

THE EVALUATION OF HUMAN HERPESVIRUS 8 INFECTION IN TOBAGO

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University of Pittsburgh, 2008

HHV-8 seropositivity was associated with prevalent prostate cancer among African-Caribbean Tobago men (Odds ratio [O.R.] = 2.24; 95% confidence interval [C.I.], 1.29-3.90). To understand this association, HHV-8 seropositivity among African-Caribbean Tobago women, modes of HHV-8 sexual transmission, the natural history of HHV-8 seropositivity, and the relationship between HHV-8 and incident prostate cancer were examined.

A cross-sectional study was conducted in 213 Tobago women, ages 18-65 years. Age-specific rates were compared to those previously observed in men. Logistic regression analyses were performed to determine the association between HHV-8 seropositivity and sexual behaviors among women. A 9-year prospective cohort study was conducted among 407 Tobago men at risk for incident prostate cancer, ages 40-81 years. HHV-8 seroconversion and seroreversion rates and their 95% C.I. were calculated using a Poisson distribution. A case-cohort study was conducted among 90 and 407 Tobago men, ages 40-81 years, with incident prostate cancer and at risk for incident prostate cancer, respectively. Cox proportional hazards modeling for case-cohort design was used to examine the association between baseline HHV-8 seropositivity and incident prostate cancer. All serum/plasma were tested for HHV-8 seropositivity by immunofluorescence assay.

Among women, HHV-8 seroprevalence was 14.1%, with no difference with men of similar age (p -value = .741). There was a significant but minimal association between HHV-8 seropositivity and age ≤ 17 years at first sexual intercourse among women (O.R. = 2.51, 95% C.I. = 1.09-5.78). Among men at risk for incident prostate cancer, HHV-8 seroconversion and seroreversion rates were 0.5 (95% C.I., 0.22-

0.99) and 2.52 (95% C.I., 1.09-4.96) per 100 person-years, respectively. There were inverse associations between baseline HHV-8 seropositivity and screen-detected incident prostate cancer when age and baseline prostate cancer screening results (Hazard ratio [H.R.] = 0.454, 95% C.I., 0.221 – 0.933) and age (H.R. = 0.517, 95% C.I., 0.262 – 1.020) were considered.

Sexual activity may not be the predominant mode of HHV-8 transmission among women. HHV-8 is probably acquired at younger ages, < 40 years among men. HHV-8 seropositivity may not be related to prostate cancer incidence. The public health significance of these studies is to reduce HHV-8 infection and prostate cancer in Tobago.

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PREFACE

I would like to thank the staff in the Tobago Health Studies Office in Scarborough, Tobago, Trinidad & Tobago for their help in providing me the data I needed for my dissertation. Also, I would like to thank my Ph.D. committee members, family (especially, my mother Alberta McDonald Skipper), and friends for their support through this Ph.D. degree process.

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1.0 INTRODUCTION/SPECIFIC AIMS

Human herpesvirus 8 (HHV-8) is the causal agent of the vascular tumor Kaposi's sarcoma (KS) and primary effusion lymphoma and has been associated with multicentric Castleman's disease¹. The seroprevalence of HHV-8 infection varies geographically and is considered endemic in African and Mediterranean countries where KS is relatively common. African countries have the highest overall HHV-8 seroprevalence and KS incidence, followed by Mediterranean countries²⁻⁷. North America, Northern Europe, and Asia have the lowest HHV-8 seroprevalence with little to no KS⁸. For the Caribbean island of Tobago, a high seroprevalence of HHV-8 infection was detected in prostate cancer free African-Caribbean men, ≥ 40 years of age (22.9%), and this value was even higher when men with prostate cancer were considered (39.9%)⁹. The reasons behind these high rates of HHV-8 infection observed in Tobago men, particularly men with prostate cancer, are not known, considering that this country reports a low to no incidence of known HHV-8 malignancies such as KS^{10, 11}. The study of the epidemiology of HHV-8 infections may help explain the high prevalence of prostate cancer (15% of men aged 50-79) observed in this Tobago population, as well the etiologic mechanisms.

In HHV-8 endemic populations, sexual and non-sexual transmission have been shown to be modes of transmitting this virus. In hyper-endemic KS areas like Sub-Saharan Africa, non-sexual exchange (for example, saliva exchange) has been shown to be the predominant mode of HHV-8 transmission¹². In Tobago, the predominate mode of HHV-8 transmission is unknown, as well as the seroprevalence in Tobago women is unknown. The knowledge of women seroprevalence at various ages may shed light on the route of transmission of the virus in Tobago. We propose to conduct a cross-sectional study in 213 Tobago women, 18-65 years of age, in order to examine HHV-8 seropositivity rates

in this population. We hypothesize that age-specific HHV-8 seropositivity rates will be similar to Tobago men's rates due to similar environmental exposures. We want to explore in this study population whether certain sexual practices are associated with increased risk for acquiring this herpes virus. We hypothesize that HHV-8 seropositivity rates will be higher in women who have many sexual partners, have been previously diagnosed with a sexually transmitted disease, sexually active at a younger age, participate in oral sex activities, and use no barrier methods for protection during sexual activity. HHV-8 infection by sero-status will be determined by a modified serological assay HHV-8 monoclonal antibody-enhanced immunofluorescence assay (IFA)¹³ which has been reported to have a sensitivity of 89.9% and specificity of 97.5% in comparison to other serological assays based on a latent-class analysis¹⁴. Sexual activity was ascertained from The Centers for Disease Control and Prevention's Sexual Lifestyle Questionnaire that was administered to study participants in a private setting.

In order to better understand possible relationships between HHV-8 and prostate cancer, the natural of history of HHV-8 infection should be investigated. Therefore, we propose to conduct a 9-year prospective cohort study that examines patterns of change in HHV-8 sero-status (seroconversion and seroreversion) and the frequency of persistent HHV-8 seropositivity among 501 Tobago men, ages 40-81 years, at risk for incident prostate cancer. We hypothesize low seroconversion rates, ~ 3%, due to low HHV-8 exposure after the age of 40; and, we hypothesize even lower seroreversion rates, ~ 1%, due to the virus' life-long infection. HHV-8 seroconversion is defined as HHV-8 seronegative at baseline visit to HHV-8 seropositive at subsequent visits. HHV-8 seroreversion is defined as HHV-8 seropositive at baseline visit to HHV-8 seronegative at subsequent visits. HHV-8 seropositivity will be determined by the modified IFA described in the previous paragraph. The examination of HHV-8 seroconversion, seroreversion, persistent seropositivity rates in men at risk for incident prostate cancer will provide knowledge of the pattern of HHV-8 infection in the Tobago population.

In HHV-8 infected individuals, HHV-8 may contribute to the inflammatory process in the prostate. This virus has been shown to infect prostatic epithelial cells¹⁵. The expression of HHV-8 proteins (LANA-1, vIL-6, and K8.1), as well as evidence of local inflammation (macrophage/monocyte

marker and B-cell marker), have been found in the prostate¹⁶. Because of its presence in the prostate, HHV-8 may contribute to prostate cancer through chronic inflammation, a persistent inflammatory response to the virus. Therefore, it is important to examine whether HHV-8 seropositivity is associated with prostate cancer risk, especially in a population where 11% prostate cancer prevalence was detected at initial screening¹⁷. We propose to conduct a case-cohort study among 501 men at risk for incident prostate cancer (sub-cohort, the controls) and 116 screen-detected incident prostate cancer men (the cases) in order to determine whether baseline HHV-8 seropositivity is associated with incident prostate cancer. Based on Hoffman et al. study results, we hypothesize screen-detected incident prostate cancer cases are 2-fold more likely to be HHV-8 seropositive at baseline visit than men at risk for incident prostate cancer (the sub-cohort). Examining possible relationships between HHV-8 seropositivity and incident prostate cancer will provide information on whether HHV-8 infection plays a role in the progression of prostate cancer.

In conclusion, examining HHV-8 seropositivity among Tobago women, identifying possible modes of sexual transmission of HHV-8, studying the natural history of HHV-8 seropositivity, and investigating the relationship between HHV-8 and incident prostate cancer will allow us to evaluate HHV-8 infection and its possible relationship with prostate cancer in Tobago. These aims will help us to determine whether further studies are needed in examining relationships among HHV-8 infection, inflammation, and prostate cancer.

Specific Aims

Aim 1 (Paper 1): To examine HHV-8 infections among African-Caribbean women living in Tobago

From a Tobago Cervical and Oral Cancer Screening Study, specimens were collected from 213 healthy African-Caribbean Tobago women 18 to 65 years old. We propose to conduct a cross-sectional study that will examine the following items:

- a) To examine age-specific rates of HHV-8 seroprevalence in Tobago women 18-65 years of age.

- *Hypothesis:* We hypothesize that HHV-8 seropositivity will increase with age due to cumulative exposure to sexual practices and possibly other environmental factors.
- b) To compare age-specific HHV-8 seroprevalence rates in African-Caribbean Tobago women with rates in African-Caribbean Tobago men, aged 40-65 years.
 - *Hypothesis:* We hypothesize that HHV-8 seroprevalence will be similar in older men and women due to similar environmental exposure (for example, childhood exposure).
- c) To determine if HHV-8 seropositivity in African-Caribbean Tobago women is associated with sexual behavior.
 - *Hypothesis:* We hypothesize that seropositivity will be higher in women who have many sexual partners, have been previously diagnosed with a sexually transmitted disease, sexually active at a younger age, participate in oral sex activities, and use no barrier methods for protection during sexual activity than women who do not participate in these activities.

Aim 2 (Paper 2): To measure the incidence of HHV-8 seroconversion and seroreversion, and to examine persistent HHV-8 seropositivity in African-Caribbean men at risk for incident prostate cancer in Tobago.

From the Tobago Prostate Cancer Screening Survey of African-Caribbean Tobago men, aged 40-81 years, we propose to conduct a prospective cohort study among 501 men at risk for incident prostate cancer in order to examine the following items:

- a) To measure the incidence of HHV-8 seroconversion in seronegative men at baseline visit to subsequent visits (wave 2 visit or wave 3 visit).
 - *Hypothesis:* We hypothesize low seroconversion rate, 3%, due to low HHV-8 exposure after the age of 40.
- b) To measure the incidence of HHV-8 seroreversion in seropositive men at baseline visit to subsequent visits (wave 2 visit or wave 3 visit).

- *Hypothesis:* We hypothesize low seroreversion rate, 1%, due to the virus' life-long infection.
- c) To measure the rate of persistent HHV-8 seropositivity in men from baseline visit to subsequent visits (wave 2 visit and/or wave 3 visit).
 - *Hypothesis:* We hypothesize high persistent seropositivity rate due to the virus' lifelong infection.

Aim 3 (Paper 3): To examine baseline HHV-8 seropositivity in screen-detected incident prostate cancer men (cases) in comparison to men at risk for incident prostate cancer (sub-cohort, the controls) in Tobago.

From the Tobago Prostate Cancer Screening Survey of African-Caribbean Tobago men, aged 40-81 years, we propose to conduct a case-cohort study in 116 screen-detected incident prostate cancer cases in comparison to 501 men at risk for incident prostate cancer to examine the following:

- a) To test the association between baseline HHV8 seropositivity and incident prostate cancer in men from Tobago
 - *Hypothesis:* We hypothesize prostate cancer incident cases are 2 times more likely to be HHV-8 seropositive at the baseline visit than men at risk for incident prostate cancer.

2.0 BACKGROUND

2.1 HERPESVIRIDAE

The family Herpesviridae consists of viruses that affect humans and animals such as primates, various mammals, bony fishes, and invertebrates. Herpesviridae has three subfamilies of common ancestry that are grouped on biological properties: Alphaherpesvirinae, Betaherpesvirinae, and Gammaherpesvirinae (see Table 1). The subfamily Alphaherpesvirinae affects a wide range of hosts, has a short reproductive cycle, destroys infected cells efficiently, spreads rapidly in culture, and can establish latent infection in the sensory ganglia¹⁸. The alphaherpesviruses that affect humans include herpes simplex virus 1 (e.g., oral cold sores), herpes simplex virus 2 (e.g., genital herpes), and varicella-zoster viruses (e.g., chicken pox and shingles).

The subfamily Betaherpesvirinae, affects a restricted range of hosts, has a long reproductive cycle, grows slowly in culture, and can establish latent infection in secretory gland, lymphoreticular cells, kidneys, and other tissues¹⁸. The betaherpesviruses that affect humans are cytomegalovirus and human herpesviruses 6 (e.g., exanthema subitum) and human herpesvirus 7.

The subfamily Gammaherpesvirinae infection occurs primarily in T and B lymphocytes and to a lesser extent in epithelial and endothelial cells. Latency is usually established in lymphoid tissue¹⁸. The gammaherpesviruses that affect humans are divided into the genera Lymphocryptovirus (Epstein Barr virus) and Rhadinovirus (human herpesvirus 8, also known as Kaposi's sarcoma associated herpesvirus). HHV-8 has commonly been compared to Epstein-Barr virus (EBV) because they both cause

lymphoproliferative diseases and tumors^{1, 19-22} as well as have similar tropism for B cells, endothelial cells, macrophages, and keratinocytes²³⁻²⁵. However, HHV-8's homology is closest to the gammaherpesvirus, herpesvirus saimiri, a virus that affects squirrel monkeys^{8, 26}.

Table 1. Family Herpesviridae's Human Associated Diseases

Alphaherpesvirinae	
<i>Herpes simplex 1 (HSV-1)</i>	Orolabial infections, gingivostomatitis, encephalitis
<i>Herpes simplex 2 (HSV-2)</i>	Genital infections, encephalitis
<i>Varicella-zoster (HHV-3)</i>	Chicken pox & Shingles
Betaherpesvirinae	
<i>Human Herpesvirus 5 (HHV-5)</i>	Cytomegalovirus infection
<i>Human Herpesvirus 6 (HHV-6A & 6B)</i>	Exanthema subitum
<i>Human Herpesvirus 7 (HHV-7)</i>	Sub-clinical infection; occasionally exanthema subitum & febrile convulsions
Gammaherpesvirinae	
<i>Epstein-Barr virus (HHV-4)</i>	Mononucleosis, Hodgkin's lymphoma, nasopharyngeal carcinoma, Burkitt's lymphoma, gastric carcinoma, T & natural killer (NK) lymphoma, and Leiomyosarcoma
<i>Human Herpesvirus 8 (HHV-8)</i>	Kaposi's sarcoma (KS), primary effusion lymphoma (PEL), and multicentric Castleman's disease (MCD)

2.1.1 Seroprevalence of Human Herpes Viruses

The seroprevalence of common human herpes viruses and herpes-associated diseases varies geographically (Table 2). In the U.S., the alphaherpesviruses human herpes simplex 1 and 2 (HSV-1 and -2) have a seroprevalence of 80% in individuals < 40 years of age and 21.9% in adults, respectively (NHANES II & III)²⁷. In some northern European, Mediterranean, Caribbean, African, and Asian countries, HSV-1 seroprevalence has been reported to be over 95% in the population²⁷. As for the varicella-zoster virus, a 4 million yearly incidence of the chicken pox infection has been reported in the

U.S.; however, this incidence has recently declined due to the introduction of the varicella vaccine in 1995²⁷.

With the betaherpesviruses, cytomegalovirus has a U.S. seroprevalence of 58.9% compared to Mediterranean, Asian, and South American countries' seroprevalence ranging from 77% to 99% in the population²⁷. As for the gammaherpesviruses, HHV-8 has been reported to have a seroprevalence ranging from 3% to 5.2% in U.S. healthy individuals^{4, 14} in comparison to Mediterranean and African countries having a significantly higher seroprevalence, ranging from 11.5% in West Sicily, Italy to 87% in Botswana, Africa²⁻⁷. Epstein Barr virus has a seroprevalence of 90% worldwide.

Table 2. Human Herpes Viruses Seroprevalence in General Population

	<i>U.S. Seroprevalence (overall)</i>	<i>Non-U.S. Seroprevalence</i>
Herpes simplex 1 (HSV-1)	80%	> 95%
Herpes simplex 2 (HSV-2)	21.9%	
Varicella-zoster (HHV-3)	~80%	
Human Herpesvirus 5 (HHV-5)	58.9%	52% - 99% (Grenoble, France – Ankara, Turkey)
Epstein-Barr virus (HHV-4)	90.0%	90%
Human Herpesvirus 8 (HHV-8)	3.0% - 5.2%	11.5% - 87% (West Sicily, Italy - Botswana, Africa)

2.1.2 Malignancies of Human Herpes Viruses

Some herpes viruses have been associated with human malignancies and/or cancer-causing diseases, in particular, the subfamily Gammaherpesvirinae (Table 1). For example, Epstein Barr virus, a gammaherpesvirus that causes mononucleosis, has been associated with Hodgkin's lymphoma, nasopharyngeal carcinoma, Burkitt's lymphoma, gastric carcinoma, T & natural killer (NK) lymphoma, and Leiomyosarcoma²⁷. HHV-8, also a gammaherpesvirus, has been associated with Kaposi's sarcoma (KS), primary effusion lymphoma (PEL), and the cancer-causing multicentric Castleman's disease (MCD).

As for other subfamily herpes viruses, studies have suggested that the betaherpesvirus HHV-6 may play a possible co-factorial role in cancer development for the following cancers and cancer-causing diseases: non-Hodgkin's lymphoma, Hodgkin's disease, S100-positive, T-cell chronic lymphoproliferative disease, oral salivary gland carcinoma tissue, and other neoplasia^{18, 27-29}. However, the subfamily Gammaherpesvirinae contain the only known herpes viruses that cause malignancies in humans.

2.2 HISTORY OF HHV-8

In 1872, a Hungarian dermatologist Moritz Kaposi described an "idiopathic multiple pigmented sarcoma" of the skin that affected men of Mediterranean descent³⁰; this KS was later called "classical KS." In 1920's, more KS cases with an aggressive form were identified in central and eastern African populations^{31, 32}; this form was later called "endemic KS." In 1972, Giraldo et al. identified a herpes-like particle in KS lesions which was thought to be cytomegalovirus (CMV)^{32, 33}. CMV DNA was never identified³²; however, the Giraldo et al. study led to the hypothesis that KS might have a viral etiology³³. Decades later, an increase incidence of KS cases was observed worldwide, including in the U.S., among homosexual men during the 1980's human immunodeficiency virus (HIV) pandemic³⁴⁻³⁶, a new emerging sexually transmitted disease that causes acquired immunodeficiency disease syndrome (AIDS). KS cases that resulted from this sexually transmitted disease were called "AIDS-associated KS." In 1994, Moore and Chang discovered an unknown herpes virus from KS lesions of AIDS patients using representational differences analysis¹. This new herpes virus was called Kaposi's sarcoma associated herpesvirus or human herpes virus 8 (HHV-8). Later in 1995, HHV-8 was found to be associated with two lymphoproliferative diseases, primary effusion lymphoma (PEL) and multicentric Castleman's disease (MCD).

2.3 BIOLOGY OF HHV-8

2.3.1 Genetic Structure of HHV-8

HHV-8 is an envelope, double-strand, DNA virus that structurally has a DNA containing nucleocapsid core, a middle proteinaceous tegument, and an outer lipid bi-layer envelope. The lipid bi-layer envelope has many glycoproteins attached to its outer layer that are responsible for functions such as viral cell entry and replication as well as assembly, maturation, migration, and release of viral particles^{8, 18, 37}. The conserved structural glycoproteins (common for all herpes viruses) are glycoproteins B (gB), H (gH), L (gL), M (gM) and N (gN) which encode open reading frames (ORFs) 8, 22, 47, 39, and 53, respectively^{20, 37, 38}. Open reading frames (ORFs) are part of the HHV-8 genome that contains sequences of bases (A,G,C,T) that can encode a protein. The glycoproteins unique to HHV-8 are K8.1A, K8.1B, K1, K14, and K15 which are expressed during lytic replication^{20, 37}. HHV-8-encoded virion envelope glycoproteins B and K8.1A are important for viral entry in target cells through their interaction with heparan sulfate, a binding receptor on the target cell²⁴.

HHV-8's DNA is approximately 165 kbps (kilobase pairs). The genome consists of a long unique region (approximately 140 kbps) flanked by terminal repeat sequences. The long unique region contains the open reading frames. There are 81 to more than 90 open reading frames (ORFs) of which sixty-six to sixty-eight have similar homology to the non-human, gammaherpesvirus herpesvirus saimiri^{8, 19, 20, 39-41}. The terminal repeat regions consist of multiple copies of an 801 bp sequence that is approximately 85% G+C content. The terminal repeats vary in number at each end of the genome and contain packaging and cleavage signal sequences^{8, 20, 39}.

2.3.2 HHV-8 Specific Genes

HHV-8 has been reported to have more than 90 separate ORFs each one encoding for a separate protein. Majority of these genes are homologues with other herpesviruses and are therefore described as conserved genes. HHV-8 genes that do not have a herpesvirus homologue have been given a “K” prefix (i.e., ORF K1 to ORF K15) in order to identify genes unique to HHV-8. These unique HHV-8 genes have been found to consist primarily of cellular homologues for genes such as IL-6, bcl-2, macrophage inhibitory proteins, cyclin-D, IL-8 receptor, etc.^{20, 40, 42}.

HHV-8's gene expression is dependent on whether the virus is in latency (virus is not replicating) or in a lytic cycle (virus is replicating producing infectious progeny). Latent and lytic stages are common in all herpes viruses and these stages contribute to the virus's long-life infection. With HHV-8, there are genes that are expressed in either the latent and lytic cycle and contribute to the survival of the virus and/or the progression to cancers like Kaposi's sarcoma (KS) and primary effusion lymphoma (PEL). However for cancer progression, individuals who are immunodeficient who usually acquire HHV-8 associated diseases and malignancies^{8, 40}.

During latency, there are a few genes that are expressed once HHV-8 is well-established in the infected B cell, macrophage, endothelial cell, spindle cell, epithelial cell, or keratinocytes nucleus as a circular DNA episome^{19, 23-25}. In individuals with KS, these latent gene expressions increase with KS lesion stage indicating an increase in HHV-8 latently-infected cells⁴³. For both non-KS and KS persons, no viral particles are produced during latency^{39, 44}.

Even though latent genes' expression is minimized during latency, they play an important role in the progression of lymphoproliferative malignancies and diseases through their growth and/or anti-apoptotic signaling⁴³. The major latent genes (proteins) are the following: ORF73 (LANA-1), ORF72 (viral cyclin D), ORF K13 (viral FLIP protein), ORF K12 (Kaposin A), ORF K11.5 (viral IRF2), ORF K10.5 (LANA-2), and ORF K15 (LAMP). Latency-associated nuclear antigen (LANA) and v-FLIP proteins are responsible for preventing programmed cell death (apoptosis); and, viral cyclin D, Kaposin

A, and LAMP proteins are responsible for stimulating cellular growth. Both these processes allow the survival of HHV-8 infected cells. In addition, viral interferon regulatory factor 2 (v-IRF-2) prevents transcription factors, like nuclear factor kappa B (NF- κ B) and cellular interferon regulatory factors 1 and 3 (IRF-1 & IRF-3), from regulating the immune system's response to viral infection. All these lytic gene expressions contribute to tumor progression by enhancing the survival or cellular growth of HHV-8 infected cells (Table 3).

Table 3. HHV-8 Latent Genes and their Functions

HHV-8 Gene	HHV-8 Protein	Cellular Homology	Functions
ORF73	LANA-1	None	Blocks apoptosis, stimulates cellular transformation, downregulates <i>p53</i> and targets <i>Rb</i> (tumor suppressors)
ORF72	v-Cyc D	Cyc D ₂	Stimulates cell cycle progression
ORF K13	v-FLIP	FLIP	Inhibits apoptosis, promotes tumor progression
ORF K12	Kaposin A	None	Stimulates cellular transformation
ORF K11.5	v-IRF-2	IRFs	Inhibits <i>NF-κB</i> and cellular IRFs-1 and -3 mediated transactivation, causes apoptosis of T-cell receptor, stimulates Jurkat cells and double-stranded RNA protein kinase
ORF 10.5	LANA-2	None	Inhibits <i>p53</i> -mediated transactivation and apoptosis in only B cells
ORF K15	LAMP	None	Interacts with growth control proteins

Abbreviations: v = viral, Cyc = cyclin, IRFs = interferon regulatory factors, LANA = latency-associated nuclear antigen, HHV-8 = human herpes virus 8, ORF = open reading frame, Rb = retinoblastoma protein, NF- κ B = nuclear factor kappa B, FLIP = FLICE-inhibitory protein, RNA = Ribonucleic acid

References^{40, 43 45}

Of the latent proteins, LANA-1 is probably the most important latent protein because it is highly expressed in all HHV-8 associated malignancies and diseases^{39, 46}. Because of this expression, this protein has been considered as a reliable marker and many serological assays have used this protein in examining latent HHV-8 infections. LANA-1 is also important to the maintenance of the viral episomal DNA, therefore, maintaining latent viral persistence in infected cells^{39, 40, 46}. This maintenance of latency is achieved through LANA-1's ability to repress the replication and transcription activator (Rta), a protein (ORF50) that is necessary for the initiation of lytic replication⁴⁷. In addition, this protein can also repress

or activate transcription by interacting with the tumor suppressor genes *p53* and retinoblastoma protein (*Rb*)^{40, 48, 49}. The tumor protein *p53* is a phosphoprotein that prevents the formation of tumors by 1) inducing cell cycle arrest so that DNA repair can occur and 2) promoting apoptosis in order to stop the proliferation of defective cell. *Rb* is a protein that prevents damaged cells from progressing through the cell cycle. By repressing *p53* and *Rb*, aberrant cellular proliferation continues which this continuation may lead to cancer.

When HHV-8 is activated from latency to lytic replication, the majority of the viral genes are expressed during this active stage. The major HHV-8 lytic proteins associated with viral pathogenesis are: ORF K2 (viral interleukin-6), ORF74 (viral G-protein-coupled receptor), ORF K6 (macrophage inflammatory protein 1), ORF K4 (macrophage inflammatory protein 2), ORF K4.1 (macrophage inflammatory protein 3), ORF K9 (viral interferon regulatory factors 1), ORF K10.5-10.6 (viral interferon regulatory factors 3), ORF K1 (transforming & immunomodulatory proteins), ORF16 (viral BCL-2), ORF K7 (viral inhibitor of apoptosis proteins), ORF K3 (modulator of immune recognition 1), ORF K5 (modulator of immune recognition 2), and ORF K14 (viral OX₂). These lytic genes play an important role in pathogenesis of lymphoproliferative diseases and malignancies through their cellular proliferative inductions, pro-inflammatory abilities, angiogenic activities, and anti-apoptosis mechanisms. For example, viral interleukin 6 (v-IL-6) cytokine induces cellular proliferation. Viral macrophage inflammatory proteins 1-3 (v-MIP 1, v-MIP 2, v-MIP 3) and viral OX₂ (v-OX₂) engage in inflammatory activities. Viral G-protein-coupled receptor (v-GPCR), v-IL-6, and v-MIPs contribute to angiogenesis, a process that induces blood vessel growth that may contribute to tumor expansion. Viral interferon regulatory factor 1 (v-IRF-1), v-IL-6, viral BCL-2 (v-BCL-2), and viral inhibitor of apoptosis (v-IAP) proteins prevent apoptosis, therefore, support cellular proliferation. Viral interferon regulatory factor 3 (v-IRF-3), transforming & immunomodulatory proteins, and modulator of immune recognition proteins 1 and 2 (MIP-1, MIP-2) have activities that promote HHV-8 lytic replication cycle. Collectively, these lytic protein's functions aid in the progression of HHV-8 associated malignancies and diseases (Table 4).

