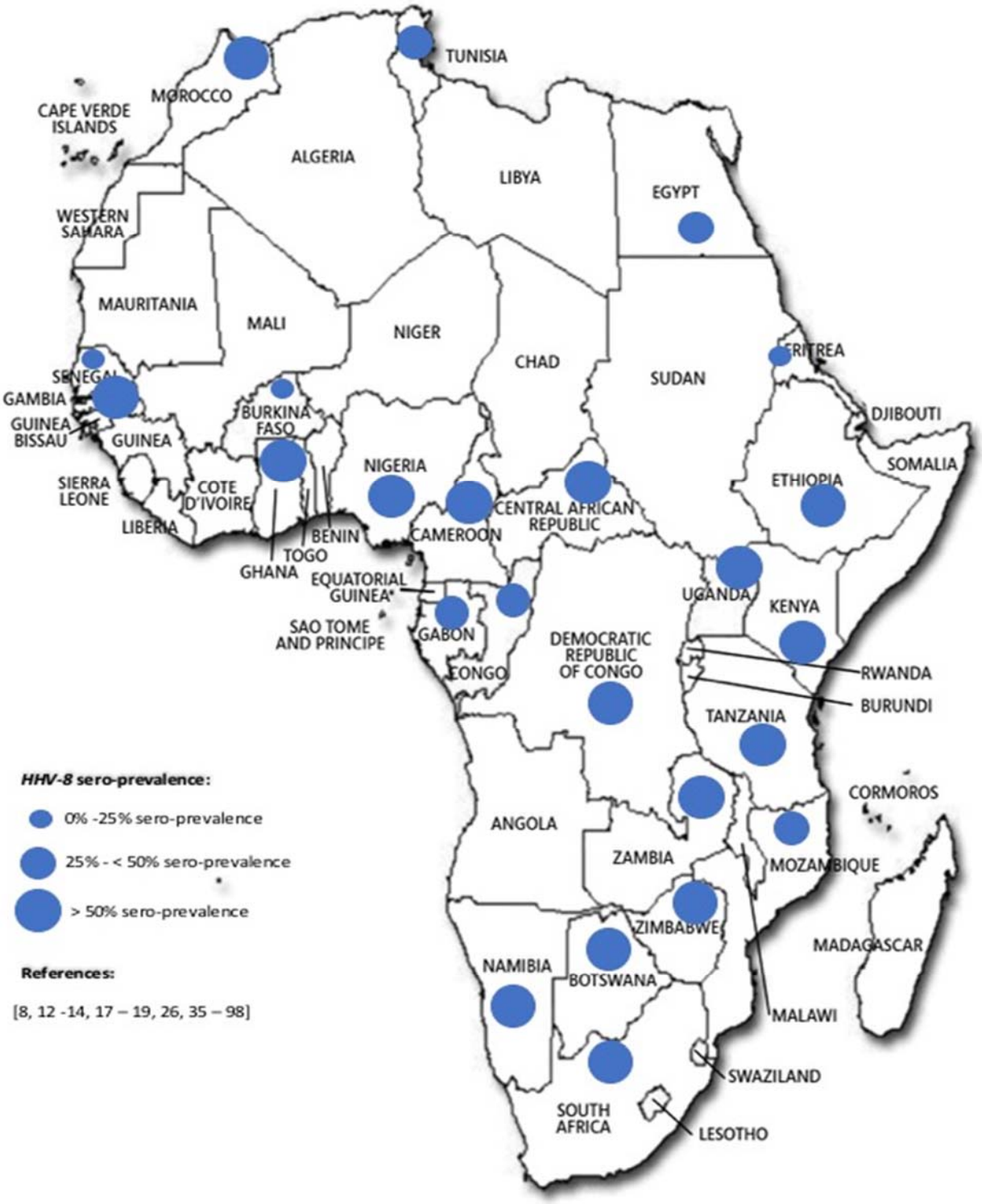

Figure 3.2: A representative of the highest HHV-8 seroprevalence observed in one population in African countries (1998 – 2017).

3.3.3 A Comparison of HHV-8 Seroprevalence among Selected Population across African Regions

From the available data, HHV-8 infection among children was observed to be significantly different across the African regions, with children in Central Africa being the most infected. The burden of infection was also significant in HIV-infected children compared to HIV non-infected children in Southern Africa (Table 1). Across the different African regions, HHV-8 antibodies were generally detected in a higher frequency in no-pregnant women than in pregnant women of comparable age, except in West Africa, were pregnant women were more infected than non-pregnant women (Table 2). No studies meeting the inclusion criteria were available for North Africa. Regarding geographical setting, there is no clear picture on the distribution of HHV-8 between rural and urban populations within and across African regions. For example, the urban population was marginally more infected than the rural population in Southern and East Africa, while this is not the case in west Africa. Across regions, the urban population in West Africa was the most infected. Data for North and Central Africa were inadequate to permit meaningful comparisons (Table 3).

Table 3.1: Relative HHV-8 infection burden in children, and proportion of HIV positive, HIV negative, and children with KS among the child population in Africa.

African Region	Percentage of children infected with HHV-8	Percentage of HIV positive children infected with HHV-8 of all children with HHV-8	Percentage of HIV negative children infected with HHV-8 of all children with HHV-8	Percentage of children with KS and infected with HHV-8 of all children with HHV-8	Chi-Square Value	p-value (Significant at p ≤ 0.005)
Southern	31.4 (342/1092)	52.6 (180/342)	21.1(72/342)	26.3 (90/342)	90.5	<0.0015
Central	49.5 (329/665)	No data	No data	No data	Not done	Not done
West	11.4 (49/429)	18.6 (80/429)	81.3 (349/429)	No data	128.8	0.0001
East	20.7 (945/4557)	No data	No data	1.6 (15/945)	Not done	Not done
North	43.3 (122/282)	No data	No data	5.3 (15/282)	Not done	Not done
Chi-Square value	367.2	98.3	279.1	34.9	34.9	0.401
p-value	<0.00001	-	-	-	-	-

Data on chidren described in selected studies were summed up for each region and analysed. The percentages of HIV positive children, HIV negatives children, and children with KS are calculated based on the total number of children with HHV-8 pooled from the extracted studies in the different African regions. Data from North Africa were not sufficient for meaningful comparable analysis and were omitted.

Table 3.2: Relative HHV-8 infection burden in women of comparable age (25 – 45 years) in Africa.

African Region	Percentage of women infected with HHV-8	Percentage of pregnant women infected with HHV-8 of all women with HHV-8	Percentage of non-pregnant women infected with HHV-8 of all women with HHV-8	Chi-Square Value	p-value (Significant at $p \leq 0.005$)
Southern	28.7 (4196/14612)	8.2 (343/4196)	91.8 (3853/4196)	155.93	<0.00001
Central	6.2 (287/4626)	27.5 (79/287)	72.5 (208/287)	100.00	<0.00001
West	1.7 (151/8491)	61.0 (92/151)	39.3 (59/151)	79.20	0.00001
East	26.1 (2280/8729)	22.2 (501/2280)	78.0 (1779/2280)	80.73	0.00001
Chi-Square value	41.49			-	-
p-value	<0.00001	<0.0001	<0.00001	-	-

Data on women described in selected studies were summed up for each region and analysed. The percentages of pregnant and non-pregnant women infected with HHV-8 were calculated based on the total number of women with HHV-8 pooled from the extracted studies in the different African regions. Data from the North Africa region were not sufficient for meaningful comparable analysis and were omitted.

Table 3.3: Relative HHV-8 infection burden in rural and urban populations in Africa

African Region	Percentage of HHV-8 seropositivity in rural setting	Percentage of HHV-8 seropositivity in urban setting	Chi-Square Value	p-value (Significant at $p \leq 0.005$)
Southern	5.4 (207/3781)	3.3 (191/5710)	764.03	0.00001
Central	20.0 (516/2579)	No data	Not done	Not done
West	31.8 (77/241)	42.0 (26/62)	2.4462	0.119
East	29.0 (829/2409)	42.5 (450/1060)	84.52	0.0
Chi-Square value	52.30	50.21	Not done	Not done
p-value	<0.00001	<0.0001	Not done	Not done

Data on the rural and urban populations described in the selected studies were summed up for each region and analysed. Data from North African region were not sufficient for meaningful comparable and were omitted.

3.3.4 HHV-8 Genotypes Distribution in Africa

Based on the *K1* gene, 33.9% (18/53) of African countries had data on genotypes, while 28.3% (15.53) of countries had data on *K15* genotypes (Figure 3.4). Genotypes A, A5, B, C, F and Z were identified at frequencies of 5.3% (2/38), 26.3% (10/38), 42.1 (16/38), 18.4% (7/38), 5.3% (2/38) and 2.6% (1/38), respectively; genotypes P, M and N of the K15 gene were identified at frequencies of 52.2% (12/23), 39.1% (9/23) and 8.7% (2/23), respectively (Figure 3.4). Of the 18 countries from which *K1* genotypes were reported, at least two genotypes were reported in each. Genotype Z was reported from only one study in Zambia involving children (Kasolo et al., 1999). All other genotypes were reported in both children and adults. From the available data, there appears to be no trend in the distribution of genotypes across the continent. Worthy of note is the description of K15 genotypes N in Southern Africa (South Africa and Zambia [Kasolo et al., 1999; Alagiozoglou et al., 2000], and is not clear whether *K15* genotype N is restricted to Southern Africa. Data were reported for a few countries on intra-genotypic variants but data on inter-genotypic recombinants appears to be scanty (Poole et al., 1999; Betsem et al., 2014; Zong et al., 2001 and Lacoste et al., 2000).

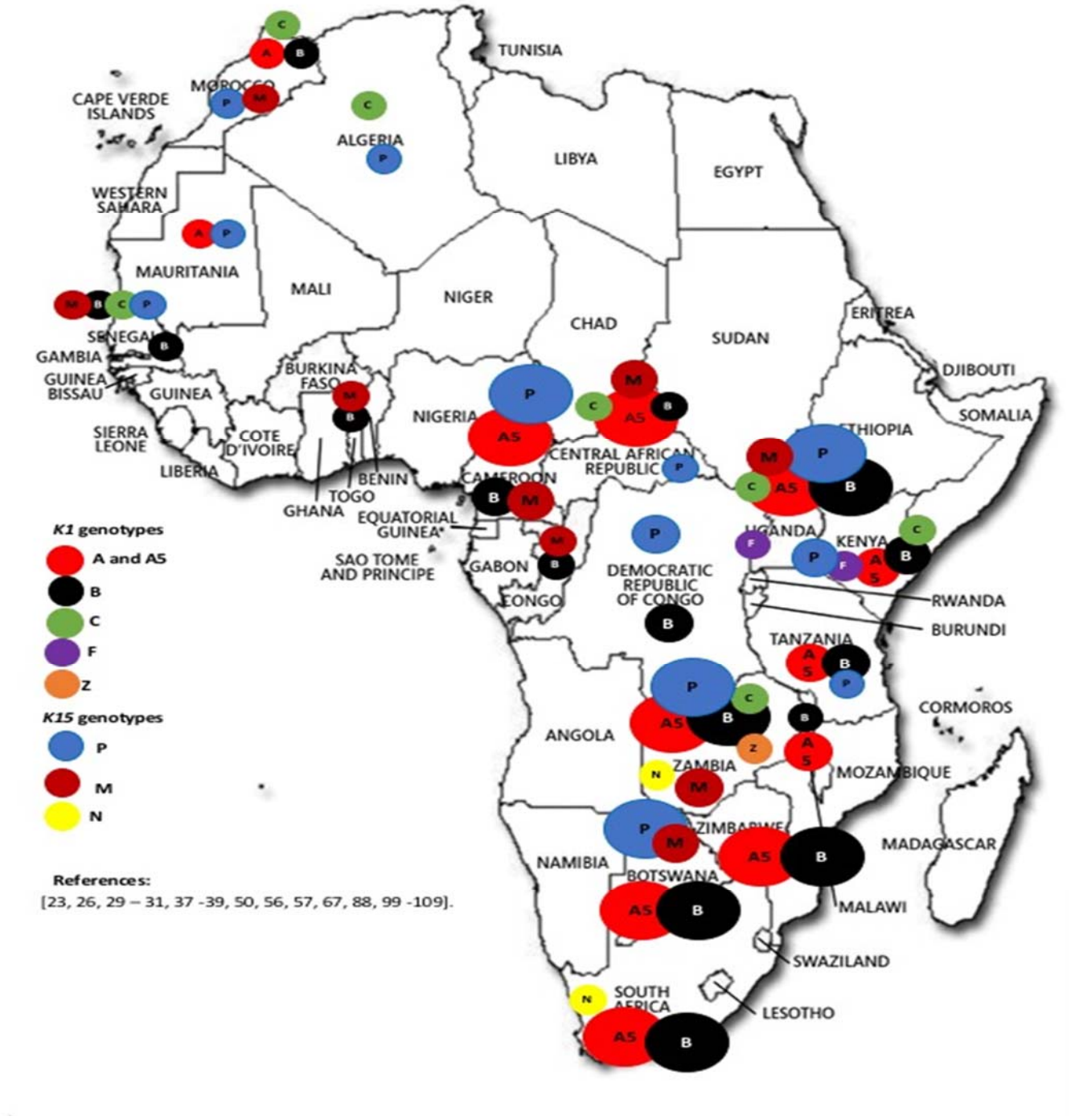

Figure 3.3: Map of Africa showing the occurrence of HHV-8 genotypes. There appears to be a general distribution of different genotypes across the continent. Countries without indications had no published data on HHV-8 genotypes between 1998-2017.

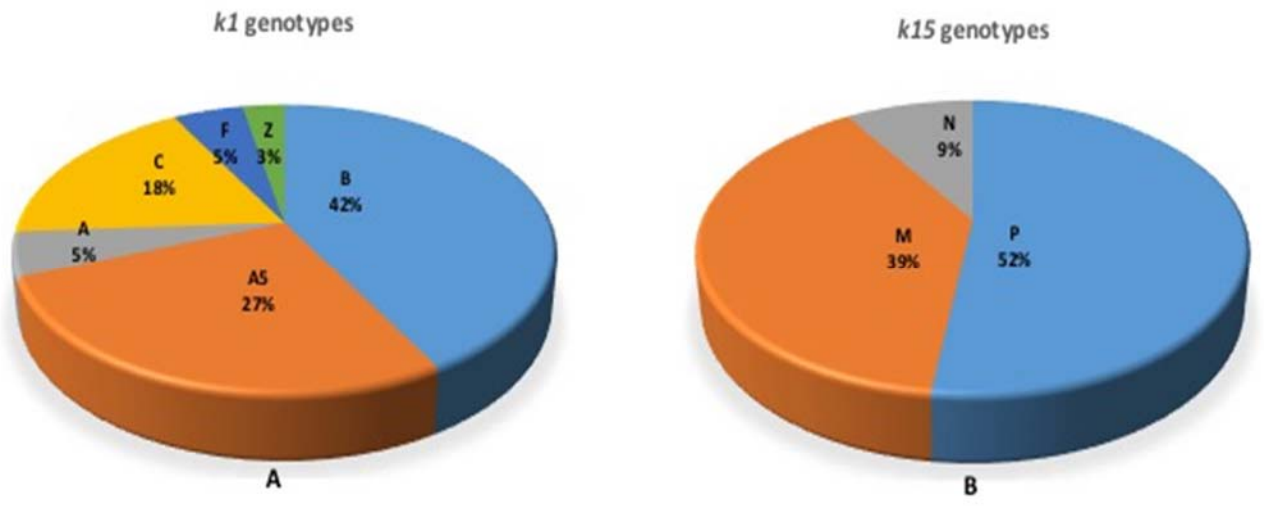

Figure 3.4: Proportional representation of HHV-8 genotypes in Africa. (A) Represents *K1* genotypes; (B) represents *K15* genotypes. *K1* genotypes is the most prevalent followed by genotypes A5, while *K15* genotypes P is most prevalent genotypes followed by genotype M.

Table 3.4: Characteristics of studies on Southern African countries included in the analysis

References	Study type	Country of study	Country of participant	Study Population	Sample size	Type of Assay for sero-prevalence detection	No of Males	No of Females	No of Children	Percentage sero-prevalence	Source of DNA/antibodies for serology and genotyping	*K1* Genotypes	*K15* Genotypes
Whitby 2004	Retrospective	Ecuador	Botswana	Rural based volunteer (Bantu)	160	K8.1 ELISA + LANA IFA	N/M	N/M	N/M	ELISA=76 % IFA =90%	Blood (sera& buffy coat)	A, B	P
Whitby 2004	Retrospective	Ecuador	Botswana	Rural based volunteer (San)	156	K8.1 ELISA + LANA IFA	N/M	N/M	N/M	ELISA=88 % IFA =79%	Blood (sera & buffy coat)	B	P, M
Simbiri 2014	Retrospective	Botswana	Botswana	HIV-AIDS hospital based patients	13	LANA	N/M	N/M	0	60%	X	X	X
Engel 2000	Retrospective	USA	Botswana	Community based (San)	155	IFA and ELISA	81	69	N/M	87%	Serum/Plasma	X	X
Engel 2000	Retrospective	USA	Botswana	Community based (Bantu)	161	IFA and ELISA	89	91	N/M	76%	Serum/Plasma	X	X
Kasolo 2007	Prospective	Zambia	Zambia	FI &Endemic KS children, hospital based	200	X	0	0	200	X	Biopsy & whole blood	A, B, C, Z	P, M
Wojcicki 2008	Prospective	Zambia	Zambia	Hospital based	1425	IFA	0	1425	0	39.87%	Plasma	X	X
Zong 1999	Retrospective	USA	Zambia	AIDS-KS patients; hospital based	5	X	5	0	0	X	Biopsy	B1	X
Poole 1999	Retrospective	USA	Zambia	AIDS-KS patients; hospital based	5	X	5	0	0	X	Biopsy	X	P
Ablashi 1999	Retrospective	USA	Zambia	Hospital based patient	40	ELISA	22	18	0	9-44%	X	X	X
Olsen 1998	Retrospective	Zambia	Zambia	Hospital based patients	251	IFA and WB	98	153	0	58%	X	X	X
Kasolo 2007	Retrospective	UK	Zambia	KS patients	30	ELISA	25	5	0	74%	KS biopsy	A, B, C	P, M

He 1998	Cross section al study	USA	Zambia	Population based	378	IFA	0	378	0	48.8%	X	X	X
Olp 2015	Prospec tive	Zambia	Zambia	HIV +ve children	287	X	0	0	287	X	Biopsy & blood	A, B	P, N
Olp 2013	Prospec tive	Brazil	Zambia	Cross sectional survey	788	ELISA	N/M	N/M	N/M	14%	Plasma & Saliva	A,B, C	X
Naniche 2011	Retrosp ective	Mozambi que	Mozambique	Hospital based	136	ELISA	55	78	0	44.7%	X	X	X
Caterino de Araujo 2010	Prospec tive	Mozambi que	Mozambique	General population	752	ELISA	263	489	0	21.4%	X	X	X
Zong 2001	Retrosp ective	USA	South Africa	AIDS-KS patients	6	X	N/M	N/M	N/M	X	PBMCs/ly mph nodes	A, B	N*
Bulter 2009	Retrosp ective	South Africa	South Africa	Population based	427	ELISA	0	0	427	9.0%	X	X	X
Sitas 1999	Retrosp ective	England	South Africa	Hospital based cancer patients	3293	IFA	N/M	N/M	N/M	32%	Serum	X	X
Wilkinson 1999	Retrosp ective	South Africa	South Africa	Hospital based	136	IFA, WB and ELISA	N/M	N/M	36	38-75%	Serum	X	X
Isaacs 2016	Retrosp ective	South Africa	South Africa	AIDS-KS + Endemic KS patients	86	X	53	33	0	X	KS biopsy	A, B	X
Maskew 2011	Prospec tive	South Africa	South Africa	Cross sectional HIV + naïve patients	416	ELISA	0	416	0	28.5%	Plasma	X	X
Bohlius 2015	Prospec tive	South Africa	South Africa	Hospital based	N/M	ELISA	N/M	N/M	N/M	48%	Plasma	X	X
Babatyi 2008	Retrosp ective	South Africa	South Africa	Pregnant women, hospital based	1740	ELISA	0	1740	0	44.6	X	X	X
Malope 2010	Retrosp ective	USA	South Africa	Pregnant women, with KS	1228	ELISA	0	1228	0	36.1%	X	X	X
Malope 2010	Retrosp ective	USA	South Africa	Pregnant women, without KS	1228	ELISA	0	1228	0	19.9%	X	X	X
Alagiozoglou 2000	Retrosp ective	South Africa	South Africa	AIDS-KS; hospital based	40	X	30	10	0	X	Biopsy	X	N*
Wojcicki 2004	Retrosp ective	South Africa	South Africa	Survey	2191	ELISA	0	2191	0	39.0%	Serum	X	X

Lampinen 2000	Retrospective	Zimbabwe	Zimbabwe	Women with/without KS and HIV/AIDS	41	ELISA	0	41	0	85%	Serum/ PBMCs, vaginal ,endocervical,	A	X
Chokunonga 2000	Retrospective	Zimbabwe	Zimbabwe	AIDS patients	**3571**	ELISA	2131	1440	145	18.2%	X	X	X
Campbell 2003	Retrospective	Zimbabwe	Zimbabwe	AIDS-KS patients	65	X	50	15	0	X	Blood	A,B	X
White 2008	Prospective	Zimbabwe	Zimbabwe	AIDS-KS patients	65	X	50	15	0	X	Blood	A, B	X
De Santis 2002	Retrospective	Malawi	Malawi	Hospitalized patients and healthy volunteers	272	ELISA, K8.1	109	143	20	61%	X	X	X
Beyari 2004	Retrospective	Malawi	Malawi	AIDS-KS patients	5	X	3	2	0	X	Mouth rinse and blood	A, B	X
Cook 1999	Prospective	UK	Malawi	AIDS-KS	22	IFA	11	11	0	X	Mouth rinse and blood	A, B	X

Note: X indicates no data exist on that parameter. There are 9 countries in southern African countries, of which 6 countries have published data based on the search criteria and date of search. N/M indicates not mention, N*-first report of subtype N using ORF75 gene adjacent to K15 gene.

Table 3.5: Characteristics of studies on Central African countries included in the analysis

References	**Study type**	**Country of study**	**Country of participant**	**Study population**	**Sample size**	**Type of Assay of assay for sero-prevalence detection**	**No of males**	**No of females**	**No of children**	**Percentage sero-prevalence**	**Source of DNA/antibodies for serology &genotyping**	***K1* Genotypes**	***K15* Genotypes**
Mbondji-Wonje 2013	Retrospective	Cameroon	Cameroon	HIV +ve and HIV – ve individuals from the rural setting	516	ELISA and IFA	N/M	N/M	N/M	61%	Plasma	X	X
Lacoste 2000	Prospective	France	Cameroon	AIDS-KS patients	49	X	36	18	0	X	KS biopsy	A	M, P
Jacky 2015	Retrospective	Cameroon	Cameroon	Hospital setting	16	ELISA	N/M	N/M	N/M	42.8%	Plasma	X	X
Tornesello 2010	Prospective	Cameroon	Cameroon	AIDS-KS patients	68	X	3	65	0	X	KS biopsy	A	P, M

Stolka 2014	Prospective	Cameroon	Cameroon	HIV +ve, -ve, KS patients	1177	ELISA	N/M	N/M	0	80%	Plasma	X	X
Betsem 2014	Retrospective	Cameroon	Cameroon	Rural setting of all aged groups	2063	LANA	1,096	967	0	37.2%	KS biopsy	A, B	P
Serraino 2001	Prospective	Cameroon	Cameroon	Population based	293	ELISA	129	164	0	50.5%	X	X	X
Ignacio 2016	Prospective	USA	Cameroon	AIDS-KS patients	38	ELISA	38	0	0	61%	X	X	X
Ignacio 2016	Prospective	USA	Cameroon	General population	3123	ELISA and IFA	N/M	N/M	N/M	29.6%	X	X	X
Fouchard 2000	Retrospective	Cameroon	Cameroon	Hospital based	3	X	0	3	0	X	Blood , KS biopsy, normal skin	A, B	X
Bestetti 1998	Retrospective	Cameroon	Cameroon	Pregnant women and prostitutes	274	IMP	N/M	274	N/M	38-57%	Serum	X	X
Gessain 1999	Retrospective	France	Cameroon	Hospital based-mother-child pair survey	258 32	IFA IFA	X X	X 32	258 X	27.5% 47%	Plasma Plasma	X X	X X
Rezza 2000	Retrospective	Cameroon	Cameroon	Serosurvey-Hospital based	292	IFA	129	65	98	39.8-61.8%	Seum	X	X
Tornesello 2010	Prospective	Italy	Cameroon	AIDS-KS patients	3	X	3	0	0	X	KS biopsy	A	P, M
Lacoste 2000	Prospective	France	Central Africa Republic	AIDS-KS + HIV +ve MCD patients	49	X	N/M	N/M	0	X	KS biopsy + lymph nodes	A, B, C	M, P
Fouchard 2000	Retrospective	France	Central Africa Republic	AIDS-KS patients	3	IMP and IFA	2	1	0	IMP=100 % IFA=100%	Blood	A	X
Whitby 2004	Prospective	England	Congo	AIDS-KS patients	23	ELISA	N/M	N/M	N/M	17.4%	X	X	X
Lacoste 2000	Prospective	France	Congo	MCD-HIV +ve patients	1	X	1	0	0	X	Tonsil	B	M
Melser 2015	Prospective	Gabon	Gabon	Hospital based	344	ELISA	0	344	0	31%	X	X	X
Engels 2000	Retrospective	USA	Democratic Republic of Congo (Zaire)	Community based Case-control	321	ELISA and IFA	155	166	N/M	82%	Serum/Plasma	X	X

Poole 1999	Retrospecti ve	USA	Democrati c Republic of Congo (Zaire)	Endemic KS patients	1	X	1	0	0	X	KS biopsy	B	P

Note: X indicates no data exist on that parameter. There are 9 countries in central African countries, of which 5 countries have published data based on the search criteria and date of search. N/M indicates not mentioned.