Table 4. HHV-8 Lytic Genes and their Functions

HHV-8 Gene	HHV-8 Protein	Cellular Homology	Functions
ORF K2	v-IL-6	IL-6	Induces cellular proliferation, prevents apoptosis, contributes to tumor angiogenesis
ORF74	v-GPCR	IL-8r (or CXCL8r)	Induces cellular transformation, secretes angiogenic vascular endothelial growth factors (VEGF)
ORF K6	v-MIP (or v-CCL-1)	MIP-I alpha	Has pro-inflammatory roles and possibly has angiogenic activities, engages chemokine receptor CCR-8
ORF K4	v-MIPII (or v-CCL-2)		Has pro-inflammatory roles and possibly has angiogenic activities, engages chemokine receptor CCR-8
ORF K4.1	v-MIPIII (or v-CCL-3)		Has pro-inflammatory roles and possibly has angiogenic activities, engages chemokine receptor CCR-4
ORF K9	v-IRF-1	IRFs	Inhibits apoptosis
ORF K10.5-10.6	v-IRF-3		Blocks IFN signaling from cellular IRF-3 and IRF-7 that have anti-viral, anti-proliferative & immunomodulatory properties
ORF K1	Transforming & immunomodulatory protein	ITAM	Important for viral replication, modulates reactivation
ORF16	v-BCL-2	BCL-2	Inhibits apoptosis
ORF K7	v-IAP	Survivin-delta EX3 (IAP)	Inhibits apoptosis
ORF K3	MIR-1	None	Increase endocytosis of MHC class I from surface of infected cells (not recognized by cytolytic arm of immune system)
ORF K5	MIR-2		
ORF K14	v-OX ₂	OX ₂ (CD200)	Activates inflammatory cytokines (IL-1B, TNF-alpha, IL-6) from myeloid-lineage cells

Abbreviations: v = viral, r = receptor, IRFs = interferon regulatory factors, HHV-8 = human herpes virus 8, ORF = open reading frame, IL = interleukin, MIP = macrophage inflammatory protein, CCL = CC chemokine, IAP = inhibitor of apoptosis, GPCR = G-protein-coupled receptor, MIR = modulator of immune recognition, CXCL = CXC chemokine, MHC = major histocompatibility complex, TNF = tumor necrosis factor, IFN = interferon, ITAM = immunoreceptor tyrosine activation motif

References^{8, 19, 40 45, 50}

Out of all the HHV-8's ORFs, the lytic ORF K1 gene is the most highly divergent gene in HHV-8's genome and has been used to identify 8 known HHV-8 subtypes (A-E, M, N, P, Z) and more than 24

clades^{40, 51, 52}. This hypervariable gene is a result of several mutations that have occurred over thousands of years, possibly due to some evolutionary pressures^{53, 54}. Epidemiological studies have shown that certain K1 sequences are unique to particular ethnic groups and geographical regions^{8, 51, 53, 55-58}. However, studies have not shown whether these K1 differences increase or decrease one's risk for cancer development. The K1 gene, which encodes the transmembrane protein that is homologous to the immunoreceptor tyrosine activation motif (ITAM), is critical for HHV-8 replication; and, this gene may play an important role in viral gene expression that modulates HHV-8 reactivation^{40, 59, 60}.

For HIV-1 infected individuals, a HIV-1 gene has been implicated to increase the incidence and the aggressiveness of KS. HIV-1 infected individuals have the most aggressive form of KS. This aggressiveness is caused by the HIV-Tat protein, a transcription activator that is released by HIV-1 infected T cells^{43, 61-63}. This protein can induce the adhesion growth, migration, and invasion KS spindle cells and augment angiogenesis, therefore, affecting the development of KS⁴³. Tat can also induce the following cytokines and adhesion molecules: TNF-alpha and -beta, IL-6, ELAM-1, vascular adhesion molecule-1 (V-CAM-1), intercellular adhesion molecule-1 (I-CAM-), and monocyte chemotactic protein-1 (MCP-1), v-GPCR, v-BCL-2, v-IRF-1^{43, 64}. These cytokines and molecules aid in KS progression. Matrix metalloproteinases 2 and 9 (MMP-2, MMP-9) may also play a necessary role in KS development in HIV-1 infected individuals through its mediation of vascular and KS cell invasion and angiogenesis⁴³.

2.3.3 HHV-8 Reactivation/Lytic Replication

HHV-8 reactivation is the re-entry to lytic replication from latency. Studies have suggested that viral reactivation occurs due to immunosuppression or forms of stress; however, physiological conditions that trigger reactivation are still undefined^{44, 65}. In addition, clinical studies have shown that ongoing lytic replication is required for tumorigenesis^{66, 67}. Therefore, lytic replication is a necessary process in the development of HHV-8 associated diseases and malignancies. The switch from latency to lytic replication is an important step in the lifecycle of HHV-8 and the development of HHV-8 associated cancers.

For HHV-8, ORF 50, a gene that encodes the replication and transcription activator (Rta) protein, is necessary and sufficient to make the switch from latency to lytic replication⁶⁸⁻⁷². The protein Rta activates immediate-early and early genes such as ORF K8 (Zta or K-bZIP), its own gene ORF50, ORF 57 (post-transcriptional regulator of gene expression), genes involved in viral DNA replication, and structural genes^{39, 73, 74}. By interacting with the following viral promoters, Rta also initiates a cascade of lytic gene expressions that may play a central role in tumorigenesis: ORF K12 (kaposin), v-IL-6, v-MIP-1, v-IRF-1, v-GPCR, and ORF K1^{40, 75-80}. These protein functions are described in Table 4.

Because of these highly expressed viral genes during lytic replication, the HHV-8 is susceptible to immune surveillance in immunocompetent individuals; this noticeable expression can disrupt cancer progression. For example, in immunocompetent individuals, IRF-7, a cytokine that activates anti-viral innate immune response, can down-regulate Rta, therefore, preventing lytic replication but maintaining latency⁷⁴. Therefore, a person's immune system may be an important determinant in whether HHV-8 reactivation occurs.

Viral reactivation is an important part of HHV-8's lifecycle. The switch from latency to lytic replication is essential in the release of viral progeny and the development of HHV-8 associated diseases and malignancies.

2.4 HHV-8 ASSOCIATED MALIGNANCIES

HHV-8 is known to cause Kaposi's sarcoma (KS) and has been associated with multicentric Castleman's disease (MCD) and primary effusion lymphoma (PEL), rare lymphoproliferative diseases^{1, 81-83}. These cancers (KS and PEL) and lymphoproliferative disorder (MCD) have been observed in immunocompromised individuals; however, HHV-8's carcinogenic effects in immunocompetent populations are unknown. HHV-8 is a newly discovered virus and little is known about the biology, infection, and its carcinogenic effects in susceptible populations.

2.4.1 Kaposi's Sarcoma (KS)

Kaposi's sarcoma, the predominant HHV-8 associated malignancy, is a slow, growing vascular tumor characterized by multi-focal lesions of the skin. KS is classified as one of the following four types: 1) classic KS, 2) endemic KS, 3) iatrogenic KS, or 4) acquired immunodeficiency disease syndrome (AIDS) associated KS. Classic KS is predominately seen in elderly men of Mediterranean, eastern European, or Jewish descent^{19, 64}. This type is known to affect the lower extremities and rarely affects visceral organs^{43, 84}. Classic KS was the first type to be identified in 1872 by the dermatologist Moritz Kaposi³⁰. Endemic KS is an aggressive type observed in human immunodeficiency virus (HIV) negative children and young adults from Africa^{32, 43, 64, 85, 86}. This endemic form involves visceral and/or lymphatic organs^{32, 85, 86}. Iatrogenic KS is a mild type that is seen in post-transplant patients who are on immunosuppressive therapy, in particular of Italian, Saudi Arabian, and Ashkenazi or Sephardic Jewish descent^{39, 43, 64, 87-89}; KS is usually resolved when treatment is reduced or terminated^{39, 40, 90}. AIDS-associated KS is the most aggressive form found in HIV-infected persons, usually homosexual men. This aggressive form affects the skin and visceral organs, causing a systemic manifestation^{19, 43}. Progression of KS lesions and visceral dissemination can lead to organ dysfunction or failure, therefore, resulting in death⁴⁰.

To treat classic and endemic KS, simple excision or radiation is used for single or multiple lesions, respectively³⁹. For recurrent disease, a combination of radiation, surgery, and chemotherapy are used as the forms of treatment in classic and endemic KS individuals³⁹. As for iatrogenic KS, modification, reduction, or termination of immunosuppressive therapy is used for the regression of KS lesions^{39, 64}. For AIDS-associated KS, highly active antiretroviral therapy (HAART) has been successful in reducing KS^{39, 91}. This reduction may be attributed to an "immune reconstitution" in HIV-infected patients⁹¹.

2.4.2 Primary effusion lymphoma (PEL)

Pleural effusion lymphoma, also called body cavity-based lymphoma (BCBL), is a fatal non-Hodgkin's lymphoma that has a pleural, peritoneal, or pericardial effusion presentation, often in the absence of a tumor mass^{19, 32, 39}. However, a solid tumor mass can develop in the lymph nodes, lungs, or gastrointestinal tract^{39, 87}. The lymphoma cells are usually monoclonal and of B-cell origin³². Interleukin 10 (IL-10) and v-IL-6 may contribute to the growth of these lymphoma cells³². PEL is mainly seen in AIDS patients. Compared to KS spindle cells, PEL lymphoma cells have a higher copy of HHV-8 DNA, 50-150 per cell^{19, 39, 87}.

2.4.3 Multicentric Castleman's disease (MCD)

Multicentric Castleman's disease is a rare lymphoproliferative disorder regulated by an over-expression of the inflammatory cytokine interleukin 6 (IL-6)^{19, 92}. This disorder is histologically characterized by expanded germinal centers that include B cell and vascular proliferations³². There are two types of MCD: 1) plasma cell variant, and 2) hyaline vascular variant. Plasma cell variant type is found in AIDS patients and is associated with HHV-8 infections³². Clinical presentations of both types are fever, anemia, and hypergammaglobulinemia possibly due the elevated levels of IL-6⁶⁴. MCD is the often the precursor of non-Hodgkin's lymphoma^{8, 93}.

2.4.4 Other Possible Associated Diseases

Studies have suggested that HHV-8 plays a role in the following diseases: multiple myeloma^{94, 95}, angiosarcoma⁹⁶⁻¹⁰⁰, pemphigus vulgaris and pemphigus foliaceus^{101, 102}, sarcoidosis¹⁰³, angioimmunoblastic lymphoma¹⁰⁴, pulmonary hypertension¹⁰⁵, angiolymphoid hyperplasia with eosinophilia¹⁰⁶, skin carcinomas in immunocompromised individuals¹⁰⁷, primary central nervous system lymphoma¹⁰⁸,

post-transplantation lymphoproliferative disorders^{109, 110}, pulmonary inflammatory myofibroblastic tumor¹¹¹ and prostate cancer⁹. Studies have demonstrated negative findings of HHV-8's relationships with these diseases and lymphoma^{64, 83, 112-122}. However, HHV-8's relationship with most of these diseases and cancers still remain inconclusive; therefore, more studies are needed to evaluate these relationships.

2.5 LABORATORY DETECTION OF HHV-8

Several laboratory methods have been developed to examine the distribution of HHV-8 infection in different risk groups and geographical areas. These methods include 1) the polymerase chain reaction (PCR) assay which is used to detect HHV-8 DNA in various bodily fluids and tissues and 2) serological assays which are used to examine antibodies against HHV-8 antigens in human serum. In comparison to the PCR, HHV-8 serological assays are more sensitive for detecting current and past HHV-8 infections. However, there is no gold standard assay in examining HHV-8 infections.

2.5.1 Polymerase chain reaction (PCR)

HHV-8 PCR has been useful in showing the causal relationship between HHV-8 DNA and HHV-8 associated malignancies (KS, PEL, and MCD)^{1, 81-83, 123}. This method has also been used in determining possible modes of transmission through the detection of HHV-8 DNA in bodily fluids such as saliva, semen, and peripheral blood¹²⁴⁻¹³¹. In addition, the PCR method has allowed the prediction of KS development in asymptomatic HIV-positive individuals¹²³. Therefore, the PCR has been a useful tool in understanding the biology of HHV-8 even though it is considered a less sensitive method in determining HHV-8 infection, possibly due to the quality of the samples, in comparison to serological assays.

To perform the PCR, studies have used the following primers in looking for the presence of HHV-8 DNA in bodily fluids and tissues: ORFs 8, 25, 26, 64, 65, 72, 75, and K1 as well as the

glycoprotein B^{123, 132}. ORFs 26 and 72 have shown to be the most sensitive and therefore are commonly used as primers in detecting HHV-8 DNA¹²³. The functions of proteins coded by genes detected by these common HHV-8 PCR primers are displayed in Table 5.

2.5.2 Serological Assays

There are several serological assays that have been developed to examine different HHV-8 lytic and latent antigens in order to determine HHV-8 sero-status: immunofluorescence assay (IFA), Western Blot, and enzyme-linked immunosorbent assay (ELISA). IFA, which includes mouse monoclonal antibody-enhanced immunofluorescence assay, is the oldest HHV-8 assay (available in 1996) and it detects latent (LANA) and lytic HHV-8 antigens⁵⁵. The advantages of the IFA are the high sensitivity for HHV-8 lytic antigens (> 97%) and the inexpensive cost; however, this method is time-consuming, not easy to use in large studies, requires extensive quality control, and is subjective in interpretation of sero-status results^{8, 132, 133}. A modified monoclonal antibody-enhanced immunofluorescence assay for antibodies against lytic antigens will be used in this research study¹³.

As for the ELISA, HHV-8's whole viral lysates, synthetic peptides, or recombinant peptide carrier protein conjugates has been used to determine HHV-8 seroprevalence in populations^{132, 133}. The most common HHV-8 genes used for the ELISA are ORFs 65, a small capsid antigen, and K8.1A, a glycoprotein associated with the virion envelope. ELISAs produce more objective results than IFAs; however, they still require quality controls in their assay and they are less sensitive (83%-87%) than IFAs¹³². As for the Western blot, it can detect latent antigen LANA and the glycoprotein K8.1. However, the Western blot is difficult to conduct and it is less sensitive (80%) than IFAs and ELISAs¹³²; therefore, it is rarely used in studies to examine HHV-8 seroprevalence in populations.

Studies have used the following HHV-8 antigens to examine HHV-8 seroprevalence in study populations: ORFs 6, 8, 9, 25, 26, 39, 59, 65, 68, 73, K8.1A, and K8.1B¹²³. The most immunogenic HHV-

8 antigens are ORFs 73 (LANA-1), 65, and K8.1; these antigens are commonly used in serological assays¹²³. The functions of these commonly used antigens are displayed in Table 5.

Table 5. Most Common HHV-8 Assays

HHV-8 Gene	HHV-8 Protein	Assay Used
ORF 26	34 k-Da minor capsid protein	PCR, IFA, ELISA
ORF 65	Small, basic, highly antigenic viral capsid protein	IFA, ELISA
ORF 72	v-Cyc D, latent protein	PCR
ORF 73	LANA-1, latent protein	WB, IFA, ELISA
ORF K8.1	Late-lytic gene that encodes for 2 HHV-8 specific glycoproteins (K8.1A and K8.1B)	WB, IFA, ELISA

Abbreviations: ORF = open reading frame, WB = Western blot, IFA =

immunofluorescence assay, ELISA = enzyme-linked immunosorbent assay,

PCR = polymerase chain reaction, LANA = latency-associated nuclear antigen,

Cyc = cyclin

The sensitivity and specificity of an assay are important in determining HHV-8 infection in populations; however, a gold standard assay has not been identified. The sensitivity and specificity of HHV-8 assays have been determined by comparing each assay's results to known HHV-8 seropositive and seronegative control samples. For example, the sensitivity and specificity of lytic versus latent antigen assays differ. The lytic antigen assays has been shown to be more sensitive (> 95%) than the latent antigen LANA assay⁸. The lower sensitivity of the latent antigen assay may be due to antibodies against lytic antigens preceding antibodies against latent antigens¹²³. As for the comparison of the different types of serologic assays, Spira et al. have found that mouse monoclonal antibody-enhanced immunofluorescence assay (mIFA) for antibodies against lytic antigens is the most sensitive (97%), followed by combined peptide enzyme-linked immunosorbent assay (ORF 65 and ORF K8.1 peptide) (93%), ORF K8.1 peptide ELISA (87%), ORF 65 peptide ELISA, mIFA latent (83%), Advanced

Biotechnologies ELISA kit prepared from sucrose gradient-purified HHV-8 whole virions (80%), and the least sensitive ORF 65 immunoblot (or Western blot) (80%)¹³². Pellet also found that mIFA (conducted at the University of Pittsburgh) had the highest sensitivity (89.9%) based on a conditional independence model by latent class analysis compared to 5 other serological assays that examined HHV-8 sero-status in a U.S. blood donor population¹⁴; this assay will be used in this research study. However, the specificity of the mIFA was low compared to the other assays (97.5%); the ELISA based on induced BC3 cells and two BC3 mIFAs against latent LANA antigens and lytic antigens, respectively, had the highest specificity (100%)¹⁴.

2.6 THE EPIDEMIOLOGY OF HHV-8 INFECTION

2.6.1 HHV-8 Seroprevalence by Country

The seroprevalence of HHV-8 infection varies geographically and is considered endemic in African and Mediterranean countries where Kaposi's sarcoma (KS) is relatively common. The prevalence of HHV-8 infection has corresponded with the prevalence of KS: Africa having the highest seroprevalence and incidence of KS, followed by Mediterranean countries with intermediate seroprevalence and KS incidence, and North America and Northern Europe having the lowest seroprevalence with little to no KS. In addition, the predominant types of KS (classic, endemic, AIDS-associated, or iatrogenic) differ geographically; for example, Sub-Saharan Africa has mostly endemic and AIDS-associated KS cases, and the Mediterranean has mostly classic KS cases. Because HHV-8 is a newly discovered carcinogenic virus, many studies have been developed to evaluate HHV-8 infections in different populations and the following geographical regions: Africa, the Mediterranean, South America, the Caribbean, Asia, Europe, and the Oceania. However, assays used to examine HHV-8 infections in populations have varied. Therefore, the comparison of HHV-8 seroprevalence among different

geographical regions may not be an accurate evaluation due to differences in sensitivity and specificity of the assays unless the same assay is used to evaluate HHV-8 infection in different populations. Nevertheless, the examination of HHV-8 infections, geographically and in different ethnicities, is important in understanding the biology of HHV-8 as well as determining which populations or environments are more susceptible in acquiring HHV-8 infection and its associated diseases and malignancies.

2.6.1.1 Sub-Saharan Africa

Sub-Saharan Africa, a region not considered a part of North Africa, has the highest HHV-8 seroprevalence and KS incidence in the world. This region has 42 mainland countries and 6 islands that are divided into the following geographical parts: Central, East, South, and West Africa¹³⁴. Epidemiological studies have shown different distributions of HHV-8 infection in the general population among Central, East, South, and West Africa, seropositivity rates ranging from 5.76% to 87%. Also, the incidence of KS has been different in Central, East, South, and West Africa: 30, 23, 13.2, and 4.6 per 100,000 person-years among men and 8.6, 9.5, 5.7, and 1.4 per 100,000 person-years among women, respectively¹⁰. Because HHV-8 infection is highly endemic in Sub-Saharan Africa, high incidences of endemic and AIDS-associated KS have been observed in this region.

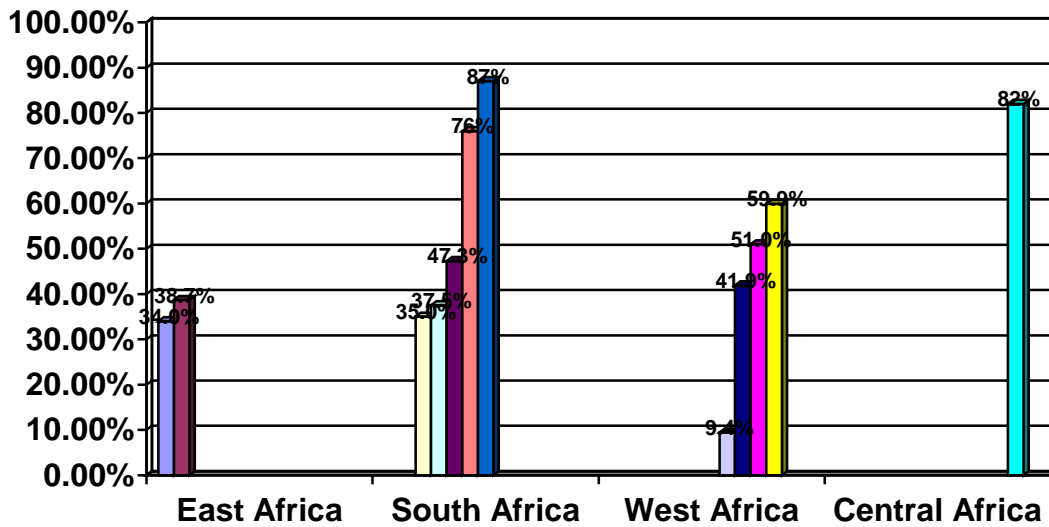
The seroprevalence of HHV-8 in Sub-Saharan Africa has ranged from 9.42% to 87% for antibodies against HHV-8 lytic antigens and 5.76% to 25% for antibodies against HHV-8 latent antigens in healthy populations (Figure 1). For example, in East Africa, two studies have found a similar seroprevalence for antibodies against HHV-8 lytic antigens in Uganda: 34% was found in 485 mothers who took their child to a sickle cell clinic and a 38.7% was found in 62 healthy donors^{4, 135}. In South Africa, a HHV-8 seroprevalence of 35% and 14% for antibodies against HHV-8 lytic and latent antigens, respectively, was found among 2546 mothers, aged 15 to > 40 years, who attend a vaccination clinic with their child in the cities of KwaZulu and Natal¹³⁶. In Zambia, South Africa, seropositivity rates for antibodies against HHV-8 lytic antigens ranging from 37.5% in 40 healthy blood donors to 47.3% in 275

pregnant women who attended an antenatal clinic at the University Hospital in Lusaka were found^{4, 137}. However, in Botswana, a higher HHV-8 seropositivity rate of 87% for antibodies against lytic antigens was detected in the semi-nomadic San people, aged 18-55; and a lower rate of 76% was found in the Bantus people, aged 16-64². Intermediate HHV-8 seropositivity rates in East Africa and intermediate to high seropositivity rates in South Africa reflect the incidence of KS observed in these areas².

As for West Africa, high HHV-8 seropositivity rates but surprisingly low incidences of KS have been reported in their healthy populations. For example, in Garoua, North Cameroon, 51% and 25% seroprevalence for antibodies against HHV-8 lytic and latent antigens, respectively, was found in 292 individuals, aged 5 to 40, who attended a general medical outpatient ward¹³⁸. In South Cameroon, an overall seroprevalence of 59.9% for antibodies against HHV-8 lytic antigens were observed in 92 families from an isolated village in the Ntem region³. However, in Ghana and Burkina Faso, a lower seroprevalence against lytic HHV-8 antigens, 41.9% and 9.42%, respectively, was found in comparison to Cameroon. The reasons for the intermediate rates of HHV-8 infection but low KS in West Africa are unknown.

For Central Africa, a higher HHV-8 seropositivity rate has been reported for this area in comparison to West and East Africa. In the Democratic Republic of Congo, a seroprevalence of 82% for antibodies against HHV-8 lytic antigens was detected in individuals, aged 6-78, without KS. This high seropositivity rate in Central Africa parallels the high incidence of KS (30 per 100,000 person-years among men) reported for this area, the highest KS incidence in the world.

In conclusion, the differences in HHV-8 seropositivity rates among and within the Sub-Saharan nations may reflect different environmental factors that contribute to the susceptibility of HHV-8 infections as well as the development of KS in their populations (Figure 1).



Note: East Africa's HHV-8 seroprevalence of 34% and 39.7% represent Kampala, Uganda and Uganda (city not identified) populations, respectively. South Africa's HHV-8 seroprevalence of 35%, 37.5%, 47.3%, 76%, and 87% represent KwaZulu/Natal, Zambia (city not identified), and Lusaka, Zambia populations, Botswana Bantu, and Botswana San, respectively. West Africa's HHV-8 seroprevalence of 9.42%, 41.9%, 51%, and 59.9% represent Burkina Faso, Ghana, North Cameroon, and South Cameroon populations, respectively. Central Africa's HHV-8 seroprevalence of 82% represents the Congo

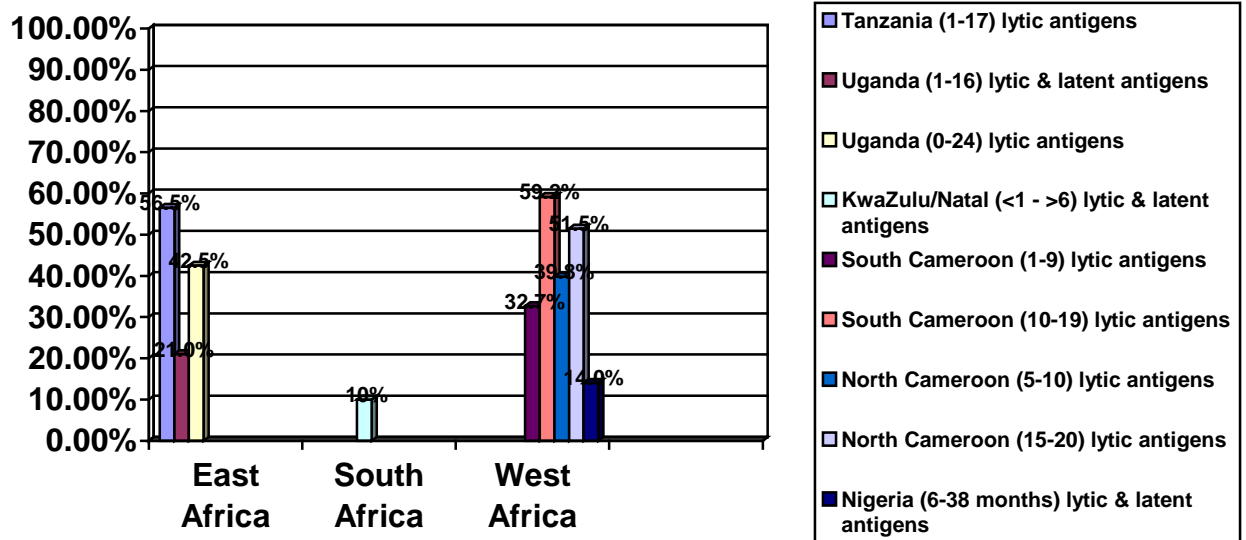
Figure 1. Seroprevalence for Antibodies against HHV-8 Lytic Antigens in Sub-Saharan Africa General Population

HHV-8 infection is prevalent among infants and children in the Sub-Sahara region (Figure 2). For example, in Tanzania, East Africa, a seroprevalence of 56.5% was observed for antibodies against HHV-8 lytic antigens in 798 children/adolescents, aged 1-17, who participated in a HIV survey; only 2 persons were HIV positive¹³⁹. In Kampala, Uganda, East Africa, HHV-8 seroprevalence of 21% for antibodies against lytic and latent antigens was found in 600 children/adolescents, aged 1-16, who attended a sickle cell clinic¹³⁵. Also, in Uganda, seroprevalence of 42.5% and 33% for antibodies against HHV-8 lytic and latent antigens, respectively, was found in 212 children/young adults, aged 0-24, who attended an outpatient department¹⁴⁰.

In South Africa, a lower HHV-8 seroprevalence was detected in children. For example, in KwaZulu and Natal, South Africa, 2501 children, < 1 to > 6 years of age, who attended a vaccination clinic, had an overall seroprevalence of 12% and 7% for antibodies against HHV-8 lytic and latent antigens, respectively¹³⁶. Of these children, seroprevalence of 10% and 7% for antibodies against HHV-8 lytic and latent antigens, respectively, was observed in 2297 HIV negative individuals; however, higher seroprevalence of 31% and 8% for antibodies against HHV-8 lytic and latent antigens, respectively, was observed in 153 HIV positive individuals¹³⁶.

As for West Africa, HHV-8 seroprevalence of 32.7% for antibodies against lytic antigens in 162 children, aged 1-9, and a seroprevalence of 59.2% in 147 children/adolescents, aged 10-19, were observed in individuals who participated in a survey in the isolated village of Ntem, South Cameroon³. In North Cameroon, 39.8% and 25.5% HHV-8 seroprevalence for antibodies against lytic and latent antigens, respectively, was observed in 292 children, aged 5-10, who attend a general medical outpatient ward; and, 51.5% and 23.7% HHV-8 seroprevalence for antibodies against lytic and latent antigens, respectively, was observed in adolescents/young adults, aged 15-20, who also attend an outpatient ward¹³⁸. As for Nigeria, a seroprevalence of 14% for antibodies against lytic and latent antigens was observed in 184 infants, aged 6-38 months, who participated in a randomized community survey¹⁴¹.

In conclusion, epidemiological studies have shown that HHV-8 infections are prevalent among pre-pubertal children, adolescents, and young adults. For pre-pubertal children, this prevalence may be due to saliva exchange, the probable mode of HHV-8 transmission in this population.



Note: References^{3, 135, 136, 138-141}

Figure 2. HHV-8 Seroprevalence among Children/Adolescents from Sub-Saharan Africa

Majority of studies conducted in Sub-Saharan Africa have demonstrated that the seroprevalence of HHV-8 increases with age. In Tanzania, East Africa, seroprevalence of 42.2% to 67.7% for antibodies against HHV-8 lytic antigens was observed in individuals aged 0-4 to aged 10-17, respectively. In KwaZulu/Natal, South Africa, seroprevalence of 7.8% to 23.4% for antibodies against HHV-8 lytic antigens and seroprevalence of 5.3% to 9% for antibodies against HHV-8 latent antigens were observed in children ages < 1 year to ages > 4 years, respectively¹³⁶. In North Cameroon, West Africa, seroprevalence for antibodies against HHV-8 lytic antigens increased from 39.8% in children, aged 5-10 years, to 61.8% in adults, aged 30-40 years¹³⁸. In South Cameroon, HHV-8 seroprevalence for antibodies against lytic antigens increased from 32.7% in children, aged 1-9, to 78.9% in adults, aged ≥ 50 ; however, HHV-8 seroprevalence in this population peaked in individuals, aged 20-29, (88.9%)³. As for gender, there were no differences in HHV-8 seroprevalence observed in the Sub-Saharan Africa populations^{3, 135, 138-140}.

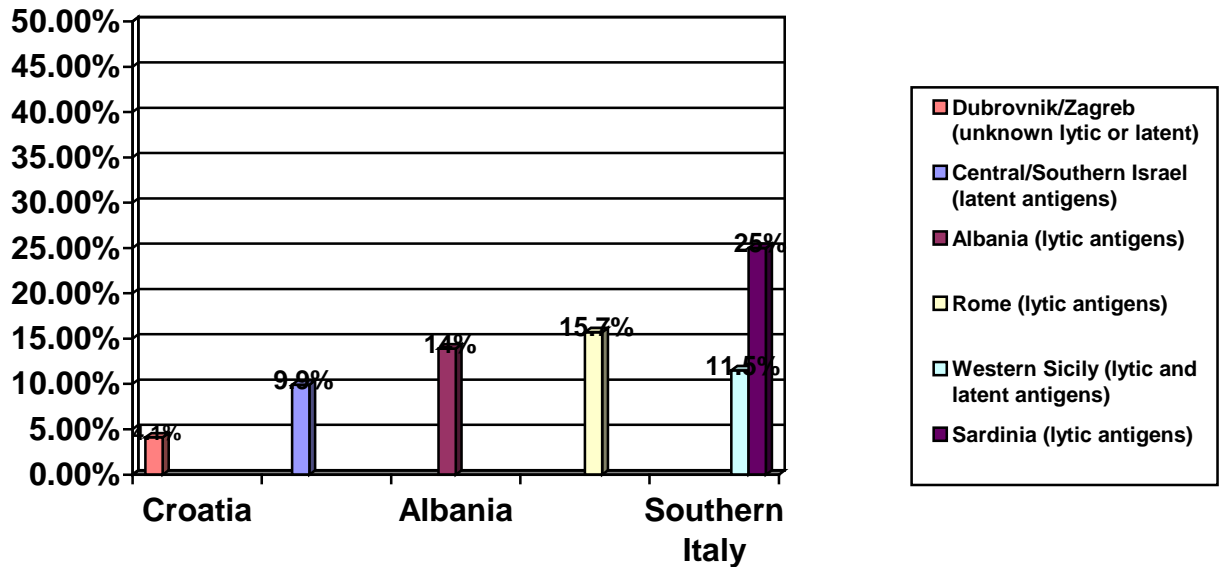
2.6.1.2 Mediterranean Countries

The Mediterranean countries have moderate levels of HHV-8 infection as well as KS incidence/prevalence in their populations in comparison to Sub-Saharan Africa. The Mediterranean area is made of 21 states along the coastline of the Mediterranean Sea which includes Southern Europe, Northern Africa, and Western Asia¹³⁴. The incidence of KS and the seroprevalence of HHV-8 vary by geographical region as well as by ethnicity. For example, studies have shown that Southern Italy has a higher KS incidence and HHV-8 seroprevalence than Northern Italy¹⁴²⁻¹⁴⁴. In addition, studies have shown Italians having a higher KS incidence and HHV-8 seroprevalence than Jewish people from Israel^{144, 145}. The seroprevalence of HHV-8 in the Mediterranean area reflects mostly the incidence of “classic KS”, a form that affects mostly elderly men. Even though the incidence of KS is higher among men, studies have shown no gender differences in HHV-8 seroprevalence in the population^{5-7, 142-144, 146}.

The seroprevalence of HHV-8 in the Mediterranean region has ranged from 4.1% to 31% in the general population (Figure 3). In Croatia, HHV-8 seroprevalence of 4.1% was found among blood donors in whom low incidence/prevalence of KS has been reported in this country¹⁴⁷. In Malta, a seroprevalence of 7% for antibodies against HHV-8 lytic antigens and 8.5% for antibodies against lytic and latent antigens were detected in elderly men, aged 71-100, and hospitalized men, aged ≥ 65 , respectively, in Malta; the incidence of KS has been reported to be 2.2 per 100,000 person-years among men and 1.8 per 100,000 person-years among women^{2, 143}. As for Israel, a seroprevalence of 9.9% were detected in families who were relatives of hepatitis B positive blood donors; the incidence of KS has been reported to be 1.96 per 100,000 in men and 0.7 per 100,000 in women¹⁴⁵.

In Italy, HHV-8 seroprevalence has shown to increase from Northern Italy to Central Italy and from Central Italy to Southern Italy. For example, in Northern Italy, a seroprevalence of 11.9% and 14.6% for antibodies against HHV-8 lytic and latent antigens, respectively, was observed in 343 healthy individuals, aged ≥ 55 , from the Province of Mantua; the incidence of KS in this area has ranged from 1.7 to 5 per 100,000 person-years in men and 0.2 to 2.8 per 100,000 person-years in women¹⁴². As for Central Italy, a seroprevalence of 15.7% for antibodies against HHV-8 lytic antigens was detected in a random

sample of 248 low KS risk patients, aged >18, attending a dermatology clinic in Rome; the incidence of KS has been reported to be 0.5-1.5 per 100,000 person-years⁶. However, in Southern Italy, the seroprevalence of HHV-8 appears to be highest in the Mediterranean regions, ranging from 8.5% to 31%, with a KS incidence ranging from 6.2-8.8 per 100,000 person-years among men and 2.1-2.5 per 100,000 person years among women¹⁴³.

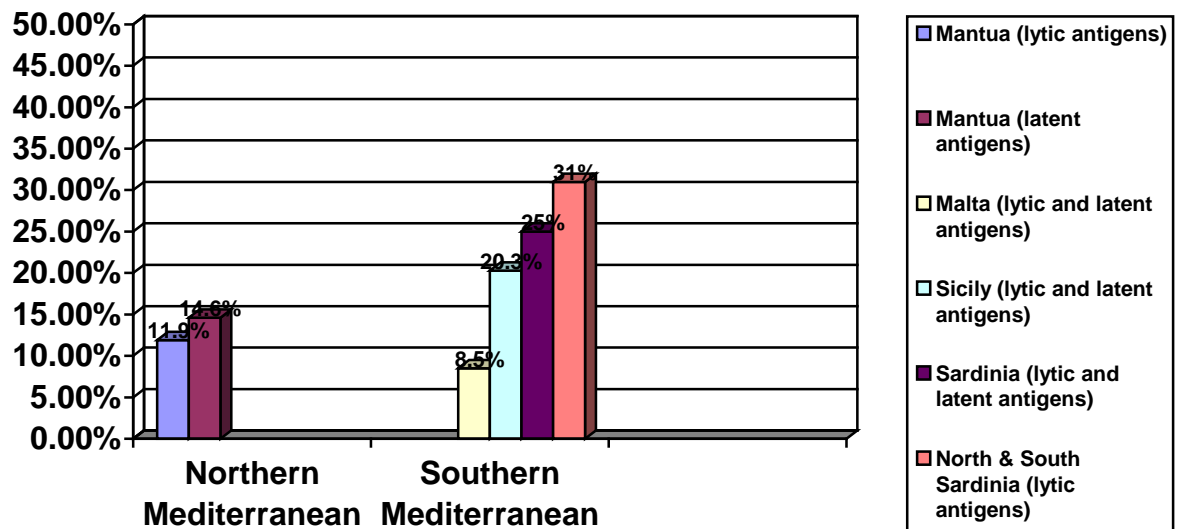


Note: References^{5-7, 145}

Figure 3. HHV-8 Seroprevalence in the Mediterranean General Population

In Southern Italy, the seroprevalence of HHV-8 in the general population has differed greatly among the cities. For example, in Western Sicily, a seroprevalence of 11.5% for antibodies against HHV-8 lytic and latent antigens were observed in 970 individuals from the general population, aged 1 to 70. However, a higher HHV-8 seroprevalence of 20.3% for antibodies against lytic and latent antigens was detected in elderly outpatients in Sicily¹⁴³. In Pomigliano d'Arco, Campania, South Italy, a seroprevalence of 14.8% for antibodies against HHV-8 latent antigens was detected in 351 heterosexual individuals that had no KS or HIV. Cattani et al. showed a seroprevalence of 18.3% for antibodies against HHV-8 lytic antigens among 517 healthy individuals from South and Central Italy who attended a Rome outpatient

lab⁷. Cattani et al. also found a 25% HHV-8 seroprevalence for antibodies against lytic antigens in 150 healthy individuals from Sardinia who attended an outpatient lab⁷. Vitale et al. also detected a similar HHV-8 seroprevalence of 25% for antibodies against lytic and latent antigens as Cattani et al. study population; however, Vitale et al. study participants were elderly outpatients, aged ≥ 65 , from Sardinia¹⁴³. In Northern and Southern Sardinia, a higher seroprevalence, 31% for antibodies against HHV-8 lytic antigens, was found in 297 KS and HIV negative individuals that were hospitalized at a dermatological clinic¹⁴⁴. These variations in HHV-8 seroprevalence for antibodies against lytic antigens were also seen in studies conducted in Albania: 13.8% in healthy lab outpatients and 20% in individuals participating in a HIV screening program or infection control program. The wide range of HHV-8 seropositivity rates may correspond with the incidence of KS observed in the population.

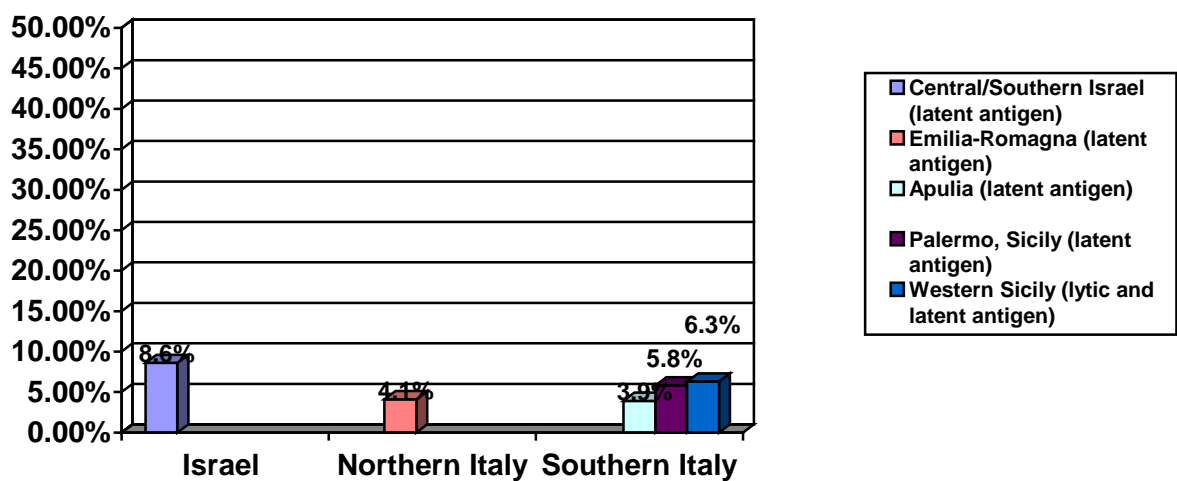


Note: References¹⁴²⁻¹⁴⁴

Figure 4. HHV-8 Seroprevalence in the Mediterranean Older Populations, aged ≥ 50 years

HHV-8 infections have also been seen in children in the Mediterranean area (Figure 5). In Israel, children, aged 2-14, were found to have a HHV-8 seroprevalence of 8.6% for antibodies against lytic antigens¹⁴⁵. As for Italy, HHV-8 seroprevalence in children and adolescents were less than 11%. For example, in Northern (Emilia-Romagna), Southeastern (Apulia), and Southern Italy (Sicily), Whitby et al.

found a seroprevalence of 4.1%, 3.9%, and 5.8% for antibodies against HHV-8 lytic antigens, respectively, in 567 hospitalized, children, aged < 1 to 15, without HIV or KS¹⁴⁸. Perna et al. detected a seroprevalence of 6.3% for antibodies against HHV-8 lytic and latent antigens in children, aged 1-15, from Sicily, South Italy, a seroprevalence similar to Whitby et al. study population⁵. However, in individuals, aged 16-20, from Sicily, Perna et al. found a slightly higher seroprevalence of 8% for antibodies against HHV-8 lytic and latent antigens in comparison to children, aged 1-15⁵. Healthy children, aged 0-14, from Rome (Central Italy), Albania, and Sardinia (South Italy), collectively, had seroprevalence of 9.7% for antibodies against HHV-8 lytic antigens; but, children < 1 year of age had a higher HHV-8 seroprevalence of 10.3%⁷. As for the Western Balkan countries, Chironna et al. found a higher HHV-8 seroprevalence of 28% and 18% against 2 antigens in refugees, aged 1-25, from Albania and Kosovo, respectively¹⁴⁹. However, healthy children from Alexandria, Egypt, aged < 1-25, had the highest HHV-8 seroprevalence, 44.7% and 8.5% for antibodies against lytic and latent antigens, than children from other Mediterranean areas¹⁵⁰. In these children/adolescent populations, the differences of HHV-8 seropositivity rates may reflect the cultural behaviors that contribute to HHV-8 transmission, for example, saliva exchange. The increase in HHV-8 seroprevalence from adolescence to young adulthood may be due to a combination of saliva exchange and sexual behavior.



Note: References^{5, 145, 148}

Figure 5. HHV-8 Seroprevalence among Children, aged 1-15 years, from the Mediterranean

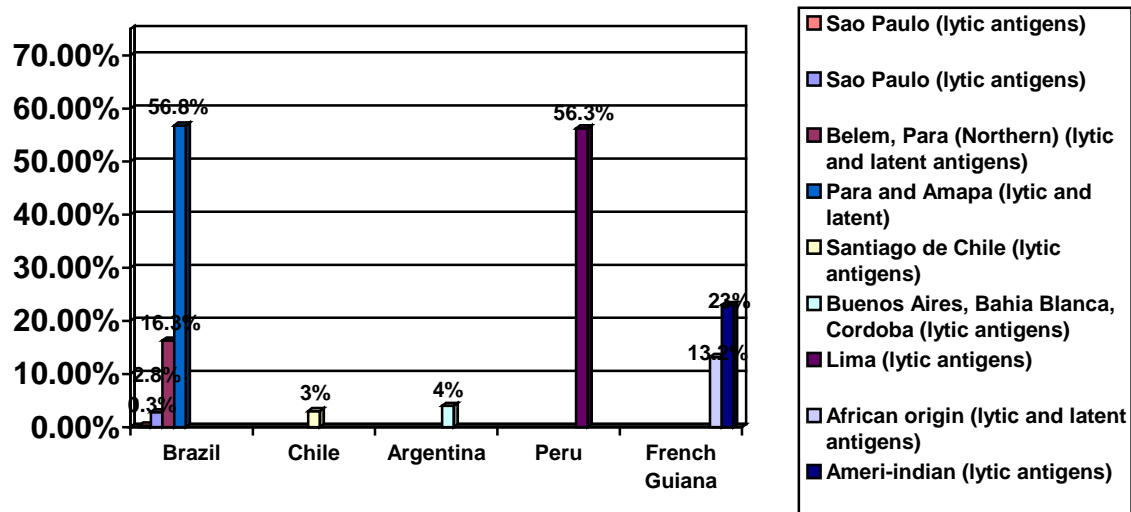
Studies that examined HHV-8 infections in the Mediterranean region have shown that HHV-8 infections increase with age in Southern Italy, Israel, and Egypt. In Southern Italy, Serraino et al. have shown a significant increase in HHV-8 seroprevalence for antibodies against lytic antigens in individuals residing in Northern Sardinia, from 23.2% in 50-64 year olds to 30.7% in ≥ 65 year old people (p-value=0.03); however, in Southern Sardinia, this increase was not observed in these age groups, 30% in both groups¹⁴⁴. In Western Sicily, seroprevalence of 5.4%, 8%, and 22.3% against lytic and latent HHV-8 antigens were observed in 1-5, 16-20, and 51-70 age groups, respectively⁵. Also, an increase in HHV-8 seroprevalence for antibodies against latent antigens was found in Pomigliano d' Arco, Campania, from 14.5% in 35-55 year olds to 17.5% in ≥ 75 year olds; however, this increase was not significant (p-value=0.4)¹⁴⁶. As for Northern and Central Italy, increase in HHV-8 seropositivity was not consistent with increasing age^{6, 142}.

In Israel, Davidovici et al. has shown that HHV-8 seropositivity rate for antibodies against latent antigens significantly increase from 8.6% in 2-14 year olds to 18% in ≥ 55 years olds (p-value=.011)¹⁴⁵. As for Alexandria, Egypt, a dramatic increase in HHV-8 seropositivity rate was observed in < 1 to > 12 year olds, from 16.6% to 58%, respectively, for antibodies against lytic antigens and 7.1% to 10%, respectively, for antibodies against latent antigens¹⁵⁰. Increasing HHV-8 seropositivity rate with increasing age may suggest cumulative exposure, sexual and/or asexual (for example, saliva exchange for asexual).

2.6.1.3 South America

For the continent of South America, the distribution of HHV-8 infections has been the widest compared to other countries, from a seroprevalence of 0.3% to 56.8% (Figure 6). This wide distribution is partially due to the incidence of KS in the population, in particular, the Amazon basin where endemic KS is found⁸. In addition, this distribution may be a reflection of the economic status of their country. For example, a low seroprevalence of 0.3% and 1.1% for antibodies against HHV-8 lytic and latent antigens,

respectively, were found among women working in a mentally handicap institution in Sao Paulo, Brazil¹⁵¹. Among blood donors in Sao, Paulo, Brazil, a slightly higher HHV-8 seropositivity rate of 2.8% for antibodies against HHV-8 lytic antigens was found¹⁵². Argentina's and Santiago de Chile's blood donors had about the same low seropositivity rates for antibodies against HHV-8 lytic antigens, 4% and 3%, respectively¹⁵². However, among low-income families from Northern Brazil, a seroprevalence of 16.3% for antibodies against HHV-8 lytic and latent antigens was detected¹⁵³. As for French Guiana, a seropositivity rate of 13.2% for antibodies against lytic and latent antigens was found among individuals of African origin who participated in a Human T-cell Leukemia Virus 1 (HTLV-1) study¹⁵⁴; but, a higher seroprevalence of 23% for antibodies against lytic and latent antigens was found among Amerindians in this same country¹⁵⁵. A significantly higher seropositivity rate of 56.25% for antibodies against HHV-8 lytic antigens was found among blood donors from Lima, Peru; this high rate most likely parallels the recent classic KS report of 2.54 cases per 10,000 people¹⁵⁶. A similarly high HHV-8 seroprevalence of 56.8% for antibodies against lytic and latent antigens were detected among Amerindians from Para and Amapa, Brazil; however, the prevalence of KS in this population is unknown¹⁵⁷. Therefore, KS and possibly economic status may explain the low to high incidence of HHV-8 infections in South American countries.



Note: References^{151, 152 153-157}

Figure 6. HHV-8 Seroprevalence in South American Populations

Low to high HHV-8 seropositivity rates were also identified among children residing in South America. In Sao Paulo, Brazil, HIV negative and positive children, < 2 of age, who were born to HIV-1 mothers had a seropositivity rate of 0% and 10.3%, respectively, for antibodies against lytic antigens¹⁵¹. However, in Rio de Janeiro, Brazil, a seropositivity rate of 7.59% and 30.7% for antibodies against lytic and latent antigens was found in healthy children and children with HIV, aged 0-12, respectively¹⁵⁸. In Northern Brazil, 12% and 17.4% HHV-8 seropositivity rates were found among children, aged < 10, and adolescent, aged 11-20, from low-income families¹⁵³. But, higher seropositivity rates for antibodies against lytic and latent antigens were found among Amerindian children: 44.4% in < 2 year olds, 35% in 2-9 year olds, and 51.4% in 10-19 years¹⁵⁷. The seroprevalence of HHV-8 among healthy children of a particular region is comparable to the seroprevalence found among healthy adults.

In French Guiana, HHV-8 seroprevalence of 2.1% for antibodies against lytic and latent antigens was detected among children of African origin 2-4 years of age and it increased to 16.1% in adolescents, aged 15-19¹⁵⁴. However, in Kazanji et al. study, a higher seropositivity rate for antibodies against lytic antigens was found among Amerindian children, 18.4% in < 6 years to 23.3% in 16-20 year olds¹⁵⁵.

Differences in seroprevalence rates between these two French Guiana populations may be related to cultural or possibly genetic differences that increase one's susceptibility of acquiring this virus.

As for age, studies conducted in French Guiana showed HHV-8 seropositivity rates increased with age, from 5-18% in pre-pubertal children to > 34% in individuals > 50 years of age^{154, 155}. In Northern Brazil, the seropositivity rate found in low income families increased from 12% in < 10 year olds to 33.3% in individuals, aged > 50¹⁵³. In Para and Amapa, Brazil, a significant increase in HHV-8 seropositivity rate was found among Amerindians, from 44.4% in < 2 year olds to 82.3% in > 50 years (p-value < 0.001)¹⁵⁷. However, in Lima, Peru, HHV-8 seropositivity rates, 65% to 50%, were consistent among the 18 to \geq 45 year old blood donors¹⁵⁶. In counties that had low HHV-8 seroprevalence, their rates did not increase with age^{152, 158}. As for gender differences, there were no significant differences in HHV-8 seropositivity rates found in South American populations except for in children who attended a university hospital in Rio de Janeiro, Brazil (p-value = 0.0002)¹⁵⁸.

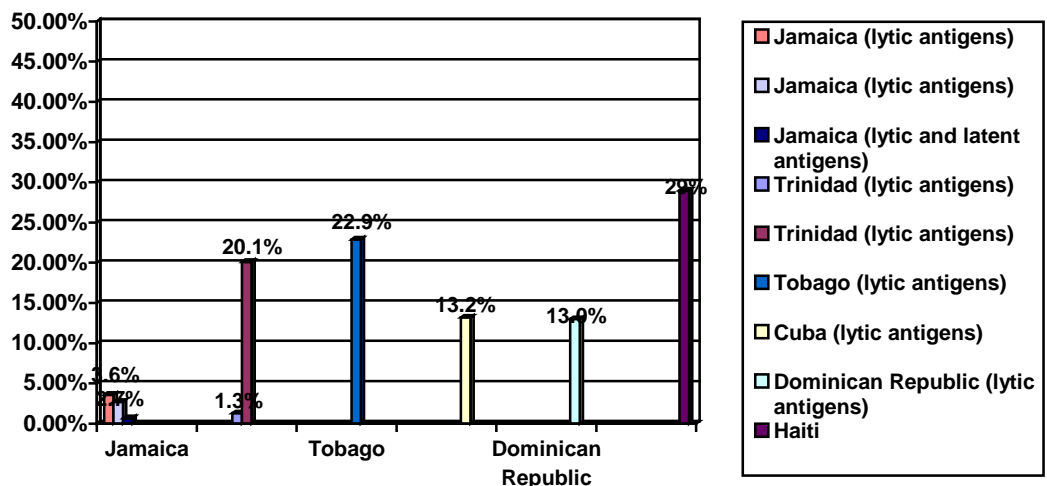
2.6.1.4 Caribbean Islands

The Caribbean islands, which are composed of 28 territories located in the Caribbean Sea, have been reported to have moderate levels of HHV-8 infections. However, the prevalence of KS is unknown for most of this area; the International Agency for Research on Cancer has reported little to no KS in this region¹⁰. However, Jamaica has been reported to have low incidence of KS^{159, 160}. In Tobago, no KS cases have been reported since the establishment of the cancer registry¹¹.

Few studies that have been conducted in Caribbean populations have reported seropositivity rates ranging from 1.3% to 29.0% (Figure 7). For example, in Havana City, Cuba, HHV-8 seroprevalence of 16.9% for antibodies against HHV-8 lytic antigens was found among 379 individuals who participated in a hospital-based case-control study¹⁶¹. Among individuals who had blood collected from Haiti (n = 52) and the Dominican Republic (n = 40), a seroprevalence of 29% and 13%, respectively, for antibodies against lytic and latent antigens was found¹⁶². As for Jamaica, seroprevalence of 3.6% for antibodies against HHV-8 lytic antigens was found in 250 healthy blood donors from Jamaica⁴. A similar HHV-8

seropositivity, 2.7%, was found among 1010 donors in the Jamaica Transfusion Study¹⁶³. However, a lower HHV-8 seropositivity of 0.68% for antibodies against HHV-8 lytic and latent antigens was found in 146 women at a gynecology clinic in Jamaica¹⁵⁹. The differences in HHV-8 seropositivity between the genders in Jamaica may be due to differences in lifestyle behaviors that increase one's risk for acquiring HHV-8 infection.

In Trinidad, a low seropositivity rate of 1.3% for antibodies against lytic antigens was reported in 160 healthy blood donors from Trinidad⁴. However, Hoffman et al. found a significantly higher HHV-8 seropositivity rate of 20.1% for antibodies against lytic antigens among 174 Trinidadian men without prostate cancer who participated in a case-control study⁹. The reason for the differences in HHV-8 seropositivity rates in Trinidad may due to 1) the IFA being more sensitive than the whole virus ELISA, a seroprevalence of 20.1% opposed to 1.3%, respectively; and, 2) the study populations may be different. A similar HHV-8 seropositivity rate, 22.9%, was found among 140 Tobagonian men without prostate cancer, Trinidad's neighbor island⁹. The moderate levels of HHV-8 infection but no KS may reflect the health status of the population, environmental factors that affect the development of KS, or an under-report of KS in HHV-8 positive individuals.