Table 3.6: Characteristics of studies on West African countries included in the analysis

Reference s	**Study type**	**Countr y of study**	**Country of participa nt**	**Study populatio n**	**Sampl e size**	**Type of Assay for sero-prevalenc e detection**	**No of male s**	**No of female s**	**No of childre n**	**Percentag e sero-prevalenc e**	**Source of DNA/antibodi es for serology &genotyping**	***K1* Genotype s**	***K15* Genotype s**
Ukonu 2011	Prospective	Nigeria	Nigeria	Hospital based	180	ELISA	60	120	0	70%	Plasma	X	X
De Sanjose 2009	Comparativ e	USA	Nigeria	General population	1085	K8.1 or LANA ELISA	0	1085	0	46.02%	Plasma	X	X
Ogoina 2011	Cross sectional	Nigeria	Nigeria	AIDS patients with KS KS patients without HIV	71	ELISA	23	48	0	62%	Plasma	X	X
Pfeiffer 2010	Retrospectiv e	Nigeria	Nigeria	Populatio n based	1880	ELISA	718	1162	0	83.1%	X	X	X
Simpore 2004	Prospective	Burkina Faso	Burkina Faso	Pregnant women (HIV ive, - ve and AIDS paients)	429	ELISA IFA	0	0	429	11.4%	Serum	X	X
Collenberg 2006	Prospective	Burkina Faso	Burkina Faso	Hospital based	683	ELISA	191	492	0	12%	X	X	X
Cook 1999	Retrospectiv e	UK	Gambia	AIDS-KS patients	9	X	8	1	0	X	KS biopsy	B	X
Ariyoshi 1998	Retrospectiv e	Ghana	Gambia	Hospital based	N/M	ELISA and IFA	N/M	N/M	N/M	73-83%	Serum	X	X
Ablashi 1999	Retrospectiv e	USA	Ghana	Rural and urban setting	62	ELISA	47	15	0	41.9%	X	X	X
Aidoo 2001	Retrospectiv e	Ghana	Ghana	Hospital based	410	ELISA	365	45	0	33.5-43.0	Serum	X	X

Adjei 2008	Retrospective	Ghana	Ghana	Hospital based	3275	ELISA	2573	702	0	65.6%	X	X	X
Whitby 2004	Prospective	UK	Ghana	AIDS-KS patients	56	ELISA	N/M	N/M	N/M	23.2%	X	X	X
Lacoste 2000	Retrospective	France	Togo	HIV + MCD patients	1	X	1	0	0	X	Spleen	B	M
Lacoste 2000	Retrospective	France	Senegal	HIV +MCD	2	IMP and IFA	2	0	0	X	KS biopsy	B,C	P,M
Gaye-Diallo 2001	Retrospective	Senegal	Senegal	HIV + pregnant women	407	IFA	0	407	0	14%	Serum	X	X
Lacoste 2000	Retrospective	France	Mauritania	MCD	1	X	1	0	0	X	Lymph node	A	N/M

Note: X indicates no data exist on that parameter. There are 15 countries in western African countries, of which 7 countries have published data based on the search criteria and date of search. N/M indicates not mentioned.

Table3.7: Characteristics of studies on East African countries included in the analysis

References	**Study type**	**Country of study**	**Country of participant**	**Study of population**	**Sample size**	**Type of Assay for sero-prevalence detection**	**No of males**	**No of females**	**No of children**	**Percentage sero-prevalenc**	**Source of DNA/ antibodies for serology &genotyping**	***K1* Genotypes**	***K15* Genotypes**
Pfeiffer 2010	Retrospective	Tanzania	Tanzania	Hospital based	248	ELISA	97	151	0	33.8%	X	X	X
Mwakigonja 2007	Prospective	Sweden	Tanzania	Hospital based	78	ELISA	24	54	0	69%	Serum	X	X
Mbulatieye 2003	Prospective	Tanzania	Tanzania	Hospital based	798	ELISA	357	379	62	54-93%	Serum	X	X
Mwakigonja 2008	Prospective	Sweden	Tanzania	Hospital based	184	ELISA + IFA	157	27	0	89%	Serum	X	X
Whitby 2004	Prospective	UK	Tanzania	Hospital based	23	ELISA	N/M	N/M	N/M	17.4%	X	X	X
Poole 1999	Retrospective	USA	Tanzania	AIDS-KS patients	2	X	2	0	0	X	KS biopsy	A, B	P
Lavreys 2003	Retrospective	Kenya	Kenya	Hospital based	633	ELISA	0	633	0	44.1%	X	X	X
Ignacio 2016	Prospective	USA	Kenya	KS patients	39	ELISA	39	0	0	16%	X	X	X
Baeten 2002	Retrospective	Kenya	Kenya	Population based	1061	ELISA	1061	0	0	42%	X	X	X

Taylor 2004	Prospective	Kenya	Kenya	Population based female prostitute	174	ELISA	0	174	0	32%	X	X	X
Whitby 2004	Prospective	UK	Kenya	Hospital based	23	ELISA	N/M	N/M	N/M	17.4%	X	X	X
Tornesello 2010	Prospective	Kenya	Kenya	AIDS-KS	68	X	5	1	62	X	KS biopsy	A, B, C, F	P
Senba 2011	Prospective	Kenya	Kenya	Hospital based	228	LANA	0	228	0	63.5%	X	X	X
Pfeiffer 2010	Retrospective	Kenya	Kenya	Hospital based	3196	ELISA	N/M	N/M	2040	42.4%	X	X	X
Mayama 1998	Retrospective	UK	Uganda	Hospital based	215	ELISA, IFA and WB	76	139	35	33-52%	Serum	X	X
De The 1999	Retrospective	Uganda	Uganda	Hospital based	118	IFA	0	15	103	88%	Serum	X	X
Wawer 2001	Retrospective	USA	Uganda	Random section-population based	523	ELISA and IFA	239	283	0	36.1-45.0%	Serum	X	X
Meng 1999	Prospective	Uganda	Uganda	AIDS-KS	4	X	3	1	0	X	KS biopsy	A, B	X
Kajumbula 2006	Retrospective	Uganda	Uganda	KS patients	56	X	N/M	N/M	N/M	X	Blood	A, B, C, F	X
Nalwoga 2015	Prospective	Uganda	Uganda	Cross-sectional; mother and child study	2391	ELISA	0	1164	1227	15-69%	Serum	X	X
Kakoola 2001	Retrospective	UK	Uganda	Endemic KS patients and blood donors	30 118	ELISA, IFA, WESTERN BLOT	25 98	5 18	N/M N/M	N/M 74%	KS biopsy Plasma	A, B, C	P, M
Tornesello 2010	Prospective	Italy	Uganda	AIDS-KS + Endemic KS	14	X	13	1	0	X	KS biopsy	A, B, C	P
Poole 1999	Retrospective	USA	Uganda	AIDS-KS patients	2	X	2	0	0	X	KS biopsy	B	P
Serraino 2001	Prospective	Uganda	Uganda	Hospital based	315	ELISA	42	273	0	44.8%	X	X	X
Shebl 2011	Retrospective	Uganda	Uganda	Population based	2681	ELISA	1448	1233	0	56.2%	X	X	X
Wakeham 2011	Retrospective	Uganda	Uganda	Population based	1915	ELISA	0	1915	0	32%	X	X	X
Bulter 2009	Retrospective	Uganda	Uganda	Population based	427	ELISA	0	0	427	9.0%	X	X	X

Pfeiffer 2010	Retrospective	Uganda	Uganda	Population based	3196	ELISA	N/M	N/M	N/M	70.6%	X	X	X
Ablashi 1999	Retrospective	USA	Uganda	Hospital based	62	ELISA	44	18	0	45.7%	X	X	X
Biryahwaho 2010	Retrospective	Uganda	Uganda	Survey	2681	ELISA	N/M	N/M	N/M	55.4%	X	X	X
Mbulaiteye 2008	Retrospective	Uganda	Uganda	Hospital based	461	ELISA	0	228	233	43%	X	X	X
Mbulaiteye 2006	Retrospective	Uganda	Uganda	Hospital based	600	ELISA	0	600	0	N/M	Saliva and buffy coat	A, B, C	X
Cook 1999	Retrospective	UK	Uganda	Hospital based	7	X	N/M	N/M	N/M	X	PBMCs and biopsy	A, B, C	X
Zong 2002	Retrospective	USA	Uganda	Hospital based	3	X	N/M	N/M	N/M	X	Biopsy	A, B	X
Whitby 2004	Prospective	UK	Uganda	Hospital based	23	ELISA	N/M	N/M	N/M	38.7%	X	X	X
Grossman 2002	Retrospective	Ethiopia	Ethiopia	Hospital based	202	LANA	N/M	N/M	N/M	39.1%	Serum	X	X
Lemma 2009	Retrospective	Ethiopia	Ethiopia	Hospital based	400	LANA	0	400	0	53%	X	X	X
Enbom 1999	Retrospective	Eritrea	Eritrea	Hospital based	511	IFA	0	327	184	2-26%	Serum	X	X

Note: X indicates no data exist on that parameter. There are 12 countries in eastern African countries, of which 5 countries have published data based on the search criteria and date of search. N/M indicates not mentioned.

Table 3.8: Characteristics of studies on North African countries included in the analysis

References	**Study type**	**Country of study**	**Country of participant**	**Study of population**	**Sample size**	**Type of Assay for sero-prevalence detection**	**No of males**	**No of females**	**No of children**	**Percentage sero-prevalence**	**Source of DNA/ antibodies for serology &genotyping**	***K1* Genotypes**	***K15* Genotypes**
Lacoste 2000	Retrospective	France	Algeria	HIV+ MCD	**1**	X	1	0	0	X	Spleen	C	P
Andreoni 1999	Prospective	Egypt	Egypt	Hospital based	246	ELISA IFA	126	0	196	42.4% 7-45%	X	X	X
Andreoni 2002	Prospective	Egypt	Egypt	Hospital based	86	IFA	0	0	86	41.9%	X	X	X
Lahlaoui 2012	Prospective	Egypt	Egypt	Hospital based	196	ELISA	N/M	N/M	N/M	44.7%	X	X	X

Mbulaiteye 2008	Retrospectiv e	Egypt	Egypt	Random selection	965	ELISA	N/M	N/M	N/M	24.2%	X	X	X
Serraino 2001	Prospective	Egypt	Egypt	Population based	236	ELISA IFA	120	116	0	42.4% 42-43%	X	X	X
Hannachi 2012	Prospective	Tunisia	Tunisia	Hospital based Pregnant women	553	ELISA	0	553	0	13.8%	X	X	X
El Kissi 2011	Prospective	Tunisia	Tunisia	Hospital based	N/M	ELISA	N/M	N/M	N/M	28.7%	X	X	X
Lahlaoui 2012	Prospective	Tunisia	Tunisia	Hospital based	60	ELISA	26	34	0	17%	X	X	X
El Kassimi 2003	Prospective	Morocc o	Morocco	KS patients	26	ELISA	N/M	N/M	N/M	92%	X	X	X
Duprez 2006	Prospective	Morocc o	Morocco	AIDS-KS	28	X	20	8	0	X	Biopsy	A, B, C	P, M

Note: X indicates no data existed on that parameter. There are 7 countries in northern African countries, of which 4 countries have published data based on the search criteria and date of search. N/M indicates not mentioned.

3.4 DISCUSSION AND CONCLUSIONS

3.4.1 Discussion

Studies on infection with cancer associated viruses, such as HHV-8 are important in regions such as Africa, where there is a significant proportion of the continent is infected with immunosuppressive agents as HIV. This review aimed at providing an update on the epidemiology and prevalent genotypes of HHV-8 in Africa within 1998 – 2017.

Our analyses show that HHV-8 is generally highly endemic in the entire African continent, indicating that the risk factors for infection persist throughout the continent. Reports suggest that there is a variety, and non-uniformity in the modes of transmission of HHV-8 among populations in Africa. For example, horizontal transmission from mother to child through chewed food (saliva) (Minhas et al., 2008; Hannachi et al., 2012; Phipps et al., 2014), sexual intercourse (Rezza et al., 2000; Mbulaiteye et al., 2000; Ogoina et al., 2011), other infections such as malaria (Wakeham et al., 2013), and blood transfusion (Gobbini et al., 2012; Hannachi et al., 2012) have been identified as modes of transmission. Generally, there is relatively lower rate of infection in children in Africa, which reflects findings that the rate of infection increases with the older age group (Mbulaiteye et al., 2005; Adjie et al., 2008). Most of the countries in Central, Southern and East African regions of the continent harbor huge burdens of HHV-8 infection. Geographic and cultural factors have been proposed as key determinants of HHV-8 transmission in some of these regions (Plancoulaine et al., 2004; Malope et al., 2008; Malope et al., 2010), also accounting for the variablility in prevalence across and within geographic regions (Pfeiffer et al., 2010). Additionally, a meta-analysis involving studies undertaken in 32 countries in sub-Saharan Africa, Australia, North and South America, Europe and Asia, demonstrated that HIV-infection is associated with a significant increase in HHV-8 co-infection globally, and in all population groups (Rohner et al., 2016). This scenario thereby coexists with the high HIV endemicity in Central, East and Southern Africa, and the probability of co-infection is therefore significantly increased in the said population. Hence, with immunosuppression from HIV infection, it is plausible that cases of Kaposi's sarcoma will rise, although this could be mitigated with antiretroviral

therapy (Olp et al., 2015). This further emphasizes the additional benefit of access to antiretroviral therapy for HIV infected individuals.

Our analysis revealed that HHV-8 genotypes A5 and B based on the *K1* gene and genotype P and M based on the *K15* gene are the most circulating genotypes in Africa. Genotype B is associated with endemic KS, while genotype P has been reported to be highly transmissible. Previous reports (Hayward et al., 1999; Zong et al., 2002; Whitby et al., 2004) have confirmed the clustering patterns of HHV-8 genotypes with ethnic composition of the population and geographical location, and these might have arisen through ancient human migrations and genetic polymorphisms. The classification of HHV-8 into genotypeshas not generally followed a unified approach. Some studies have used more than one gene for viral grouping, and some investigators has shown a link between gene regions; for example, Tornesello et al., 2010 reported a link between ORF26 and *K1*. Intra-genotype variants have also been reported; for example, *K1* A5 and *K15* M based on allele differences (Poole et al., 1999; Betsem et al., 2014; Kakoola et al., 2001). A5 viruses are closely related to viruses found mostly in viral populations that form the A1-4 genotypes. Prevously, A5 was thought to have emerged from recombination. All in one, previous studies have demonstrated the clustering patterns of HHV-8 genotypes with geography and ethnicity, and these may have emerged through ancient human movement and genetic polymorphisms, respectively (Zong et al., 2001; Lacoste et al., 2000).

3.4.2 Strength of the study

The strength of this study is the use of a large sample size, which involved reporting on HHV-8 seroprevalence and circulating genotypes for all 53 African countries.

3.4.3 Conclusion

This is patently, the first systematic review on the seroprevalence and genotype distribution of HHV-8 considering all African countries. The findings suggest that the entire continent is endemic for HHV-8, and co-infection with HIV would be common in those African countries endemic for HIV. Perceiving that there is high heterogeneity in the testing strategies employed in the detection of HHV-8 antibodies, there is need for an acceptable harmonized protocol to enhance the possibility of comparisons of

seropositivity across studies. Considering genotypes A5, B, P and M are highly prevalent in Africa, it is recommended that full length genomes of these genotypes from Africa be classified to brace the rationale selection of genes for the design and formation of vaccine candidates.

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CHAPTER FOUR

SEROPREVALENCE OF HHV-8 IN NORTHERN SOUTH AFRICA

ABSTRACT

Background: Human herpes virus type 8 (HHV-8) is an oncogenic virus and a key determinant for Kaposi's sarcoma development, particularly in individuals with prolonged immune suppression, for example HIV infected individuals. South Africa is highly endemic with HIV, with the potential for an epidemic of Kaposi's sarcoma. For a better understanding of the consequence of HHV-8 and HIV co-infection, it is important to establish the burden of HHV-8 infection in South Africa, particularly in regions such as Northern South Africa (Limpopo Province) which has a relatively high prevalence of HIV but where data on HHV-8 is non-existent.

Objective: The main objective of this component of the study was to establish and determine the sero-prevalence of HHV-8 ORFK8.1 lytic and HHV-8 ORF73 latent antibodies in Northern South Africa.

Methodology: Three thousand, five hundred and one plasma (3501) samples were retrospectively collected between September 2015 to February 2016, from a cross section of HIV infected individuals from the five districts comprising the Limpopo Province of South Africa. Plasma was tested for antibodies to HHV-8 K8.1 and ORF73 antigens using an in-house protocol developed by the Viral Oncology Section of the National Cancer Unit, USA. A sample was considered positive for HHV-8 antibodies if at least one of the antigen is detected. Antibody distribution in the study population was assessed according to gender; age group; racial population groups; districts; an immune suppression measured by CD4+ cell count; HIV viral load and year of sample collection. Differences among variables were assessed for significance at 95% confident interval, with a p value of less than 0.05 considered significant.

Results: HHV-8 prevalence of 19.0% (reactive to either lytic K8.1, ORF73 or both K8.1/ORF73) was detected in the study population. Antibodies to HHV-8 K8.1 antigen were detected in 8.6% of the study population, antibodies to HHV-8 ORF73 were detected in 2.1% of the study population, while antibodies for HHV-8 K8.1 and HHV-8 ORF73 were detected in 8.2% of the study population. Significant differences were observed for age groups, racial population groups, districts HIV viral load and year of sample recruitment (p=<0.0001, p=<0.0001, p=<0.0001 p=0.0300 and p=0.0385) respectively. Associations were found between both antigens in comparison to the

different variables such as age group, racial population groups and districts (R^2 value ranging between 0.886 and 1.0)

Conclusion: There is a high prevalence of HHV-8 in Northern South Africa. Age groups, racial population groups, districts and HIV viral load and year of sample recruitment are associated with the acquistion of HHV-8 infection. This is the first study to report on the seroprevalence of HHV-8 considering all districts from Northern Soith Africa.

Keywords: HHV-8 seroprevalence, ORFK8.1 and ORF73 antigens, Northern South Africa.

4.1 INTRODUCTION

HHV-8 is an oncogenic virus and a key determinant for Kaposi's sarcoma development, particularly in individuals with prolonged immune suppression, for example HIV infected individuals. In African countries where human herpes virus type 8 (HHV-8) infection is very common in the general population. Thus, defining the various risk factors associated with this infection remains complex

South Africa is highly endemic with HIV, with the potential for an epidemic of Kaposi's sarcoma. In 1991, Rakkin reported that, the HIV epidemic in South Africa has lead to a 50-fold increase towards the risk of HIV patients developing KS. Moreover, the incidence of KS in South Africa between 1988 – 1996 was reported at an increase of about 3 folds and continues to increase as HIV prevalence increases (Sitas et al., 2000). Also, between 1992 – 1996, the South African Cancer Registry reported an increased ratio of 7:1 between male and female. The role of HIV as a predatory factor for HHV-8 infection in South Africa cannot be fully justified because some reports show associations while other reports do not (Rakhin et al.,1991; Sitas et al.,1998). In Egypt, HHV-8 seroprevalence was 14.2% among those less than 15 years of age, and 24.2% and 72.8%, Mbulaiteye (2008). In China, HHV-8 seroprevalence ranged between 9.8 – 32.3% (Zhang et al., 2017). However, the majority of these studies used the IFA screening method, which was tested using ELISA lytic or ELISA latent antibodies but not both. Therefore, in this study, detection of both the lytic K8.1 and latent ORF73 antibodies to HHV-8 will be done. Additionally, HIV prevalence for the general population visiting public sector health instutions in Northern South Africa has been used as a reliable source to pinpoint the HIV epidemic in South Africa. Understanding HHV-8 infection in this general population will provide a reasonable and comparable impact to the govenment health policy.

Although it is obvious that various patterns of KS exist in countries where HHV-8 is prevalent, the route in which humans acquire the virus is still unknown. This indicates the necessity for epidemiological studies to provide knowledge of these emerging non-sexual transmission patterns. This idea is also backed-up by data indicating HHV-8 seropositivity in American children and adolescents (Anderson et al., 2008). Studies in Africa have suggested that, in South Africa, HHV-8 infection in children may be

acquired from non-sexual routes, such as vertical transmission (Dedicoat et al., 2004).

Based on serological assay and molecular characterization, the prevalence of HHV-8 is high within the Mediterranean population and among countries within sub-Saharan Africa (Oettle et al., 1962; Biggar et al., 1984; Hjalgrim et al., 1996). Furthermore, the seroprevalence also varies by geographical locations. HHV-8 infects the general population and it has been observed that, some demographic and clinical parameters play an important role in HHV-8 transmission within the general population. In an HIV infected population, HHV-8 seroprevalence ranged from 30-48% in North America while rising to above 60% in sub-Saharan Africa (Dedicoat and Newton, 2003; Ablashi et al., 1999; Martin et al., 1998; Gao et al., 1996).

It is not clearly understood whether HIV facilitates the distribution of HHV-8 in the study setting. HHV-8 shedding will differ in relation to antibody titre, as well as in relation to HIV infection. Although literature shows a possible relationship, additional information is needed to understand this tendency. For a better understanding of the consequence of HHV-8 and HIV co-infection, it is important to establish the burden of HHV-8 infection in South Africa, particularly in regions such as Northern South Africa (Limpopo Province) which has a relatively high prevalence of HIV but where data on HHV-8 is non-existent.

In this chapter, we focused on determining the seroprevalence of HHV-8 ORFK8.1 lytic and HHV-8 ORF73 latent antibodies from an HIV infected population, as well as determining the different risk factors associated with HHV-8 infection in Northern South Africa.

4.1.1 Objectives

The overall objective was to determine the seroprevalence of HHV-8 in Northern South Africa.

The specific objectives were:

1. To determine the prevalence of antibodies to HHV-8 ORFK8.1 and HHV-8 ORF73 antigens in the study population.

2. To determine the distribution of antibody response to HHV-8 ORFK8.1 and ORF73 antigens according to gender, age groups, racial population groups, districts, $CD4^+$ cell count, HIV viral load and year of HIV sample recruitment.

4.2 METHODOLOGY

4.2.1 Ethical considerations

Ethical clearance was obtained from the University of Venda's Health Safety and Research Ethics committee and permission to use public hospitals and clinics was obtained from the Limpopo Department of Health, Polokwane. This study is nested with other studies which are ongoing and has as research project number: SMNS/15/MBY/23/0710. Volunteers were provided with consent forms which they signed prior to sample collection. Specific research codes were assigned to each samples collected to ensure patient confidentiality.

4.2.2 Study areas, study size estimation and sample collection

4.2.2.1 Study area

The Limpopo Province of South Africa which comprised of Capricorn (1528); Waterberg (659); Sekhukhune (867); Mopani (320) and Vhembe (307), districts were used for the study. Northern South Africa is the entry path to the rest of Africa and it shares borders with Botswana to the West, Zimbabwe to the North and Mozambique to the East. Furthermore, towards the Eastern region lies the Kruger National Park.