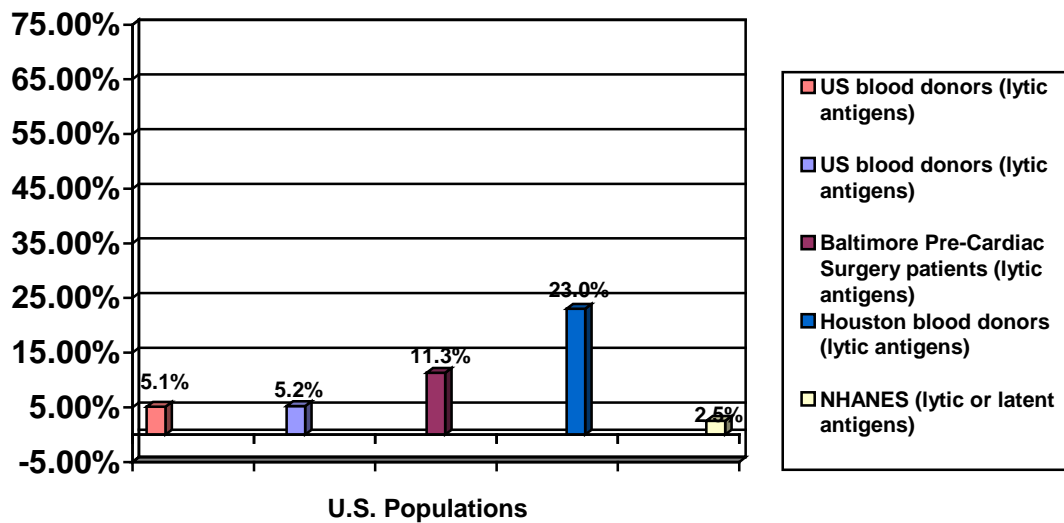
Note: References^{4, 9, 159, 161-163}

Figure 7. HHV-8 Seroprevalence in Caribbean Populations

As for HHV-8 infections rates among children residing in the Caribbean, no study has been conducted in the population.

2.6.1.5 North America

In North America, HHV-8 seropositivity rates and the incidence/prevalence of KS have been estimated to be low. Majority of these HHV-8 infections and KS cases have been estimated to come from HIV-infected individuals¹⁶⁴. The incidence of KS has been 3 and 0.7 cases per million men and women, respectively^{8, 165}. But, in non-HIV individuals, studies have found HHV-8 seropositivity rates ranging from 2.5% to 23% in U.S. populations (Figure 8). For example, Pellet et al. found an overall HHV-8 seroprevalence of 3.0-3.5% in blood donors from 6 U.S. major cities¹⁴. Engels et al. found a HHV-8 seroprevalence of 2.5% for antibodies against either latent or lytic among individuals sampled from the National Health and Nutrition Examination Survey III in the U.S.¹⁶⁶. Hoffman et al. found a seroprevalence of 5.1% for antibodies against lytic antigens among U.S. blood donors⁹. As Hoffman et al, Ablashi et al. found a similar seropositivity rate for antibodies against lytic antigens, 5.2%, among healthy individuals from the U.S.⁴. Engels et al. found 5.9%, 2.7%, and 6.7% HHV-8 seropositivity rates for antibodies against lytic antigens and 5.4%, 6.1%, and 4.4% for antibodies against latent antigens among New York residents of the Jewish, Protestant, and Catholic faiths, respectively¹⁶⁷. But, in Baltimore, Maryland, a seroprevalence of 11.3% for antibodies against lytic antigens was found among pre-cardiac surgery patients¹⁶⁸. An even higher seroprevalence for antibodies against lytic antigens, 23%, and latent antigens, 5%, was found in blood donors from Houston, Texas¹⁶⁹. These differences of HHV-8 seropositivity rates observed among different study populations may be due to 1) environmental and/or cultural differences, 2) genetic susceptibility in acquiring this virus, or 3) different assays used to examine HHV-8 infection in the study population.



Note: References^{4, 9, 166, 168, 169}

Figure 8. HHV-8 Seroprevalence in U.S. Populations

Similar HHV-8 seropositivity rates found in adults were also observed in U.S. children. Matro et al. found a seropositivity rate for antibodies against lytic and latent antigens of 4% among 552 children, aged 6 months to 17 years, who attended a hospital in Atlanta and North Georgia area¹⁴¹. But, Baillargeon et al. detected a higher seroprevalence of 26% for antibodies against lytic and latent antigens from 123 healthy children, aged 1-13, from South Texas¹⁷⁰. These differences in seropositivity rates may be attributed to ethnicity and/or socioeconomic differences. Majority of the children from South Texas were 84.5% Hispanic, usually of low socioeconomic status. The hospitals around the Atlanta and North Georgia area are surrounded by affluent neighborhoods. Therefore, the children attending the Atlanta hospitals may be of a different demographic than the South Texas children; these different environments may affect their susceptible to acquiring HHV-8.

As for examining age with HHV-8 seropositivity rates, only two studies examined this association in North American populations. From the Atlanta area, HHV-8 seropositivity rates were 4.2%, 6%, 1.2%, and 2% among children, 6-11 months, 5-6, 11-12, and 15-17 years of age, respectively; these

rates did not significantly increase with age¹⁴¹. In South Texas, seropositivity rates were 18.4%, 30.6%, and 26.1% among children ages 0-5, 6-11, and > 12, respectively; these rates appear to increase with age¹⁷⁰. The pattern of rate differences between these two populations may suggest differences in cumulative exposure to HHV-8. As for gender differences with HHV-8 seropositivity, this association was not documented in these North American study populations.

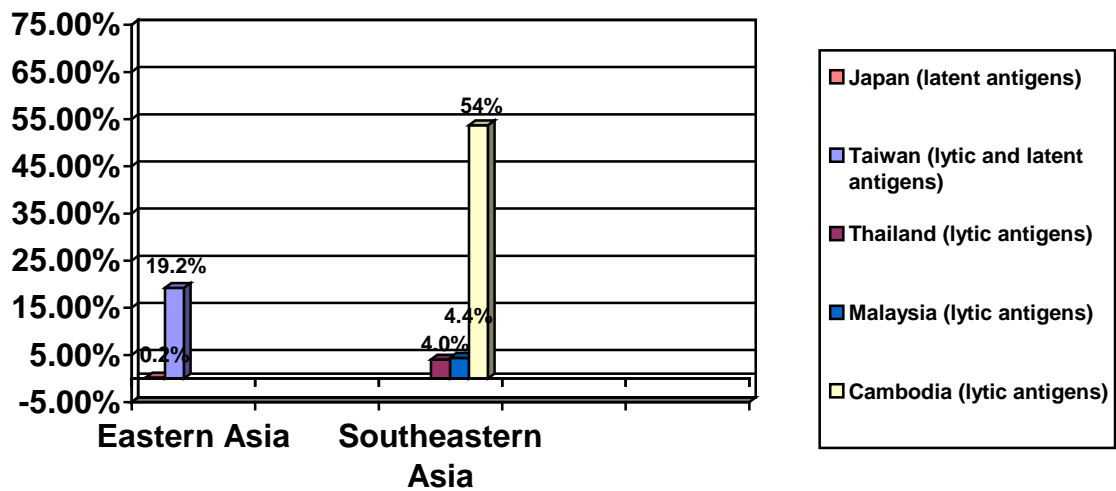
2.6.1.6 Northern Europe

In Northern Europe, HHV-8 seropositivity rates and the incidence/prevalence of KS have been estimated to be low, similarly to North America. For example, seropositivity rates have been estimated to be < 5% in the population; and, annual rates of KS has been estimated to be 0.14 cases per million⁸. Because of “reported low” seropositivity rates and incidence of KS in the population, few studies have been conducted in examining HHV-8 infections among healthy individuals. Tedeschi et al. conducted a population-based sero-survey among 520 individuals, aged 30-60 years, from Sweden in which seropositivity rates of 14.4% and 1.7% for antibodies against lytic and latent antigens, respectively, were found¹⁷¹. Seropositivity rates in this study population did not increase with age. In addition, there were no significant gender differences in HHV-8 seropositivity¹⁷¹. Tedeschi et al. have demonstrated that moderate rates of HHV-8 infection are present in Northern Europe’s general population, rates that are similar to parts of the Mediterranean and the Caribbean¹⁷¹.

2.6.1.7 Asia

The distribution of HHV-8 infections in Asia, which includes Eastern and Southeastern Asia, has varied greatly, from 0.2% in Eastern Asia to 53.7% in Southeastern Asia (Figure 9). In Eastern Asia, a seroprevalence of 0.2% for antibodies against HHV-8 latent antigens were found among blood donors from Japan¹⁷². But in Taiwan, a seroprevalence of 19.2% for antibodies against lytic and latent antigens were found among healthy blood donors, aged 31-40 years¹⁷³.

As for Southeastern Asia, a wider distribution of HHV-8 seropositivity rates was observed. In Thailand, a seropositivity rate of 4% for antibodies against lytic antigens was found among healthy adult blood donors. In Malaysia, a similar rate as Thailand, 4.4% for antibodies against lytic antigens, was found among healthy blood donors, aged 9-85years⁴. But, in Cambodia, a seroprevalence of 53.7% for antibodies against lytic antigens was detected among individuals, aged 4-69 years, who participated in a *Schistosoma mekongi* cross-sectional study¹⁷⁴. Differences in seropositivity rates among these Asian countries may be attributed to cultural and/or environmental differences that increase one's risk for acquiring this virus. However, little to no cases of KS has been reported in this region¹⁰.



Note: References^{4, 172-174}

Figure 9. HHV-8 Seroprevalence in Asian Populations

As for HHV-8 infections among children from Asia, low to high seropositivity rates have also been observed. In Eastern Asia, a seropositivity rate of 3.5% for antibodies against lytic antigens was found in South Korean children, aged 1-15 years¹⁷⁵; and, seropositivity rates of 3-4% and 12.1% for antibodies against lytic and latent antigens were detected among Taiwanese children/adolescents, < 10 and 11-20 years of age, respectively¹⁷³. As for Southeastern Asia, seropositivity rates of 68.8% and 50% for antibodies against lytic antigens were found among Cambodian children/adolescents < 13 and 13-17

years of age, respectively¹⁷⁴. The seropositivity rates found in Eastern and Southeastern children reflect the rates found in adults from the same geographical area.

As for HHV-8 seropositivity with age, Sarmati et al. found a significant decrease with seropositivity and increasing age among Cambodian children/adolescents: 68.8%, 50%, and 24.4% in < 13, 13-17, and >17 year olds, respectively (p-value < 0.001)¹⁷⁴. However, Haung et al. found inconsistent patterns of HHV-8 seropositivity rates with age in Taiwan; seropositivity rates increase from 3% in < 5 year olds to 19.2% in 31-40 year olds, but decrease to 13.5% in > 70 year olds¹⁷³. The reasons for the inconsistent and decreasing pattern of HHV-8 seropositivity rates with increasing age in Asia are not known. As for gender differences, Sarmati et al. found no difference in seropositivity rates among Cambodian women and men¹⁷⁴; other studies conducted in Asia did not examine this association.

2.6.1.8 Oceania

As for the Oceania area, only one study has been conducted that examined HHV-8 seropositivity rates in the general population. In this study, a seroprevalence of 0.3% for antibodies against lytic and latent antigens were detected in Vanuatu, Southwest Pacific; an area that is consisted of 4 islands: Aneityum Island, Etafe Island, Pantecost Island, and Santos Island¹⁷⁶. The low seroprevalence of 0.3% in the Southwest Pacific was attributed to one HHV-8 seropositive individual from the Santos Island. This low HHV-8 seropositivity rate observed in this country also reflects the low to no incidence of KS reported in this geographical region.

2.6.2 Seroprevalence in High-Risk Populations

Studies have shown that certain groups are at high risk for acquiring HHV-8 infection as well as developing KS in comparison to the general population. These high risk groups are the following: individuals infected with HIV/AIDS or other sexually transmitted diseases, homosexual males, injection

drug users, and organ transplant recipients. Studies that examined these groups in different geographical areas are listed below.

2.6.2.1 HIV/AIDS Infected Persons

The rates of HHV-8 infection have been high among individuals infected with HIV/AIDS in comparison to healthy persons from non-KS endemic area (Figure 10). For example, in Japan, seroprevalence of 10.5% for antibodies against latent antigens was detected among HIV positive men opposed to 0.2% among healthy blood donors¹⁷². In Northern Thailand, a seroprevalence of 28.1% for antibodies against HHV-8 lytic antigens was observed in HIV positive couples versus 18.3% in HIV negative couples¹⁷⁷. In Sao Paulo, Brazil, a seroprevalence of 8% and 1.2% for antibodies against lytic and latent antigens, respectively, were found in HIV positive mothers opposed to 0.3% and 1.1% for antibodies against lytic and latent antigens, respectively, in healthy women¹⁵¹. In the U.S., a seroprevalence of 18.2% against HHV-8 lytic antigen was found in HIV positive women with high-risk sexual behavior in comparison to a seroprevalence of 11.6% detected among their negative counterparts¹²⁶; and, a seroprevalence of 23% for antibodies against HHV-8 lytic and latent antigens among HIV positive homosexual men versus 10.7% in HIV negative homosexual men were found¹⁷⁸.

In KS endemic areas, differences in HHV-8 seropositivity rates between HIV infected and non-infected persons are inconsistent (Figure 10). In Ghana, West Africa, a seroprevalence of 45.5% for antibodies against HHV-8 lytic and latent antigens was observed in HIV positive individuals opposed to 32.3% in HIV negative individuals, aged 10-70 years¹⁷⁹. But, in Uganda among sexually active adolescent, aged 15-19 years, HIV negative individuals had a seropositivity rate of 40% for antibodies against HHV-8 lytic and latent antigens opposed to 28.9% in HIV positive individuals¹⁸⁰. Also, in Zambia, Olsen et al. found no association between HIV and HHV-8 from participants, aged 14-84 years, who participated in a hospital-based HIV seroprevalence study¹⁸¹. A possible explanation for HHV-8 seropositivity differences between HIV and non-HIV persons in KS endemic areas is that HHV-8 is

transmitted sexually and asexually in these populations unlike non-KS endemic area where sexual transmission is predominant in acquiring HHV-8.

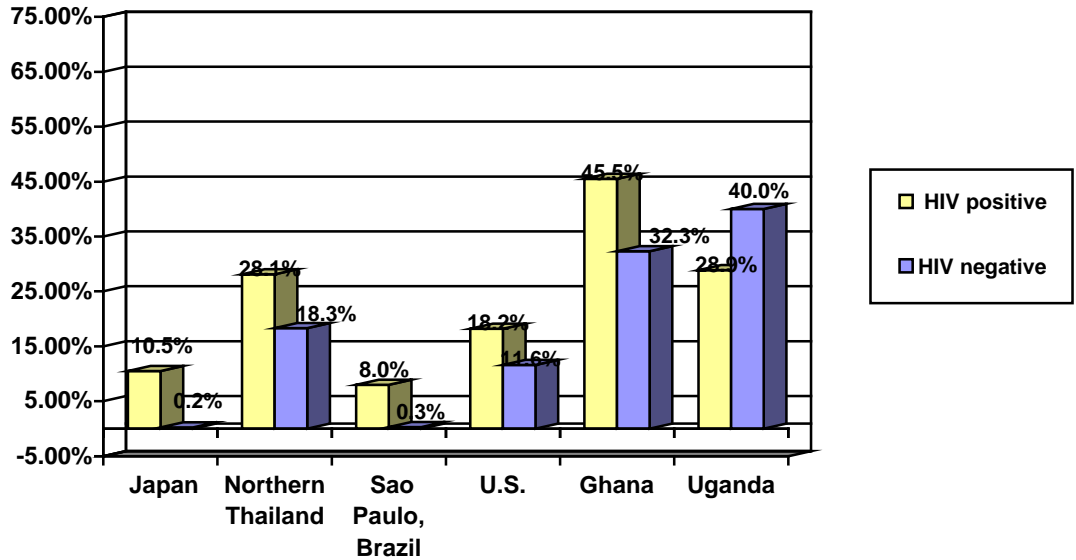


Figure 10. HHV-8 Seropositivity Rates between HIV Infected and Non-HIV Persons in Different Geographical Areas

HIV/AIDS has also increased the incidence/prevalence of KS throughout the world. In countries like the U.S, KS was rarely seen in the population until the onset of the 1980s AIDS epidemic among homosexual men. During this epidemic, AIDS-KS was estimated to ~ 40% among homosexual men in San Francisco^{40, 182}. A retrospective study showed that HHV-8 infection, seroprevalence of 24.6%, was present among homosexual men in San Francisco in 1978, a time before the AIDS epidemic¹⁸³. Therefore, HHV-8 may have been present in high-risk sexual behavior population well before the AIDS epidemic in the U.S.

As for countries where KS (classical and endemic) was prevalent before the AIDS epidemic, the incidence/prevalence of KS has also increased as a result of this epidemic, AIDS-KS. With the development of highly active anti-retroviral therapy (HAART) and safe sex educational programs, the incidence of KS has decreased among HIV-infected patients in the United States⁹⁰. This decrease in KS

may be attributed to an “immune reconstitution” in HIV-infected patients⁹¹. However, for countries in Sub-Saharan Africa, HAART is not available due to the high financial costs of these medications; therefore, KS remains a public health problem in these HHV-8 endemic countries.

2.6.2.2 Other Sexually Transmitted Diseases Infected Persons

Since HIV infection is highly associated with HHV-8, other sexually transmitted diseases (STDs) have been examined to see if they are associated with this virus. The most studied STDs and HHV-8 relationship, excluding HIV, are hepatitis B virus (HBV), hepatitis C virus (HCV), and herpes simplex 2 virus (HSV-2). With HBV, Janier et al. and Sarmari et al. found that men who attend an STD clinic in France and men who were prison inmates in Italy, respectively, were > 2 times more likely to be HHV-8 seropositive than their HBV negative counterparts^{184, 185}. For HCV, Cannon et al. found that HIV positive and negative women with high risk behaviors from U.S. were 1.7 times more likely to be HHV-8 seropositive than HCV negative persons¹²⁶; but, Schinaia et al. found a higher risk, odd ratio of 8.9, in individuals who participated in a HIV screening and infection control program in Albania¹⁸⁶. As for HSV-2, prison inmates from Italy, individuals who were attending an outpatient ward in Northern Cameroon, and men who attended a STD clinic in France were 1.7, 2, and 7.1 times more likely, respectively, to be HHV-8 seropositive than their HSV-2 negative counterparts^{126, 184, 185}. However, several studies have found no significant association with these STDs and HHV-8^{126, 174, 184, 185, 187}.

STDs such as herpes simplex 1 (HSV-1), gonorrhea, syphilis, and Chlamydia have also been studied with HHV-8 infection. Some studies have found significant positive associations with gonorrhea and HHV-8 as well as syphilis and HHV-8. Casper et al. and Lavreys et al. found that gonorrhea-infected women who practiced high-risk sexual behaviors in the U.S. and gonorrhea-infected female prostitutes in Kenya, respectively, were > 2 times more likely to be HHV-8 seropositive than women who did not have gonorrhea^{178, 188}. Cannon et al. found that HIV positive and negative women with high risk behaviors from U.S. who had syphilis were 2 times more likely to HHV-8 seropositive than women who did not have syphilis¹²⁶. As for HSV-1 and Chlamydia, there were no significant association found with these STDs

and HHV-8^{126, 178}. Also, insignificant associations between HHV-8 and these two STDs, gonorrhea and syphilis, were found^{126, 180}.

In conclusion, inconsistent study results have been reported for HBV, HCV, HSV-2, gonorrhea, and syphilis in examining HHV-8 seropositivity risk; none of these STDs have been associated with KS development or other HHV-8 associated diseases and malignancies. The relationship between these STDs and HHV-8 infections still remain inconclusive.

2.6.2.3 Homosexual Men

In developed countries, like the U.S., homosexual men are at higher risk for acquiring HHV-8 infection as well as developing KS than the general population. For example, homosexual men who attended a STD clinic in France were 3.7 times more likely to HHV-8 seropositive than heterosexual men¹⁸⁴. This higher risk applies for not only HIV-infected homosexual men, but also, to HIV negative homosexual men in comparison to the general population^{8, 189, 190}. However, HIV-infected homosexual men are more likely to develop KS and to be HHV-8 seropositive than HIV negative homosexual men^{8, 178}. For example, among homosexual adolescents, HIV positive males had a twice as high HHV-8 seropositivity rate, 23%, than males who did not have HIV, 10.7%¹⁷⁸. Studies have examined HHV-8 seropositivity rate in homosexual men, in particular HIV-infected men, and compared their rates to other groups such as bi-sexual and heterosexual men, with or without HIV infection (Figure 11). These studies have shown that homosexual men have higher HHV-8 seropositivity rates than bi-sexual men, heterosexual men, and the general population^{178, 184, 187, 191}.

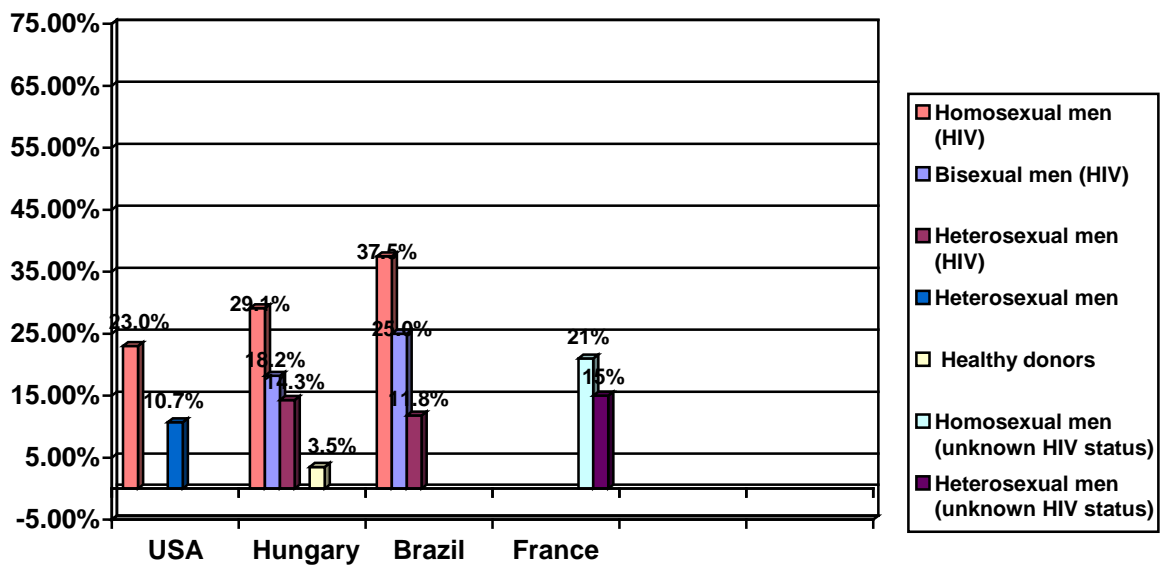


Figure 11. A Comparison of HHV-8 Seropositivity Rates among Homosexual, Bisexual, and Heterosexual Men and Healthy Donors from Different Geographical Areas

2.6.2.4 Injection Drug Users

Studies have shown that the duration of injection drug use increases one's risk for acquiring HHV-8 infection. In a study that examined HHV-8 seropositivity rates in injection drug users from San Francisco Bay area, women, heterosexual men, and men who have sex with men (MSM) who have injected drugs for > 40 years were 5.8, 3, and 3.9 more likely, respectively, to be seropositive than individuals who injected drug for ≤ 10 years¹⁹². A study conducted in women at high risk for acquiring HIV from Detroit, Baltimore, New York, and Providence, Rhode Island also found an increased risk for HHV-8 infection by duration of injection drug use; women who used injection drugs daily during the study were 3.2 more likely to be seropositive than women who were not injection drug users¹²⁶. For these two U.S. study populations, the association of injection drug use and HHV-8 seropositivity still existed after adjusting for HIV status^{126, 192}. However, in a study conducted in the Netherlands, injection drug use was not significantly associated with HHV-8 seropositivity or the frequency of injection drug use¹⁹³. The

reason for no association in the Netherlands study population is probably due to the low seropositivity rate of 2.7% found among 1179 injection drug users, unlike the San Francisco study (seropositivity rate of 10% in women, 10% heterosexual men, 23% MSM) and the U.S. multi-center study (seropositivity rate of 16.1% in women).

2.6.2.5 Organ Transplant Recipients

Because organ transplant recipients usually become immunocompromised due to immunosuppressive therapy, HHV-8 infection has become a public health concern due to possible development of KS, the iatrogenic form that is common in solid-organ transplant recipients. Organ transplant recipients who are from areas that are associated with endemic and classic KS are usually at higher risk for KS development than recipients not from those KS areas. Several studies have examined seropositivity rates in post-organ transplants recipients, in particular renal transplant patients, from different geographical areas (Table 6).

Table 6. HHV-8 Seroprevalence in Post-Organ Transplant Recipients

Author	Country	Number of Recipients	Type of Transplant	HHV-8 Serostatus for antibodies against lytic antigens	HHV-8 Serostatus for antibodies against latent antigens
Delorme et al. ¹⁹⁴	Quebec, Canada	150	Renal	0%	
Panayiotakopoulos et al. ¹⁹⁵	Athens, Greece	48	Renal	4.2%	
Garcia-Astudillo et al. ¹⁹⁶	Spain	Kidney (Renal) = 788; Liver = 231	Kidney, Liver	Kidney = 0.6% Liver = 3.4%	
Stein et al. ¹⁹⁷	Johannesburg, South Africa	430	Renal		7.3%
Alzahrani et al. ¹⁹⁸	Saudi Arabia	150	Renal		18%
Jenkins et al. ¹³	U.S.	Liver = 46, Kidney (Renal) = 19, Heart = 13, Multi-organ = 12, Lung = 10	Liver, Kidney, Heart, Multi-organ, Lung	Overall: 20% Liver = 21.7% Kidney = 15.8% Heart = 23% Multi-organ = 16.7% Lung = 20%	

Some studies have shown that the solid-organ transplant recipients had a higher seropositivity rate than the healthy study participants: 18% versus 1.7% in Saudi Arabia and 20% versus 9.9% in the U.S., respectively^{13, 198}. These study results suggest that organ transplant recipients are at higher risk for acquiring HHV-8 infection. In addition, solid-organ transplant recipients may be at higher risk for HHV-8 reactivation. Jenkins et al. have shown that 10% solid-transplant organ recipients seroconvert from pre-transplantation to post-transplantation; none of their donors were HHV-8 seropositive. The seroconversion that occurred in Jenkins et al. study may have been a reactivation based on high antibody titers after transplantation; therefore, undetectable antibodies titers at pre-transplantation were reactivated to detectable antibody titers after transplantation due to immunosuppressive conditions.

Studies have indicated that organ transplant recipients are at high risk for developing KS in areas where endemic and classic KS are common. For example, Garcia-Astudillo et al. was able to observe KS development in 0.5% of the kidney transplant recipients and 2.16% of the liver transplant recipients from

Spain within 33.7 and 10.4 months, respectively, after post-organ transplantation¹⁹⁶. As for the U.S. where endemic and classic KS is rare, KS usually occurs in 0.5% of transplant recipients¹³.

2.6.3 Prevalence of HHV-8 Subtypes (Clades)

HHV-8 has a varied genome with many subtypes (A-E and N) as well as the M and P alleles that are based on the open reading frame (ORF) K1 gene, a transmembrane protein^{51, 52}. Each of these subtypes shows predominance in different geographical areas and ethnic backgrounds^{52, 199}. Studies have reported that subtypes A and C are found in Europe, Middle East, America, and Asia^{53, 55, 58}; subtype B is found in Africa^{8, 51, 55}; subtype D is found in the Pacific Islands²⁰⁰; subtype D and E are found in native South American Indian population⁵⁵; and, subtype N is found in South Africa^{56, 57}. As for the Caribbean islands, subtypes A, B, and C have been found in Cuba²⁰¹; but for the other islands, HHV-8 subtypes have not been reported, yet. The subtype differences found in these different geographical regions support the idea that HHV-8 has been in the population for a long time, possibly over 35,000 years^{56, 199, 202}. The link between these subtypes and the development of HHV-8 associated diseases and malignancies are not known^{51, 53, 58, 203-205}.

2.6.4 Transmission

HHV-8 can be transmitted sexually or non-sexually. Identifying the presence of HHV-8 DNA has been useful in studying routes of HHV-8 infection. HHV-8 DNA has been identified in peripheral blood mononuclear cells, urine, semen, prostate, and saliva as well as the rectal, vaginal, and cervical sites^{91, 124, 125, 127, 128, 136, 150, 191, 206-211}. Studies have shown that saliva has the highest viral load followed by peripheral blood mononuclear cells in comparison to other sites. These data suggest that saliva and peripheral blood mononuclear cells are primary sites involved in viral shedding (i.e. viral replication)^{127, 207, 211}. Because of

the prevalence of viral shedding at these sites, saliva and blood are considered the main routes of HHV-8 infection in the population^{124-130, 207, 211}.

2.6.4.1 Sexual Transmission

Several studies have shown HHV-8 infection is associated with sexual behaviors such as number of sex partners, sex with an HIV partner, and the number of sex partners with KS^{56, 130, 182, 212, 213}. In non-endemic KS areas, sexual transmission appears to be the predominant mode of HHV-8 infection. For example, in the U.S., individuals who have been infected with a sexually transmitted disease or identified as “men having sex with men” (MSM) have higher HHV-8 seropositivity rates, 11%-23%, in comparison to U.S. healthy blood donors, 3% - 5.3%^{4, 9, 14, 126, 178}. The exact methods of acquiring HHV-8 among these “high-risk sexual behavior” individuals have been examined. Recent data suggests that HHV-8 transmission may occur through the exchange of infectious semen, in particular, among HIV-1 infected individuals^{12, 214}.

For geographic regions where KS is endemic, high-risk sexual behaviors and sexually transmitted diseases such as HIV/AIDS correspond with an increase in the prevalence of HHV-8 infection and KS in that population. For example, in Ghana, a seropositivity rate of 45.5% was reported among HIV positive individuals as opposed to 32.3% in non-infected persons¹⁷⁹. However, in Uganda among sexually active adolescents, a higher HHV-8 seropositivity rate of 40% was observed in HIV negative individuals as opposed to 28.9% in HIV-infected persons¹⁸⁰. The findings from the study conducted in Uganda suggest that sexual transmission may not be the only mode of acquiring HHV-8 infection. Therefore, both sexual and non-sexual transmissions are probable modes of acquiring HHV-8 infection in KS endemic areas.

2.6.4.2 Non-sexual Transmission

Studies have suggested that HHV-8 can be transmitted non-sexually. In KS endemic areas, HHV-8 seropositivity rates have ranged from 9.7% in 0-14 year old children from Italy to 39.8% in 5-10 year old children from Sub-Saharan Africa^{7, 138, 157}. These rates, especially among pre-pubertal children,

suggest non-sexual modes of HHV-8 transmission. Saliva exchange, for example, premastication of foods or mother's saliva to clean wounds, is most likely the probable non-sexual route of transmission among pre-pubertal children. For infants, vertical transmission due to HHV-8 shedding into cervicovaginal secretions may be one route of HHV-8 infection^{12, 215}; however, studies suggest that this transmission route might be rare, due to the low HHV-8 viral load detected in cervicovaginal secretions^{12, 215}. Saliva is thought to have the highest HHV-8 viral load compared to any other body site or fluid^{127, 207, 211}. Therefore, saliva may be the predominant route of acquiring HHV-8 infection. Because of the high HHV-8 seropositivity rates observed among children, non-sexual transmission has been identified as the predominant mode of HHV-8 infection in KS endemic areas^{8, 55}.

Studies have also reported that organ transplantation and parenteral transmission are routes of non-sexual HHV-8 transmission. For example, with organ transplantation, HHV-8 infection as well as KS have been shown to be transmitted from donors to organ recipients^{54, 216}. For parenteral transmission, some studies suggest that HHV-8 can be transmitted through blood transfusion and injection drug use^{192, 217, 218}. These non-sexual routes of transmission may be a major concern for endemic KS areas.

2.6.4.3 Familial Transmission (non-sexual)

HHV-8 infection has also been shown to be transmitted among 1st degree family members (mother, father, and siblings). Studies have found highly significant familial correlation in HHV-8 seropositivity rates between mother and child in French Guiana, South Cameroon, rural Tanzania, South Africa, and Central and Southern Israel study populations^{3, 136, 139, 145, 154}. Significant associations in HHV-8 seropositivity rates have also been found between siblings in French Guiana, South Cameroon, and rural Tanzania^{3, 139, 154}. Studies conducted in Israel and rural Tanzania found significant associations in HHV-8 seropositivity rates between father and child and between spouses^{139, 145}; however, these relationships were insignificant in French Guiana and South Cameroon^{3, 154}. The associations between parents and child and between siblings suggest that saliva or interpersonal contacts (exchange of bodily fluids of broken

skin) may be routes of HHV-8 transmission¹⁵⁴. As for populations that found an association of HHV-8 infection between spouses, sexual transmission may be the probable mode of transmitting this virus.

Studies have also found familial associations with KS and HHV-8 seropositivity rates among 1st degree family members. For example, He et al. found that not all mothers infected with KS had children infected with KS; but, all children infected with KS had mothers infected with KS¹³⁷. Guttman-Yassky et al. reported that classic KS patients' 1st degree family members were > 6-fold more likely to be HHV-8 seropositive than the hospital controls²¹⁹. In conclusion, these two studies have demonstrated that HHV-8 infected or KS individuals can affect the HHV-8 sero-status of their 1st degree relatives; however, this route of this transmission is not known.

2.6.5 Genetic Susceptibility

Based on familial clustering studies, two studies have suggested that some individuals are genetically susceptible to HHV-8 infection and KS development. In the French Guiana HHV-8 infection familial clustering study, Plancoulaine et al. found that individuals who were homozygous for the human genome D allele, DD, were predisposed to HHV-8 infection²²⁰. From this study, Plancoulaine et al. has concluded that a possible recessive gene controls the susceptibility or resistance to HHV-8 infection, particularly in childhood, in endemic populations²²⁰. In the Israel KS familial clustering study, Guttman-Yassky et al. found that four immunocompetent siblings from a large consanguineous family that were clinically diagnosed with classical KS had GC genotype at the -174 interleukin 6 (IL-6) locus, a cytokine that may a role in KS pathogenesis; and, three of the four siblings had the DRB1*11 HLA allele, a possible risk factor for KS development²²¹⁻²²³. In addition, seven out of eight family members without classical KS were HHV-8 seropositive; all of the family members except for the father had the GC genotype; and, most of the family members had the DRB1*11 HLA allele²²³. From this study, Guttman-Yassky et al. has concluded that HHV-8 infected individuals with the IL-6 GC genotype and/or DRB1*11 HLA allele may be at higher risk for KS development²²³.

2.6.6 Possible Environmental Risk Factors

Some studies have examined environmental factors that may contribute to acquiring HHV-8 infection and/or KS development in high risk HHV-8/KS populations^{135, 224-226} (Table 7). However, these environmental factors' influence on HHV-8 infection and KS development are controversial.

Table 7. Possible Environmental Co-factors for HHV-8 Infection and KS Development

Co-factors	Possible Influences
Bloodsucking Insects	Increase HHV-8 infection and KS development
Volcanic areas	Increase KS development
Increase Iron Exposure	Increases KS development
Amyl nitrite capsules/inhaled nitrites	Increase KS development
Cigarette smoke	Decreases KS development
Use surface water (associated with poor hygiene)	Increases HHV-8 infection
Aqueous and organic extracts of natural products (i.e., plants, marine invertebrates, and fungi)	Cause reactivation of HHV-8

2.7 PROSTATE CANCER

Prostate cancer is a global health issue with 679,023 new cases worldwide in 2002¹⁰. However, little is known about the etiology or prevention of this disease. In the United States, prostate cancer is one of the most common cancers and the 2nd leading cause of cancer death in men²²⁷. It has been estimated that 218,890 men will be diagnosed and 27,050 men will die from this malignancy in the year of 2007²²⁷. Based on these statistics, prostate cancer is a public health problem in the U.S. However, for African-American men, having the highest incidence and mortality rate than any other ethnic group, prostate cancer is a public health burden²²⁷.

This prostate cancer burden is not only observed in the U.S., but also, in other countries, in particular, among men of African descent. For the African-Caribbean male population of Tobago, 11% prostate cancer prevalence rate at initial screening was estimated from the population-based longitudinal Tobago Prostate Screening Survey¹⁷. The reason for this high rate is not known. A case-control study conducted among African-Caribbean men from Tobago suggests that HHV-8 infection may possibly be a risk factor for prostate cancer⁹. Further epidemiological studies are needed to examine the relationship between HHV-8 infection and prostate cancer development.

2.7.1 Biology of Prostate Cancer

The prostate gland is a male organ that is located in front of the rectum and under the urinary bladder. The gland is composed of two parts: an epithelium and a connective tissue stroma that is comprised of smooth muscle. The prostate's function is to produce a small amount of seminal fluid in the male's semen; the remaining seminal fluid is produced by the seminal vesicles.

The development of the prostate gland is regulated by peptide cytokines and sex steroids like estrogen and androgens²²⁸. Androgens, the most important part in prostate development, are male hormones that are present during fetal development into adulthood. Androgens (i.e, testosterone) along with growth factors (i.e, transforming growth factor and epidermal growth factors) and stromal cells signaling play an important role in maintaining a healthy prostate through the counterbalance of apoptosis and cellular proliferation^{229, 230}. Disruption of this balance in prostate epithelial cells can lead to the development of prostate cancer.

2.7.1.1 Pathogenesis

The pathogenesis of prostate cancer is complex and not well-defined. This process usually starts when the DNA of normal prostate epithelial cells are altered or mutated, therefore causing uncontrollable cellular growth due to improper or the lack of cellular repair. This uncontrollable growth can develop into

a preneoplastic lesion, followed by a primary tumor, and finally a growth that can metastasize, causing invasion and destruction of other body tissues and organs (i.e, bone). This cellular process, from normal prostate epithelial cells to malignant tumor, can occur in several known pathways such the inactivation of tumor suppressor genes, the activation of oncogenes, the lack of apoptosis, or angiogenesis as well as pathways that are not yet known²³⁰. These known pathways are described below.

2.7.1.2 Oncogenes/Tumor Suppressor Genes

Oncogenes are created through the genetic alteration of pro-oncogenes, normal regulatory genes. This alternation can occur through mutation of the coding region or translocation of two genes²³¹. Oncogenes are thought to be positive regulators of the cell cycle. Oncogenes that may contribute to the development of prostate cancer include *ras* oncogene, *c-myc*, *EIF3S3*, *ERBB2*, *PSCA*, and the *AR* (androgen receptor) gene.

Tumor suppressor genes play significant role in the prostate epithelial cellular growth. These genes are negative regulators of the cell cycle; therefore, their function is to inhibit abnormal cellular proliferation in the prostate. This inhibitory function can be lost when a tumor suppressor gene is inactivated, possibly due to allelic deletions, mutations, or loss of expression, allowing for the progression of prostate cancer due to uncontrolled growth of the prostate epithelial cells. Tumor suppressor genes that may play a role in the development of prostate cancer are *RBI* (retinoblastoma gene), *TP53*, *DCC* (deleted in colon carcinoma gene), *BRCA1*, *p16*^{*MST1/CDKN2*}, *p21*^{*WAF1/CIP1*}²³². The most studied tumor suppressor genes are *RBI* and *TP53*. The loss of pRB function requires the inactivation of both gene copies of a diploid cell, the “two-hit” theory. As for *TP53*, the inactivation of one allele is required for its function to be lost which leads to prostate cancer.

2.7.1.3 Apoptosis

Apoptosis is defined as programmed cell death and plays an important role in controlling the growth of prostate epithelial cells. This process contributes to the maintenance of a homeostatic

environment in the prostate by creating a balance with cellular proliferation. If apoptosis is suppressed, tumor progression can occur if DNA damaged or mutated prostate epithelial cells continue to proliferate uncontrollably. The proteins that play a role in the apoptotic process are listed in Table 8^{230, 233-235}.

Table 8. Regulators of Apoptosis

Types	Examples
Oncogenes/ Tumor Suppressor genes	p53, bcl-2/bax, myc
Growth factor & Growth factor receptors	NGF/NGFR, TNF-alpha/FAS, TGF-beta/TGFR
Intracellular Signal Transducers	Protein kinase C and Ca ²⁺
Extracellular matrix regulators & Signal transducers	Fibronectin and transmembrane integrin receptors
Endonucleases	Ca ²⁺ - and Mg ²⁺ - dependent DNase and cytoplasmic proteases typified ICE

The bcl-2 family is considered the most important mediators of apoptosis because they can suppress and induce this process²³⁶. The *TP53* gene functions as a tumor suppressor by regulating apoptosis, cell cycle checkpoints, DNA replication and repair which ultimately maintains genomic stability²³⁶. If the p53 gene is altered, aberrant epithelial cells are created, resulting in the progression of prostate cancer.

2.7.1.4 Angiogenesis

Angiogenesis, the formation of new blood vessels, is a process involved in embryonic development, wound healing, and reproduction²³⁷. In addition, angiogenesis aids in the development of tumors and metastases by expanding its vascular set-up through degradation of basement membrane by protease, proliferation and migration of endothelial cells, cell adhesions, and formation/expansion and survival of blood vessels^{238, 239}. Studies have shown that angiogenesis positively correlated with Gleason score (measurement of tumor differentiation), tumor stage, metastases formation, and survival; however, this association was not found with serum prostate specific antigen (PSA) levels²⁴⁰⁻²⁴⁶.

Before angiogenesis can occur, normal endothelial cells must switch to the angiogenic phenotype²⁴⁴. Angiogenic stimulators such as acidic fibroblast growth factor (aFGF), basic fibroblast growth factor (bFGF), cyclooxygenase-2 (COX-2), and vascular endothelial growth factor (VEGF) make this switch possible in response to some stress such as inflammation or hypoxia^{238, 244, 247}. The most studied angiogenic stimulator is VEGF, a cytokine important in vasculogenesis and angiogenesis.

The angiogenic process can also be prevented by the following inhibitors: thrombospondin-1 (TSP-1) upregulated by p53, interferon alpha and beta, angiostatin and endostatin^{237, 244}. Because of their abilities to arrest tumor growth, these inhibitors have been targeted for antiangiogenic therapy for prostate cancer.

2.7.2 Prostate Cancer Screening/Treatment

Prostate Cancer screening is consisted of two examinations: serum prostate-specific antigen (PSA) determination and digital rectal examination (DRE). The American Cancer Society and the American Urological Association recommend these two tests annually in men starting at the age of 50 with ≥ 10 life-expectancy years, and the age of 45 in men who have a family history of prostate cancer or of African descent.

PSA is a serine protease that is secreted by prostate epithelial cells. Serum PSA concentrations increase when prostate's glandular structure is disrupted by inflammation, hyperplasia, or neoplasia²⁴⁸. For prostate neoplasia, serum PSA determination has been considered as the best marker in determining prostate cancer progression, with a sensitivity reported to be 71%-81%²⁴⁹⁻²⁵¹. Individuals who have a serum PSA concentration of ≥ 4 ng/ml have been considered to be at high risk of developing prostate cancer; ≥ 4 ng/ml is the recommended cutoff in referring men for a prostate biopsy, a procedure used to detect prostate cancer. However, this ≥ 4 ng/ml cutoff has been questioned, especially in individuals at high risk for prostate cancer, for example, African-American men. A study conducted in men with "normal" serum PSA concentrations had detected prostate cancer in 15.2% of these men²⁵². Because of

these missed prostate cancer diagnosis, some researchers have suggested lowering the serum PSA concentration cut-point in high-risk populations. Also, to distinguish between benign and malignant tumors, alternative methods such as PSA Velocity, Free PSA Measurement, and PSA Density have been suggested to use instead of total serum PSA concentration in determining prostate cancer risk. These recommended changes for the current serum PSA determination's standard are still an on-going debate.

DRE is a procedure in which prostate abnormalities such nodules, induration, or irregular areas are evaluated. If any of these abnormalities are noted, prostate biopsy is usually recommended in order to determine prostate cancer status. DRE and serum PSA determination are recommended to be used together as screening tools for prostate cancer; this combination gives a higher detection rate for localized prostate cancer, 78%, opposed DRE and serum PSA alone, 56% and 75%, respectively²⁵¹.

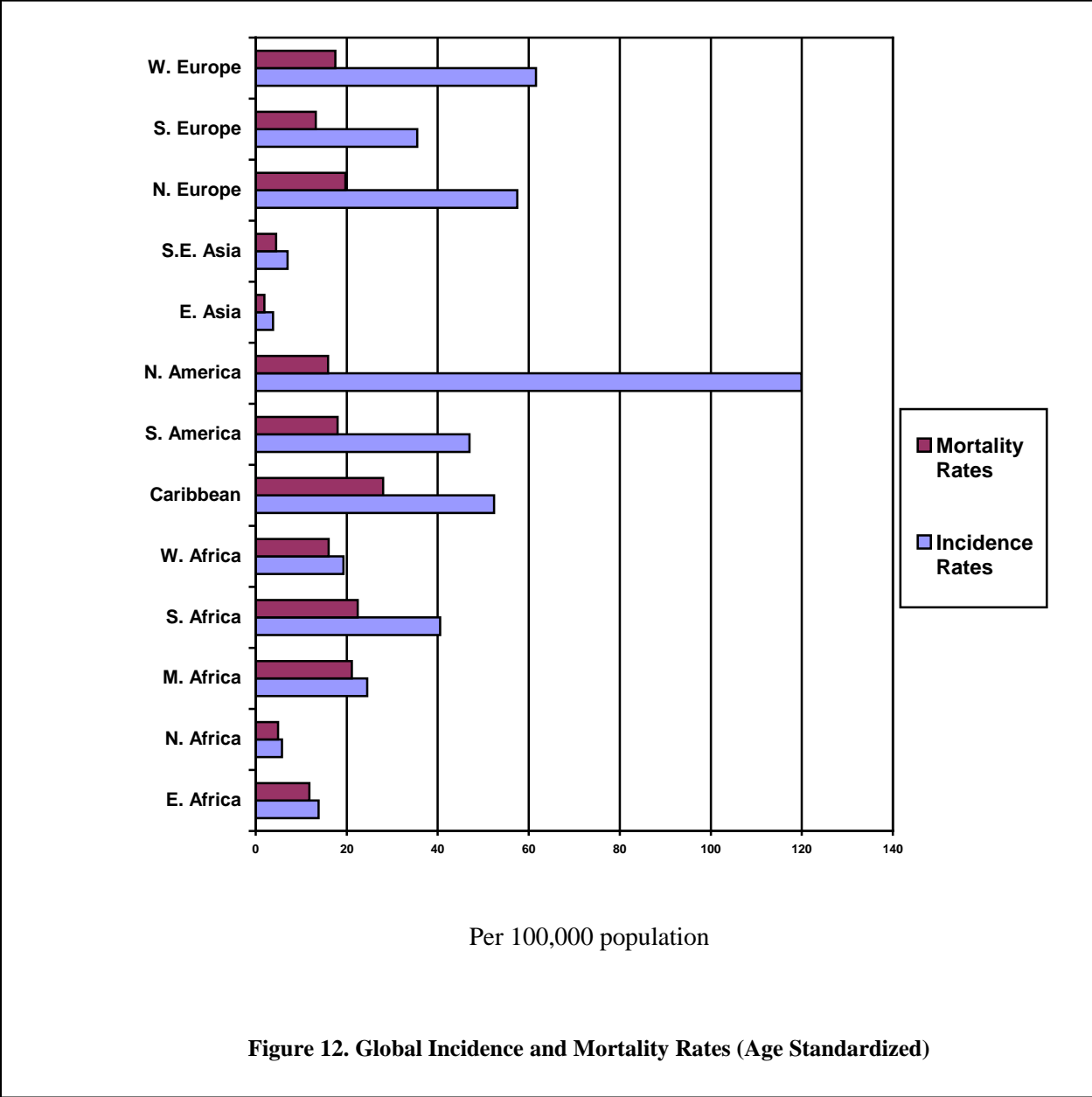
Prostate biopsy, a procedure used to detected prostate cancer, is recommended to be performed on individuals who have a serum PSA concentration of ≥ 4 ng/ml and/or an abnormal DRE. Transrectal Ultrasound-Guided Biopsy (TRUS) with random systematic parasagittal sextant biopsies has been used as the standard protocol in performing prostate biopsies. This needle-biopsy procedure takes six samples of the peripheral zone of the prostate, the area where most cancers have been thought to form^{253, 254}. These samples will be used to determine the presence or absence of prostate cancer as well as the grade of the tumor (well, moderately, poorly differentiated). However, this standard sextant biopsies technique has been reported to have a false negative rate of 15% to 31%^{255, 256}. Studies have found by increasing the biopsy core, the detection rate increase by 30% to 35%^{257, 258}. Therefore, increasing biopsy sampling (i.e., 10-core biopsy) will improve the sensitivity of this procedure.

As for symptoms related to prostate cancer, men are usually asymptomatic in the early stages; however, symptoms such as frequent urination, hematuria, or impotence may be experienced at an aggressive stage. As for treatment, it is determined on whether the prostate cancer is localized or distant metastasis. If the prostate cancer is localized, the following treatment options are available: radial retropubic prostatectomy (RRP), external beam radiotherapy (XRT), hormone therapy, brachytherapy, or watchful waiting. Radial retropubic prostatectomy which is an excision of the prostate, seminal vesicles,

and adjacent tissue has been the preferred surgical procedure due to its low mortality, acceptable morbidity, and high disease-free survival rates²⁵⁹. As for advanced prostate cancer cases, monotherapy with antiandrogens, androgen receptor antagonists, have been used; however for androgen-independent prostate cancer cases, there is no cure.

2.7.3 Rates of Prostate Cancer

Prostate cancer is the 2nd most common cancer diagnosed in men in U.S.; the 2nd most common cancer diagnosed in men in the European Union; and, the 4th most common cancer diagnosed in men worldwide²⁶⁰. According to the International Agency for Research on Cancer, North America had the highest rates of prostate cancer; and, Eastern Asia had the lowest rates of prostate cancer in 2002 while, the mortality rates were highest in the Caribbean region and, lowest in Eastern Asia¹⁰ (Figure 12).



2.7.4 Risk Factors

The etiology of prostate cancer remains unknown; however, studies have identified risk factors that contribute to the development of this disease. Race, older age, family history, diet, and the

presentation of high-grade prostatic intraepithelial neoplasia (HGPIN) in the prostate are known risk factors for prostate cancer development. Other factors like benign prostatic hyperplasia (BPH), obesity, smoking, and viral infections have been examined as possible risk factors in prostate cancer development; however, their contribution to this disease development is still unclear.

2.7.4.1 Race

African-American men have the highest incidence of prostate cancer; and, Asian and American Indian men have the lowest incidence of prostate cancer than any other racial or ethnic group. For example, African-American men were found to have > 65-fold greater risk in comparison to mainland Chinese men and 2-fold greater risk in comparison to White Americans^{227, 261}. Also, African-American men have a higher prostate cancer mortality rate than White Americans. These higher incidence and mortality rates may be related to the higher serum PSA levels, higher androgen levels, more advanced prostate cancer, worse Gleason score (the grade of prostate cancer), and higher recurrence rates of prostate cancer found among African-American men than their white counterparts^{9, 262-268}. African-American men have not only displayed these high rates of prostate cancer, but also, men of African descent from other countries such as Tobago, Jamaica, England, and South America^{260, 269-271}. The high prostate cancer rates observed among black men, in particular, African-American men, suggest that genetic as well as environmental factors play a role in the etiology of prostate cancer.

Studies have suggested that genetic differences in the androgen receptor, the gene encoding type 2 steroid 5-alpha reductase (SRD5A2), and possibly vitamin D binding protein gene may increase African-American men's risk for prostate cancer. For the androgen receptor, African-American men have been found to have shorter repeats of CAG and GGC gene lengths found on exon-1 of the Xq11-12 chromosome in comparison to White and Asian men²⁷²⁻²⁷⁴. These repeats control the transcriptional activation of the androgen receptor, therefore, increasing androgen activity which increases prostate cancer risk^{272, 273}. As for the SRD5A2 gene, it converts testosterone (an androgen) to dihydrotestosterone (DHT), a "more potent" hormone in the prostate epithelial cell which affect cellular proliferation, cellular

differentiation, and the lack of cellular apoptosis^{272, 275, 276}. In Reichardt et al. study, African-American men were found to have specific polymorphic alleles (TA repeat alleles) of the SRD5A2 gene opposed to White and Asian men; these alleles may elevate enzyme activity that increase dihydrotestosterone levels, therefore, increasing prostate cancer risk in African-American men²⁷⁷. For the vitamin D binding protein gene, high levels of vitamin D has been postulated to decrease prostate cancer risk^{261, 278}. Polymorphic variations of the vitamin D binding protein gene between Black and White Americans were found²⁷⁸; however, its association with prostate cancer remains unknown.

Environmental factors such as diet may also play a role in African-American men's higher risk for prostate cancer. Studies suggest that African-American men large consumption of dietary fats, low zinc levels, low selenium, and low consumption of lycopene-containing foods which increase their risk for developing prostate cancer^{229, 261, 279-281}. For example, dietary fats increase androgen which is responsible for the development and growth of the prostate^{263, 282}. Zinc which is an essential mineral is considered an antioxidant that participates in cellular growth and replication²⁸³ as well as mitochondrial apoptosis which lower prostate cancer risk²⁸⁴. Selenium is a trace element that may inhibit cellular proliferation, induce apoptosis, and modulate androgen regulated gene²⁸⁵. Lycopene, a carotenoid, may inhibit cellular growth²⁸⁶. Consumption differences of these dietary items have been found between African-American men in comparison to White and Asian men²⁶¹.

2.7.4.2 Age

The incidence of prostate cancer increases with age. Men who are 75 years of age have > 100-fold higher risk for prostate cancer than men who are 45 years of age²⁸⁷. In the U.S. from 2000-2004, incidence rates of 8.4%, 27.3%, and 36.7% were found in men between the ages of 45-54 years, 55-64 years, and 65-74 years, respectively²²⁷. The mortality rates of prostate cancer also increase with age. In the U.S. from 2000-2004, a mortality rate of 6.6%, 20.8%, and 41.8% were found in men between the ages of 55-64 years, 65-74 years, and 75-84 years, respectively²²⁷. Therefore, age is an important risk factor for prostate cancer.

2.7.4.3 Family History

Studies have indicated that men with 1st degree relatives (father, brother, or son) with prostate cancer are at higher risk than men with no family history of prostate cancer. Whittlemore et al. reported that men with a family history of prostate cancer were at a 2 to 3-fold higher risk for prostate cancer development^{287, 288}. The Massachusetts Male Aging Study reported a 3.78 higher risk in men with a prostate cancer family history than men without a prostate cancer family history^{260, 289}. Steinberg et al. reported that men with a 1st degree, 2nd degree, and both 1st and 2nd degree relatives had a 2-fold, 1.7-fold, and 8.8-fold higher risks of developing prostate cancer, respectively^{290, 291}. In addition, men with hereditary prostate cancer are presented with this disease at an earlier age than other prostate cancer cases²⁹². As a result of this earlier presentation, the American Cancer Society has recommended an earlier screening age (begin at or before the age of 45) for men with a strong family history of prostate cancer. Studies have identified prostate cancer susceptibility genes that are associated with hereditary prostate cancer in men²⁹²; these genes are listed in Table 9.