4.2.2.2 Study size and sample recruitment

A total of 3501 plasma samples were collected for the study. Demographic as well as geographical data collected included age, gender, ethnicity, geography, $CD4^+$cell count, HIV viral load and year of sample collection. Samples were collected for a duration of 6 months. About 500 μl of plasma was available for each participant. The samples were transported on ice to the Molecular HIV genetics research laboratory of

the University of Venda, where the samples where then stored at -80°C awaiting use.

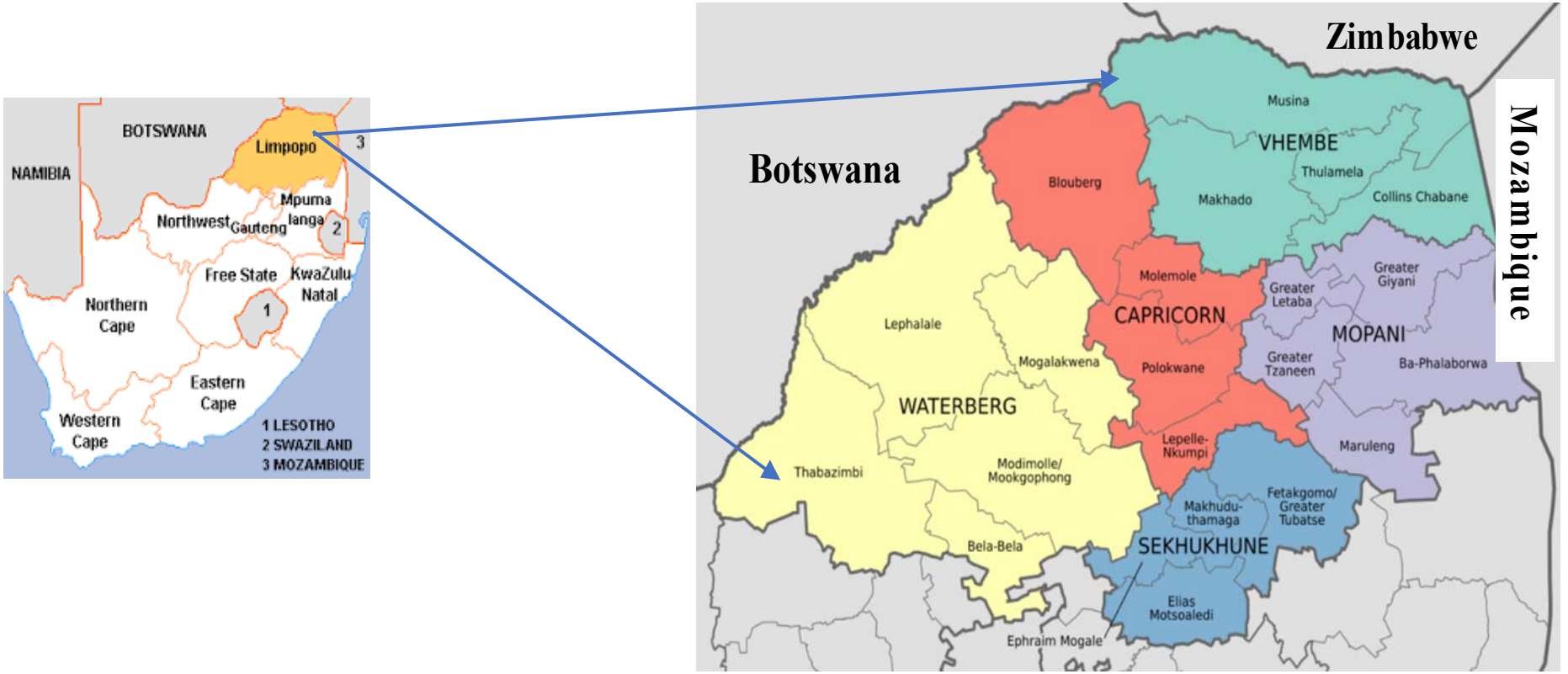


Figure 4.1: Map of Limpopo Province, showing the 5 main districts and countries which share borders with South Africa. The green bullet indicates the 5 districts from which samples were collected and the box coloured purple is the University of Venda were samples were transported to for processing and analysis .

4.2.3 Overview of serological assay for human herpes virus type 8 screening

Several assays have been established for the detection of HHV-8 seroepidemiology but some are more sensitive, specific and less costly than others. The most commonly used antigens in HHV-8 assays are: ORFK8.1 which is a lytic phase of glycoprotein expressed on the cell surface of the virion; and latency associated nuclear antigen (LANA), which is encoded by ORF73. ORF73 is a protein which is expressed during latency and inhibits p53, which if not prevented from expressing, has the ability to trigger apoptosis (DNA damage). HHV-8 infection is best described by the presence of HHV-8 antibodies.

4.2.4 HHV-8 serology

The HHV-8 assay used in this study is a recombinant peptide of ELISA to ORFK8.1 and ORF73 proteins developed by the Viral Oncology Section (VOS), National Cancer Institute (NCI), USA. Assays ORFK8.1 and ORF73 have a high performance accuracy with a sensitivity of 98.78% and 89.02% respectively, and specificity of 98.79% and 97.57% respectively. Plates were designed and coated with samples from patients with KS (positive control) and samples from an American blood donor population (negative control). These controls were coated in triplicate per plate. All ELISA plates to be screened for both recombinant peptides were transferred from Viral Oncology Section, AIDS and Cancer Virus Program, Science Application International Corporation (SAIC-Frederick), National Cancer Institute (NCI-Frederick), Frederick, Maryland, USA NCI to the HIV/AIDS Molecular Genetic Research Laboratory of the University of Venda, South Africa.

4.2.5 ORFK8.1 ELISA preparation and screening procedures

Recombinant ORFK8.1 protein was diluted 1/5000 in 0.05M Sodium Carbonate/ 0.05M Sodium Bicarbonate, pH 10.0 (Invitrogen, Carlsbad, CA) and 100 µl of diluted protein was added to each well of a flat– bottom, 96-well, non-sterile Dynex Immulon 4 HBX microtitre plate (VWR, Bridgeport, NJ). Each plate was covered with a plate sealer and incubated overnight at 4°C. The wells were then washed 3 times with 350

µl wash buffer (300ml PBS Ph7.4 10x, 0.005% Tween 20 (Sigma Aldrich), 2700ml distilled water) using an automated plate washer (model ELx405 Bio-Tek Instruments). Assay buffer of a volume of 280 µl was used to block each well [(2.5% BSA [Sigma-Aldrich], 2.5% normal goat serum (Equitech-Bio, Kerrville, TX), 0.005% Tween 20 (Sigma-Aldrich) and 0.005% Triton-X 100 (Sigma-Aldrich) in dulbeccos PBS)] for 3h at 37°C. Plates containing assay buffer were stored at -80°C for transportation and subsequent use.

Plates were defrosted for an hour at room temperature. They were washed 3 times with 350 µl wash buffer using an automated plate washer. Diluted test and control sera (final volume of 100 µl at 1/20 dilution in assay buffer) were dispensed into designated wells and incubated at 37°C for 90 minutes. Unbound antibodies were removed by washing the plates five times with wash buffer. The conjugate was prepared by diluting Goat anti- human IgG (g) alkaline phosphatase (Reserve APTM, KPL, Gaithersburg, MD) at 1:15000 with assay buffer and 100 µl of diluted conjugate added to each well. Plates were then covered and incubated at 37°C for 30 minutes. After 5 washes, 100 µl of substrate, [{1 step p-nitrophenyl phosphate}, disodium salt (Pierce, Rockport, IL)] was added to detect antigen–antibody complexes and incubated for 25 minutes in the dark. After incubation, 50ul of a 3N NaOH was added to each well to stop the reaction and the absorbance was read at 405nm using a spectra-fluorometer (Versa max; softmax pro software Version 6.3).

4.2.6 ORF73 ELISA preparation and screening procedures

Recombinant ORF73 protein (0.5 mg/mL) was diluted 1/500 in phosphate buffer saline (PBS) (Invitrogen, Carlsbad, CA) and 100 µl of diluted protein was added to each well of a flat-bottomed, 96-well, non- sterile Dynex Immulon 4 HBX microtitre plate (VWR, Bridgeport, NJ). Each plate was covered with a plate sealer and incubated overnight at 4°C. The wells were then washed 3 times with 350 µl of in-house ELISA wash (300ml PBS Ph7.4 10x, 0.005% Tween 20 (Sigma Aldrich), 2700ml distilled water) using an automated plate washer (model ELx405 Bio-Tek Instruments). Assay buffer of a volume of 280 µl was used to block each well (2.5% BSA [Sigma-Aldrich], 2.5% normal goat serum [Equitech-Bio, Kerrville, TX], 0.005% Tween 20 [Sigma-Aldrich] and 0.005% Triton-X 100 [Sigma-Aldrich] in Dulbecco's PBS]) for 3h at 37 °C.

Plates containing assay buffer were stored at -80°C for transporting and subsequent use.

Plates were defrosted for an hour at room temperature. They were washed 3 times with 350 µl wash buffer using an automated plate washer. Diluted test and control sera (final volume 100 µl at 1/100 dilution in assay buffer) were dispensed into designated wells and incubated at 37°C for 90 minutes. Unbound antibodies were removed by washing the plates five times with wash buffer. The conjugate was prepared by diluting Goat anti- human IgG(g) alkaline phosphatase (Reserve APTM, KPL, Gaithersburg, MD) at 1:5000 with assay buffer and100 µl of diluted conjugate added to each well. Plates were covered and incubated at 37°C for 30 minutes. After 5 washes in 350 µl wash buffer, 100 µl of substrate [1 step p-nitrophenyl phosphate, disodium salt (Pierce, Rockport, IL)] was added to each well to detect antigen–antibody complexes and incubated for 30 mins at room temperature in the dark. After incubation, 50ul of a 3N NaOH was added to each well to stop the reaction and the absorbance was read at 405nm using a spectra-fluorometer (Versa max; softmax pro software Version 6.3).

4.2.7 Cut-off values for HHV-8 assay

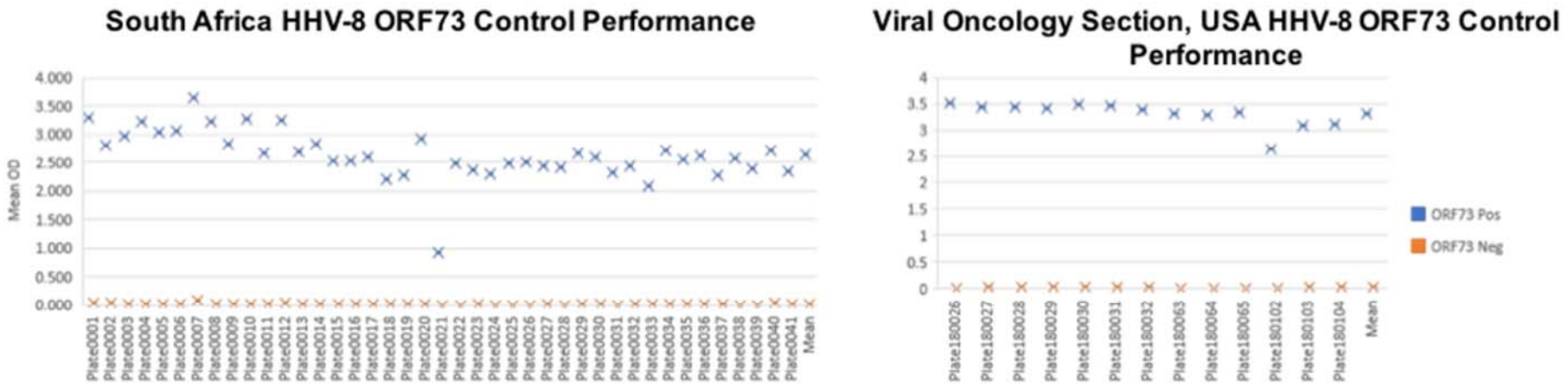
From each assay which was on account of a plate-to-plate variation, a cut-off was calculated. For the ORFK8.1 ELISA, the cut off was calculated as the mean of the negative controls plus 0.95 for each plate. For the ORF73 assay, a cut-off was calculated at the mean of the negative controls plus 0.35 for each plate. These values were based on the variations observed with the blanks, positives and negative controls per plate.

4.2.8 HHV-8 assay quality control and intra-plate quality control

For the assay results to be considered valid, the mean of the two blanking wells was calculated and required to be between -0.05 and 0.05. The mean of the blank wells was subtracted in turn from each negative and positive control value. For ORFK8.1, each negative control value needed to have an optical density value of between 0 and 0.2 after subtracting the mean of the OD of the two blank wells. For ORF73, each negative control should have an optical density value of between 0 and 0.1 after subtracting the mean OD of the two blank wells. For both ORFK8.1 and ORF73, each

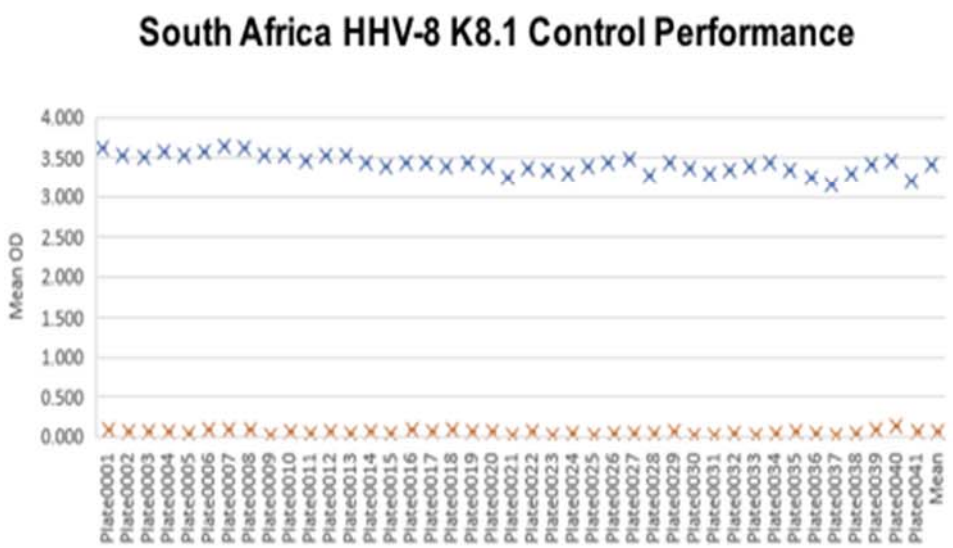
positive control value needed to have an OD value greater than 1.5 after subtracting the mean of the OD of the blank wells.

For assay validity, OD analysis for the positive and negative controls showed comparable performance with controls from NCI and UNIVEN (Figures 4.2 and 4.3). For analysis with outlier ODs (less than the expected), the experiments were repeated and ODs were in concordance with the other ODs.

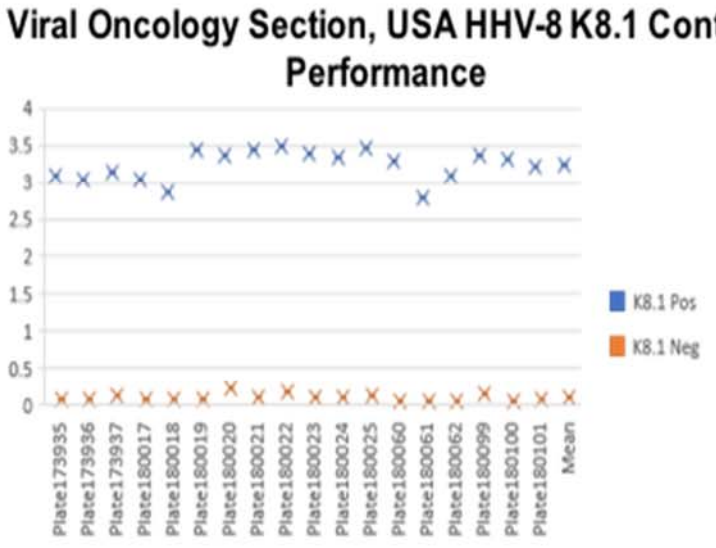


A B

Figure 4.2 Assay validity for mean OD values for ORF73 experiment performed in South Africa **A** compared with that performed in the USA **B.** The OD ranged between 2.500 – 3.500. The validation indicates comparable outcomes in validity for the University of Venda and National Cancer Institute site.



A



B

Figure 4.3 Assay validity for mean OD values for K8.1 experiment performed in South Africa, **A** compared with that performed in the USA, **B.** The OD ranged between 3.00 – 3.500. The validation indicates comparable outcomes in validity for the University of Venda and National Cancer Institute site.

4.2.9 Data Preparation for statistical analysis

4.2.9.1 Data clean-up

All ELISA data were entered into an excel spread sheet and checked for mislabels of codes or codes which exist more than once. Parameters such as age, gender, racial population groups, districts, $CD4^+$ cell count, HIV viral load, and year of sample recruitment were entered for each corresponding sample ID.

4.2.9.2 Data analysis

Descriptive statistics was used. Data were entered on an excel spread sheet and analyzed using Pearson Chi square analysis within PRISM (Version 11.0). Univariant and multivariant analyses were also performed to check for association between the different outcomes. Proportions were compared with the chi-square test, while for level of statistical significance, $P<0.05$ was considered and for level of association Pearson Coefficient was used. Results of assays for ORFK8.1 and ORF73 were analyzed separately and then combined: a subject was defined as seropositive for HHV-8 if they tested positive for K8.1 or ORF73. For a sample to be considered positive for HHV-8 infection, the creteria below must be applicable:

HHV-8 Sero-positivity = [ORFK8.1 only or ORF73 only or both ORFK8.1 + ORF73].

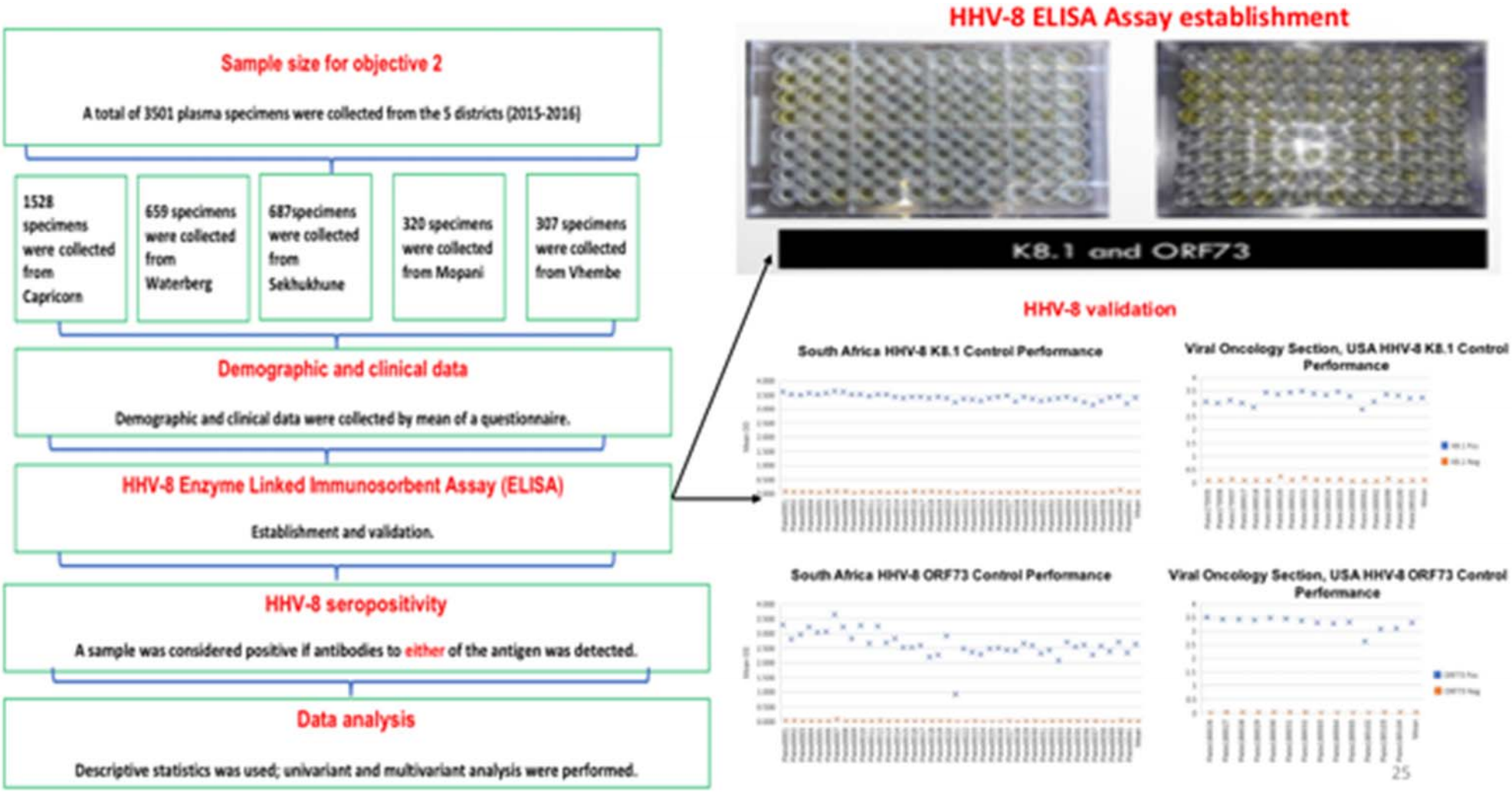


Figure 4.4: Flow diagram of a successful establishment of HHV-8 ELISA assay.

4.3 RESULTS

4.3.1 Demographic characteristics of the study participants

Plasma for each of the 3501 individuals was tested for antibodies of HHV-8. Of the 3501 participants, 2449 (70.0%) were females, 1026 (29.3%) were males while 26 (0.7%) participants had no demographic data. The mean age of the study participants was 45.0 years (range, 1-86 years). Table 4.1 represents details of the demographic and clinical data for the study participants.

Table 4.1: Demographic and clinical characteristics of the study participants

Characteristics	**Frequency (%)**
Gender	**n = 3501**
Female	2449 (70.0)
Male	1026 (29.3)
Unknown	26 (0.7)
Age (years)	**n = 3501**
<14	26 (0.7)
15 - 24	91 (2.5)
25 - 34	261 (7.4)
35 - 44	555 (15.8)
45 - 55	1677 (47.9)
>55	865 (24.7)
Unknown	26 (0.7)
Racial population groups	**n = 3109**
Nguni (Zulu, Xhosa, Ndebele and Swazi)	434 (0.5)
Sotho (Tswana, Pedi and Sotho)	2004 (0.6)
Shangaan-Tsonga	287 (12.4)
Venda	258 (57.2)
Asian	19 (8.2)
White	87 (7.4)
Coloured	20 (2.5)
Unknown	392 (11.19)

Districts	**n = 3486**
Capricorn	1528(43.6)
Mopani	320 (9.1)
Sekhukhune	687 (19.6)
Vhembe	292 (8.3)
Waterberg	659 (18.8)
Unknown	15 (0.4)
CD4+ cell counts (cells/mm^3)	**n = 1150**
< 350	836 (28.9)
>350	314 (8.96)
Unknown	2351 (67.15)
HIV Viral load (Copies/ml)	**n = 1098**
<1000	460 (13.14)
>1000	638 (18.22)
Unknown	2403 (68.63)
Year of HIV sample recruitment	**n = 3501**
2015	1500 (42.8)
2016	2001 (57.2)

Note: All participants were HIV-1 positive.

4.3.2 Overall HHV-8 sero-prevalence in the study population

Of the 3501 participants: 2449 (70.0%) were female; 1026 (29.3%) were males and 26 (0.7%) had no demographic data. The mean age was 45.0 years (1-86 years). From the total of 3501 participants screened: 662 (19.0%) were reactive to: ORFK8.1antigens 299 (8.6%); ORF73 75 (2.1%) and both antigens 288 (8.2%). A significant number of the subjects were reactive to both antigens (p=0.0001).

4.3.3 To determine the prevalence of antibodies to K8.1 and ORF73 antigens of HHV-8 in the study population.

The seroprevalence of antibodies to K8.1 and ORF73 were common in the population and the difference for either of these antibodies present was highly statistically significant (χ^2= 4.910, p= 0.0001).

4.3.4 To determine the distribution of antibody response to ORFK8.1 and ORF73 antigens according to gender; age groups; racial population groups; districts; CD4^{+} cell count; HIV viral load and year of sample recruitment.

A significant difference (0.0001) and a perfect correlation (1.0) were observed with age groups. Additiona, significant differences were observed with; districts (0.0061; 0.0032; 0.0041 and 0.00092); racial population groups (0.0025; 0.0188; 0.0057 and 0.004). Year of sample recruitment (0.0385). Table 4.2 shows analyses based on univariant and multivariant.

The distribution of HHV-8 antibodies according to gender, showed that either bother male and female either or the antigens was detected. However, no significant difference no association was observed (see table 4.2; figure 4.5).

The distribution of HHV-8 antibodies according to age groups, illustrated that an increase in age has an implication that HHV-8 has an influence on the acquisition of infection. Signifcance difference and association were observed (see table 4.2; figure 4.6).

The distribution of HHV-8 antibodies according to districts in comparison with HIV prevalence in the district were of almost the same level. This ia an indication that district position does not restrict contraction of HHV-8. Signifcance difference and association were observed (see table 4.2; figure 4.7).

Table 4.2: Distribution of HHV-8 seropositivity and risk factors

Characteristics	Number tested (%)	K8.1 reactivity (%)	Chi-Square, P- and correlation values K8.1	ORF 73 reactivity (%)	Chi-Square, P- and correlation values ORF73	K8.1 and ORF73 reactivity (%)	Chi-Square, P- and correlation values K8.1 and ORF73	K8.1 or ORF73 reactivity (%)	Chi-Square, P- and correlation values K8.1 or ORF73
Gender									
Female	2449(69.95)	212 (8.65)	0.128, 0.720	54 (2.20)	0.287, 0.594	200(8.16)	0.044, 0.833	466 (19.02)	0.0801, 0.777
Male	1026 (29.31)	85 (8.28)		20 (1.94)		86 (8.38)		191 (18.61)	
Missing data	26 (0.74)	2 (7.69)	-	1 (3.84)	-	2 (7.69)	-	5 (19.2)	-
Age groups									
<14	26 (0.74)	2 (7.69)	0.5088, <0.0001 **(S),** 0.998	0 (0.00)	0.5007, 0.0997 0.609	1(3.85)	0.5131, <0.0001 **(S),** 0.9941	3(11.53)	0.5126, <0.0001 1.0 **(PC)**
15 - 24	91 (2.59)	6 (6.59)		0 (0.00)		4 (4.39)		10 (10.98)	
25 - 34	261 (7.45)	24 (9.19)		9 (3.45)		22 (8.43)		55 (21.07)	
35-44	555 (15.85)	49 (8.83)		9 (1.62)		49 (8.83)		107 (19.27)	
45-55	1677 (47.90)	138 (8.23)		37 (2.21)		148 (8.82)		323 (19.26)	
>55	865 (24.71)	79 (9.13)		19 (2.19)		61(7.05)		159 (18.38)	
Missing data	26 (0.74)	2 (7.69)	-	1 (3.84)	-	2 (7.69)	-	5 (19.20)	-
Year of sample recruitment									
2015	1500 (42.84)	121 (8.06)		29 (1.93)		135 (9.00)	2.081,	285 (8.14)	0.014,

2016	2001 (57.15)	178 (8.89)	0.754, 0.0385 **(S)**	46 (2.29)	0.456, 0.459	153 (7.64)	0.149	377 (10.76)	0.905
CD4+ cell count (cells/ml)									
<350	836 (28.9)	84 (10.04)	0.053, 0.818	16 (1.91)	0.000, 0.990	93 (11.12)	0.589, 0.442	193 (5.51)	0.160, 0.688
>350	314 (8.96)	33 (10.50)		6 (1.91)		30 (9.55)		69 (1.97)	
Missing data	2351 (67.15)	182 (7.74)	-	53 (2.25)	-	165 (7.01)	-	400 (11.42)	-
HIV viral load (copies/mm^3)									
<1000	460 (13.14)	56 (12.17)	2.169, 0.140	11 (2.39)	0.605, 0.436	57 (12.39)	1.313, 0.251	124 (3.54)	4.7044, 0.0300 **(S)**
>1000	638 (18.22)	60 (9.61)		11 (9.40)		65 (10.18)		136 (3.88)	
Missing data	2403 (68.63)	183 (7.61)	-	53 (2.20)	-	166 (6.90)	-	402	-
Racial population groups									
Nguni (Zulu, Xhosa, Ndebele and Swazi)	434 (12.39)	70 (16.13)	0.3008, 0.0025 **(S),** 0.905	20 (4.61)	0.3124, 0.0188 **(S),** 0.7823	62 (14.29)	0.299, 0.0057 **(S),** 0.867	152 (4.34)	0.2752, 0.004 **(S),** 0.886
Sotho (Tswana, Pedi and Sotho)	2004 (57.24)	125 (6.24)		25 (1.25)		104 (5.19)		254 (7.25)	
Shangaan-Tsonga	287 (8.19)	59 (2.56)		16 (5.57)		51 (17.77)		126 (3.59)	
Venda	258 (7.37)	26 (10.08)		3 (1.16)		46 (17.83)		75 (2.14)	
White	87 (2.49)	5 (5.74)		2 (2.29)		4 (4.59)		11 (0.31)	

Asian	19 (0.54)	3 (15.79)		0 (0.00)		0 (0.00)		3 (0.08)	
Coloured	20 (0.57)	1 (5.00)		0 (0.00)		1 (5.00)		2 (0.05)	
Missing data	392 (11.19)	10 (2.55)	-	9 (2.29)	-	20 (5.10)	-	39 (1.11)	-
Districts									
Capricorn	1528 (43.64)	145 (9.49)	19.55, 0.00061 **(S),** 0.7283	31(2.03)	15.87, 0.0032 **(S),** 0.7064	150 (9.82)	15.29, 0.0041, **(S),** 0.9973	326 (9.31)	23.6836, 0.000092, **(S),** 0.9881
Waterberg	659 (18.82)	37 (5.61)		8 (1.21)		49 (7.44)		94 (2.68)	
Sekhukhune	687 (19.62)	78 (11.35)		11 (1.60)		57 (8.31)		146 (4.17)	
Mopani	320 (9.14)	20 (6.25)		15 (4.69)		22 (6.88)		57 (1.62)	
Vhembe	292 (8.34)	19 (6.51)		10 (3.42)		10 (3.42)		39 (1.11)	
Missing data	15 (0.43)	-	-	-	-	-	-	-	-

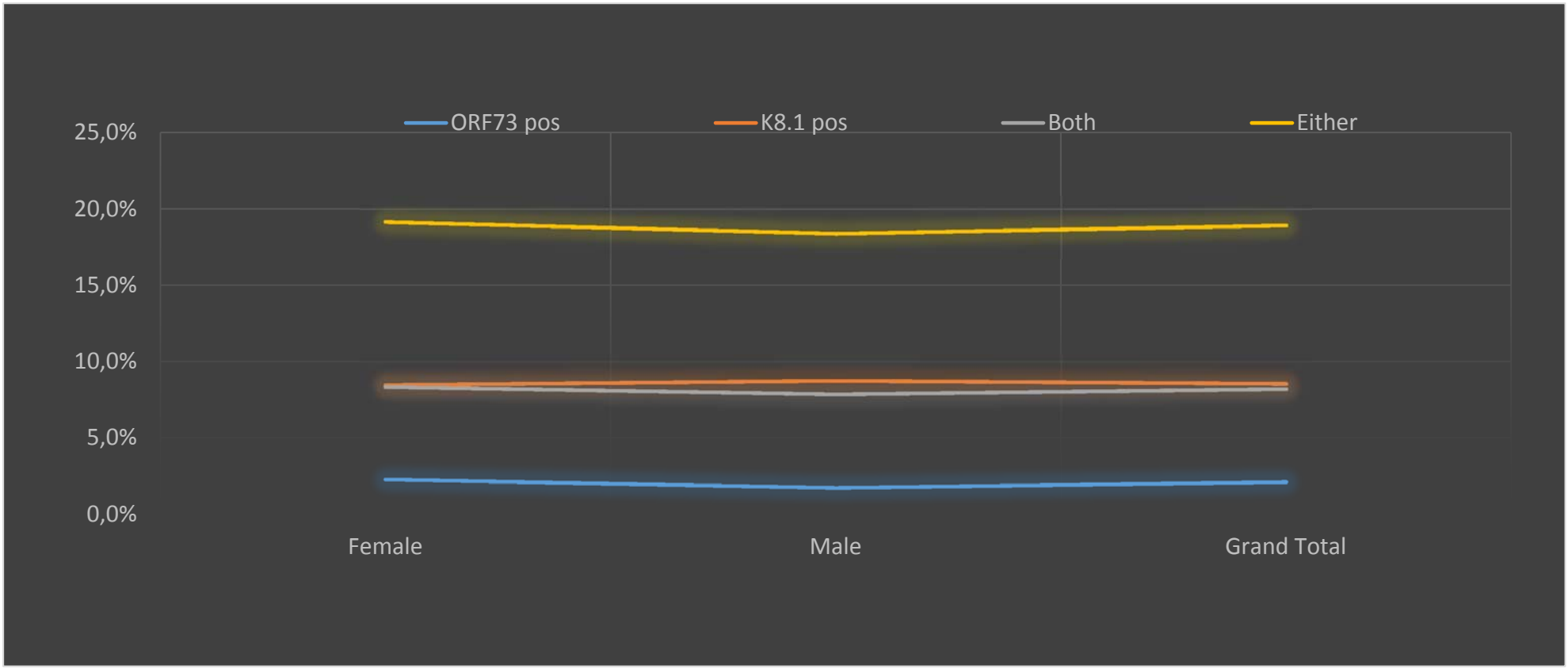


Figure 4.5: Distribution of HHV-8 antibodies according to gender.

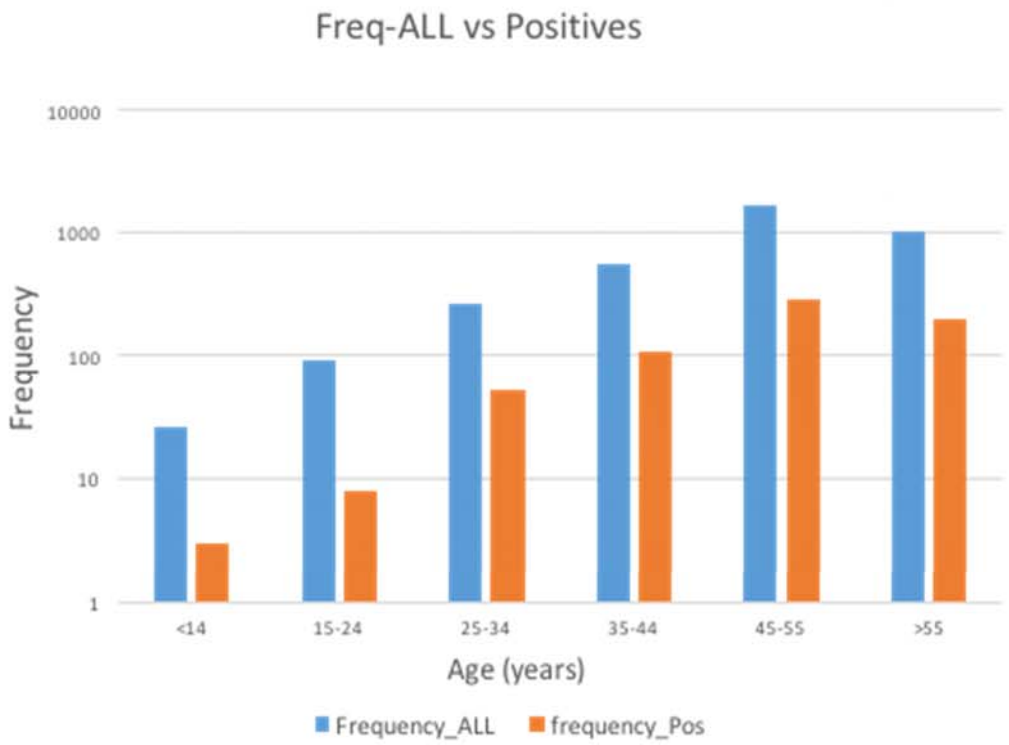


Figure 4.6: Distribution of HHV-8 antibodies according to age groups.

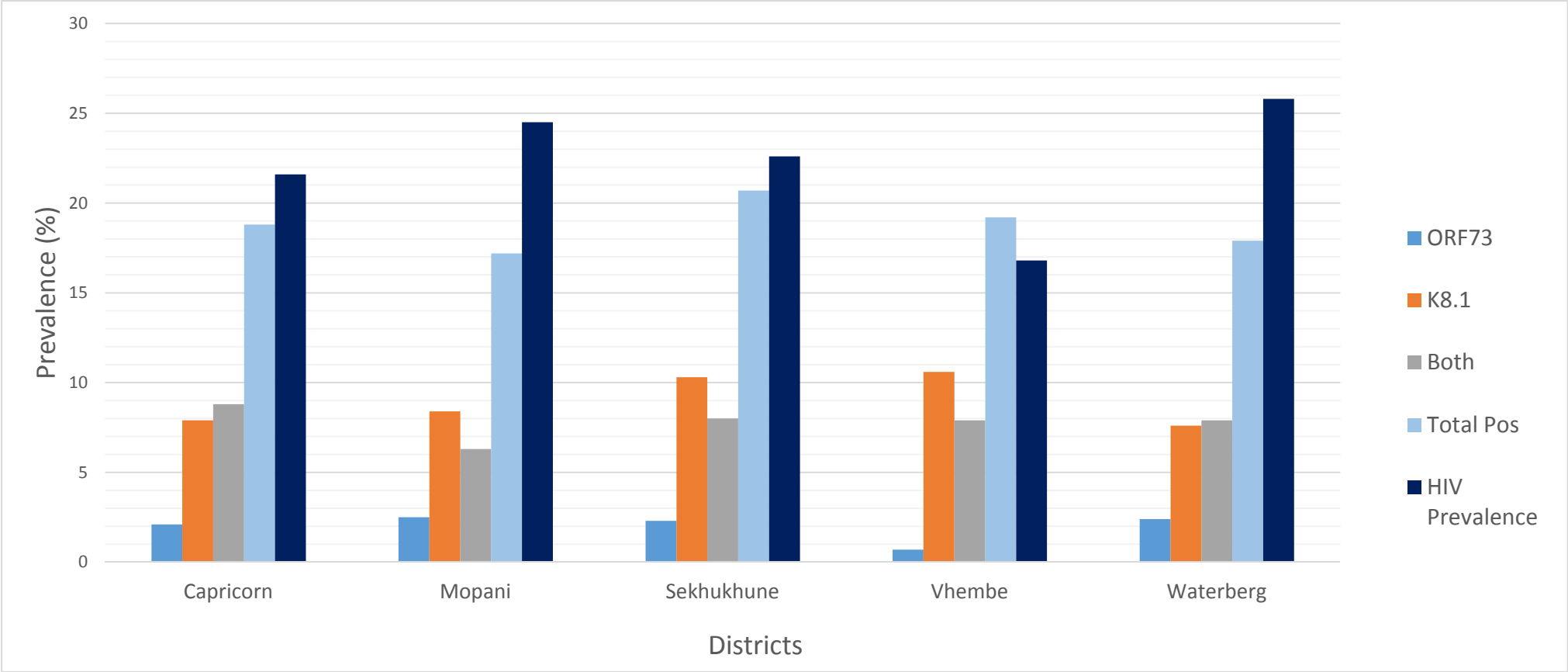


Figure 4.7: HHV-8 reactive antibodies prevalence compared to HIV prevalence in the 5 districts in Northern South Africa.

4.4 DISCUSSION AND CONCLUSIONS

4.4.1 Discussion

Infection with co-pathogens is one of the agents contributing to persistent inflammation in HIV-infected individuals. One of the viruses which appears most often with HIV co-infection is HHV-8. The mechanism by which HHV-8 undergoes its association is by establishing persistent inflammation in HIV-infected patients (Masia´et al., 2014). Several studies on HHV-8 infection have been reported to be associated with various risk factors such as; gender, age group, racial population groups, districts. Although HIV prevalence in Northern South Africa has been described, there was no data on the burden of HHV-8 in this part of the country. In the present analysis we evaluated risk factors associated with HHV-8 infection in Northern South Africa.

4.4.1.1 HHV-8 Seroprevalence

In this study, coated HHV-8 ELISA (ORFK8.1 and ORF73) plates were used to investigate the seroprevalence of HHV-8 from an HIV-infected population in a laboratory-based retrospective approach. Antibodies to HHV-8 antigens was found in 19.0% (662/3501). An expression of K8.1 antigen by the virus is associated with active replication, while that of ORF73 is associated with the dormant stage of HHV-8. The importance of the expression of latent ORF73 or lytic K8.1 antibodies is not clear. In this study, most subjects had significantly higher lytic K8.1 as compared to latent ORF73. With all participants being HIV positive, conclusions can not be made as of yet if HIV infection promotes increased shedding of HHV-8 or whether HIV-infected individuals are more susceptible to infection by HHV-8. A study with a control group (HIV negative individuals) is recommended for a better conclusion.

4.4.1.2 HHV-8 and Socio-Demographic Factors

Our analysis showed association between HHV-8 and age. The association between age and HHV-8 observed in this study may be attributed to the broad age range studied. Several studies in Africa have shown a stronger association between HHV-8 infection and age. However, in most of these studies the association is clear when both children and adults form part of the study and therefore the age range is broader.

A study by Malope et al., 2008 reported on a clear association between children and their mothers, but our findings were not focused on comparison. The biological significance of this finding is still unclear.

Although the number of females in this current study is about twice the number of the males, the prevalence observed in both groups presented no significant association between lytic K8.1 and latent ORF73 HHV-8 antibodies. This observation is in agreement with previous studies conducted in HIV-1 infected individuals of Northern Nigeria and the Southern region of Cameroon (Ogoina et al., 2010; Pedergnana et al., 2012). Also, having an infected member of the family of any age group or gender is an important stake factor for HHV-8 infection (Mbulaiteye et al., 2006), but this aspect was not investigated in this study.

4.4.1.3 HHV-8 and Districts Factors

Significant variation in the seroprevalence of HHV-8 among the districts within Northern South Africa was observed. Though Capricorn District had a very high seroprevalence, a significant difference in HHV-8 infection amongst the different districts was observed. However, the prevalence for HHV-8 was almost similar among the other districts. Our findings are in line with a study from in Uganda, which observed a marginal regional association with only one of the 8 regions compared to the Central Region showing a significant difference (Biryahwaho et al., 2010).

Globally, racial population groups have been associated with HHV-8 infection (Kouri et al., 2005; Cao et al., 2014; Zheng et al., 2017). In South Africa, racial population groups are indicative of different social practices and geographical origins (Maskew et al., 2011), as well as organization of one's host genes. In our study, we observed a significant difference of antibodies of HHV-8 between the different racial population groups. Variations between different racial population groups could also be as a result of host genetic variation. During the apartheid period, most South African residential areas were divided based on different racial population groups, as well as the original location of the residents. With these classifications, some of the variations observed today could be better explained by differences in cultural practices. Also environmental risk factors, such as insect vectors and the source of household water can also lead to the variation to HHV-8 infection (Mbulaiteye et al., 2005; Whitby et al., 2007).

In 2005, Tanzi and colleagues reported on the high risk of HHV-8 seroprevalence association with close contacts to streams and rivers. Unfortunately, our study design did not provide data on location of participants. The present study was not set to explore variations further. Novel studies are needed to provide answers to ethnicity as well as geographical variations in HHV-8 prevalence observed in this study.

4.4.1.4 HHV-8 and Immunological Factors

An elevation in HHV-8 patient antibodies with high HIV viral loads was found, hence leaving the body exposed to infections. At CD4+ cell counts >500, which is indicative of a good immune system, the exposure to HHV-8 infection increased, similarly, at viral load >1000 copies/ml the same observation was seen. This implies an increase in the infection rate. Lu and colleagues (2016) reported a contradiction of our findings.

Other associations are difficult to explain, such as year of recruitment of samples which from our finding showed a significant difference. Indicating the year of recruiting study participants might be a risk factor for both HIV and HHV-8 infection, and although this may be linked to sexual behavior, it could also be related to the common practice of poor hygiene and geographical factors. Further studies are highly recommended to shed more light on this aspect.

4.4.2 Strength and limitations of the study

Strengths of this study include: its massive sample size, screening of samples for both antigens (ORFK8.1 and ORF73), diversity since patients came from all districts within Northern South Africa, a variety of racial population groups and also the fact that children were part of the study.

Despite these strengths, this study also had several limitations which include: lack of complete data set for HIV viral load and CD4 + cell counts, which might reveal a higher prevalence of HHV-8 DNA in HHV-8 seropositive patients. Lack of information such as: the sexual orientation (gay or bisexual); family members; level of education; history of blood transfusion; substance abuse; history of any sexually transmitted diseases other than HIV; the number of sexual partners; stage of the participants KS status as well as data on HIV/HHV-8 viral load which could help in a better correlation and interpretation of our findings.

Since the study is retrospective and all samples were of HIV origin and not of HHV-8 cohort, there was no control over sample and data collection.

4.4.3 Conclusion

There is a high seroprevalence of HHV-8 in Northern South Africa. Age group, racial population groups, districts HIV viral load and year of sample recruitment were detected to be associated with HHV-8 infection in the study population. The data is useful in the consideration of whether to include HHV-8 screening in addition to HIV testing to all HIV positive patients.

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CHAPTER FIVE:

A META ANALYSIS ON THE ASSOCIATION BETWEEN HHV-8 SEROPOSITIVITY AND GEOGRAPHICAL SETTING IN SOUTHERN AFRICA.

ABSTRACT

Background: HIV infection is an important factor in developing Kaposi's sarcoma (KS). However, literature remains unclear if HIV-positive individuals are at a greater risk of co-infection with human herpes virus type 8 (HHV-8) which is the causative agent of KS. Also, it is also unknown if the geographical setting (urban or rural) is an underlying cause of HHV-8 in HIV infected patients. Literature was systematically searched between the year 1998 to 2018 and reports on HHV-8 seropositivity, gender and geographical setting were included. The study was aimed at performing a systematic review and meta-analysis to evaluate the association between HHV-8 seropositivity and geographical setting in Southern Africa, in comparison with our findings obtained in chapter 4 which was carried out in a rural setting of South Africa.

Objective: To evaluate the association between HHV-8 seropositivity and geographical setting within Southern African countries.

Methodology: We searched PubMed, Medline, Web of Science, Embase and conference proceedings from 1998 to 2018. We included observational studies that recruited participants from both urban and rural settings and reported on HHV-8 seroprevalence. Estimates of the odds ratio (OR) with 95% confidence interval (CI) were pooled by the fixed/ random-effects model.

Results: A total of 18 studies, with 16,708 participants were included in the meta-analysis. More than half of the participants were female (9,215). A significant difference was observed with studies conducted within the urban setting (p=0.0001; OR 1.71; 1.56, 1.89 at 95% CI). There was no significant difference observed with studies conducted in the urban setting (p=0.0001; OR 1.55, 1.88; 1.71 at 95% CI). A significant difference was observed when the seroprevalence of the urban setting was compared with the rural setting; mixed population (p=0.0001; OR 1.33; 1.24, 1.44 at 95% CI).

Conclusion: There is a strong association between HHV-8 seropositivity and urban setting.

Keywords: HHV-8, HIV, meta-analysis, Southern Africa, rural and urban settings.

5.1 INTRODUCTION

Human herpes virus type 8 (HHV-8) is an ancient virus, which was discovered before HIV/AIDS. At the start of the AIDS pandemic, literature reported that, cases with HIV disease were at a growth risk for neoplastic occurrences. HHV-8 being the etiological agent of KS, has been recognized as the origin of the AIDS pandemic and serve as a label. In the presence of HIV, there is weakness in the immune system, hence providing a robust event for co-infection with other viruses such as HHV-8. Furthermore, Berai and colleagues in 1990 reported approximately 20,000 times fold of HIV patients exposed to HHV-8 infection and which later progresses to KS which is the cancer stage.

Sexual intercourse, geographic and cultural factors are key determinants of HHV-8 transmission (Malope-Ngokong et al., 2010), and prevalence varies across geographic regions and within regions (Pfeiffer et al., 2010; Dollard et al., 2010). A meta-analysis involving studies done in 32 countries in sub-Saharan Africa, Australia, North and South America, Europe and Asia, demonstrated that HIV-infection is associated with a significant increase in HHV-8 co-infection globally, in all population groups (Rohner et al., 2016). In additionally, Maskew et al (2011) reported a 48% KSHV seropositivity in a group of individuals initiating antiretroviral therapy in 2008/2009 in Johannesburg, while Malope-Kgokong et al. (2010), reported a similar prevalence (44.6%) among pregnant women from several localities in Gauteng Province, South Africa based on a 2001 sampling.

Geographical differences, one factor which can have an impact on HHV-8 infection has not been fully elucidated, although a correlation between higher KSHV seroprevalence and geographical setting has been reported in Botswana (Engel et al., 2000). Understanding geographical differences is essential in the development of public health interventions to reduce HHV-8 infection. The current study performed a meta-analysis of HHV-8 prevalence in the context of HIV infection across rural and urban settings in Southern Africa. Furthermore, the seroprevalence pattern of HHV-8 worldwide is inconsistent with African countries reported to be highly variable. The reason to this elevated variation is due to the fact that there is no standard routine test for screening KSHV, hence leading to different approaches and findings.