Table 9. Possible Prostate Cancer Susceptibility Genes

Gene	Chromosome position	Associations
Hereditary Prostate Cancer I (<i>HPCI</i>) gene	1q24-25	- Found in 6% of hereditary prostate cancer cases
Prostate (<i>PCAP</i>) gene	1q42.2-43	- Found among men in Southern and Western Europe
Human Prostate Cancer (<i>HPCX</i>) gene	Xq27-28	- Associated with late-onset prostate cancer - Found in 16% of hereditary prostate cancer cases - Not found in African-Americans
<i>HPC2/ELAC2</i>	17p12	- Linked to a Utah family
<i>HPC20</i>	20q13	- Associated with late-onset prostate cancer

Note: References²⁹²⁻²⁹⁹

Studies have also suggested these genes may have been acquired through x-linked or autosomal-recessive inheritance^{292, 300}.

2.7.4.4 Prostatic Changes

HGPIN is considered a pre-malignant lesion for prostate carcinogenesis³⁰¹. HGPIN precedes the onset of prostate cancer within 10 years³⁰²; and, it increases with age which this increase also correlates with prostate cancer development³⁰²⁻³⁰⁴. In addition, HGPIN is multi-focal like prostatic carcinoma and they are both found in the same peripheral zone³⁰⁵. HGPIN has a phenotype and genotype that is between normal prostate epithelium and prostate carcinoma³⁰⁶. African-American men, aged 50-60 years, have been found to have a higher frequency of HGPIN in comparison to their White counterparts³⁰⁶⁻³¹⁰; and, mainland Japanese men have been found to have a lower frequency than American men^{306, 311, 312}. These higher and lower rates of HGPIN parallels the high and low incidences of prostate cancer found in African-American and Asian men, respectively.

2.7.4.5 Diet

Studies have suggested that diet may increase prostate cancer risk in men. Foods like dietary fats, red meat, dairy products, and soy products as well as selenium, vitamin E (alpha-tocopherol), vitamin D, and lycopene have been indicated to have an affect on prostate cancer development^{229, 279, 285}. Possible effects of these agents are listed in Table 10.

Table 10. The Association between Higher Intake of Food Items and Prostate Cancer Risk

Food Items	Function(s)	Increased or Decreased Prostate Cancer Risk
Dietary fats	Increase androgen levels	Increased risk
Selenium	Inhibits cellular proliferation, induces apoptosis, and modules androgen genes	Decreased risk
Vitamin E (alpha-tocopherol)	Has anti-androgenic activity	Decreased risk
Vitamin D	Inhibits proliferation on prostate cancer cells	Decreased risk
Lycopene	Inhibits cellular growth	Decreased risk
Soy	Inhibits cellular proliferation and has anti-angiogenesis and antioxidant properties	Decreased risk

Note: References^{229, 279, 285-287}

Large-scale clinical trials such as the Nutritional Prevention of Cancer Trial, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Trial, the Beta-Carotene and Retinol Efficacy Trial, and most recently SELECT have been conducted to examine whether certain dietary factors like selenium and alpha-tocopherol can be used as chemopreventive agents in reducing prostate cancer risk²⁸⁵.

2.7.4.6 Other Possible Risk Factors

The relationship between prostate cancer risk and other possible risk factors such as benign prostatic hyperplasia (BPH, an overgrowth of the prostate), obesity, physical activity, and viruses have not been well-defined; studies have reported conflicting results. Therefore, more research is needed to study the effects of BPH, obesity, or viruses on the development of prostate cancer.

2.8 HHV-8, PROSTATE CANCER, AND INFLAMMATION

Chronic inflammation may play a role in the pathogenesis of prostate cancer. Chronic inflammation is a persistent inflammatory response that is triggered by an infectious agent, environmental factor, (i.e., diet, physical or chemical injury, or hormonal exposure), or a combination of both^{313, 314}. Chronic inflammation triggered by an infectious agent has caused the development of the following known cancers: Hepatitis B & C (liver cancer), *Helicobacter pylori* (stomach cancer), Schistosomes (bile duct/bladder cancer), human papillomaviruses (cervical carcinoma), human polyomaviruses (mesotheliomas, brain tumors), Epstein-Barr virus (B-cell lymphoproliferative diseases and nasopharyngeal carcinoma), Human T-cell Leukemia Virus-1 (T-cell leukemias), and HHV-8 (KS, primary effusion lymphoma)^{315, 316}. As for the development of prostate cancer, evidence has shown that inflammation may be associated with prostate cancer. For example, studies have found anti-inflammatory agents such as aspirin or non-steroidal anti-inflammatory drugs (NSAIDs) reducing prostate cancer risk

~15-20^{314, 317-320}. These anti-inflammatory studies support the presence of inflammation in the prostate; therefore, the relationship between chronic inflammation and prostate cancer should be examined.

2.8.1 Infectious agents and Prostate Cancer

Studies have suggested that infectious agents (certain bacteria and viruses) may contribute to the development of prostate cancer^{314, 321}. For example, sexually transmitted diseases (STDs) such as *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, and *Treponema pallidum* (syphilis) and non-sexually transmitted diseases such as *Propionibacterium acnes* and *Escherichia coli* are known to infect the prostate and cause acute or chronic bacterial prostatitis, inflammation of the prostate^{314, 322-327}. In addition, studies have identified DNA from viruses like human papillomavirus (HPV), human herpes simplex virus (HSV-2), cytomegalovirus (CMV), Epstein-Barr Virus (EBV), and HHV-8 in the prostate which may also contribute to chronic inflammation^{15, 209, 314, 328-332}. Chronic inflammation has been hypothesized as another pathway to prostate carcinogenesis through the recruitment of macrophages, lymphocytes, and mast cells which elicit inflammatory cytokines that can 1) cause cellular and genomic damage and 2) increase tissue repair, cellular replication, and angiogenesis in the prostate^{314, 321, 331, 333-337}.

Epidemiological studies have found a significant positive association between prostate cancer and the following STDs: HPV type 18, syphilis, gonorrhea, and HHV-8^{9, 331, 338, 339} (see Table 11). Based on a longitudinal study of men who attended a std clinic, serum PSA levels, a marker of prostate cancer progression, have been found to be increased in men who were diagnosed with a std, $\geq 40\%$ increase, than their negative counterparts³⁴⁰. However, the influence of STDs on serum PSA levels remains unclear due to conflicting study results on the relationship between chronic prostatitis caused by these infections and prostate cancer^{339, 341, 342}. As for the relationship between inflammatory cells and prostate cancer, there have been no epidemiological studies conducted yet that have examined this relationship. In conclusion,

the association between these infectious agents and prostate cancer development still remains inconclusive.

Table 11. Significant Positive Associations between Infectious Agents and Prostate Cancer

Infectious Agents	Odds Ratio (OR)/Relative Risk(RR) (95% Confidence Interval)
History of any STDs ³³⁹	RR= 1.44 (1.24-1.66)
HPV type 18 ³³⁸	RR= 2.59 (1.17-5.75)
History of syphilis ³³⁹	RR= 2.30 (1.34-3.94)
History of gonorrhea ³³⁹	RR= 1.36 (1.15-1.61)
HHV-8 ⁹	OR= 2.24 (1.29-3.90)

2.8.2 HHV-8 and Prostate Cancer

HHV-8 may be a contributor in the inflammatory process in the prostate. Previous studies have shown the presence of HHV-8 DNA in prostatic tissue as well as in semen^{15, 209, 332}. HHV-8 is known to express viral proteins in infected cells during latency (i.e, LANA-1) and lytic replication (i.e, v-IL-6). LANA-1 is known to block apoptosis, stimulate cellular transformation, downregulate *p53*, and target *Rb* (tumor suppressor); and, viral IL-6 (v-IL-6) is known to induce cellular proliferation, prevent apoptosis, and contribute to tumor angiogenesis. A recent study has shown the expression of HHV-8 proteins (LANA-1, vIL-6, and K8.1) in normal prostates; and, there was evidence of local inflammation (macrophage/monocyte marker and B-cell marker) in the prostate¹⁶. This data supports the presence of HHV-8 in the prostate and its possible role in the inflammatory process. The presence of these viral expressions may contribute to the development of prostate cancer by injuring prostate epithelial cells, which result in proliferative inflammatory atrophy (PIA) which leads to the prostate carcinogenesis³¹⁴.

Viral IL-6 (v-IL-6) may play an important role in the development of prostate cancer. This cytokine is a homology of human IL-6 (hIL-6) and it is expressed during HHV-8's early-lytic replication stage^{8, 39, 40 343}. Studies have reported that viral IL-6 shares between 24% to 62% similar amino acid sequence to hIL-6^{8, 39}. These IL-6s also have similar functions which are to "bestow upon the infected cell

resistance to immune mediated apoptotic stimuli, thus ensuring the survival and propagation of the viral pathogen”³⁴⁴. However, vIL-6 stimulates multiple cellular pathways to induce cellular proliferation⁴⁰; and, it can bind directly to glycoprotein 30 (gp30) to initiate signaling through several pathways (i.e. Jak-STAT) unlike hIL-6 that also needs IL-6R alpha, a co-receptor^{8, 40}. Because of the IL-6’s similar properties and functions, HHV-8’s vIL-6 may be a major contributor to the development of prostate cancer in some individuals.

2.8.3 HHV-8 and Prostate Cancer Studies

The relationship between HHV-8 infection and prostate cancer has been examined in many study populations. Hoffman et al. found significant association between HHV-8 seropositivity and prostate cancer in Tobago (Tobago cases versus Tobago controls and Tobago cases versus Trinidad controls) and in Pittsburgh, Pennsylvania (Pittsburgh cases versus US blood donors)⁹. However, inverse associations were found between HHV-8 seropositivity and prostate cancer in U.S. populations^{345, 346} (Table 12). A nested case-control study conducted among men in the U.S. Health Professional Follow-up Study found a significant inverse association between prostate cancer and HHV-8 seropositivity (O.R. = 0.70, 95% C.I., 0.52 – 0.95), with seropositivity of 13.5% and 18% for the cases and controls, respectively³⁴⁷. Studies that found a found positive but non-significant association were conducted in populations in Pittsburgh, Pennsylvania and the Bologna region in Italy^{9, 345}. The reason for differences in association between HHV-8 and prostate cancer among Tobago, Pittsburgh, Italy, other U.S. populations, and Finland may be related to lifestyle or genetic differences in the populations.

Most of these studies used the same serological assay, mIFA, which detects antibodies against HHV-8 lytic antigens^{9, 13, 345, 347}.

Table 12. HHV-8 Seropositivity and Prostate Cancer Studies

	Prostate Cancer Cases (n/N) (% HHV-8 +)	Controls (n/N) (% HHV-8 +)	Odds Ratio (95% Confidence Interval)
Tobago cases vs Tobago controls (Hoffman 2004)	55/138 (39.9%)	32/140 (22.9%)	2.24 (1.29-3.90)
Tobago cases vs Trinidad controls (Hoffman 2004)	55/138 (39.9%)	35/174 (20.1%)	2.63 (1.56-4.50)
Pittsburgh cases vs US blood donors (Hoffman 2004)	20/100 (20%)	9/177 (5.1%)	4.67 (1.91-11.65)
Pittsburgh cases vs Pittsburgh cancer controls (Hoffman 2004)	20/100 (20%)	13/99 (13%)	1.65 (0.77-3.54)
Finland cases vs Finland controls (Korodi 2005)	3/163 (1.8%)	7/288 (2.4%)	0.74 (0.19-2.88)
Italy cases vs Italy BPH controls (Jenkins 2007)	4/10 (40%)	13/34 (38.2%)	1.08 (0.27-4.33)
Washington, DC cases (Black) vs Washington, DC BPH controls (Black) (Jenkins 2007)	7/41 (17.1%)	19/98 (19.0%)	0.88 (0.35-2.24)
U.S. White cases vs U.S. White controls (Jenkins 2007)	19/104 (18.7%)	20/80 (24.4%)	0.71 (0.36-1.43)
U.S. Black cases vs. U.S. Black controls (Jenkins 2007)	17/95 (17.5%)	21/75 (27.5%)	0.56 (0.28-1.14)

Note: In bold, numerator not given

3.0 PUBLIC HEALTH SIGNIFICANCE

Prostate cancer is a global health issue with 679,023 new cases detected in 2002 worldwide¹⁰. African-American men are known to have the highest incidence and mortality rate than any other ethnic group, incidence rate 60% higher and mortality rate 2-fold higher than Whites in the U.S.²²⁷. High rates of prostate cancer have also been observed in the African-Caribbean population of Tobago, an 11% prevalence rate at initial screening¹⁷. Prostate cancer is a public health problem, particularly in men of African descent; therefore, possible etiologies of this cancer need to be examined in high-risk populations.

HHV-8 infection varies geographically, ranging from 3% in the U.S. to 87% in Botswana where KS is endemic. However, little is known about this newly discovered virus' biology, pathogenesis, and carcinogenic effects. Hoffman et al. demonstrated that men with prostate cancer have a significantly higher HHV-8 seropositivity rate than men without prostate cancer, 39.9% and 22.9%, respectively (p-value = .003)⁹. Studies have shown that HHV-8 DNA and its viral proteins are present in prostatic tissue^{15, 16}. However, HHV-8's influence on prostate cancer development remains unknown.

To understand the relationship between prostate cancer and HHV-8, we propose to examine HHV-8 seropositivity among Tobago women, identify possible modes of sexual transmission of HHV-8, study the natural history of HHV-8 seropositivity, and investigate the relationship between HHV-8 and prostate cancer risk. This study "The Evaluation of HHV-8 Infection in Tobago" will be an important resource in understanding HHV-8 and possible relationships between HHV-8 infection and prostate cancer. We hope that this study will provide knowledge that assists in the development of appropriate interventions to prevent HHV-8 infection and possibly prostate cancer in at risk populations.

**4.0 PAPER 1: HUMAN HERPESVIRUS 8 (HHV-8) SEROPREVALENCE AMONG
TOBAGO WOMEN AND THE ROLE OF SEXUAL LIFESTYLE BEHAVIOR**

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(Under review by The Journal of Medical Virology)

4.1 ABSTRACT

Human herpesvirus 8 (HHV-8) infection is present in 22.9% of Tobago men. However, seroprevalence and modes of transmission of HHV-8 among Tobago women are not known. HHV-8 seropositivity rates in Tobago women were examined and compared rates to Tobago men of similar ages. To assess possible modes of transmission, sexual behavior among Tobago women was examined to determine its association with HHV-8 seropositivity.

A cross-sectional study was conducted in 213 Tobago women, ages 18-65 years, who participated in the Tobago Cervical and Oral Cancer Screening Study. HHV-8 seropositivity was determined by a monoclonal immunofluorescence assay. Age-specific rates were compared to those previously observed in men. Logistic regression analyses were performed to determine the association between HHV-8 seropositivity and sexual behavior among the women.

HHV-8 seroprevalence among Tobago women was 14.1% (95% C.I., 10 - 19%), with no significant differences with men of similar age (p-value = .741). Age \leq 17 years at first sexual intercourse was found to have a minimal significant association with HHV-8 seropositivity (O.R. = 2.51, 95% C.I. = 1.09-5.78) in women.

HHV-8 age-specific rates were similar between genders. Sexual activity may not be a major contributor to HHV-8 infection among Tobago women.

4.2 INTRODUCTION

Human herpesvirus 8 (HHV-8), a member of the Family *Herpesviridae* and subfamily *Gammaherpesvirinae*, is the causal agent of Kaposi's sarcoma (KS) and primary effusion lymphoma and has been associated with multicentric Castleman's disease^{1, 81-83}. HHV-8 seroprevalence varies geographically and is considered endemic in Mediterranean countries where seropositivity rates are as high as 31% in Southern Italy¹⁴⁴, and in Sub-Saharan Africa, where seropositivity reaches 87% in Botswana Africa². KS is relatively common in these areas: incidence is as high as 8.8 and 30 per 100,000 population per year in Italy and Sub-Saharan Africa, respectively^{2, 10, 143, 144}. In the United States and parts of Northern Europe where KS is not prevalent, low to moderate HHV-8 seropositivity rates (3% to 23%) have been reported^{4, 8, 9, 14, 169}.

HHV-8 infection is known to be a sexually transmitted disease (STD); however, the presence of HHV-8 infection among pre-pubertal children suggests that HHV-8 can also be transmitted non-sexually. Studies have suggested non-sexual transmission (i.e., saliva exchange) as the primary mode of HHV-8 transmission in hyper-endemic KS areas like Sub-Saharan Africa^{8, 12, 55}. However, in non-KS endemic areas like the U.S. and Northern Europe, sexual transmission is believed to be the predominant mode of HHV-8 transmission among high-risk populations such as men having sex with men^{12, 166, 348, 349}.

In the Caribbean islands, six studies^{4, 9, 159, 161-163} have examined HHV-8 infection; however, only two studies^{161, 163} examined possible modes of HHV-8 transmission. To have a better understanding of HHV-8 infection in the Caribbean island of Tobago, we used rates from a previously conducted cross-sectional study among 215 healthy Tobago women, 18-65 years of age. In this population, we examined HHV-8 seropositivity rates, and investigated possible modes of transmission by analyzing available information on sexual behavior.

4.3 METHODS

4.3.1 Study Population

The Tobago Cervical and Oral Cancer Screening Study is a cross-sectional study conducted among healthy women to examine the prevalence of oral and cervical human papillomavirus (HPV) infections in Tobago³⁵⁰. Between July and August 2004, study participants were recruited by posters, flyers, television, radio public announcements, presentations in churches, seminars with health care workers at the Tobago hospital, and word of mouth. Women who had a terminal illness, did not sign an informed consent, or were not the ages 18-65 years were excluded from participation in the study. Two-hundred sixteen women were approached, and 215 of them agreed to participate in the screening. Blood, cervical, and oral epithelial cells were collected at the study visit. Of the 215 women, blood samples were available for all except 2 study participants; as a result, 213 women were included in the present study. Demographic and health information, family medical history, and sexual lifestyle behaviors were collected through the Tobago Cervical and Head and Neck Cancer Health Assessment which included the standardized questionnaire by the University of Pittsburgh Head and Neck Cancer Program as well as elements from the Centers for Disease Control and Prevention's Sexual Lifestyle Questionnaire³⁵⁰. The sexual lifestyle part of the assessment was self-administered in a private room, and then enclosed in a sealed envelope by the participant. At the end of the study, all of the participants' assessments were mailed to the University of Pittsburgh for data entry and data analysis.

HHV-8 seropositivity rates of Tobago men, ages 40-65 years, were used as a comparison. These men represented the control group in a case-control study on the association between HHV-8 seropositivity and prostate cancer conducted in Tobago between 1997 and 2000⁹. Control male participants were drawn from a population-based prostate cancer screening study¹⁷, and had normal digital rectal examination (DRE) and serum prostate-specific antigen (PSA) values < 4.0 ng/mL¹⁷. An additional 97 men, ages 40-65 years, from the Tobago prostate cancer screening study (n = 3201) were

added to the existing controls (n = 62), giving a total of 159 men between the ages 40-65 years; their seropositivity rates were compared to those observed in a subset of the Tobago women (n = 122) belonging to the same age range (40-65 years)⁹.

Demographic information for men was collected from the Tobago Prostate Cancer Screening Survey questionnaire. Sexual lifestyle behaviors were not collected during this survey. All blood samples were tested for antibodies against HHV-8 lytic antigens at the University of Pittsburgh.

All study participants signed an informed consent that was approved by the University of Pittsburgh Biomedical Institutional Review Board (IRB) and the IRB of the Division of Health and Social Services, Tobago House of Assembly.

4.3.2 Laboratory Methods

A modified HHV-8 monoclonal antibody-enhanced immunofluorescence assay (mIFA) that assessed lytic antigens using the BCBL-1 cell line, as described elsewhere¹³, was used to test blood specimens (plasma from Tobago women & serum from Tobago men) for HHV-8 seropositivity at the University of Pittsburgh. A HHV-8 seropositive result was reported for specimens that gave fluorescence at the dilution cut-off value of 1:100. For each mIFA run, known HHV-8 positive and negative sera were included. All blood specimens were tested in duplicates per lab run on 2 different days. A 10% random sample of blood specimens were tested twice in a blinded fashion per assay run. Agreement between duplicates was substantial³⁵¹ (Kappa_{intra-batch} = 0.78, 95% confidence interval [C.I.], 0.38 – 1.00, based on n = 33 sample pairs; Kappa_{inter-batch} = 0.61, 95% C.I., 0.46 - 0.77, based on n = 202 sample pairs with non-missing mIFA test results from the first two laboratory runs). HHV-8 antibody titers were also determined by mIFA on serially diluted serum samples (1:100 to 1:51,200). All blood specimens that were analyzed by mIFA were assessed microscopically by the same reader.

4.3.3 Data Analysis

The overall frequency and age-specific frequency distribution were used to measure the seroprevalence of HHV-8 infection among the Tobago women. Pearson's chi-square test was used to examine whether there were any differences in HHV-8 seropositivity rates among these age groups (18-29, 30-39, 40-49, 50-59, 60-65 years). HHV-8 antibody titer of women who tested HHV-8 seropositive was examined by age groups. Fisher's exact test was used to determine whether there was a difference in antibody titer (low, < 800 versus high, \geq 800) between younger (ages 18-39 years) and older (ages 40-65 years) women.

The Mantel-Haenszel chi-square test was used to determine if there was a difference in the overall seroprevalence of HHV-8 infection between Tobago women and men of comparable age, after the Breslow-Day test for homogeneity was conducted. The Pearson's chi-square test or Fisher's exact test (if appropriate) was used to examine whether there were any differences in age-specific HHV-8 seropositivity rates for each age group (40-49, 50-59, 60-65 years) between genders.

Logistic regression analyses were performed to assess the independent contribution of each study variable (age, marital status, history of cancer, oral and cervical HPV detection, and sexual lifestyle behavior variables) to HHV-8 sero-status, and to analyze the interaction of age and the study variables on HHV-8 sero-status.

Nine sexual behaviors plus results from HPV oral and cervical screening listed in Table 13 were scored (0 or 1) based on a prior hypothesis of their likelihood of having a positive association with HHV-8 seropositivity. A score of 0 was given to the reference group (no risky behavior and/or no presence of HPV DNA); a score of 1 was given to the exposed group that had the risky behavior and/or presence of HPV DNA (Table 13). The studied variable "Number of partners in the past 12 months" was collapsed to none or one partner (score = 0) in comparison to two or more partners (score = 1). Women who did not answer all 9 sexual behavior questions and had no HPV oral or cervical results were excluded from this analysis. To alleviate converging problems in the logistic regression model, sexual behavior scores were

divided in the following categories: category 1 (scores 1-4), category 2 (score 5), category 3 (score 6), category 4 (score 7), and category 5 (scores 8-11).

All data analyses were conducted with SPSS version 12.0; an alpha level of 0.05, two-sided, was set for all the analyses.

4.4 RESULTS

4.4.1 Study Population Characteristics

A total of 213 Tobago women, ages 18-65 years, was included in the study. The median age was 41.0 years (25-75% percentile, 35-48 years). Of these women, 83.1% identified themselves as African-Caribbean, 7% as mixed race, and 1.9% as East-Indian; 8% of the women did not report their race. The majority of women reported that they were married at the time of interview (51.6%).

The HHV-8 sero-status of 159 Tobago men, ages 40-65 years, were compared to that of the subset of Tobago women of comparable age (n = 122). The median age of the male study population was 59.0 years (25-75% percentile, 53-63 years). The median age of the female subset study population was 47.0 years (25-75% percentile, 45-53 years). The majority of the female subset and of the men reported that they were married at the time of the interview (62.3% and 71.7%, respectively).

4.4.2 HHV-8 Seroprevalence

An overall seroprevalence of 14.1% (95% C.I., 10 - 19%) for antibodies against HHV-8 lytic antigens was found among Tobago women. HHV-8 seroprevalence point estimates varied across age groups, with the lowest prevalence observed in the age group 30-39 years, and the highest prevalence observed in the age group 60-65 years (Figure 13). However, there were no significant differences in

HHV-8 age-specific seropositivity rates among the age groups (18-29, 30-39, 40-49, 50-59, 60-65 years) (p-value = .835).

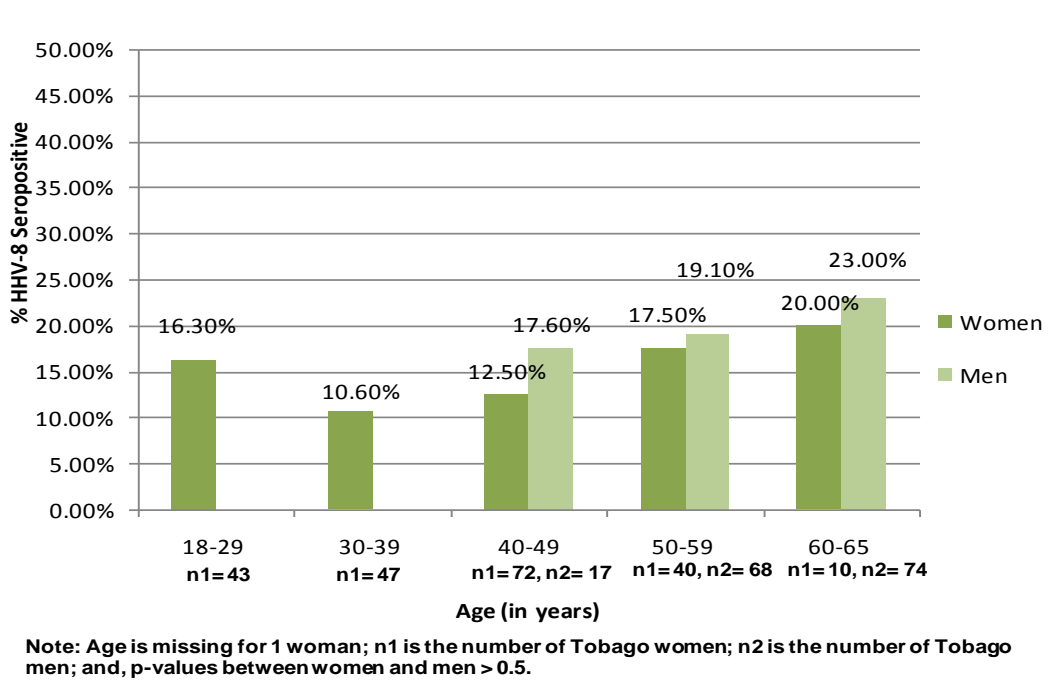


Figure 13. HHV-8 Seropositivity Rates According to Age in Tobago Women and Men

4.4.3 HHV-8 Antibody Titers Distribution

There were 30 HHV-8 seropositive women; their IgG antibody titer against HHV-8 lytic antigens ranged from 1:100 to 1:6400 (Figure 14). The median antibody titer was 400 (25-75% percentile, 200-800) among the seropositive women. Overall, women, ages 40-65 years, were more likely to have an antibody titer of ≥ 800 (8.2%, n = 10/122) than women, ages 18-39 years (0%, n = 0/90) (p-value = .006).

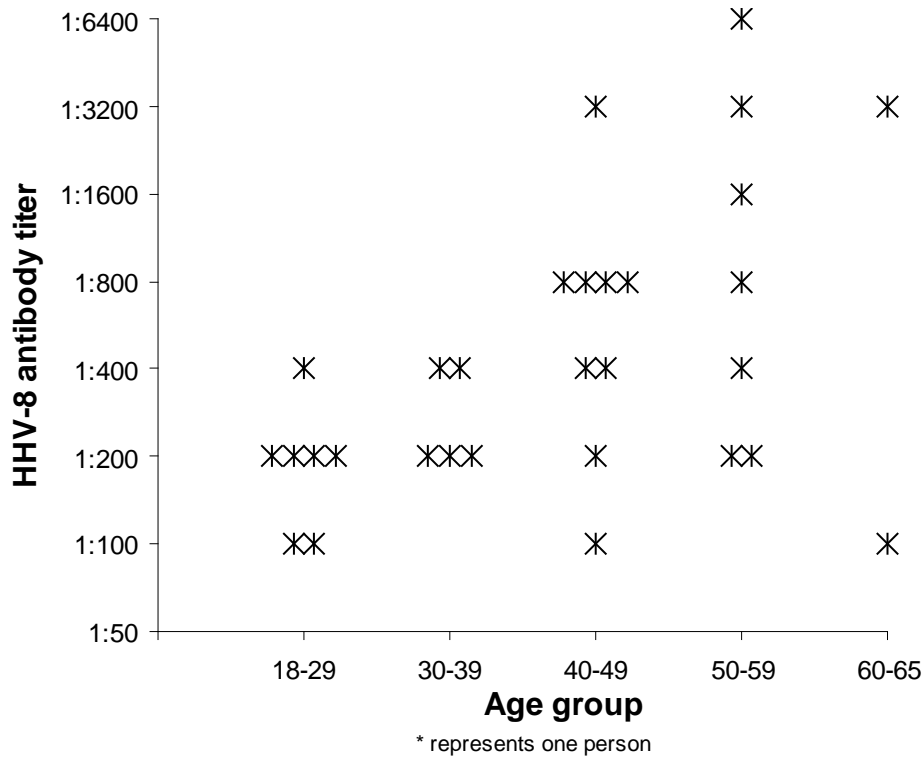


Figure 14. The Frequency of HHV-8 Antibody Titers in HHV-8 Seropositive Tobago Women by Age Groups (N = 30)

4.4.4 Comparison of HHV-8 Seropositivity Rates between Tobago Women and Men

Across age groups 40-65 years, HHV-8 seropositivity rates were 14.8% (95% C.I., 9.0 - 22.3%) in women and 20.8% (95% C.I., 14.7 - 27.9%) in men. Adjusting for age, the test of homogeneity (p-value = .946) and differences in HHV-8 seropositivity between men and women were not statistically significant (p-value = .741) (Figure 13). However, in each age group, Tobago women had a lower HHV-8 seropositivity rate than their male counterparts (Figure 13).

4.4.5 Risk Factors for HHV-8 Infection

HHV-8 seropositivity was analyzed in relation to sexual lifestyle behavior in women (Table 13). HHV-8 seropositivity was not statistically associated with any sexual behavior variables, except for “age at first sexual intercourse”. Tobago women who reported to have their first sexual intercourse at ≤ 17 years of age were 2.51-fold more likely (95% C.I., 1.09-5.78) to be HHV-8 seropositive than women who reported to have their first sexual intercourse at ≥ 18 years of age. In addition, marital status, history of cancer, and HPV DNA detected in oral and cervical cavity were not associated with HHV-8 sero-status.

No significant interactions were observed between age and any studied variable.

Out of the 9 sexual behavior variables plus 2 HPV screening variables listed in Table 13, the combined sexual behavior scores (including HPV screening results) ranged from 1 to 9 with a mean score of 6.3 (95% C.I., 6-6.5) among 115 women. In the logistic regression model, the number of women in the combined sexual behavior score categories were the following: 15 in category 1 (scores 1-4), 15 in category 2 (score 5), 30 in category 3 (score 6), 35 in category 4 (score 7), and 20 in category 5 (scores 8-11). There was no statistically significant association between HHV-8 seropositivity and the combined sexual behavior scores after adjusting for age (p-value = .282). There was no statistically significant association with HHV-8 seropositivity when the combined sexual behavior scores were divided into two categories: low score of < 6 (6.7% seropositive, n = 2/30) and high score of ≥ 6 (17.6% seropositive, n = 15/85) (p-value = .231).

Table 13. Potential Risk Factors for HHV-8 Infection in Tobago Women

Variables (Reference/ Exposed Groups)	Number in Reference/Exposed Groups	Odds ratio*	95% Confidence Interval
Marital Status (married/widow vs. single/other)	118/92	.74	.31-1.76
History of Cancer (no/yes)	198/8	2.17	.40 – 11.94
Age at 1st sexual intercourse (age \geq 18 vs. age \leq 17)	106/87	2.51	1.09 – 5.78
Number of lifetime partners (one partner vs. 2 or more partners)	29/164	.81	.28 – 2.33
Ever diagnosed with a STD (no vs. yes)	175/21	1.84	.61 – 5.50
Sexual intercourse in the past 12 months (no vs. yes)	25/170	1.65	.42 – 6.42
Number of partners in the past 12 months (none vs. one partner; none vs. two or more partners)	17/153	.76	.19 – 3.13
	17/43	.79	.17 – 3.76
Condom used during vaginal sex (sometimes/frequently vs. no)	104/82	.86	.37 – 2.00
Ever had oral sex (no vs. yes)	59/128	.63	.26 – 1.55
Condom used during oral sex (sometimes/always vs. no)	16/111	1.18	.25 – 5.73
Partner performed oral sex (no vs. occasionally/frequently)	33/153	.61	.22 – 1.69
HPV detected in oral cavity (no vs. yes)	197/15	1.58	.42 – 5.97
HPV detected in cervix (no vs. yes)	137/75	.92	.4 – 2.11

Note. - * = age-adjusted, HPV= Human papillomavirus, STD= sexually transmitted disease, vs. = versus

4.5 DISCUSSION

This study indicates that HHV-8 infection is present among Tobago women at a frequency similar to that previously reported in men^{3, 9, 144, 166}. The seropositivity rate observed among these women is comparable to rates found among women in the Mediterranean area^{6, 7, 142}. As for KS, this incidence has been reported to range from 0.2 to 2.8 per 100,000 population per year among women in the Mediterranean region^{142, 143, 145}. In Tobago, no cases of KS have been reported for women and men during the period of 1994-1997^{10, 11}. The lack of KS in Tobago despite the moderate rates of HHV-8 infection may be due to the immune status of the population, genetic make-up that prevents disease development/progression to clinical stage, possible environmental factors (for example, diet), under-reporting of KS to health officials, or a small population size.

A gradual increase in HHV-8 seropositivity was observed among Tobago women, from ages 30-39 years to ages 60-65 years. Women ages 18-29 years had a higher HHV-8 seropositivity in comparison to women ages 30-49 years; this higher seropositivity among younger women may be due to a cohort effect. Women ≥ 40 years of age had a higher prevalence of antibody titer $\geq 1:800$ than women ages ≤ 39 years. The reason for the higher antibody titers among older women may be due to cumulative exposure to HHV-8. As for the male controls in Hoffman et al. study, the overall median antibody titer among these seropositive men (n = 15/159), ages 40-65 years, was lower (median = 400) in comparison to women (n = 18/122) of similar ages (median = 800)⁹. The reason for the lower median antibody titers among men in comparison to women is not known.

As for gender and HHV-8 seropositivity with age, the overall HHV-8 seropositivity rate as well as the rates across age groups (40-49, 50-59, 60-65 years) were higher in Tobago men in comparison to Tobago women; however, these seropositivity rates did not significantly differ. This lack of significant gender difference in seropositivity rates is consistent with studies conducted in Sub-Saharan Africa³, Italy¹⁴⁴, and the United States^{166, 169}.

Few studies have examined HHV-8 infection in Caribbean populations. Studies conducted in Dominican Republic¹⁶², Cuba¹⁶¹, and Haiti¹⁶² found moderate HHV-8 seropositivity rates for antibodies against HHV-8 lytic antigens, 13%, 16.9%, and 29%, respectively. These seropositivity rates are similar to rates found in Tobago. Also, no gender difference in HHV-8 seropositivity rate found in the Cuba study¹⁶¹ is consistent to the present study's finding; the study conducted in Dominican Republic and Haiti did not report gender seropositivity rates¹⁶².

In the present study, HHV-8 seropositivity rates were higher than rates reported in other Caribbean populations. For example, in Jamaica, three studies reported low HHV-8 seropositivity rates of 0.68% among women attending gynecology clinics, 2.7% among blood donors (ages 18 to 63 years), and 3.6% among blood donors (ages 18 to 64 years); in these studies, a whole virus enzyme-linked immunoassay and/or immunofluorescence assay testing for antibodies against lytic and latent HHV-8 antigens were used^{4, 159, 163}. In Trinidad, HHV-8 seropositivity rate of 1.3% among female and male blood donors (ages 18 to 64 years) was found by using a whole virus enzyme-linked immunoassay⁴. The low HHV-8 seropositivity rates found in Trinidad and Jamaica populations in comparison to the present study population may be due to differences in study populations or in the sensitivity of the serological assay used to examine HHV-8 infection. The serological assay, mIFA, used in the present study has been reported to have a sensitivity of 89.9% and specificity of 97.5%¹⁴. Based on studies that used serological assays to examine patterns of change in HHV-8 antibody responses, antibody titers have shown to decrease over time in some individuals^{352, 353}. Therefore, HHV-8 serological assays may not be sensitive enough to detect all HHV-8 exposed individuals. The decline in antibody titers suggests that HHV-8 infection may be underestimated in cross-sectional studies.

In this study, a significant association between HHV-8 sero-status and age at first sexual intercourse was found among women. A study conducted among female prostitutes and age-matched controls in Oviedo and Barcelona, Spain found a similar association between HHV-8 seropositive status and age at first sexual intercourse³⁵⁴; but, this association was not confirmed by studies conducted among U.S. women^{126, 355}. A study conducted in Cuba which included women and men did not find an

association between HHV-8 seropositivity and age at first sexual intercourse¹⁶¹. The minimal association between HHV-8 seropositivity and age at first sexual intercourse reported in the present study suggests that sexual activity may not be the predominate mode of HHV-8 transmission in Tobago.

Sexual transmission may not be the only mode of HHV-8 transmission in Tobago. In this study, several variables that assess sexual lifestyle behaviors were not associated with HHV-8 sero-status among the women. In addition, the detection of HPV DNA in oral or cervical cavity was not associated with HHV-8 seropositivity. The lack of associations between seropositivity and several sexual behavior variables is consistent with previous studies that examined similar sexual lifestyle behaviors and HHV-8 sero-status among women^{166, 355}.

Non-sexual transmission of HHV-8 may play a role in Tobago. Evidence of HHV-8 infection among pre-pubertal children in other populations suggests non-sexual routes of HHV-8 transmission, and point at saliva as the probable route of transmission^{7, 12, 138, 157}. Saliva is known to have the highest viral load and viral shedding in comparison to other sites such as peripheral blood mononuclear cells, urine, semen, and prostate^{127, 207, 211, 356}. Due to the age of the present study population, ≥ 18 years of age, evidence of non-sexual transmission through oral transmission was not observed. However, to evaluate whether oral transmission through sexual behaviors was associated with HHV-8 sero-status, women who participated in oral sexual activities and/or had the presence of HPV DNA in their oral cavity were examined and found to have higher risk for HHV-8 infection than their counterparts. Tobago women were less likely to be seropositive if they ever had oral sex or if they had partners who performed oral sex on them; in contrast, they were more likely to be seropositive if they did not use condoms during oral sex or if HPV was detected in their oral cavity. However, none of these associations were statistically significant.

There are some limitations in the present study. One limitation is that for Tobago men, sexual lifestyle behavior data were not available. A recent study has shown sexual lifestyle behaviors such as duration of sexual activity in years, the number of lifetime sex partners, and co-infection with other STDs to be associated with HHV-8 seropositive status among heterosexual men¹⁶⁶. Another limitation is the

limited power to examine the association between HHV-8 sero-status and sexual lifestyle behaviors among women. Sexual lifestyle behavior variables were missing in some of the women's responses; however, this missing data was less than 14% for most variables.

The present study is the first to examine the relationship between several sexual lifestyles behaviors and HHV-8 infection in the Caribbean. A good estimation of HHV-8 seroprevalence among women, ages 18 to 65 years, based on a 95% confidence interval width of < 10% was calculated. This study had enough study participants to detect a doubling of HHV-8 seropositivity (from 15% to 30%) with 80% power to test at alpha level of 0.05, two-sided.

In conclusion, the present study provides evidence that HHV-8 infection is present in Tobago. However, sexual activity may not be a major contributor in acquiring HHV-8 infection. Non-sexual transmission of HHV-8 may also occur in Tobago; however, evidence of this transmission remains not known. Understanding possible modes of HHV-8 transmission will help design programs aimed at interrupting this viral infection in populations. Further studies, especially longitudinal studies, are needed to examine HHV-8 infection, transmission, and its relationship with possible associated malignancies in Tobago.

**5.0 PAPER 2: THE NATURAL HISTORY OF HUMAN HERPESVIRUS 8
SEROPOSITIVITY IN A COHORT OF TOBAGO MEN OF AFRICAN DESCENT, 1997-
2007**

(Will submit to a peer-reviewed journal)

5.1 ABSTRACT

Human herpesvirus 8 (HHV-8) is the causal agent of Kaposi's sarcoma (KS) and primary effusion lymphoma (PEL). In populations at high risk for KS, in particular, HIV/AIDS patients and organ transplant recipients, the natural history of HHV-8 infection has been examined. However, for healthy populations, the natural history of HHV-8 infection is not known. To study the natural history of HHV-8 infection among a presumably healthy adult population, HHV-8 seroconversion and seroreversion rates as well as persistent seropositivity were examined among African-Caribbean men in Tobago.

A random sample of 501 Tobago men at risk for incident prostate cancer, ages 40-81 years, was selected from the Tobago Prostate Cancer Screening Survey. The inclusion criteria were men who had a serum specimen at baseline visit and at wave 2 and/or wave 3 visits. Men diagnosed with prostate cancer at the baseline visit were excluded. Demographic information was collected, and serum prostate specific antigen (PSA) levels and digital rectal examination (DRE) were recorded. Serum specimens available for each study visit were tested for HHV-8 seropositivity by monoclonal antibody-enhanced immunofluorescence assay (mIFA) at each study visit. HHV-8 seropositivity was examined at baseline visit, wave 2, and wave 3 visits. Pearson's chi-square, Fisher's exact, or Mann-Whitney tests were used to compare HHV-8 seropositivity with baseline characteristics. Logistic regression was used to see whether there was a difference in baseline HHV-8 seropositivity among men who had blood collected at all study visits in comparison to men who had blood collected at only two study visits. HHV-8 seroconversion (seronegative at baseline visit to seropositive at subsequent visit) and seroreversion (seropositive at baseline visit to seronegative at subsequent visit) rates and their 95% confidence intervals were calculated using a Poisson distribution. To evaluate persistent seropositivity, the Kaplan-Meier approach was used to examine the seroreversion event-free survival to estimate persistent seropositivity.

Of the 501 men identified as at risk for incident prostate, 407 (81.2%) study participants were included in the analysis. The frequency of HHV-8 seropositivity was 16.5% (95% C.I., 13.0% - 20.4%), 18.5% (95% C.I., 14.1% - 23.6%), and 16.6% (95% C.I., 12.8% - 21.0%) at baseline visit, wave 2 visit,

and wave 3 visit, respectively. The HHV-8 seroconversion rate was 0.50 per 100 person-years (95% C.I., 0.22-0.99); and, the HHV-8 seroreversion rate was 2.52 per 100 person-years (95% C.I., 1.09-4.96). Among HHV-8 seropositive men at baseline visit, the rate of persistent seropositivity was 0.856 (standard error = 0.05) at 5.5 years after baseline visit.

We found a low HHV-8 seroconversion rate and a moderate seropositivity rate at each study visit among Tobago men, aged 40-81 years. These data suggest that HHV-8 infection is present in Tobago, however; this viral infection is probably acquired at a younger age, < 40 years. We also found a moderate seroreversion rate among Tobago men; this pattern of change suggests that a more sensitive serological assay is needed. Further studies are needed to understand HHV-8 infection and possible risk factors for acquiring HHV-8 infection in Tobago.

5.2 INTRODUCTION

Human herpesvirus 8 (HHV-8), a gammaherpesvirus, causes life-long infection that can result in disease (i.e, Kaposi's sarcoma [KS], primary effusion lymphoma [PEL], or multicentric Castleman's disease [MCD])^{1, 81-83} which is usually seen in immunocompromised individuals. KS, the most common HHV-8 associated malignancy, is prevalent in Sub-Saharan Africa and Mediterranean populations where high and moderate HHV-8 seropositivity rates have been reported, respectively. To understand the natural history of HHV-8 infection, patterns of antibody responses against HHV-8 latent and/or lytic antigens have been examined in populations at high-risk for HHV-8 associated diseases such as populations infected or at risk for HIV/AIDS^{213, 352, 357-360}, organ transplant recipients^{13, 353, 361-367}, blood transfusion patients¹⁶⁸, and chronically ill individuals³⁵³, in which the frequency of HHV-8 seroconversion (0.7-52%) and seroreversion (0.4-82%) has been observed. However, in healthy populations, these patterns of change in HHV-8 sero-status over time, as well as the determinants of such change, have not been studied.

In the Caribbean island of Tobago, HHV-8 seropositivity rates of 39.9% and 22.9% among African-Caribbean men with and without prostate cancer, respectively, were detected⁹. To examine patterns of change and persistent antibody responses against HHV-8 lytic antigens, we conducted a prospective cohort study among prostate cancer-free Tobago men, aged 40-81 years, who participated in the longitudinal, population-based Tobago Prostate Cancer Screening Survey. By examining HHV-8 seroconversion and seroreversion rates and persistent seropositivity levels in a presumably healthy population, we hope to have a better understanding of the natural history of HHV-8 infection in the Tobago population.

5.3 METHODS

5.3.1 Study Design and Study Population

A cohort study was conducted to examine HHV-8 seroconversion, seroreversion, and persistent seropositivity rates among a presumably healthy cohort of men from the Caribbean island of Tobago. The study population included men of African descent who participated in the longitudinal, population-based Tobago Prostate Cancer Screening Survey in Tobago¹⁷. This ongoing longitudinal study, initiated in 1997, consists of men who participated at a baseline visit (October 1997 to August 2003), followed by wave 2 visit (February 1999 to August 2003), and/or wave 3 visit (May 2004 to March 2007). The study inclusion criteria were men ages 40-81 years, who participated at baseline visit, who had blood collected at baseline visit and at wave 2 and/or wave 3 visits, who were not diagnosed with prostate cancer by prostate biopsy at the baseline visit, and signed an informed consent approved by the Institutional Review Boards of the Tobago Division of Health and Social Services and the University of Pittsburgh.

At each study visit, demographic information and medical history were ascertained, serum specimen was collected, and DRE was performed by a Tobago physician. Study participant's demographic information and medical history questionnaires were mailed to the University of Pittsburgh. Serum specimens were stored in a -20°C freezer at the Tobago Health Studies office in Scarborough, Tobago and later shipped and stored in a -80°C freezer at the University of Pittsburgh. The study participants' serum specimens were used for serum PSA and HHV-8 testing.

In this cohort, the Tobago Prostate Cancer Screening Survey database contained information on 3,380 study participants (Figure 15). Out of these unique study identifications, 2,688 men were considered prostate cancer-free (no diagnosis of prostate cancer) at baseline visit based on serum prostate specific (PSA) level of < 4 ng/mL, normal or minimally abnormal digital rectal examination (DRE) result, and/or no confirmed prostate cancer biopsy diagnosis at baseline screening. Among the men at risk for incident prostate cancer, 2,002 men were identified in the Tobago Prostate Cancer Screening biological

database as having serum collected at baseline visit and at a wave 2 visit and/or wave 3 visit. Of these 2,002 men, a simple random sample of 501 men was selected to investigate the natural history of HHV-8 infection among Tobago men of African descent.

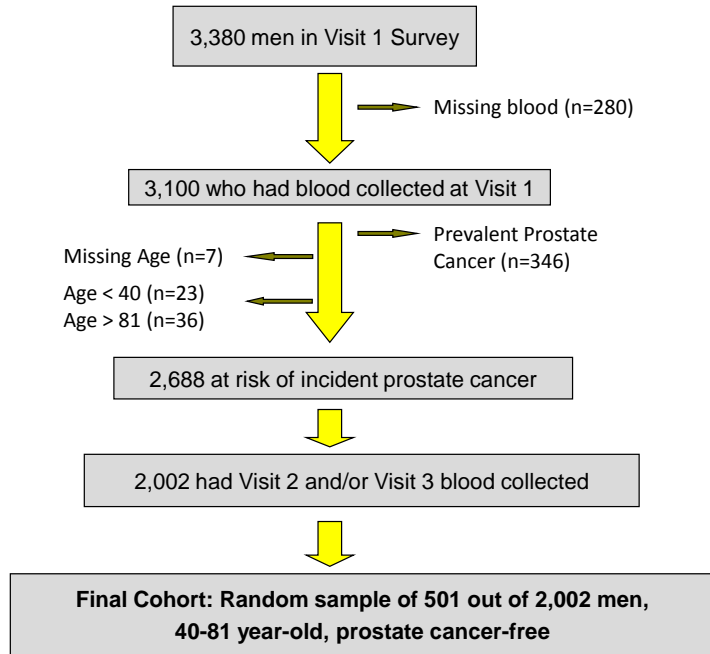


Figure 15. Flowchart of Eligibility Criteria of Tobago Men at Risk for Incident Prostate Cancer

5.3.2 Laboratory Methods

Serum specimens were tested for antibodies against HHV-8 lytic antigens with a modified HHV-8 monoclonal antibody-enhanced immunofluorescence assay (mIFA), as described elsewhere¹³, using the BCBL-1 cells that contain a doxycycline-inducible RTA gene³⁶⁸. The cutoff value for a HHV-8 seropositive result was specific fluorescence at dilution of 1:100. For each mIFA run, known HHV-8 positive and negative sera were included; and, for each participant, the serum specimens for each of his study visits were tested on the same IFA slide. Each serum specimen was tested in duplicate on separate

days in which the slide order was different on these 2 testing days. If there were disagreements in HHV-8 test results on the 2 different lab days, a 3rd lab day run was performed to determine the HHV-8 sero-status of the specimen. All serum specimens that were analyzed by mIFA were assessed microscopically in a blinded fashion by the same reader. Laboratory analysis used only frozen serum specimens thawed once and never re-frozen.

5.3.3 Data Analysis

Baseline characteristics listed in Tables 14 and 15 were recorded and compared in the following groups using Pearson's chi-square, Fisher's exact, or Mann-Whitney tests: 1) men included the sampling scheme in comparison to men excluded from the sampling scheme, and 2) study participants with a baseline HHV-8 test result in comparison to study participants with no baseline HHV-8 test result. The frequency of HHV-8 seropositivity at baseline, wave 2, and wave 3 visits was examined, and stratified by the date of laboratory analysis (\leq April 6, 2008 and $>$ April 6, 2008). The lab stratification was performed in order to examine possible effects from performance drift manifesting on April 6 as a change in split sample test-retest reproducibility. Age-specific seropositivity was also examined across age groups (40-49, 50-59, 60-69, 70-81 years). The Pearson's chi-square, Fisher's exact, or Mann-Whitney tests were also used to examine whether there were any significant associations between baseline HHV-8 seropositivity and baseline characteristics. Logistic regression was used to see whether there was a difference in baseline HHV-8 seropositivity among men who had blood collected at every study visit (baseline, wave 2, and wave 3 visits) in comparison to men who had blood collected at only two study visits (baseline and wave 2 visits, baseline and wave 3 visits).

HHV-8 seroconversion was defined as a HHV-8 seronegative specimen at baseline visit that became HHV-8 seropositive at wave 2 or wave 3 visit. HHV-8 seroreversion was defined as a HHV-8 seropositive specimen at baseline visit that became HHV-8 seronegative at wave 2 or wave 3 visit. The HHV-8 seroconversion and seroreversion rates were calculated as the incidence rate per 100 person-years.

The 95% confidence interval of HHV-8 seroconversion and seroreversion rates were estimated using the Poisson distribution. The overall HHV-8 seroconversion and seroreversion rate was examined and stratified by the date of laboratory analysis. Pearson's chi-square, Fisher's exact, or Mann-Whitney tests were used to examine whether there were any differences in baseline characteristics between the HHV-8 seroconverters and persistent HHV-8 seronegative men and between HHV-8 seroreverters and persistent HHV-8 seropositive men.

For the non-HHV-8 seroconverters and non-HHV-8 seroreverters, the follow-up time in this cohort was considered as the interval time between the first date of serum specimen collected to the last date of serum specimen collected, wave 2 or wave 3 visit. For HHV-8 seroconverters, the follow-up time was the time elapsed from the first serum specimen's collection date to the midpoint in time between the last seronegative specimen's collection date and the first seropositive specimen's collection date. For HHV-8 seroreverters, the follow-up time was the time elapsed from the first serum specimen's collection date to the midpoint in time between the last seropositive specimen's collection date and the first seronegative specimen's collection date.

The frequency of persistent HHV-8 seropositivity, defined as HHV-8 seropositive at baseline visit to wave 2 and/or wave 3, was examined among the Tobago men. The Kaplan-Meier approach was used to examine the seroreversion event-free survival to estimate persistent seropositivity at different time points.

All data analyses were conducted at alpha level 0.05 in SPSS 15.0 for Windows.

5.4 RESULTS

5.4.1 Baseline Characteristics

There were significant differences in baseline characteristics between Tobago men included in the sampling scheme (n = 2002) in comparison to men excluded from the sampling scheme (n = 686). Men who were not part of the sampling scheme were significantly older in age, had a higher serum PSA, had a higher frequency of abnormal DRE, had a lower frequency of married/ever married, and a higher frequency of ever smoke cigarettes > 6 months (p-values < 0.03) (Table 14).

Table 14. Comparison of Baseline Characteristics of Tobago Men Excluded and Included in the Sampling Scheme

	Excluded from the Sampling Scheme (N=686)	Included in the Sampling Scheme (N=2002)	P-value
Median Age (in years)	54 years	52 years	.005
Median Serum PSA (ng/mL)	1.1 ng.mL (n=675)	1.1 ng/mL (n=1978)	.015
Abnormal DRE	20.4% (n=108/529)	15.9% (n=284/1786)	.016
Married/Ever Married	79.8% (n=541/678)	83.4% (n=1660/1990)	.032
≤ 11 years of education	72.9% (n=496/680)	74.0% (n=1471/1989)	.604
Ever had Gonorrhoea	21.9% (n=146/667)	20.6% (n=403/1954)	.488
Ever had Syphilis	3.7% (n=24/643)	4.5% (n=86/1897)	.389
Ever smoke cigarettes > 6 months	49.9% (n=340/682)	39.8% (n=792/1989)	.000
Diagnosed with Prostatitis	1.4% (n=9/651)	1.5% (n=28/1893)	.859
Diagnosed with Benign Prostatic Hyperplasia (BPH)	6.6% (n=44/670)	7.4% (n=145/1951)	.455

Note: PSA = prostate specific antigen, DRE = digital rectal examination, N and n = the number of study participants

Of the 501 prostate cancer-free African-Caribbean men in Tobago, a baseline HHV-8 sero-status result was not missing on 415 men (82.8% of the study population); eight of these men had a pending HHV-8 test result wave 2 visit or wave 3 visit. When baseline study characteristics were compared between men with non-missing and missing baseline HHV-8 test results, there was a significant difference in median age, gonorrhoea diagnosis, and benign prostatic hyperplasia (BPH) (p-values < 0.03); however, no significant difference was found with serum PSA level, marital status, level of education, DRE, syphilis diagnosis, ever smoke cigarettes > 6 months, and prostatitis (p-values > 0.1) (Table 15). As for the duration of follow-up time in the study cohort, a median follow-up time of 5.14 years (range: 0.42 to 9.18 years) and 5.30 years (range: 1.10 to 7.67 years) was observed among the study participants with a non-missing and missing baseline test results, respectively; however, these follow-up times were not significantly different (p-value = 0.602) (Table 15). Therefore, the final analysis included 407 study participants with non-missing HHV-8 test results at baseline and at every subsequent wave attended.