Meta-analysis is a statistical procedure that looks for a common trend among several independent studies to generate a common finding and the main purpose for meta-analysis is to establish significant difference among different studies. Studies involving meta-analysis approach for HHV-8 seroprevalence in the context of Africa have been reported. However, studies reporting on HHV-8 seroprevalence using meta-analysis in Southern Africa is of non-existence. Moreover, information on the distribution pattern of HHV-8 seroprevalence across the region has not been fully understood. Therefore, the aim of this current chapter was to perform meta-analyses and to compare HHV-8 infection within an HIV cohort from rural Northern South Africa (chapter 4) with other rural and urban settings of HHV-8 infection within HIV positive patients in the Southern African region.

RESEARCH QUESTION:

Is human herpes virus 8 (HHV-8) infection more common in the rural or urban setting?

5.3 OBJECTIVES

The primary objective was to perform a meta analysis on the association between HHV-8, HIV seropositivity and geographical setting in Southern Africa.

The specific objectives were to determine the association of HHV-8 infection in an HIV population of rural Northern South Africa with urban and rural settings in Southern Africa.

5.4 METHODOLOGY

Online databases such as PubMed, Medline, Web of Science, Embase and conference proceedings were searched for published articles on “HHV-8” and “HIV”. Published data were downloaded and reported according to PRISMA (Preferred Reporting Items for Systematic review and Meta-analysis) guidelines (Moher et al., 2009).

5.4.1 Eligibility criteria

All studies on HHV-8 seropositivity in individuals, for all age groups, rural and urban settings were considered. Publication per study did not need to contain results for both

adults and children, urban and rural. HHV-8 infection could be defined as the presence of antibodies to HHV-8 recombinant peptides in plasma or serum or by the detection of HHV-8 genome in saliva, plasma, serum or PBMCs. All studies were in English.

5.4.2 Exclusion criteria

The exclusion criteria were defined to maintain heterogeneity. Heterogeneity is defined as the variation in study outcomes between different studies. The following exclusion criteria were considered: individuals with KS, studies on heterosexual transmission of HHV-8; studies carried out outside of Southern Africa; HIV status of participants not reported and duplicate published datasets.

5.4.3 Information sources and searches

Online databases mentioned above were electronically searched for studies between 1988 to 2018. Initial searches were conducted using “KSHV” or “HHV-8” and “HIV”. For all searches, PubMed advanced search builder, is a Medicine library which enables the researcher to search items according to the field that was used, then followed by “KSHV” or “HHV-8” and children or adults, and then “KSHV” or “HHV-8” and rural or urban settings. Furthermore, Medical subject headings (MESH) and terms of important articles were reviewed and searches carried out using “herpesvirus 8, human” [MeSH Terms] OR “human herpesvirus 8” OR KSHV and “HIV” [MeSH Terms] OR HIV (All fields) and Southern Africa [MeSH Terms] OR Southern Africa (All Fields) OR urban [MeSH Terms] OR rural [MeSH Terms] OR rural or urban [All fields] OR individuals of all age groups [MeSH Terms]. The purpose for searches from numerous databases was to ensure thoroughness of search items in order that none was left out. Only published articles in English were used.

5.4.4 Data collection and processing

Downloaded articles were reviewed and the following variables were recorded on an excel spread sheet: first author; year of publication; study location; study setting; if objective and analysis was restricted to study settings, prevalence of HHV-8-HIV co-infection in rural or urban settings; study size; cohort description; HIV test; HIV prevalence; HHV-8 assay; HHV-8 seroprevalence; Definition of HHV-8 seropositivity; unadjusted odd ratios (OR) at 95% confidence intervals (CI) for HHV-8 and adjusted odd ration at CI for HHV-8; adjusted factors.

5.4.5 Statistical analysis

Adjusted ORs and 95% CI were extracted per article. If a publication did not provide an adjusted OR, an unadjusted OR was calculated from the raw data of the article. Unadjusted odd ratios are the best inference when comparing three or more independent groups or variables. Odd ratios and 95% CI were recorded to at least 1 decimal place and maximum to 3 decimal places. Due to diversity between studies, a random-effect model was used for analysis. This model is also called variance component and helps to provide a hierarchical linear analysis. Furthermore, using an I^2 statistics, a forest plot was generated to assess the degree of heterogeneity between studies. Interpretation of I^2 statistics was based on 4 categories of defining heterogeneity at different levels. The different levels are thus:

No heterogeneity = 0%.
Low heterogeneity = 25%
Moderate heterogeneity = 50%
High heterogeneity = 75%

The purpose for these classifications based on heterogeneity, was to minimise the risk of sampling error and obtain a true representation of variation based on the different studies. For example, where an I^2 statistics value is 50%, it indicates that half of the total variability between studies is caused by true variation between articles and not due to sampling errors. For statistical significant difference, p value was calculated at 95% CIs. For significance, $p< 0.05$ was considered. All statistical analyses were performed using R software, version 3.4.3.

5.4.6 Definitions of some terms used.

i. **I^2 statistics:** describes the percentage of variation across studies that are due to heterogeneity rather than a chance event.

$$\mathbf{I^2 = 100\% \times (Q-df)/Q.}$$

Where Q stands for Cochran's heterogeneity and df stands for degree of freedom.

ii. **Random effect model:** is also called a variance component and it is a model used to generate linear regression model with emphasis on the the rank of the study.

iii. **Heterogeneity:** is a term that signifies diversity and in the the context of meta-analysis, it is referred to the variation in study outcomes between various studies.

iv. **Forest plot:** also called a blobbgram is a graphical display of estimated results from a scientific study addressing the same question, representing a meta-analysis of the results of randomized trials.

v. **Egger test:** is a test for the Y intercept = 0 from a linear regression of normalized effect estimate against precision.

vi. **Beggs test**: is a test based on interdependence of variance and effect size. This bias indicator makes fewer assumptions than that of Egger but it is less sensitive than the Egger test.

Table 5.1: Studies identified to investigate HHV-8 prevalence in HIV positive individuals in urban and rural settings

References	Year of Publication	Study description	Study location	Study size	KSHV assay	KSHV +seroprevalence (%) KSHV+/Male	KSHV+/Female	Definition of KSHVseropositivity	[1]Adjusted ORs at 95% CI
Southern Africa Studies done in the Urban setting									
Wilkinson	1998	Urban	South Africa	110	IFA	56.6	62.5	Positive for IFA	1.27 (0.54, 2.98)
Engel	2000	Urban	Botswana	155	ELISA	43.2	40.5	Positive for ELISA	0.89 (0.47, 1.69)
Minhas	2008	Urban	Zambia	684	IFA, ELISA	28.9	33.1	Positive for both IFA and ELISA	1.22 (0.88, 1.68)
Bulter	2009	Urban	South Africa	1479	ELISA	31.4	68.6	Positive for ELISA	4.79 (4.09, 5.59)
Maskew	2011	Urban	South Africa	404	K8.1 and ORF73 ELISA	16.3	31.4	Positive for both IFA and ELISA	2.35 (1.68, 3.29)
Simbiri	2014	Urban	Botswana	22	ELISA	NM		Positive for ELISA	NM
Etta	**2018 (unpublished)**	**Rural**	**South Africa**	**3501**	**K8.1 and ORF73 ELISA**	**18.4**	**19.3**	**Positive for either K8.1 or ORF73**	**1.06 (0.88, 1.28)**
Southern Africa Studies done in the Rural setting									
Albashi	1998	Rural	Zambia	40	ELISA	36.4	39.0	Positive for ELISA	1.11 (0.31, 4.03)
He	1998	Rural	Zambia	378	ELISA	0.00	48.4	Positive for ELISA	NM
Olsen	1998	Rural	Zambia	251	ELISA	39.7	39.8	Positive for ELISA	1.00 (0.51, 1.68)
DeSantis	2002	Rural	Malawi	272	ELISA	47.7	63.0	Positive for ELISA	0.25 (0.14, 0.43)
Dedicoat	2004	Rural	South Africa	2497	Elisa	10.1	13.0	Positive for ELISA	1.33 (1.04, 1.71)
Malope	2007	Rural	South Africa	2466	K8.1 and ORF73 ELISA	0.00	47.8	Positive for either K8.1 or ORF73	NM
Malope	2008	Rural	South Africa	1146	ELISA	47.5	46.0	Positive for ELISA	0.94 (0.74, 1.19)
Malope	2010	Rural	South Africa	1740	K8.1 and ORF73 ELISA	0.00	32.6	Positive for either K8.1 or ORF73	NM

Caterino	2010	Rural	Mozambique	1504	ELISA	26.2	18.8	Positive for ELISA	0.65 (0.51, 0.84)
Bohlius	2014	Rural	South Africa			48			
Etta	**2018 (unpublished)**	**Rural**	**South Africa**	**3501**	**K8.1 and ORF73 ELISA**	**18.4**	**19.3**	**Positive for either K8.1, ORF73**	**1.06 (0.88, 1.28)**

OR, odds ratio; CI, confidence intervals. [1]Adjusted odds ratio not quoted in the publication article. The study highlighted (Etta 2018) was used in comparison with studies from both geographical settings.

5.5 RESULTS

Using the electronic databases mentioned in the methods, the search terms yielded the following results: 300 articles in English for KSHV or KSHV and Southern Africa or KSHV or KSHV in rural or urban settings or KSHV or KSHV for all individuals of different age groups. Using an article grid format, the search results for 300 articles were imported into Microsoft Excel. Duplicates were deleted leaving 200 unique records for screening and analysing. All published works considered were further screened for relevance and 77 were excluded from the analysis because they did not fit the inclusion criteria. The full text for 123 articles were read and 17 studies met the inclusion criteria and were further used for analysis. A total of 17 studies were used for odd ratio computation and meta-analysis. The 18th study (Etta et al., 2018 unpublished) was used for comparison. Only studies in English were considered. These studies include: [He et al., 1998; Olsen et al., 1998; Wilkinson et al., 1998; Albasi et al., 1999; Engel et al., 2000; DeSantis et al., 2002; Dedicoat et al., 2004; Whitby et al., 2004; Minhas et al., 2008; Malope et al., 2007; Malope et al., 2008; Bulter et al., 2009; Caterino de Araujo et al., 2010; Malope et al., 2010; Maskew et al., 2011; Simbiri et al., 2014; Bohlius et al., 2015 and Etta et al., 2018 (unpuplished)].

5.5.1 Human herpes virus type 8 seroprevalence in HIV infected individuals in rural settings compared with those in the urban setting.

Seventeen studies met the inclusion criteria and from the 9 known Southern African countries; 5/9 (55.6%) reported on the the 17 studies earlier mentioned. Theses countries include: Botswana; Malawi; Mozambique; South Africa and Zambia (Table 1). All participants were HIV positive and the testing strategy varied among the studies. KSHV seroprevalence ranged from 9.0 – 90.0%. All studies reported that HIV-co-infection was associated with an increased point estimate in the odds of KSHV seropositivity in both settings.

5.5.1.1 HHV-8 seroprevalence of HIV infected individuals in rural Northern South Africa compared with those in other urban settings within Southern Africa

The random effect summary odd ratio for KSHV seropositivity in HIV positive individuals in the urban setting was OR 1.71 (1.56, 1.88) at 95% CI. The proportion of the total variation between the various studies estimates was due to heterogeneity

based on the I^2 statistics, I^2= 96.56%. The percentage of heterogeneity between studies in the urban setting ranged from 0.89 – 4.79 at 95% confident interval indicating that the findings in the studies are similar. There was a maximal significant difference between the studies, p=0.0001 (Figure 1).

5.5.1.2 HHV-8 seroprevalence of HIV infected individuals in rural Northern South Africa compared with those in other rural settings within Southern Africa

The random effect summary odd ratio for KSHV seropositivity in HIV positive individuals in the rural setting was OR 1.01 (0.91, 1.12) at 95% CI. The proportion of the total variation between the various studies estimates was due to heterogeneity based on the I^2 statistics, I^2= 0.00%. The percentage of heterogeneity between studies in the urban setting ranged from 0.25 – 1.33 at 95% confident interval indicating that the findings in the studies are not similar. No significant difference was observed among the studies p=0.093 (Figure 2).

5.5.1.3 HHV-8 seroprevalence of HIV infected individuals in rural Northern South Africa compared to both rural and urban within Southern Africa

The random effect summary odd ratio for KSHV seropositivity in HIV positive individuals from both settings was OR 1.33 (1.24, 1.44) at 95% CI. The proportion of the total variation between the various studies estimates was due to heterogeneity based on the I^2 statistics, I^2= 79.91%. The percentage of heterogeneity between studies with both study settings ranged from 0.25 – 4.79 at 95% confident interval. An association between KSHV and geographical settings was observed, p=0.0001 (Figure 3).

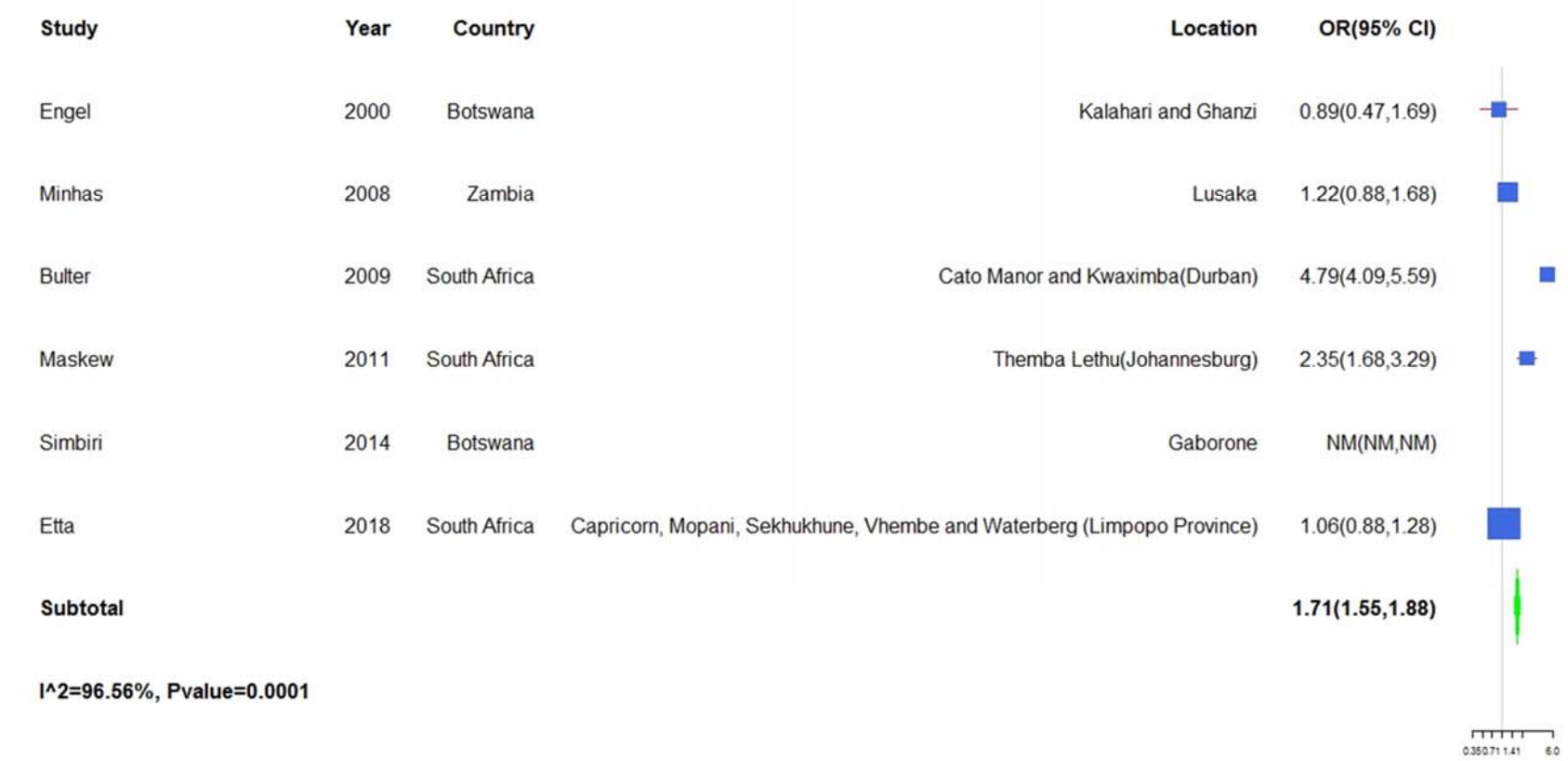

Figure 5.1: A Forest plot illustrating the association between HHV-8 seropositivity and studies from the **urban** setting between male and female from Southern Africa. The blocks and horizontal lines represent the odd ratios (OR) of the association between HHV-8 seropositivity and the rural setting with their 95% confidence intervals (CI) for each study. The diamond below the analysis is the pooled point estimate of the studies included in each population group, and the width of the diamonds represent the 95% CI of the pooled OR. P: p-value from meta-regression analysis.

Figure 5.2: A Forest plot illustrating the association between KSHV seropositivity and HIV infection in a **rural** setting within Southern Africa. The blocks and horizontal lines represent the odd ratios (OR) of the association between KSHV seropositivity and the rural setting with their 95% confidence intervals (CI) for each study. The diamond below the analysis is the pooled point estimate of the studies included in each population group, and the width of the diamonds represents the 95% CI of the pooled OR. P: p-value from meta-regression analysis.

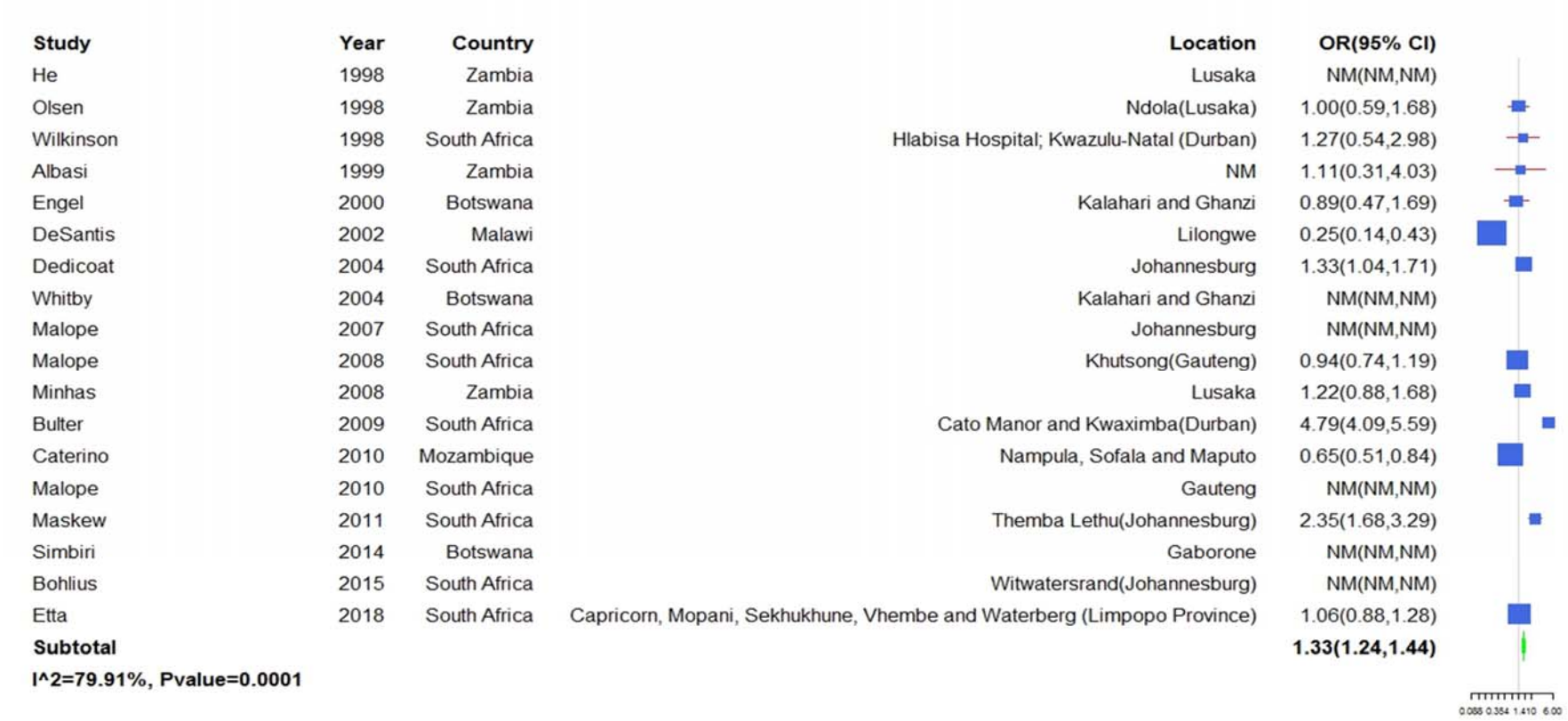

Figure 5.3 A Forest plot illustrating the association between KSHV seropositivity and HIV infection for both **urban and rural** settings within Southern Africa. The blocks and horizontal lines represent the odds ratios (OR) of the association between KSHV seropositivity and from both settings with their 95% confidence intervals (CI) for each study. The diamond below the analysis is the pooled point estimate of the studies (79.91%), included in each population group, and the width of the diamonds represents the 95% CI of the pooled OR. There was a significant association p-value =0.0001 from meta-regression analysis.

5.6 DISCUSSION AND CONCLUSIONS

5.6.1 Discussion

Studies on KSHV seroprevalence and meta-analysis are important in regions such as Southern African countries, where a significant population of that region is infected with HIV. This study was performed to provide the trend of KSHV infection in Southern Africa.

A study by Butler and colleagues in 2011, reported on the acquisition of KSHV infection as primary through non-sexual routes. A factor such as geographical setting was also one of their concepts

From our analyses, we observed that geographical settings do play a role in the acquisition of KSHV infection, indicating that geographical setting which is one of the risk factor to KSHV infection persists. Moreover, we found evidence that the urban setting had a high point estimate (96.56%), odd ratio (1.71) and a maximal significant difference (p=0.0001). This ia an indication of an association with KSHV infection, which supported findings from Bukina Faso (Collenberg et al., 2006).

On the other hand, our analysis involving the rural setting, showed no significant difference (p=0.0001) which is in contrast with a recent study carried out in the East of Africa, precisely Uganda where they reported a very high prevalence within the rural setting (Newton et al., 2017).

Further to this, in a mixed comparison analysis for both settings, a significant difference and a high I^2 statistics value, I^2= 79.91% were observed; supporting the fact that geographical setting which is one of the risk factor to KSHV infection persists through Southern Africa. The discrepancy between rural and urban setting in which one was significant and the other may be attributed to other underlying confounding factors in KSHV acquisition in the different study population bearing in mind that males, especially men who have sex with men (MSM), have always shown high prevalence of KSHV infection (DHS, 2015; Engel et al., 2007), while in our studies in the rural settings, mostly women were involved (Table 1). Furthermore, most studies used various serology tests

for the KSHV infection. With this variation, comparison between studies was not 100% effective. Moreover, within our analysis, the same test was used and hence comparison was based on the odd ratio whether study tested with the same assay.

The current analysis suffers from a relative lack of data (few studies) dispersed across the continent, hence limiting our appreciation of the trend of KSHV infection in the geographical setting. However, despite the limitation, the strength of our finding includes the fact that though the sample size was small, a significant difference was observed, indicating that the geographical setting has an effect on KSHV infection. This is the first study to have performed a meta-analysis on KSHV seroprevalence and geography setting within Southern Africa. Thus, providing fundamental data for further meta-analytical studies.

5.6.2: Conclusion

In conclusion, HIV infected individuals in the urban settings have more chances of being infected with KSHV, with high significant differences associated with KSHV seropositivity. In addition to this, we conclude by stating that HIV/KSHV co-infection is common in the urban settings than in the rural, but a large sample size with many studies is highly recommended to support these findings. Our findings postulate that geographical settings have an influence on KSHV seropositivity.

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CHAPTER SIX

GENETIC CHARACTERIZATION OF HHV-8 FROM MOUTH WASH DNA SAMPLES IN NORTHERN SOUTH AFRICA

ABSTRACT

Background: HHV-8 is characterized by high genetic variability across the entire genome. Studies on genetic diversity of HHV-8 in South Africa on the knowledge of infecting genotypes for successful vaccine development are scanty. ORF26 which is one of the genes of HHV-8 genome is a conserved. However, studies have shown some degree of diversity within the gene, leading to different genotypes and Single Nucleotide Polymorphism (SNPs). Several studies have reported using DNA from PBMCs, plasma, biopsy from a mixed population (HIV positive and HIV negative) individuals but studies using DNA from saliva has not been thoroughly assessed. Next Generation Sequencing was used to draw inferences on the genotypes of ORF26 and SNPs circulating in Northern South Africa. But on HHV-8 ORF26 from South Africa is scarce and this will be the first study to provide baseline data on HHV-8 from Northern South Africa.

Objective: The main objective of this study was to genetically characterize HHV-8 ORF26 genotypes and SNPs using DNA from mouth wash samples from both HIV positive and HIV negative individuals in Northern South Africa.

Methodology: DNA was isolated from mouth wash samples of 308 HIV positive and 226 HIV negative individuals. ORF26 amplicons of 233 bp were generated by PCR. Amplicons were purified using qubit beads. Purified amplicons were sequenced using an Illuminia MiniSeq platform. Viral genotypes were determined using Geneious version 10.0 and phylogenetic trees were constructed using MEGA version 6.0 software. Mutation/SNPS were determined using Genious software version 10.0.

Results: A prevalence of 28.1% (150/534) was detected in the study population. A significant difference was observed for gender, district, HIV status and the level of education (p=0,0003; p=0.0002; p=0.0094 and p=0.0095) respectively. Twenty-three amplicons were sequenced. Good quality nucleotide sequences, with sequence coverage ranging between 16,4683 – 86,980 were available for fifty-seven percent of the reads (13/23). Forty six percent (6/13) of the test positive sequences were genotype Q; thirty-one percent (4/13) test sequences were genotypes B; fifteen pecent (2/13) of the test sequences were genotypes E and eight percent (1/13) of the test sequence was genotype N. The two major genotypes observed in this population were genotypes Q and B.

Transition polymorphisms at a threshold of 5% (minority) was detected in 12 participants' sequences at nucleotide positions of 90 (C changed to T) and 143 (C changed to T) and transversion polymorphisms were detected in all participants' sequences at position 203 (A changed to a C). Of these changes, nucleotide positions at 90 and 143 showed non-synonymous changes (amino acid). At nucleotide position 90, Arginine was changed to Valine and at nucleotide position 143, Lysine was changed to glutamine. The nucleotide changes at position 203 had no effect on the amino acid and no mixed infection was detected in the study population

Conclusion: This study showed that gender, HIV status, level of education and districts have an influence on acquiring HHV-8. HHV-8 genotypes Q, B, E and N are the infecting variants in the study population, with genotypes Q and B being the most prevalent types. The SNPs were observed both at the minority and the majority population and the observations at the minority level lead to non synonymous mutation.

Keywords: HHV-8 ORF26, Next Generation Sequencing, genotypes, SNPs, Northern South Africa.

6.1 INTRODUCTION

Human herpesvirus 8 (HHV-8) presents a high degree of genetic variability permitting the classification of isolates in genotypes, variants and sub variants. Genetic variability is attributed to organization of these genes on the virus (3' and 5' variability activity). Viruses with genotypes B, N, K and Q are considered to be ORF26 viruses. The distribution of HHV-8 genotypes world wide is heterogeneous with genotype B largely responsible for endemic KS (Whitby et al., 2007).

The greater number of HHV-8 infection involves ORF26 viruses because it is one of the lytic gene of HHV-8 and the genotypes mostly associated with infection is B genotype, which if not monitored can lead to endemic KS. Genotypes B represent over half of HHV-8 infection worldwide, with genotype B being the most predominant reported in Southern Africa (Tagny et al., 2010). Additional studies also reported on genotype N isolates obtained from individual sequences (Tornesello et al., 2010; Zong et al., 2002).

Several studies have reported using DNA from PBMCs, plasma, biopsy from both HIV positive and HIV negative individuals but studies using DNA from saliva have not been thoroughly assessed. The fundamental knowledge on the circulating genotypes for ORF26 virus is limited. The question still to be answered is whether both HIV positive and HIV negative individuals have this virus. And if yes, what are the most common genotypes?

Furthermore, the high frequency of ORF26 virus may not be related to the genotypes detected but instead there might be an ongoing evolution of HHV-8 genotypes B epidemic in Africa. Relentless research of HHV-8 genetic diversity for both HIV positive and negative individuals will be necessary to nip out the emergence of high frequency of ORF26 virus in genotypes B infections. As a result of the viral diversity and redistribution of viral strains globally, regular monitoring of the genetic variants infecting individuals in a particular region is important. Such monitoring will assist in providing updates on the circulating variants. These updates can play a great role in diagnosis, treatment and prevention. Furthermore, the knowledge about the viral genetic landscape in any

geographical region is of paramount importance in vaccine development, as it is often thought that if a workable vaccine is to be developed, it must take into account the genetic diversity of viruses in the particular area where it is to be used. This is also necessary because B viruses drive the epidemic in regions with the highest burden of HIV infection.

The genetic diversity of HHV-8 has been described (Isaac et al., 2016; Tornsello et al., 2010; Poole et al., 1999). However, to date, there is no report on the genetic diversity from the Northern South Africa, which encompasses the Limpopo Province. The present study was therefore carried out to provide data on the genetic diversity profile of HHV-8 ORF26 viruses in Northern South Africa.

6.2 OBJECTIVES OF THE STUDY

The main objective of the study was to characterize HHV-8 ORF26 isolates from both HIV positive and HIV negative individuals in Northern South Africa. The specific objectives were:

1. To determine the prevalence of HHV-8 using mouth wash samples
2. To genetically characterize HHV-8.

6.3 METHODOLODY

6.3.1 Ethical considerations

With the difference in sample type, see chapter 4 for ethical clearance, section 4.2.1.

6.3.2 Study area, study population and sample collection

This was a cross sectional study which involved a once-off sample collection from each participant. Five study sites; Donald Frazer Clinic (DFC), Thohoyandou Health Clinic (THC), Rethabile Health Clinic (RHC), Seshego Health Clinic (SHC) and Univen Health Clinic (UHC) were involved (Figure 6.1).

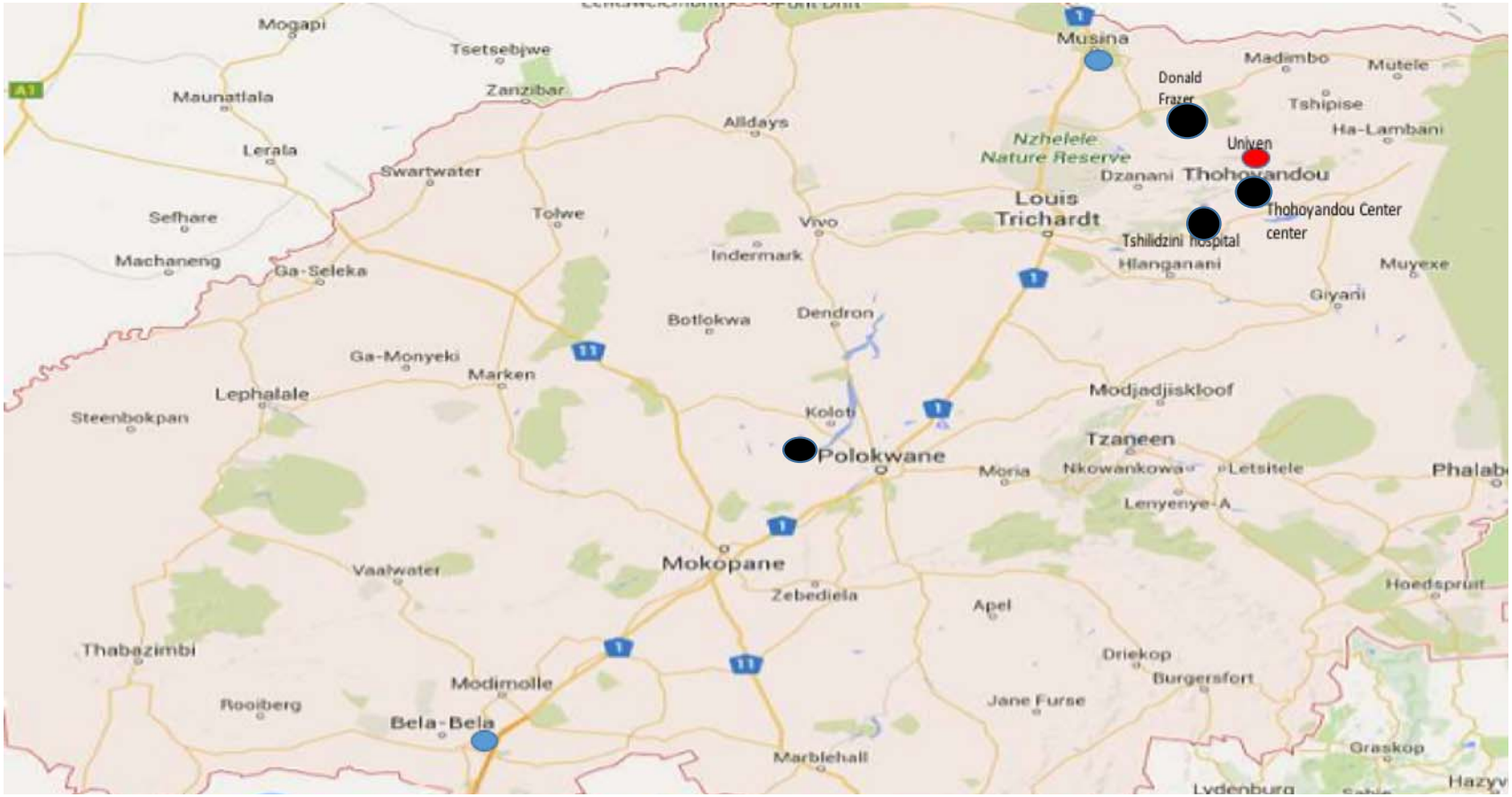

Figure 6.1: Representing map showing all the 5 study sites located within the Northern part of South Africa. The black dots represent the sites from which samples were collected, and the red dot represents the University of Venda where the samples are transported to, for processing and analyzing.

Using a sterile Nalgene tube, 3ml of mouthwash/saliva sample was collected from 534 study participants. Samples were centrifuged at 4000 rpm for 3 minutes. After centrifugation, aliquots of the supernatant were transferred into sterile eppendorf tubes and stored at -80ºC for future use. Pellets were resuspended in 200µl Phosphate Buffer Saline (PBS). DNA was extracted from the resuspended pellet of 534 participants (308 HIV infected and 226 HIV negative) using the Qiagen mini DNA extraction kit following the manufacturer's instruction (Qiagen Biosynthesis, Germany).

6.3.3 Amplification of HHV-8 ORF26 gene.

One microliter of the extracted DNA was used as template in first round PCR amplification in a 20ul reaction of 10X PCR Buffer, 1.5 mM of $MgCl_2$, 0.1mM dNTPs, 0.25 units/µl of Plantinum Taq DNA polymerase (Invitrogen) and 1um each primer (see table 6.1) was used. The first PCR products were used in the nested PCR to amplify 233bp fragment with nested primers (see Table 6.1). The thermal cycling conditions were: initial denaturation at 94ºC for 3 minutes; then 40 cycles of 94ºC for 1 minute; 58ºC for 1 minute; 72ºC for 1 minute and final elongation at 72ºC for 10 minutes and held at 4ºC. The master mix preparation and cycling conditions for nested reaction was the same as that for the first round PCR, except for the primers which were changed as shown in Figure 6.2.

6.3.4 Size verification using agarose gel electrophoresis

A 2% gel was prepared in a sterile 200ml bottle by dissolving 1g of agarose powder in 50ml of 1x TAE buffer for 2 minutes using a microwave. After dissolving, the agarose was removed from the microwave and allowed to cool on the bench. After cooling, 0.5mg/µl of ethidium bromide was added and mixed by swirling. The agarose solution was then poured onto a gel cast, containing the tray and a comb and a comb was placed to create wells for loading the amplicons. After solidification, the comb was removed, leaving wells into which amplicons were loaded. A 0.1 µl of orange 6x loading dye was then mixed with 2µl of amplicons and loaded on designated wells. Each amplicon was loaded per well. Quick load 100bp ladder was used for size verification. The gel was run at 300 amperes at 80 volts for 35 minutes. The gel was then visualized under UV transilluminator.

6.3.5 Data Preparation for statistical analysis

6.3.5.1 Data clean-up

All data were entered into an excel spread sheet and checked for mislabelled codes or codes which exist more than once. Parameters such as age group, gender, geography, level of education, marital status, HIV status, smokers and non-smokers, including comments if the participant ate prior to sample collection or not were entered for each corresponding sample ID.

6.3.5.2 Data analysis

Descriptive statistics was used. Data were entered on an excel spread sheet and analyzed using Pearson Chi square analysis within PRISM. Univariant analysis was also performed to check for association between the different parameters. Proportions were compared with the chi-square test value, while for level of statistical significance, $P<0.05$ was considered.

6.3.6 Library preparation and MiniSeq sequencing

The HHV-8 ORF26 and amplicons were purified using Ampure XP beads (Backman coulter) and quantified using Qubit 3 dsDNA HS kit with detection range from 10pg/ul to 100ng/ul. The concentration of each amplicon was diluted to 0.2ng/ul with 10mM Tris HCL. Nextera XT DNA sample preparation kit (Illuminia K.K Tokyo, Japan) fragments DNA and uses transposase to tag the DNA with sequencing adaptors was used to fragment and tagment 1ng DNA. Using the enrichment PCR, the libraries were amplified to add the illuminia index 1 (i7) and the index 2 (i5) unique barcodes for sample identification. After enrichment PCR, the libraries were again purified using Ampure XP beads. Agaroses electrophoresis E-gels was then used for size verification. After size verification, the libraries were then normalized to 4nM each to ensure equal library representation in the pool. Five microliters of each normalized library was mixed, denatured and diluted with 1.8pM with 20% PhiX as control. A final volume of the 500ul prepared libraries and PhiX were then pooled. The final pool was sequenced in an

Illumina MiniSeq machine using a high output kit of 150 cycles. ORF26 paired-end 2 x 150 bp reads for each were generated.

6.3.7 Sequence analysis

6.3.7.1 Sequence quality control evaluation

Fastq files are generated for each sample representing the two paired-end reads. The quantity of the sequence was validated using the fastQC program.

6.3.7.2 Sequence filtering, trimming and mapping

Before editing the paired sequences, NCBI BLAST tool (http://www.ncbi.nlm.nih.gov/blast), an online tool was used to blast the sequences. The purpose of blasting was to check for contamination and to confirm sequenced region. If a sequence mapped with an already existing genotype HHV-8 sequence, that sequence is considered valid, meaning it is not contaminated. The fastq files were imported into Geneious software version 10.0 for sequence filtering and trimming. The sequences were further used for downstream analysis. All sequences were trimmed at the ends as part of the assembly procedure and each sequence was assigned a quality score by the sequencing base caller. A probability limit threshold is set at 0.001% which helps to minimise error rates during trimming. The forward and the reverse reads from each sample were paired and mapped to HHV-8 ORF26 reference sequences and each consensus per sample was generated.

6.3.7.3 HHV-8 ORF26 genotype determination

Before filtering and trimming, original sequences were blasted against the public dataset using the online NCBI BLAST tool (http://www.ncbi.nlm.nih.gov/blast). The aim of this was to confirm the sequence and check for contamination. Sequences which aligned with previously genotyped HHV-8 isolates were considered not contaminated and were further used for analysis. Reference sequences were obtained from Genebank. The study sequences, previously described HHV-8 sequences from Africa and Western countries were aligned using map to reference and multiple alignment incorporated in Geneious

version 10.0 and CLUSTAL X incorporated in the MEGA program version 7.0 (Tamura et al., 2013) as well as BIOEDIT software version 7.2.5 (Hall, 1999).

Together with all the consensus sequences of test isolates and reference sequences, a phylogenetic tree was constructed. Three different softwares were used for control checks. For genotyping confirmation, an online tool, BioAfrica Oxford HHV-8 Automated Genotyping tool version 2.0 (http://bioafrica.mrc.ac.za/rega-genotype/html/indexhhv8.html) was used (http://www.geneious.com; Alcantara et al., 2009). For genotype confirmation, maximum likelihood phylogenetic tree incorporated in the MEGA version 6.0 was used, whereby patients' sequences were assembled with reference sequences. A bootstrap of 1000 replicates was used for phylogenetic tree variability.

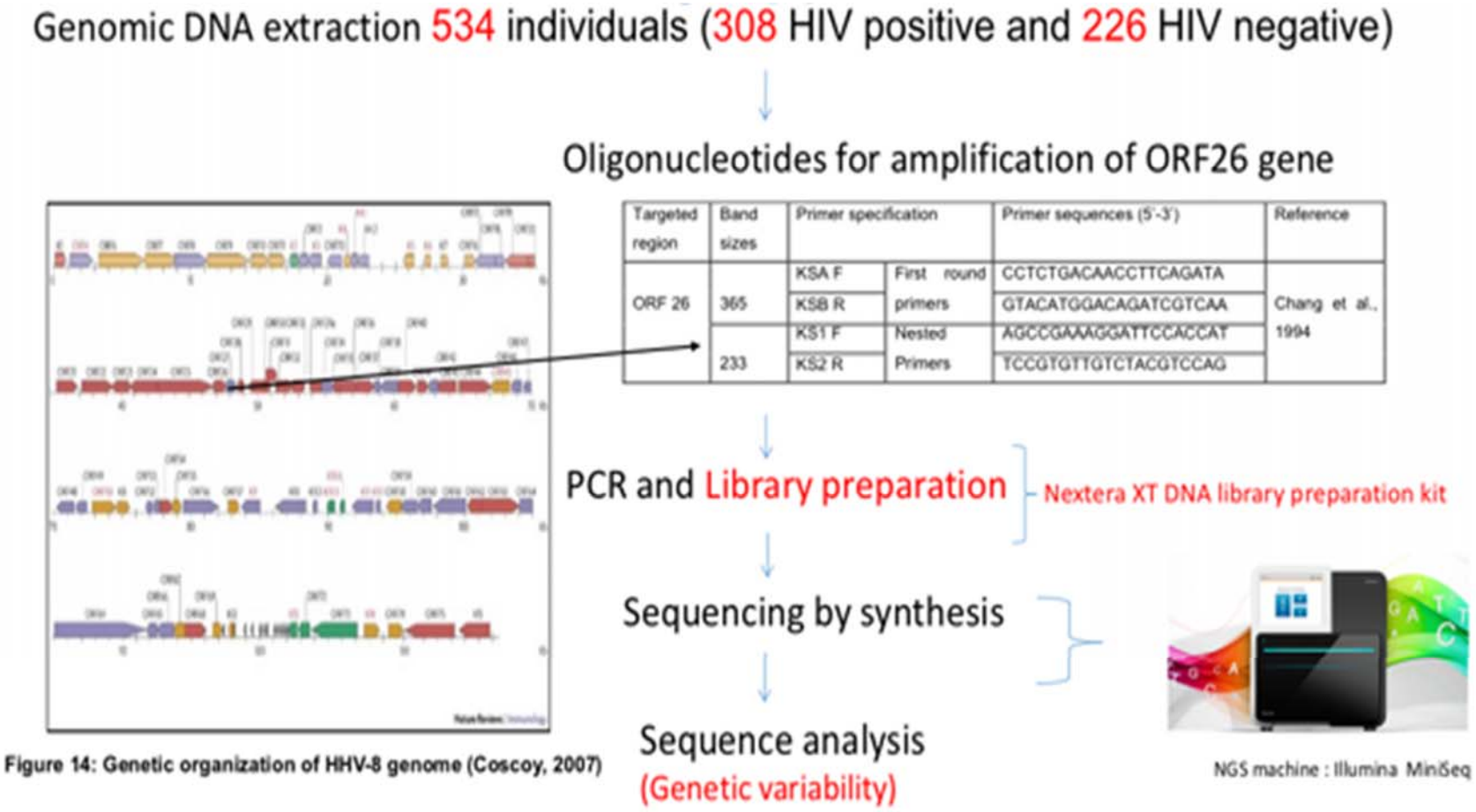

Figure 6.2 Study approach for HHV-8 ORF26 detection and sequencing

6.3.7.4 Mutation determination or Single nucleotide polymorphism determination

Mutations can either be Synonymous or Non-Synonymous. Using Geneious, mutations were detected, as well as SNPs at both minority and majority levels. At a threshold of 20% and below to 5%, that was considered as the minority population, while above 20% was considered the majority population.

6.3.7.5 Detection of mixed infection

A cut of 20% was set and the generated consensus for each sample was mapped to a reference sequence at a threshold of <20% (minority level) and checked for variations among the reads and the same was at a threshold > 20% (majority level).

6.4 RESULTS

6.4.1 Patient characteristics

Patients' demographic and clinical data, are listed on table 5.3. The median age was 38 (2 - 82).

Table 6.1. Participants' demographic characteristics.

Characteristics	**No (%)**
Total Number of participants	534 (n)
Gender	
Female (384)	71.9
Male (150)	28.1
Median Age	38(2-82)
HIV status	
HIV negative (226)	42.4
HIV positive (308)	57.6
Districts	
Vhembe (345)	64.6
Capricorn (189)	35.4
Marital status	
Single (328)	61.4
Married (164)	30.7
Widow (31)	5.8
Widower (2)	0.4
Divorced (9)	1.7
Smokers (58)	10.9
Nonsmokers (465)	87.1
Had stopped smoking (7)	1.3
Unknown (3)	0.56
Employment status	
Employed (128)	24
Unemployed (406)	76.0
Level of education	
<grade 12/ no schooling (300)	56.2
Grade 12 (160)	29.9
Tertiary level (74)	13.9
Comments on mouthwash collection	
Participants who ate before mouthwash collection (114)	21.3
Participants who ate nothing before mouthwash collection (420)	78.7

6.4.2 Screening for HHV-8 viral DNA using mouthwash samples.

6.4.2.1 Amplification of HHV-8 ORF26 gene.

From 534 mouth wash DNA samples, nested PCR was done to amplify 233bp of HHV-8 ORF26 gene. Of the 534 samples, 150 samples were successfully amplified, an implication of the presence of HHV-8 viral DNA in the samples. Figure 6.2 shows an illustration of the amplifications obtained from nested PCR.

Figure 6.3: Representative gel electrophoresed amplicons of the nested PCRs, loaded on a 2% ethidium bromide agarose-stained gel. The first lane, Lane M, is a 100base pair plus molecular weight marker, while lanes 1-9 are test specimens and lane (P) is a positive control and lane (N) is a negative control (nuclease free water). The blue arrow indicates the targeted band size.

6.4.2.2 Prevalence of HHV-8 ORF26 based on PCR screening.

A prevalence of 28.1% (150/534) was detected in the study population. The 150 positive samples were analysed based on gender, age groups, districts, education level, smokers or non-smokers, marital status, occupation and if participants ate prior to sample collection. Table 6.3 shows the demographic characteristics for all positive participants.

Furthermore, distribution of HHV-8 based on ORF26 identified, demographic, immunologic and socio-economic variables were assessed at the univariate level. Significant differences were observed for gender, districts, HIV status and level of education (p=0.0003, p=0.002, p=0.0094 and p=0.0095 respectively. (See Table 6.4)

Table 6.2: Demographic characteristics for HHV-8 ORF26 positive participants

Characteristics	No (%)
Number of positive participants	150
Gender	
Female (104)	69.3
Male (46)	30.7
Median Age	38.5
HIV status	
HIV negative (50)	33.3
HIV positive (100)	66.7
Marital status	
Single (88)	58.7
Married (45)	30.0
Widow/widower (11)	7.3
Divorced (6)	4.0
Districts	
Vhembe (DFC, UHC, THC) (81)	54.0
Capricorn (SHC, RHC) (69)	46.0
Smokers (29)	19.3
Non-smokers (121)	80.6
Employment status	
Employed (40)	26.7
Unemployed (110)	73.3
Level of education	
<grade 12/no schooling (60)	40.0
Grade 12 (70)	46.7
Tertiary (20)	13.3
Comments on mouthwash collection	
Participants who ate before mouth wash collection (46)	30.6
Participants who did not eat before sample collection (104)	69.3

Note: DFC: Donald Frazer Clinic, UHC: University of Venda Health Clinic, THC: Thohoyandou Health Clinic, SHC: Seshego Health Clinic and RHC: Rethabile Health Clinic.

Table 6.3: Distribution of HHV-8 ORF26 prevalence and risk factors.

Characteristics	Number tested	HHV-8 ORF26 detection	Chi square; p-value
Gender			
Female	384	104	X^2=13.12; p=0.0003 **(S)**
Male	150	46	
Age groups			
<25	100	23	X^2=1.450; p=0.4844
25-50	250	103	
>50	184	24	
Districts			
Vhembe	345	81	X^2=13.96; p=0.002 (S)
Capricorn	189	69	
HIV Status			
Positive	308	100	X^2=6.749.1; p=0.0094 (S)
Negative	226	50	
Marital status			
Single	323	88	X^2=3.371; p=0.338
Married	164	45	
Widows/widowers	33	11	
Divorced	9	6	
Smokers/Non-smokers			
Smokers	58	121	X^2=0.0734; P=0.787
Non-smokers	465	29	
Occupation			
Employed	128	40	X^2=0.0055; P=0.9404
Unemployed	406	10	
Level of education			
<grade 12/never gone to school	300	60	X^2=9.310; P=0.0095 (S)
Grade 12	160	70	
Tertiary	74	20	
Comments prior to sample collection			
Participant ate before sample collection	114	46	X^2=1.861; P=0.173
Participant never ate prior to sample collection	420	104	

6.4.2.11 Purification of PCR amplicons

After amplification, 23 amplicons were purified using qubit beat purification procedure and sequenced using the MiniSeq platform.

6.4.2.12 NGS validation

After the sequencing run, 26.54% PhiX was recovered, even after loading 20%. This is considered good, indicating that the quantification and pipetting steps were carried out with maximum effectiveness. The error rate on Phix is extremely low at 0.62%. This, coupled with the fact that 4.2 Gb of data was generated with 94.2% at QC (Quality Control) of 30 and above, confirms how great the run was. An optimally clustered run using MiniSeq system using a high-output kit of 2 × 150 bp takes approximately 24 hours and should yield about 2.5 -7.5 Gb of data.

6.4.2.13 FASTQC quality control.

Quality control and filtering of sequencing reads is one of the most important steps in the pre-processing of sequence reads. FASTQC helps in the determination of the quality score, the length of the reads and how good the sequences are. Sequence reads are short fragments of genetic information which are stored in a FASTQ file. For a sequence to be considered of good quality, the quality score of the reads should be above 25 and 95% of the reads should be in green and the brown compartments as shown in figure 6.3.

Quality score of reads

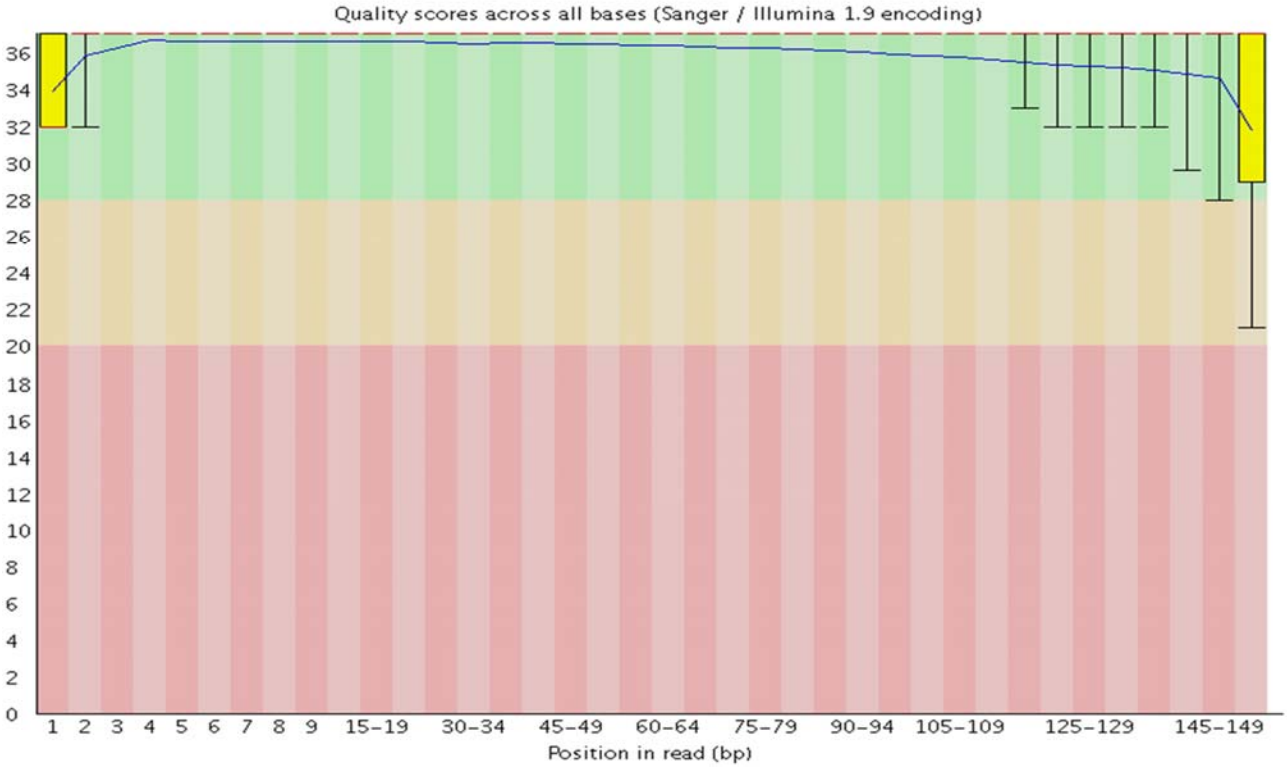

Figure 6.4: Quality control for one sample using FASTQC. (Note: the caption above this figure is an indication that samples sequenced using either Sanger or Illumina can be checked for quality control using FASTQC software.

6.4.2.14 Prevalence of HHV-8 ORF26 genotypes

Forty six percent (6/13) of the test sequences were genotype Q; thirty-one percent (4/13) test sequences were genotypes B; fifteen pecent (2/13) of the test sequences were genotypes E and eight percent (1/13) of the test sequence was genotype N. The two major genotypes observed in this population were genotypes Q and B. (Figure 6.4). All the genotype sequences are of participants from all study sites (Table 6.5). None of the variables was statistically associated with HHV-8 K1 subtypes.

Table 6.4: Demographic characteristics of ORF26 amplicons and genotypes detected from sequenced amplicons.

Serial Number	Sample ID	Gender	Age	Marital status	Districts	HIV status	ORF26 genotypes
1	AHDR300	Female	25	Single	Capricorn	Positive	B
2	AHDR325	Female	51	Married	Capricorn	Positive	B
3	OUHC47	Male	20	Single	Vhembe	Negative	B
4	ODF039	Male	45	Widower	Vhembe	Positive	B
5	ODF023	Female	29	Married	Vhembe	Positive	Q
6	ODF072	Female	29	Married	Vhembe	Positive	Q
7	OTHC024	Female	29	Single	Vhembe	Negative	Q
8	OTHC039	Male	30	Single	Vhembe	Positive	Q
9	ODF123	Male	41	Married	Vhembe	Negative	Q
10	ORHC025	Female	31	Divorced	Capricorn	Positive	Q
11	ORHC046	Female	49	Single	Capricorn	Positive	N
12	OSHC42	Female	27	Single	Capricorn	Negative	E
13	ORHC040	Female	33	Married	Capricorn	Positive	E

6.4.2.15 Analysis of HHV-8 ORF26 genotypes

Thirteen test sequences (labelled purple) were aligned with the reference sequences using Geneious version 10.0 and a phylogenetic tree was constructed. Figure 6.4 shows the various genotypes detected in the study population.

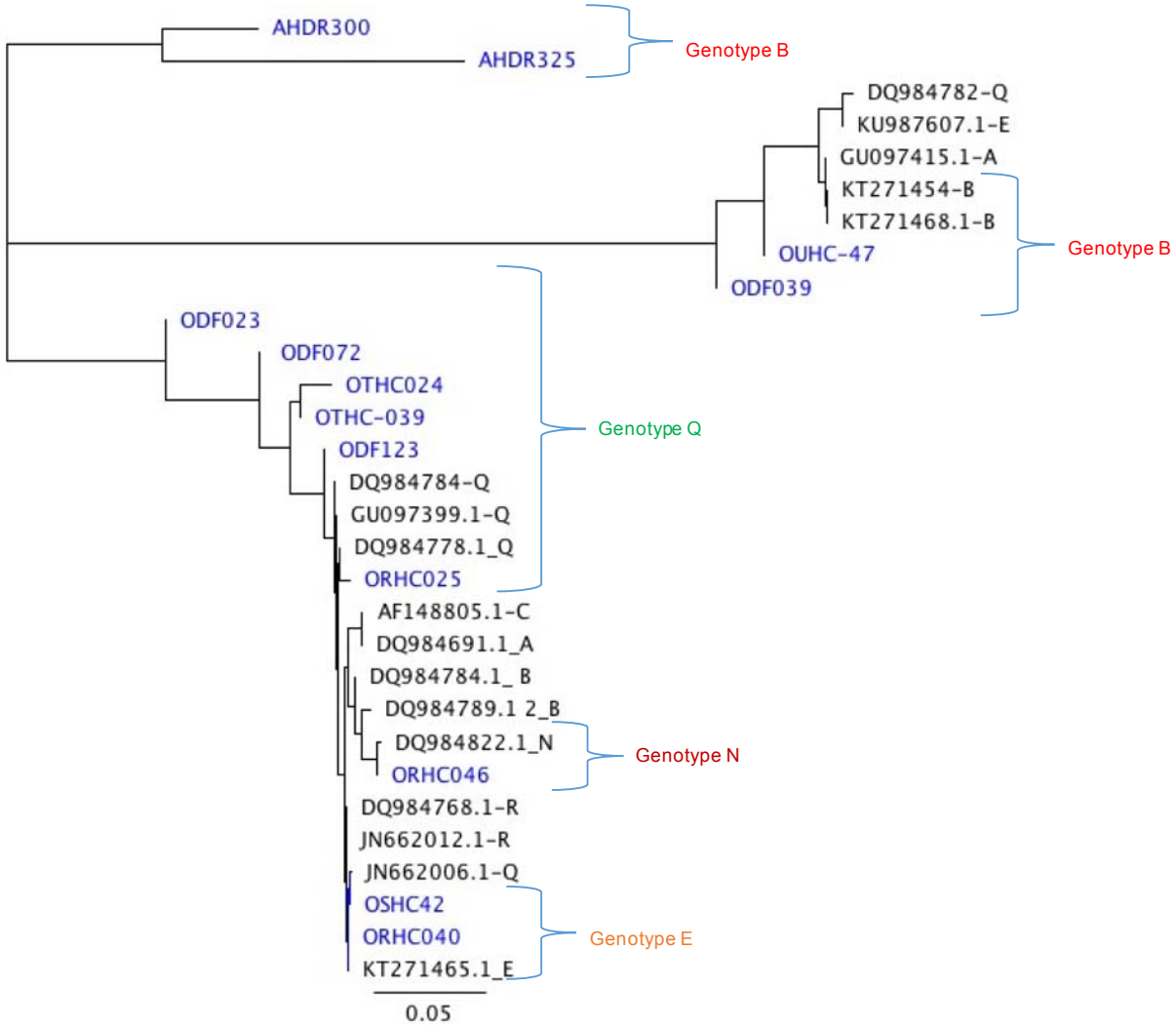

Figure 6.5: Phylogenetic tree of 13 HHV-8 ORF26 consensus sequences from ORHC (Rethabile), OTHC (Thohoyandou), AHDR (Polokwane), ODF (Donald frazer), OUHC (University of Venda) and reference sequences from South Africa, USA, Uganda, Zambia and UK. The different genotypes are represented with different colors. The tree was drawn to scale, indicating 0.05 nucleotide substitutions per site (bootstrap value > 70%). The clustering of genotypes E and Q can be best explained to the same time of KS (endemic KS) which they cause.

6.4.2.15 Single Nucleotide Polymorphism (SNPs) detection

Geneious was used to detect SNPs when test sequences were aligned with a prototype, HHV8 ORF26. Transition and transversion were the two types of SNPs detected. Transversion mutation is defined as the substitution of a pyrimidine for purine or vice versa. While transition is a point mutation that changes a purine nucleotide to another purine (A to G or G to A) or pyrimidine changes to another pyrimidine (C to T or T to C). Variant frequency is defined as that percentage of reads which are in agreement with the reference sequence at a given position. Further to this, no mixed infection was observed.

Out of 13 isolates, 9 (54%) had changes compared to the prototype and NCBI HHV-8 ORF26 consensus sequences and 4 were “wild type”. Three positions: 90 (C – T), 143 (A – C) and 203 (C – T) were the most common (see Table 6.6).

Table 6.5: HHV-8 ORF26 Single Nucleotide Polymorphisms, variant frequencies and predicted amino acids for tested isolates.

Nucleotide Positions	Nucleotide changes	Amino Acid change	Type of polymorphism	Variant frequency (%)	Sequence Coverage	Non-Synonymous change	Population level
90	C – T	A	Transition	95	16,463 - 86,980	A – V	Minority
143	A – C	K	Transition	99		K – Q	Minority
203	T – A	None	Transversion	96		None	Majority

Key words: Nucleotides :A-Adenine, C-Cytosine, T-Thymine and G-Guanine.

Amino acids: A-Argine, V-Valine, K-Lysine, Q-Qlutamine.

6.5 DISCUSSION AND CONCLUSIONS

6.5.1 Discussion

Studies have used PBMCs biopsy as a DNA template for HHV-8 ORF26 detection (Alagiozoglou et al., 2000; Campbell et al., 2000). However, the fewer studies which used saliva detected ORFK1 (Pellet et al., 1999; Brayfield et al., 2004). Good quality viral sequences were obtained from 13 of the participants. The aim was to determine the prevalence and characterize HHV-8 ORF26 gene using DNA from mouth wash, the most effective route of transmitting the virus**.**

This study utilized PCR a very sensitive method to investigate the prevalence of HHV-8 This method was used for the detection of the virus in the latent. Using PCR with the knowledge that the virus is shedded in the mouth; ensures that be it in the lytic or latent stages it will be detected.

In our finding, a seroprevalence of 28.1% was detected. Our observation may be due to individuals' habit such as smoking or geographical factors predisposing the study population to HHV-8 infection and/or KS. Examples of these factors include exposure to helminth parasites, phorbol ester plants and volcanic soils (Wakeham et al., 2011; Whitby et al., 2007). Also, the geographical setting could also play a role in acquiring this virus (Jacky et al., 2015). Hence a significant difference was found in our study.

In 2000, Lacoste and colleagues reported on the predominance of HHV-8 genotype B in Africa and more distant from genotypes A and C, which are found in Europe and the United States. Also, in 2002, Treurnicht and colleagues reported on HHV-8 ORF26 genotype B when they were working with patients' blood samples from different provinces in South Africa: Western Cape, Gauteng and Mpumalaga. According to a study by Tornesello et al (2010), genotypes B, Q, R and N are identified in sub-Saharan African countries. In our study, we observed genotype Q, B, E and N as the circulating strains of HHV-8 ORF26 in Northern South Africa.

Despite the small number of samples sequenced and HHV-8 ORF26 being a conserved gene, we observed evidence for evolutionary difference among our study

participants. The clustering pattern observed between our sequences (Northern South Africa) and global reference might suggest human migration. Although HHV-8 ORF26 which is considered a conserved region, however, some non-synonymous changes were observed. These changes occur in 4 out of the 13 samples sequenced.

Single nucleotide polymorphisms are changes in a single nucleotide that occur at a specific position within the genome. In the current study, HHV-8 ORF26 viruses were detected in 28% of the study population. It was interesting to observe that 20% of the participants had SNPs, irrespective of the threshold level of Next Generation Sequencing. It is also worth noting that 15% of the patients harboured ORF26 viruses as a minority population.

A study done in South Africa of HHV-8 ORF26, showed that B genotype had a characteristic of different polymorphisms, G changed to T at position 1055 in addition to A changed to G and A changed to C in positions 1132 and 1139 (Treurnicht et al., 2002). Another study on SNPs done in Cameroon, reported that there is a relationship between SNPs at nucleotide positions 1032 and 1055 of ORF26 (Endo et al., 2003). However, this was among numerous SNPs assessed and requires further investigation and confirmation. The SNPs that were observed in this study seemed novel. Furthermore, the genotyping of viruses using nucleotide polymorphism analysis is an important tool for investigating their epidemiology, transmission routes, virulence, and differences in clinical manifestations.

6.5.2 Strength and limitations of the study

The source from which the highest percentage of the virus can be detected (mouthwash) was used. This is the first study that established a theorem using ORF26 gene for HHV-8 genetic characterization in place of the ORFK1 gene and this is the first study to provide data for HHV-8 ORF26 in Northern South Africa. This study also used a modern sequencing technique (NGS), which can detect changes both at the majority as well as the minority levels. Though this study had numerous advantages, there were some draw backs such as few samples sequenced and no ORFK1 analysis.

6.5.3 Conclusion

The findings show that a significant number of individuals in the general population harbor HHV-8. Genotypes Q, B, E and N were detected as the prevalent genotypes in the study population, with genotype Q and B being the most prevalent types.

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CHAPTER SEVEN

HIGHLIGHTS OF THE STUDY AND FUTURE RESEARCH THRUSTS

7.1 HIGLIGHTS OF THE STUDY

The following conclusions and recommendations could be drawn from this study:

This thesis involved four sectional studies that aimed to define the seroprevalence and molecular epidemiology of HHV-8 in the African and Northern South African populations. The analysis in chapter 3 shows that HHV-8 is endemic on the entire African continent and it is being reported for the first time, suggesting a co-endemicity in regions already endemic for HIV.

The burden of HHV-8 has been established for the first time in Northern South Africa. The study also highlighted it highlighted the importance of understanding the role of HIV in HHV-8 epidemiology. While the seroepidemiology of HHV-8 in the Northern South African population is now known, more efforts should be geared towards understanding the mode of transmission of the virus. It is therefore proposed that both retrospective and prospective studies looking at children in different ages groups should be conducted. This is of paramount importance in answering the question of when an individual gets infected with HHV-8.

Urban settings in Southern Africa are associated with high HHV-8 infection.

Genotypes Q and B were the most prevalent genotypes in Northern South Africa. However, genotype A5 which has been reported as one of the most prevalent genotype in Africa was not observed in our study. Next Generation Sequencing (NGS), a very sensitive sequencing technique was used for genetic characterization and for Single Nucleotide Polymorphism detection. In comparison to Population based Sequences (Sanger) (data not shown), more SNPs were detected with NGS as opposed to Sanger Sequencing. Hence NGS is recommended for disease monitoring. A major drawback of this study is the use of a minor lytic capsid conserved gene for genetic characterization, with a small fragment size (HHV-8 ORF26, 233bp), rather than a major lytic and diverse gene (HHV-8 ORFK1, 1056bp). However, because both genes are lytic and have similar genotypes such as as genotype B and genotype N which is common with ORFK15 and is linked to ORFK1, ORF26 was used. Within the findings of this thesis, it can be concluded that genotype B, which is linked to endemic KS is prevalent in Northern South Africa and Africa in general.

Also, for the first time, a theorem on using HHV-8 ORF26 in place of HHV-8 K1 has been propsed. It was observed that, there is no genetic difference between ORF26 virus from Northern South Africa and the global consensus of ORF26 and ORFK1 viruses as mapping our test isolates with global consensus generated genotypes B and N which are ORFK1 and ORFK15 genes of HHV-8 genome. This implies that any vaccine based on genotype B or N subtype will be effective in curtailing the epidemic of HHV-8 in Northern South Africa and Africa in general.

7.2 FUTURE RESEARCH THRUSTS

For those countries within the continent on which no data on either HHV-8 seroprevalence and genotyping exist, it is recommended to carry out studies for better understanding on the seroprevalence and genotype distribution in Africa.

Furthermore, additional questions of interest include determining the importance of HHV-8 K8.1 (lytic) and HHV-8 ORF73 (latent) antibodies and the patterns of antibody detection in the general population over a period. Moreso, studies could also identify additional potential risk factors for HHV-8 infection to increase our understanding of HHV-8 transmission and epidemiology in Northern South Africa.

Characterization of HHV-8 full-length genomes from African isolates is needed for the selection of genes for vaccine construct and development. Since there are few studies on HHV-8 in Africa, further studies using more advanced technique such as NGS on the complete ORFK1 and ORF 26 genes could give more detailed information on the molecular epidemiology of HHV-8. Additional studies on the effect of HHV-8 strains on its epidemiology in relation to environmental and other stake factors should also be pursued.

More studies on plasma and mouth wash samples from the same participants for a better comparison and understanding on the epidemiology of this virus are recommended.

It is hoped that the findings presented in this thesis will help in achieving greater goals, such as government health intervention and policy, as well as aid in vaccine designing.

APPENDIX I

viruses

MDPI

Review

HHV-8 Seroprevalence and Genotype Distribution in Africa, 1998–2017: A Systematic Review

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Abstract: Human herpes virus type 8 (HHV-8) is the causative agent of Kaposi's sarcoma (KS). We systematically reviewed literature published between 1998 and 2017, according to the PRISMA guidelines, to understand the distribution of HHV-8 infection in Africa. More than two-thirds (64%) of studies reported on seroprevalence and 29.3% on genotypes; 9.5% were on both seroprevalence and genotypes. About 45% of African countries had data on HHV-8 seroprevalence exclusively, and more than half (53%) had data on either seroprevalence or genotypes. Almost half (47%) of the countries had no data on HHV-8 infection. There was high heterogeneity in the types of tests and interpretation algorithms used in determining HHV-8 seropositivity across the different studies. Generally, seroprevalence ranged from 2.0% in a group of young children in Eritrea to 100% in a small group of individuals with KS in Central African Republic, and in a larger group of individuals with KS in Morocco. Approximately 16% of studies reported on children. Difference in seroprevalence across the African regions was not significant (95% CI, $\chi^2 = 0.86$; $p = 0.35$), although specifically a relatively significant level of infection was observed in HIV-infected children. About 38% of the countries had data on K1 genotypes. K1 genotypes A, A5, B, C, F and Z occurred at frequencies of 5.3%, 26.3%, 42.1%, 18.4%, 5.3% and 2.6%, respectively. Twenty-three percent of the countries had data for K15 genotypes, and genotypes P, M and N occurred at frequencies of 52.2%, 39.1%, and 8.7%, respectively. Data on HHV-8 inter-genotype recombinants in Africa are scanty. HHV-8 may be endemic in the entire Africa continent but there is need for a harmonized testing protocol for a better understanding of HHV-8 seropositivity. K1 genotypes A5 and B, and K15 genotypes P and M, from Africa, should be considered in vaccine design efforts.

Keywords: HHV-8; seroprevalence; genotypes; systematic review; Africa

1. Introduction

Human herpes virus type 8 (HHV-8) is the causative agent of four classes of Kaposi's sarcoma (KS) [1]: endemic, classic, iatrogenic and AIDS-associated KS; of these, endemic-KS and AIDS-KS are the most aggressive. AIDS-KS has been highlighted in young homosexual men [2], while classic-KS is common in elderly Mediterranean people and individuals of Eastern Europe [3]. Furthermore [4], reported on endemic-KS, also called African endemic-KS, as common in children and young adults in sub-Saharan Africa. Iatrogenic-KS has been observed in immunosuppressed patients who had undergone a solid organ transplant [5,6].

Globally, the epidemiologic pattern of HHV-8 is uneven, but follows that of KS, and countries at high risk of KS report high prevalence of HHV-8. In a healthy population, there is great variability in the seroprevalence of HHV-8, as opposed to groups at increased risk of developing KS. HHV-8 undergoes both latent and lytic phases in its life cycle, whereby the virus remains within the host at a dormant stage until other cofactors trigger it to start replicating, leading to the lytic phase [7–9]. The mode of transmission of HHV-8 can either be vertical or horizontal, including mother to child transmission and from members of family units [10–12].

HHV-8 infection is ubiquitous in African regions with increased incidences of endemic Kaposi's sarcoma in the general population and AIDS-associated Kaposi's sarcoma in the HIV/AIDS population [13,14]. An increase in the incidence of KS has been observed due to HIV infection in Africa. However, with the scale up and improved access to combination antiretroviral therapy, it is expected that the incidence of KS may be mitigated as has been observed in the developed world [15,16]. In contrast to observations in US, studies have showed that HHV-8 seroprevalence increases from childhood to adulthood in African regions [17–19]. Treatment for HHV-8 or KS includes combined chemotherapy, such as vincristine, bleomycin and doxorubicin, radiation therapy, surgery and biological therapy that will effectively dissolve lesions whether localized or widespread, compared to a single HAART drug [20–22].

HHV-8 is characterized by high genetic variability across the entire genome, with the highest level of genetic variation observed at the 5′ and 3′ ends of the genome. HHV-8 has been classified into genotypes A, A5, B, C, D, E, F and Z based on the hypervariable regions (VR1 and VR2) of the *K1* gene; while genotypes P, M and N are based on the *K15* gene. Generally, these genotypes have been identified globally [4,23–31]. Genotypes B, Q, R and N are ORF26 genotypes, of which genotypes B and N overlap with genotypes based on *K1* and *K15* genes.

There are several ongoing vaccines and therapeutic development efforts against HHV-8 [32–34]. In this backdrop, it is important to understand the burden of HHV-8 in Africa where the infection appears to be relatively common. The current systematic review examined and analyzed data on the prevalence and molecular epidemiology of HHV-8 in all African countries from 1998–2017.

2. Methodology

2.1. Inclusion Criteria for Study Analysis

We conducted a systematic review of published full text articles on HHV-8 seroprevalence and genotypes from 53 African countries, according to the PRISMA guidelines, except for meta-analysis. Sudan and South Sudan were considered as one country. Cross-sectional, case report, retrospective, prospective and observational studies on HHV-8 seroprevalence and/or genotypes were included for analysis. Full texts in the French language were interpreted, analyzed and included in the analysis. However, there were no full text articles in other languages that met the inclusion criteria. African countries were assessed and categorized into Central, East, North, Southern and West Africa.

2.2. Relevant Literature Searches

MEDLINE, EMBASE, SCOPUS, WEB OF SCIENCE databases and conference proceedings, were searched for published data from 1998–2017. Using electronic search, reference lists were screened for relevant and additional data.

2.3. MEDLINE Search Strategy Using PubMed

The search terms used were: ((((((prevalence* OR epidemiology* OR incidence OR seroprevalence* OR seroepidemiology* OR sero-epidemiology* OR seropositivity* OR sero-positivity*)) OR ((((sero-epidemiologic studies [MeSH Terms] OR prevalence [MeSH Terms] OR incidence [MeSH Terms])))))) AND ((((((((herpesvirus 8, human [Term]) OR "human herpesrvirus 8") OR "HHV8") OR "HHV-8" OR "KSHV" OR "kaposi sarcoma associated herpesvirus") OR "kaposi's sarcoma associated

(http://www.capconcorp.com/meeting/2013/14thICMAOI/index.asp); Conference on Retroviruses and Opportunistic Infections (CROI) (www.croi2017.org).

2.8. Data Extraction

Two reviewers downloaded relevant articles from the above search options and classified each article using an article grid. These classifications were based on: the study objectives, design, sampling size, results, discussion and conclusion. For all included studies, information was extracted on: author(s), study type, country of study, country of participants, study population, sample size, sample type, number of males, number of females, number of children, type of assay, seroprevalence, source of DNA, K1 genotypes and K15 genotypes. We used 'X' for parameters with no information. The various tests used for the determination of HHV-8 seropositivity were also considered. These tests were immunofluorescence (IFA) and enzyme immunoassay (EIA), immuno-peroxidase (IMP), western blots and antigen ELISA tests (latent ORF73 and lytic K8.1). In addition, relevant articles on the genetic characterization of HHV-8 for *K1* and *K15* genes were analyzed. Figure 1 shows a PRISMA flow chart on the selection of studies included for analysis.

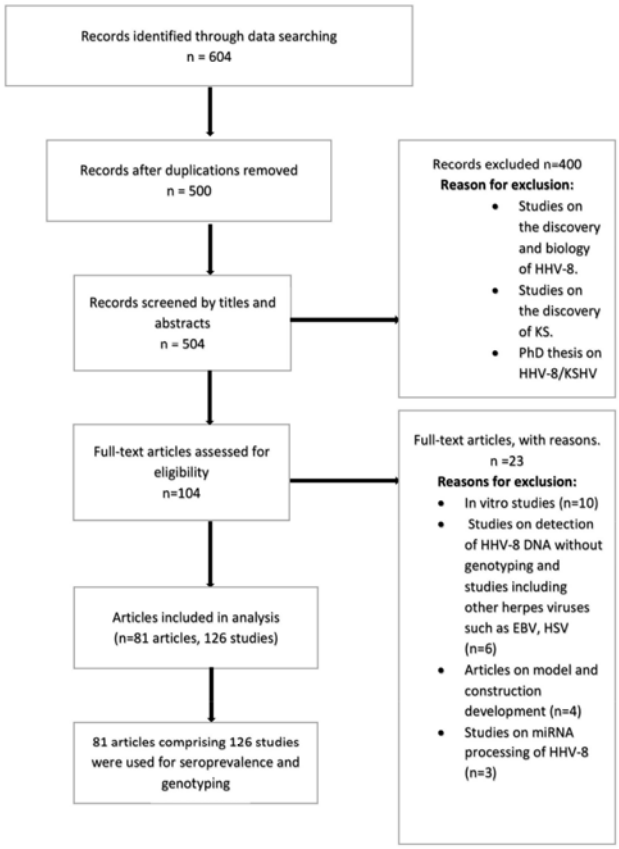

Figure 1. Flow chart on the selection of studies included for analysis.

3. Results

The use of different approaches and algorithms to determine the sero-epidemiology of HHV-8 across different populations presents challenges for comparisons. Examples of these challenges include the use of different dilution factors in plasma or serum preparation for antibody detection, the use of ELISA with different principles and interpretation guidelines, different immunofluorescence techniques, or a combination of these approaches. In addition to these, the inclusion of different populations (low and high risk), even in the same study community, increases the degree of complexity in deciphering an accurate picture of the distribution of HHV-8 infection among populations. Consequently, herein, we firstly present a descriptive analysis of the systematic literature search on studies on HHV-8 sero-epidemiology in Africa. To extract salient sero-epidemiological features, amid the challenges posed by different methodologies, we performed, where plausible, comparable analysis for studies involving children, pregnant women, and for rural and urban populations, against the backdrop of the risk factors for HHV-8 acquisition, for the different regions of Africa (Southern, Central, West, East, and North Africa). Countries were categorized as follows: Southern Africa—Botswana, Zambia, Mozambique, South Africa, Zimbabwe, Namibia, Swaziland, Lesotho, Malawi; Central Africa—Angola, Cameroon, Central Africa Republic, Chad, Congo, Gabon, Democratic Republic of Congo, Equatorial Guinea, Sao Tome & Principle; West Africa—Nigeria, Burkina Faso, Guinea, Gambia, Ghana, Mali, Senegal, Sierra Leonne, Niger, Liberia, Benin, Cote d'Ivoire, Togo, Cape Verde, Mauritania; East Africa—Tanzania, Kenya, Uganda, Ethiopia, Rwanda, Burundi, Djibouti, Eritrea, Somalia, Madagascar, Comoros; and, North Africa—Algeria, Egypt, Tunisia, Sudan (including South Sudan), Morocco, Libya, Western Sahara.

3.1. Characteristics of Studies Included in the Analysis

We identified a total of 604 articles from database searches, which were reduced to 500 after the removal of duplicates. Observational studies included for analysis were cross-sectional, prospective, retrospective and case report, which occurred at different settings such as rural, urban or hospital based. Out of the 500 articles, 81 articles met the inclusion criteria and were further analyzed (Figure 1). Some studies reported on multi-country and multi-site studies. One hundred and twenty-six studies on HHV-8 sero-epidemiology or genotypes, published from 1998–2017, were obtained from the 81 articles and met the inclusion criteria for analysis. About 64% (81/126) of the studies were available on HHV-8 seroprevalence; 29.3% (37/126) were available on HHV-8 genotypes; and 9.5% (12/126) of the studies were available for both seroprevalence and genotypes. Overall, 52.8% (28/53) of African countries had data on either seroprevalence or genotypes of HHV-8. Tables S1–S5 (Supplementary Materials) present studies from the different African regions included in the analysis.

3.2. HHV-8 Seroprevalence Distribution in Africa

About 45% (24/53) of African countries had data on HHV-8 seroprevalence only. Bearing in mind that different test strategies were applied in determining seropositivity in different populations, seropositivity reported ranged from 0.0% in a group of blood donors in Morocco by indirect immunofluorescence [35], through 2.0% in a group of young children in Eritrea by the detection of antibodies to ORF73 [36], to 100% in a small group of individuals with KS in the Central Africa Republic using immunofluorescence and immuno-peroxidase assays [37], although in a larger group of individuals with KS in Morocco the prevalence was 92% by indirect immunofluorescence [35]. Out of the 126 studies, 20 (15.8%) reported on children, in which seroprevalence ranged from 2% to 69%. Regionally, the seroprevalence across the continent ranged as follows: Southern Africa (14.0–90.0%), Central Africa (17.4–100.0%), West Africa (14.0–83.1%), East Africa (2.0–93.0%), and North Africa (0.0–92.0%). Considering the highest reported seroprevalence from the different African regions, there was no significant difference among the African regions (95% CI, $\chi^2 = 0.86$; $p = 0.35$). Figure 2 represents the relative seroprevalence of HHV-8 across Africa.

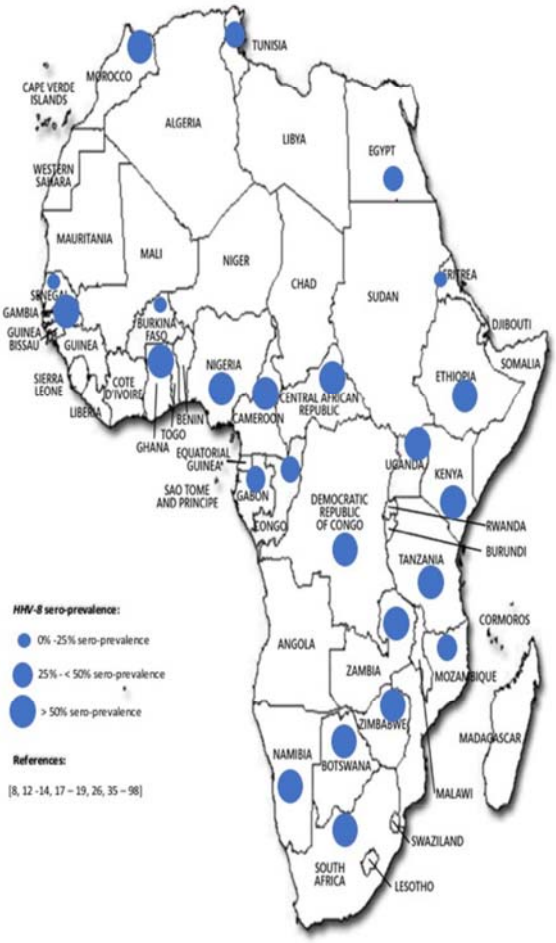

Figure 2. A representation of the highest HHV-8 seroprevalence observed in any one population in African countries (1998–2017). It should be borne in mind that the reported seroprevalences are from studied populations which employed different designs, approaches, populations, tests and interpretation algorithms, even within the same country. Thus, it could be difficult to make direct comparisons between and among studied populations [8,12–14,17–19,26,35–98].

3.3. A Comparison of HHV-8 Seroprevalence among Selected Populations across African Regions

From the available data, HHV-8 infection among children was observed to be significantly different across the African regions, with children in Central Africa being the most infected. The burden of infection was also significant in HIV-infected children compared to HIV non-infected children in Southern Africa (Table 1). Across the different African regions, HHV-8 antibodies were generally detected in a higher frequency in non-pregnant women than in pregnant women of comparable age, except in West Africa, were pregnant women were more infected than non-pregnant women (Table 2). No studies meeting the inclusion criteria were available for North Africa. Regarding geographical setting, there is no clear picture on the distribution of HHV-8 between rural and urban populations within and across African regions. For example, the urban population was marginally more infected than the rural population in Southern and East Africa, while this is not the case in West Africa. Across regions, the urban population in West Africa was the most infected. Data for North and Central Africa were inadequate to permit meaningful comparisons (Table 3).

Table 1. Relative HHV-8 infection burden in children, and proportion of HIV positive, HIV negative, and children with KS among the child population in Africa.

African Region	Percentage of Children Infected with HHV-8	Percentage of HIV Positive Children Infected with HHV-8 of All Children with HHV-8	Percentage of HIV Negative Children Infected with HHV-8 of All Children with HHV-8	Percentage of Children with KS and Infected with HHV-8 of All Children with HHV-8	Chi-Square Value	*p*-Value (Significant at $p \leq 0.05$)
Southern	31.4 (342/1092)	52.6 (180/342)	21.1 (72/342)	26.3 (90/342)	90.5	< 0.0015
Central	49.5 (329/665)	No data	No data	No data	Not done	Not done
West	11.4 (49/429)	18.6 (80/429)	81.3 (349/429)	No data	128.8	0.0001
East	20.7 (945/4557)	No data	No data	1.6 (15/945)	Not done	Not done
North	43.3 (122/282)	No data	No data	5.3 (15/282)	Not done	Not done
Chi-square value	367.2	98.3	279.1	220.0	34.9	0.401
p-value	< 0.00001	-	-	-	-	-

Data on children described in selected studies were summed up for each region and analysed. The percentages of HIV positive children, HIV negative children, and children with KS are calculated based on the total number of children with HHV-8 pooled from the extracted studies in the different African regions. Data from North Africa were not sufficient for meaningful comparable analysis and were omitted.

Table 2. Relative HHV-8 infection burden in women of comparable age (25–45 years) in Africa.

African Region	Percentage of Women Infected with HHV-8	Percentage of Pregnant Women Infected with HHV-8 of All Women with HHV-8	Percentage of Non-Pregnant Women Infected with HHV-8 of All Women with HHV-8	Chi-Square value	*p*-Value (Significant at $p \leq 0.05$)
Southern	28.7 (4196/14,612)	8.2 (343/4196)	91.8 (3853/4196)	155.93	<0.00001
Central	6.2 (287/4626)	27.5 (79/287)	72.5 (208/287)	100.00	<0.00001
West	1.7 (151/8491)	61.0 (92/151)	39.3 (59/151)	79.20	0.00001
East	26.1 (2280/8729)	22.2 (501/2280)	78.0 (1779/2280)	80.73	0.00001
Chi Square value	41.49	72.54	41.61	-	-
p-value	<0.00001	<0.0001	<0.00001	-	-

Data on women described in selected studies were summed up for each region and analysed. The percentages of pregnant and non-pregnant women infected with HHV-8 were calculated based on the total number of women with HHV-8 pooled from the extracted studies in the different African regions. Data from the North Africa region were not sufficient for meaningful comparable analysis and were omitted.

Table 3. Relative HHV-8 infection burden in rural and urban populations in Africa.

African Region	Percentage of HHV-8 Seropositivity in the Rural Setting	Percentage of HHV-8 Seropositivity in the Urban Setting	Chi-Square Value	*p*-Value (Significant at $p \leq 0.05$)
Southern	5.4 (207/3781)	3.3 (191/5710)	764.03	0.00001
Central	20.0 (516/2579)	No data	Not done	Not done
West	31.8 (77/242)	42.0 (26/62)	2.4462	0.119
East	29.0 (829/2409)	42.5 (450/1060)	84.52	0.0
Chi Square value	52.30	50.21	Not done	Not done
p-value	<0.00001	<0.0001	Not done	Not done

Data on the rural and urban populations described in the selected studies were summed up for each region and analysed. Data from the North African region were not sufficient for meaningful comparable analysis and were omitted.

3.4. HHV-8 Genotype Distribution in Africa

Based on the *K1* gene, 33.9% (18/53) of African countries had data on genotypes, while 28.3% (15/53) of countries had data on K15 genotypes (Figure 3). Genotypes A, A5, B, C, F and Z were identified at frequencies of 5.3% (2/38), 26.3% (10/38), 42.1% (16/38), 18.4% (7/38), 5.3% (2/38) and

2.6% (1/38), respectively; genotypes P, M and N of the *K15* gene were identified at frequencies of 52.2% (12/23), 39.1% (9/23) and 8.7% (2/23), respectively (Figure 4). Of the 18 countries from which K1 genotypes were reported, at least two genotypes were reported in each. Genotype Z was reported from only one study in Zambia involving children [23]. All other genotypes were reported in both children and adults. From the available data, there appears to be no trend in the distribution of genotypes across the continent. Worthy of note though is the description of K15 genotype N in Southern Africa (South Africa and Zambia) [23,99], and it is not clear whether K15 genotype N is restricted to Southern Africa. Data were reported for a few countries on intra-genotypic variants but data on inter-genotypic recombinants appears to be scanty [38,39,100,101].

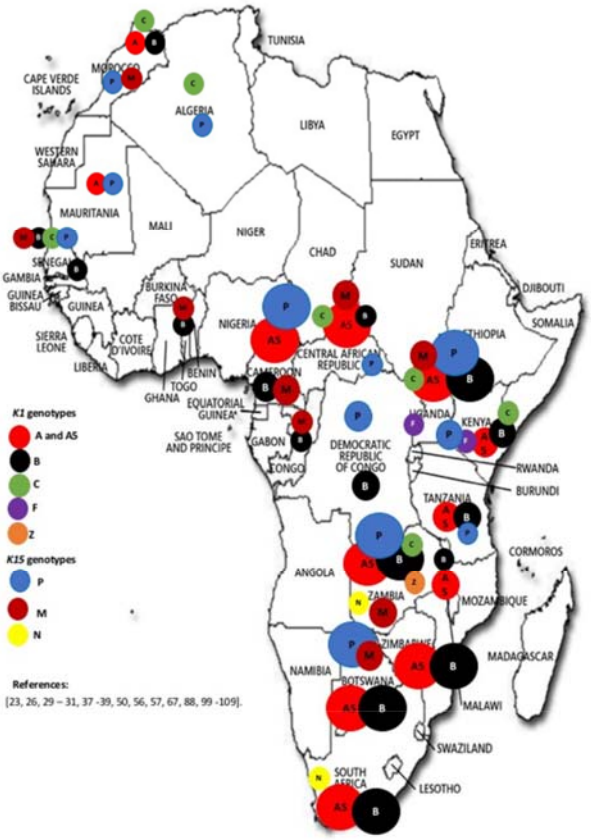

Figure 3. Map of Africa showing the occurrence of HHV-8 genotypes. There appears to be a general distribution of different genotypes across the continent. Of note is the identification of K15 genotype N in Southern Africa only (Zambia and South Africa). Countries without indications had no published data on HHV-8 genotypes between 1998–2017. The size of the circles indicates the relative proportion of occurrence of the different genotypes in the different countries. Overall, K1 genotypes A5 and B, and K15 genotypes P and M are the most common throughout the continent [23,26,29–31,37–39,42,44, 50,56,57,67,88,99–109].

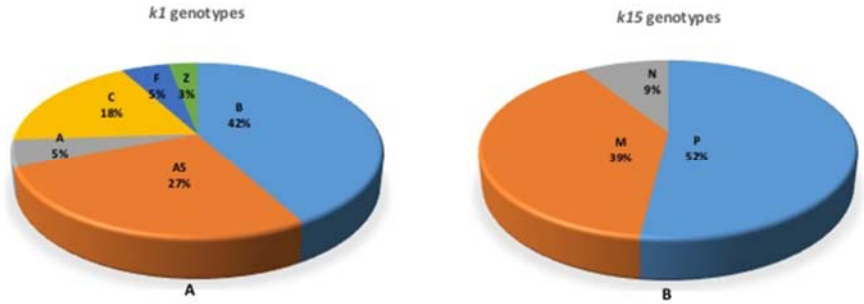

Figure 4. Proportional representation of HHV-8 genotypes in Africa. (**A**) represents K1 genotypes; (**B**) represents K15 genotypes. K1 genotype B is the most prevalent followed by genotype A5, while K15 genotype P is most prevalent genotype followed by genotype M.

4. Discussion and Conclusions

Studies on HHV-8 infection are important in regions, such as Africa, where a significant proportion of the continent is infected with immunosuppressive agents, such as HIV. This review was aimed at providing an update on the epidemiology and prevalent genotypes of HHV-8 in Africa. We observed that infection with HHV-8 is generally highly endemic in most parts of Africa, indicating that risk factors for infection persist throughout the continent. Reports suggest that there is variety and non-uniformity in the modes of transmission of HHV-8 among populations in Africa. For example, horizontal transmission from mother to child through chewed food (saliva) and contact with family members [12,42,89] sexual intercourse [62,65,87] other infections such as malaria [83], and blood transfusion [90,93] have been identified as risk factors for transmission. High seropositivity has been shown in pregnant women [64], and it has been suggested that the risk of HHV-8 seropositivity is significantly higher in children of HHV-8 seropositive mothers compared with children of HHV-8 seronegative mothers [110]. Further to this, in a sub-analysis of literature of pregnant and non-pregnant women, we found that in certain regions of Africa HHV-8 seropositivity was more frequent in non-pregnant women than in pregnant women of comparable age, while the converse was true in other regions (for example, in West Africa). This discrepancy or lack of trend may be attributed to other underlying confounding factors in HHV-8 acquisition in the different study populations and regions. Generally, there is a relatively lower rate of infection among children in Africa, which reflects findings that the rate of infection with HHV-8 in a given population increases in older age groups [71]. Most of the countries in Central, Southern and East African regions of the continent harbor huge burdens of HHV-8 infection. Geographic and cultural factors have been proposed as key determinants of HHV-8 transmission in some of these regions [12,53,54], also accounting for the variability in prevalence across and within geographic regions [71]. Additionally, a meta-analysis involving studies undertaken in 32 countries in sub-Saharan Africa, Australia, North and South America, Europe and Asia, demonstrated that HIV-infection is associated with a significant increase in HHV-8 co-infection globally, and in all population groups [111]. This scenario thereby coexists with the high HIV endemicity in Central, East and Southern Africa, and the probability of co-infection is therefore significantly increased in the said populations. Hence, with immunosuppression from HIV infection, it is plausible that cases of Kaposi's sarcoma will rise, although this could be mitigated with the improved access to antiretroviral therapy [45].

Our analysis revealed that HHV-8 genotypes A5 and B based on the *K1* gene and genotypes P and M based on the *K15* gene are the most prevalent in Africa. Genotype B is associated with endemic KS, while genotype P has been reported to be highly transmissible. Previous reports [25,27,43], indicated the clustering patterns of HHV-8 genotypes with ethnic composition of the population and

geographical location, and these might have arisen through ancient human migrations and genetic polymorphisms. The classification of HHV-8 into genotypes has not generally followed a unified approach. Some studies have used more than one gene for viral grouping, and some investigators has shown a link between gene regions; for example, [30] reported a link between ORF26 and K1. Intra-genotype variants have also been reported; for example, K1 A5 and K15 M based on allele differences [38,39,88]. A5 viruses are closely related to viruses found mostly in viral populations that form the A1-4 genotypes. Previously, A5 was thought to have emerged from recombination. However, subsequent and more robust analyses point to the emergence of the A5 genotype because of natural genetic drift and divergence between A1-4 genotypes [39,65]. It has been hypothesized [28] that the distribution of the K1-A5 genotype in African populations is because of a "very rapid and recent aggressive spread" of the K1-A5 prototype introduced into the population, aided by a selective advantage of the A5 allele. Overall, previous studies have demonstrated the clustering patterns of HHV-8 genotypes with geography and ethnicity, and these may have arisen through ancient human migrations and genetic polymorphisms, respectively [100,101]. The current analysis revealed scanty data on inter-genotype recombinants, with two studies reporting on the A/C genotype in Africa [39,101]. The A/C genotype is prevalent in Europe, United States, Asia, and the Middle East.

The current analysis, covering the period 1998–2017, suffers from a relative lack of data, dispersed across the continent, from about 50% of the countries, thereby somewhat limiting our appreciation of the burden of HHV-8 infection. However, the fact that high prevalence was noted in certain countries in all regions of the continent suggests that there is generalized infection across the geographical spectrum of Africa. The findings suggest that the entire continent is endemic for HHV-8, and co-infection with HIV may be common in those African countries endemic for HIV. Noting that there is high heterogeneity in the testing strategies employed in the detection of HHV-8 antibodies, there is need for an acceptable harmonized protocol to enhance the possibility of comparisons of seropositivity across studies. Since genotypes A5, B, P and M are highly prevalent in Africa, it is suggested that full length genomes of these genotypes from Africa be characterized to support the rational selection of genes for the design and development of vaccine candidates. This is, apparently, the first attempt at a systematic review of the seroprevalence and genotype distribution of HHV-8 comprising all African countries.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4915/10/9/458/s1. Tables S1–S5: Characteristics of studies included in the systematic review according to regions of Africa.

Author Contributions: P.O.B. conceptualized the study and analysed the data; E.M.E. performed the literature search, analysed the data, and prepared the first draft of the manuscript; D.P.A. performed literature search, analysed the data; L.G.M.-R. prepared the first draft of the manuscript; G.G. analysed the data and criticized the manuscript for intellectual content; all authors read and approved the final version of the manuscript for submission.

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