Table 15. Comparison of Baseline Characteristics of Tobago Men who had a Tested or Not Tested

Serum Specimen at Baseline Visit

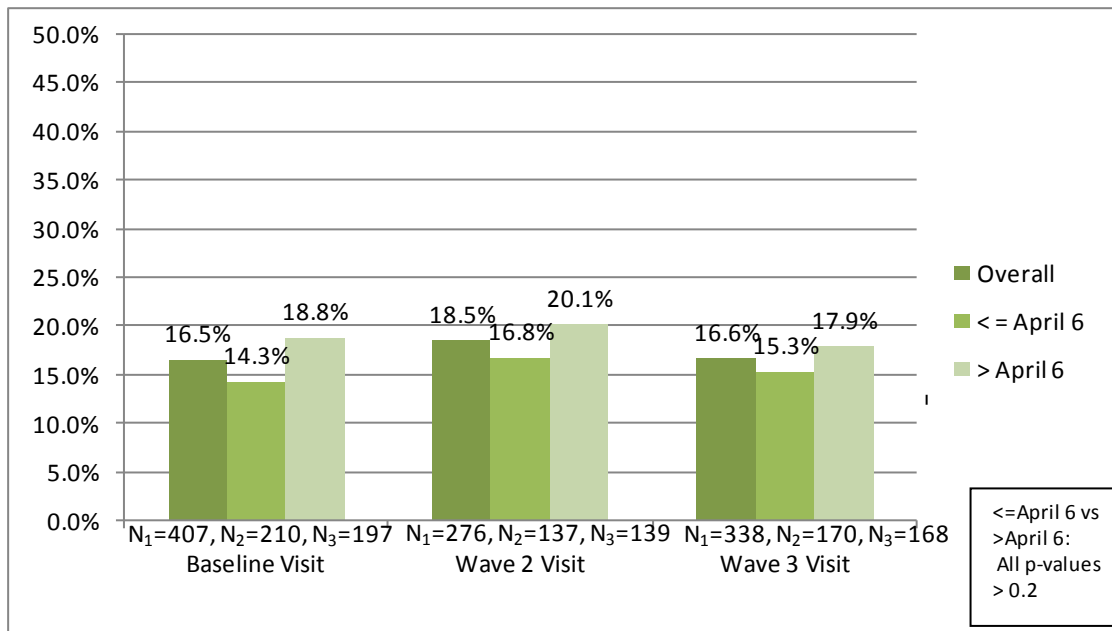
	Tested Serum Sample (N=415)	Not Tested Serum Sample (N=86)	P-value
Median Years of Follow-up (range)	5.14 years (0.42 to 9.18 years)	5.30 years (1.10 to 7.67 years)	.602
Median Age (in years)	52 years	57.5 years	.009
Median Serum PSA (ng/mL)	1.0 ng/mL	1.3 ng/mL	.108
Abnormal DRE	14.9% (n=54/363)	17.9% (n=14/78)	.495
Married/Ever Married	81.9% (n=339/414)	87.2% (n=75/86)	.234
≤ 11 years of education	78.4% (n=323/412)	72.1% (n=62/86)	.204
Ever had Gonorrhoea	18.0% (n=72/401)	30.6% (n=26/85)	.008
Ever had Syphilis	5.5% (n=22/397)	3.8% (n=3/80)	.783
Ever smoke cigarettes > 6 months	41.9% (n=173/413)	36.0% (n=31/86)	.316
Diagnosed with Prostatitis	1.3% (n=5/390)	1.2% (n=1/84)	1.00
Diagnosed with Benign Prostatic Hyperplasia (BPH)	6.2% (n=25/406)	12.9% (n=11/85)	.029

Note: PSA = prostate specific antigen, DRE = digital rectal examination, N and n = the number of study participants

5.4.2 HHV-8 Seropositivity Rates

Among 407 eligible study participants at risk for incident prostate cancer, HHV-8 seropositivity was 16.5% (95% confidence interval [C.I.], 13.0% - 20.4%), 18.4% (95% C.I., 14.1% - 23.6%), and 16.5% (95% C.I., 12.8% - 21.0%) at baseline visit, wave 2 visit, and wave 3 visit, respectively (Figure 16). HHV-8 seropositivity at each study visit did not vary significantly according to the date of laboratory analysis (p-values > 0.2) (Figure 16). The baseline age-specific HHV-8 seropositivity rates were the following: 6.9% (n = 12/175) in men 40-49 years, 16.1% (n = 18/112) in men 50-59 years, 28.0% (n = 23/82) in men 60-69 years, and 36.8% (n = 14/38) in men 70-81 years (p-value = 0.000). As for baseline

characteristics, there were no significant differences between HHV-8 seropositive and seronegative men (p-values > 0.2), except for age and serum PSA levels (p-values < 0.01) (Table 16).



Note : N₁ = the total number of study participants, N₂ = the number of study participants who had the serum specimens tested before or on April 6, 2008, N₃ = the number of study participants who had the serum specimens tested before after April 6, 2008

Figure 16. HHV-8 Seropositivity Rates, Stratified by Laboratory Analysis, among Tobago Men

(N = 407)

Table 16. Comparison of Baseline Characteristics between HHV-8 Seropositive Men (N = 67) and HHV-8 Seronegative Men (N = 340) in Tobago

	HHV-8 Seropositive Men (N=67)	HHV-8 Seronegative Men (N=340)	P-value
Median Years of Follow-up (range)	5.35 years (1.40-8.77 years)	5.00 years (.42-9.18 years)	.030
Median Age (in years)	60 years	50 years	.000
Median Serum PSA (ng/mL)	1.35 ng/mL	1.00 ng/mL	.006
Abnormal DRE	19.7% (n=12/61)	13.9% (n=41/296)	.244
Married/Ever Married	86.6% (n=58/67)	80.5% (n=273/339)	.245
≤ 11 years of education	76.1% (n=51/67)	78.6% (n=265/337)	.649
Ever had Gonorrhea	19.7% (n=13/66)	17.4% (n=57/328)	.653
Ever had Syphilis	3.2% (n=2/63)	5.5% (n=18/326)	.754
Ever smoke cigarettes > 6 months	31.3% (n=21/67)	43.5% (n=147/338)	.065
Diagnosed with Prostatitis	1.6% (n=1/63)	1.3% (n=4/320)	.595
Diagnosed with Benign Prostatic Hyperplasia (BPH)	10.8% (n=7/65)	5.4% (n=18/334)	.102

Note: PSA = prostate specific antigen, DRE = digital rectal examination, N and n = the number of study participants

There were 69, 131, and 207 men who had blood collected at baseline and wave 2 visits, baseline and wave 3 visits, and baseline, wave 2, and wave 3 visits, respectively. Their mean ages were 56.7 years, 52.4 years, and 53.6 years, respectively. There was a significant association between baseline HHV-8 seropositivity and the number of study visits men had blood collected (p-value = 0.05) (Table 17). Men who had blood collected at baseline and wave 3 visits had a lower frequency of HHV-8 seropositivity (10.7%) than men who had blood collected at all study visits (20.8%) (age & the date laboratory analysis adjusted Odds ratio [O.R.] = 0.482, 95% C.I., 0.247 – 0.942). Men who had blood collected at baseline and wave 2 visits also had a lower frequency of HHV-8 seropositivity (14.5%) than men who had blood

collected at all study visits (age & the date of laboratory analysis adjusted O.R. = 0.507, 95% C.I., 0.229 – 1.120).

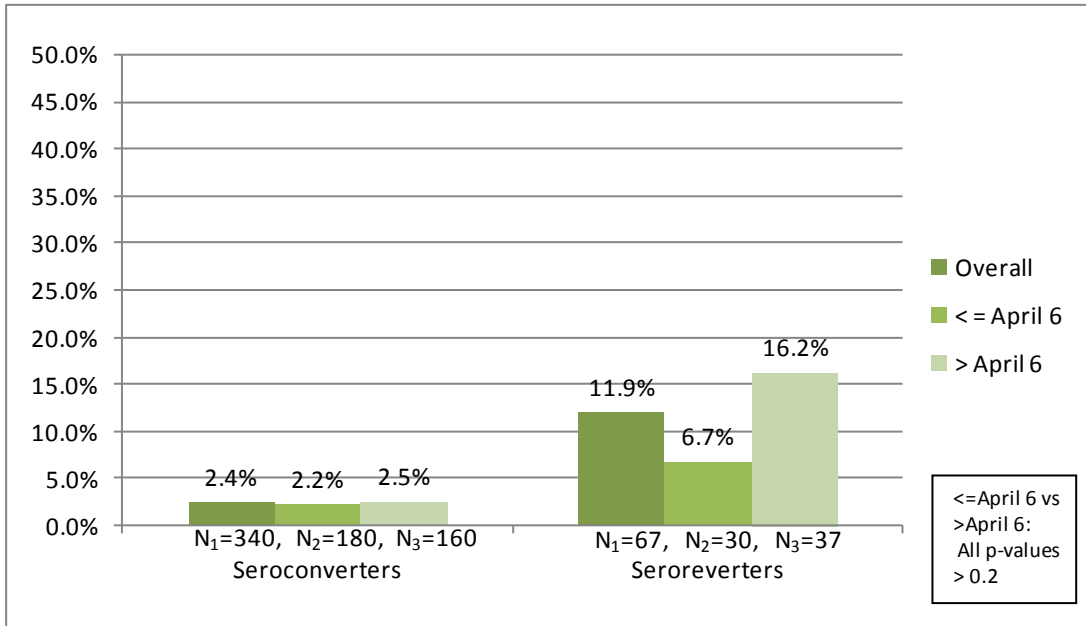
Table 17. The Association between the Number of Study Visits Blood Collected and Baseline HHV-8 Seropositivity among Tobago Men

	Percentage of HHV-8 Seropositivity	*Unadjusted Odds Ratio (95% confidence interval)	**Age-adjusted Odds Ratio (95% confidence interval)	*Age- and Lab-adjusted Odds Ratio (95% confidence interval)
Baseline and Visit 2 Only	14.5% (n=10/69)	.646 (.305 – 1.368)	.490 (.222 – 1.079)	.507 (.229 – 1.120)
Baseline and Visit 3 Only	10.7% (n=14/131)	.456 (.239 - .873)	.469 (.241 - .915)	.482 (.247 - .942)
All Study Visits	20.8% (n=43/207)	Reference	Reference	Reference

Note: * Wald's p-value = 0.05, ** Wald's p-value = 0.037, n = the number of study participants, Age is continuous, Lab-adjusted is stratified by the date of laboratory analysis (<= April 6, 2008 versus > April 6, 2008)

5.4.3 HHV-8 Seroconversion and Seroreversion

Of the 340 study participants who were HHV-8 seronegative at baseline visit, there were 8 HHV-8 seroconversions per 1599.22 person-years (incidence rate: 0.5 per 100 person-years, 95% C.I., 0.22-0.99). When examined by the date of laboratory analysis, there was no significant difference in the proportion of seroconverters identified on <= April 6, 2008 and > April 6, 2008 (p-value = 1.0) (Figure 17). When HHV-8 seroconverters and persistent HHV-8 seronegative men were compared, there were no significant differences for most of the baseline studied variables (p-values > 0.2), except for marital status and median years of follow-up (Table 18). Persistent HHV-8 seronegative men had a higher frequency of being married or ever married (81.3%) than the HHV-8 seroconverters (50%) (p-value = 0.049).



Note: N_1 = the total number of study participants, N_2 = the number of study participants who had the serum specimens tested before or on April 6, 2008, N_3 = the number of study participants who had the serum specimens tested before after April 6, 2008

Figure 17. Overall HHV-8 Seroconversion (N = 340) and Seroreversion (N = 67) Rates, Stratified by the Date of Laboratory Analysis among Tobago Men

Table 18. Comparison of Baseline Characteristics between HHV-8 Seroconverters (N = 8) and Persistent HHV-8 Seronegative Men (N = 332)

	HHV-8 Seroconverters (N=8)	Persistent HHV-8 Seronegative Men (N=332)	P-value
Median Years of Follow-up* (range)	3.15 years (.60-5.48 years)	5.00 years (.42-9.18 years)	.007
Median Age (in years)	56 years	50 years	.299
Median Serum PSA (ng/mL)	2.0 ng/mL	1.0 ng/mL	.317
Abnormal DRE	0% (n=0/7)	14.2% (n=41/289)	.599
Married/Ever Married	50% (n=4/8)	81.3% (n=269/331)	.049
Ever had Gonorrhea	0% (n=0/8)	17.8% (n=57/320)	.359
Ever had Syphilis	0% (n=0/8)	5.6% (n=18/319)	1.00
Diagnosed with Prostatitis	12.5% (n=1/8)	1.0% (n=3/312)	.097
Diagnosed with Benign Prostatic Hyperplasia (BPH)	12.5% (n=1/8)	5.2% (n=17/326)	.361

*Note: PSA = prostate specific antigen, DRE = digital rectal examination, N and n = the number of study participants, * = based on the definition of the follow-up time for HHV-8 seroconverters*

As for HHV-8 seroreversion, there were 8 HHV-8 seroreversions per 317.43 person-years (incidence rate: 2.52 per 100 person-years, 95% C.I., 1.09-4.96). When examined by the date of laboratory analysis, a higher frequency of seroreverters were identified on > April 6, 2008 in comparison to <= April 6, 2008; however, this difference was not statistically significant (p-value = 0.281) (Figure 17). When HHV-8 seroreverters and persistent HHV-8 seropositive men were compared, there were no significant differences for most of the baseline studied variables (p-values > 0.2), except for age and median years of follow-up (Table 19). Persistent HHV-8 seropositive men had a higher median age (61 years) than the seroconverters (48 years) (p-value = 0.021).

Table 19. Comparison of Baseline Characteristics between HHV-8 Seroreverters (N = 8) and Persistent HHV-8 Seropositive Men (N = 59)

	HHV-8 Seroreverters (N=8)	Persistent HHV-8 Seropositive Men (N=59)	P-value
Median Years of Follow-up* (range)	1.79 years (.55-5.46 years)	5.34 years (1.40-8.77 years)	.001
Median Age (in years)	48 years	61 years	.021
Median Serum PSA (ng/mL)	1.4 ng/mL	1.3 ng/mL	.687
Abnormal DRE	0% (n=0/7)	22.2% (n=12/54)	.327
Married/Ever Married	100% (n=8/8)	84.7% (n=50/59)	.585
Ever had Gonorrhea	12.5% (n=1/8)	20.7% (n=12/58)	1.00
Ever had Syphilis	0% (n=0/7)	3.6% (n=2/56)	1.00
Diagnosed with Prostatitis	0% (n=0/8)	1.8% (n=1/55)	1.00
Diagnosed with Benign Prostatic Hyperplasia (BPH)	0% (n=0/8)	12.3% (n=7/57)	.583

*Note: PSA = prostate specific antigen, DRE = digital rectal examination, N and n = the number of study participants, * = based on the definition of the follow-up time for HHV-8 seroconverters*

There was one participant who seroconverted from baseline visit to wave 2 visit, then seroreverted from wave 2 visit to wave 3 visit. Based on the HHV-8 seroconversion definition, this individual was classified as a HHV-8 seroconverter.

Among seropositive men at baseline visit, the frequency of persistent seropositivity was 88.1% (95% C.I., 77.8% - 94.7%). When HHV-8 seroreversion-free survival was examined, the rate of persistent seropositivity was 0.856 (standard error = 0.05) at 5.5 years after baseline (Figure 18).

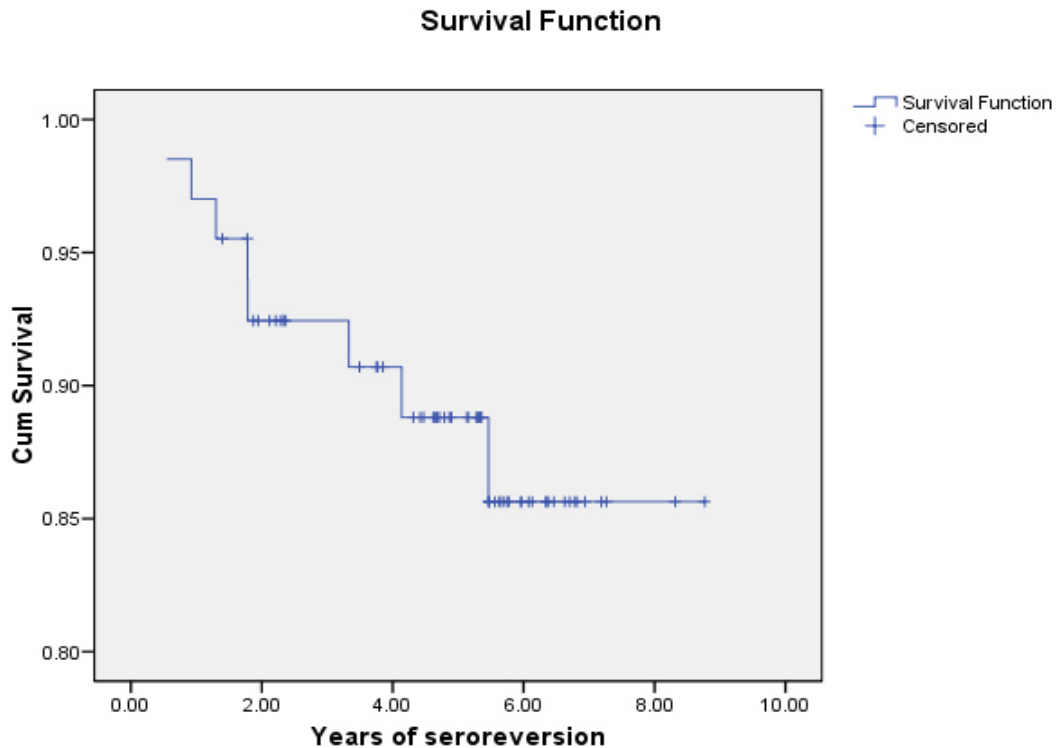


Figure 18. HHV-8 Seroreversion-free Survival among Persistent Seropositive Tobago Men at Risk for Incident Prostate Cancer (N = 67)

5.5 DISCUSSION

In this present study, a HHV-8 seroprevalence of 16.5%, 18.5%, and 16.6% against HHV-8 lytic antigens was detected at baseline, wave 2, and wave 3 visits, respectively, among African-Caribbean Tobago men at risk for incident prostate cancer. These moderate HHV-8 seropositivity rates in Tobago are consistent to rates found in Mediterranean countries^{5-7, 142, 146} and other Caribbean countries such as the Dominican Republic¹⁶² and Cuba¹⁶¹. Unlike the Mediterranean where KS is prevalent, no KS cases have been reported in Tobago since the establishment of Trinidad/Tobago cancer registry in 1994^{10, 11}. The reasons for the lack of reported KS cases in Tobago are not yet known.

In Tobago, a case-control study conducted among African-Caribbean men in the Tobago Prostate Cancer Screening Survey found a higher HHV-8 seropositivity rate of 39.9% and 22.9% in men with and without prostate cancer, respectively⁹, in comparison to seropositivity rates reported in the present study. The reason for the higher seropositivity among men without prostate cancer in the case-control study is that these men were older (mean age, 67 years) than the men in the present study (mean age, 54 years). Studies have shown that HHV-8 seropositivity increases with age^{3, 5, 138, 145, 146, 153, 157}. In the present study, baseline HHV-8 seropositivity increased from 6.9% in men 40-49 years to 36.8 % in men 70-81 years. Therefore, age may be a factor for the HHV-8 seropositivity difference between the Tobago men in the present study and the Tobago men in Hoffman et al. study.

A low HHV-8 seroconversion incidence rate of 0.5 per 100 person-years was found among Tobago men, aged 40-81 years. This seroconversion rate is comparable to incidence rates found among drug users (0.7 per 100 person-years) who were participants in the Amsterdam Cohort Study³⁵⁸ and Greek hemodialysis patients (0.28 per 100 person-years) who were participants in the Multicenter Hemodialysis Cohort on Viral Hepatitis³⁵³. In populations at high risk for sexually transmitted diseases (STDs), higher HHV-8 seroconversion rates were reported among HIV-negative homosexual men (2.6 - 3.8 per 100 person-years)^{358, 359}, HIV-infected men (6.2 per 100 person-years)³⁵⁸, and STD clinic attendees (3.2 per 100 person-years)³⁶⁰. As for other study populations, several studies have examined the frequency of HHV-8 seroconversion among post-organ transplant patients, with frequencies ranging from 2% to 15%^{13, 362-366}. Possible modes of HHV-8 transmission, sexually and/or non-sexually (i.e., HHV-8 seropositive organ donor, contaminated blood product, saliva exchange, etc.), may explain the seroconversion rates observed in populations at high risk for STDs (which includes HIV/AIDS infected individuals) as well as seroconversion rates found in organ transplant recipients. As for the Tobago population, reasons for HHV-8 seroconversion among Tobago men, aged 40-81 years, are less clear than the previously described study populations. There was no significant difference in STDs diagnosis such as syphilis and gonorrhea between HHV-8 seroconverters and persistent seronegative men; manner of fact, none of the seroconverters reported that they were ever diagnosed with these diseases. Therefore, sexual transmission

may not be the mode of acquiring HHV-8 infection for HHV-8 seroconverters in this study population. Nevertheless, a low seroconversion rate in men aged ≥ 40 years, and a moderate seropositivity rate detected at three time points in this 9-year cohort suggest that HHV-8 infection is acquired at younger ages, ≤ 39 years, in Tobago.

As for HHV-8 seroreversion, an incidence rate of 2.52 per 100 person-years was found among the Tobago men. This seroreversion rate was lower than the rate reported in hemodialysis patients in Greece, 16.4 per 100 person-years³⁵³. HHV-8 seroreversions were also reported in post-transplant patients (0.4-55%)^{168, 361-363} and HIV-infected individuals (5%-82%)^{352, 357}. In Tobago, the frequency of HHV-8 seroreversion was 11.9%; this frequency was similar to ones observed in studies conducted in post-organ transplant and HIV-infected populations. The fact that HHV-8 seroreversions are observed in high-risk KS populations suggest the following: low sensitivity of the serologic assay, restoration of participant's immune function, or poor quality of participant's serum specimen^{352, 361}. For the presumably healthy men in the present study, the observation of HHV-8 seroreversion may indicate that they had low HHV-8 antibody titers at baseline visit which became undetectable by the serological assay at a subsequent study visit. Therefore, misclassification of HHV-8 seropositivity may be occurring among persons with low antibody titers. The serological assay used in the present study, mIFA, is reported to have a sensitivity of 89.9% and specificity of 97.5%, respectively¹⁴. However, evidence of HHV-8 seropositive individuals testing seronegative at a follow-up visit for a "life-long" infection indicates that a more sensitive assay is needed. In addition, all HHV-8 seroreversion occurred within 5.5 years after baseline visit, which this action suggests that these men probably had low antibody titers at baseline visit that became undetectable within 5.5 years after the baseline visit. The moderate HHV-8 seroreversion rate among seropositive men in Tobago reflected the high persistent seropositivity found in this present study (88.1%).

Even though this Tobago cohort allowed the examination of HHV-8 seropositivity rates at three different time points and the patterns of change in HHV-8 sero-status over a 9 year period, there were some limitations to this study. First, selection bias may have been introduced in the study. There were significant differences in baseline characteristics between Tobago men included and excluded in the

sampling scheme. Men excluded in the sampling scheme were individuals who did not have serum specimens available for HHV-8 testing for more than one study visit. This unavailability may explain why these men were older in age, had a higher frequency of abnormal DRE, had a lower frequency of married or ever been married, and a higher frequency of ever smoke cigarettes > 6 months. Also, baseline HHV-8 sero-status was missing for 17.2% of the study population (n = 501). Higher median age, gonorrhea diagnosis, and benign prostatic hyperplasia (BPH) were found to be statistically different between men with missing and non-missing baseline HHV-8 test results. However, there were no differences in other baseline characteristics as well as the duration of follow-up time in the cohort.

Another limitation in the present study was that 49.1% (n = 200/407) of the men did not have blood collected at all three study visits. Men who had blood collected at baseline and wave 3 visits only and baseline and wave 2 visits only had lower HHV-8 seropositivity than men who had blood collected at all study visits. Even after adjusting for age, there was still an inverse association in HHV-8 seropositivity among the men who had blood collected at two study visits in comparison to men who had blood collected at all study visits. The reason for the higher seropositivity in men who had blood collected at all study visits is not known.

Even though the Tobago men in the present study were assumed to be healthy at baseline visit, their immune status was not known. Higher HHV-8 seropositivity rates have been found among immunocompromised individuals, for example, HIV/AIDS patients, than healthy individuals^{126, 151, 172, 177}; therefore, information on the participant's immune status may help explain HHV-8 seropositivity rates in Tobago. A fourth limitation in the present study is the variability of serological assay, mIFA, used to test serum specimens for HHV-8 seropositivity. The mIFA is a biological assay that may have variability in detecting antibodies against HHV-8 lytic antigens. To control for possible variability in the lab assay, serum specimens were tested on two separate days, in a blinded fashion, in order to test-retest reproducibility. All of the men's study visit serum specimens were tested on the same mIFA slide. In the present study, stratified analysis by laboratory date was used to compare days in which a consistent kappa statistic of > 0.85 was observed (before and on April 6, 2008) in comparison to days in which the kappa

statistic ranged from 0.54 to 1.00 (after April 6, 2008). Based on the stratified analysis, there was no significant difference in HHV-8 seropositivity by date of laboratory analysis. Finally, levels of HHV-8 antibody titers were not examined on HHV-8 seropositive men. HHV-8 antibody titers would have provided information on whether HHV-8 reactivations have occurred among Tobago men and whether low antibody titers were the reason for HHV-8 seroreversion. Also, antibody titers may explain the participant (noted as a seroconverters) who seroconverted from baseline visit to wave 2 visit, then seroreverted from wave 2 visit to wave 3 visit. In this scenario, antibody titers would have provided more information on the sensitivity of the assay.

To our knowledge, this present study was the first to investigate the natural history of HHV-8 infection in a presumably healthy cohort over a 9 year period. In this study, a low HHV-8 seroconversion rate and a moderate HHV-8 seroreversion rate were found. In addition, a moderate HHV-8 seropositivity rate was detected at each study visit. Further studies are needed to examine the natural history of HHV-8 seropositivity among men who develop prostate cancer and possible risk factors for acquiring HHV-8 infection in Tobago.

**6.0 PAPER 3: HUMAN HERPESVIRUS 8 SEROPOSITIVITY IN RELATION TO
INCIDENT PROSTATE CANCER AMONG MEN OF AFRICAN DESCENT IN
TOBAGO**

(Will submit to a peer-reviewed journal)

6.1 ABSTRACT

Human herpesvirus 8 (HHV-8) is the causal agent of Kaposi's sarcoma (KS) and primary effusion lymphoma and is associated with multicentric Castleman's disease. In the Caribbean island of Tobago, HHV-8 seropositivity has been associated with screen-detected prevalent prostate cancer among African-Caribbean men in the Tobago Prostate Cancer Screening Survey (Odds ratio [O.R.] = 2.24; 95% confidence interval [C.I.], 1.29-3.90). To further understand the association between prostate cancer and HHV-8 infection, a case-control study was conducted to examine whether HHV-8 seropositivity before prostate cancer diagnosis was associated with screen-detected incident prostate cancer in African-Caribbean men in the Tobago Prostate Cancer Screening Survey.

A case-cohort study was conducted among a random sample of 501 out of 2,002 Tobago men at risk for prostate cancer (the sub-cohort controls, including 30 screen-detected incident prostate cancer cases) and all 116 screen-detected incident prostate cancer men (the cases) who participated in the population-based Tobago Prostate Cancer Screening Survey at a baseline visit and wave 2 visit (2nd recruitment time period), and/or wave 3 visit (3rd recruitment time period). The inclusion criteria were men, ages 40-81 years, who had a serum specimen at baseline visit and at wave 2 and/or wave 3 visit, and were not screened positive for prostate cancer at baseline visit. Demographic information and medical history were collected, and serum prostate specific antigen (PSA) levels and digital rectal examination (DRE) were recorded. Serum specimens were tested for HHV-8 seropositivity by monoclonal antibody-enhanced immunofluorescence assay (mIFA).

The frequency of HHV-8 seropositivity at each study visit was examined and compared between incident prostate cancer cases in the sub-cohort and incident prostate cancer cases not in the sub-cohort and between incident prostate cancer cases and prostate cancer-free men in the sub-cohort. Cox proportional hazards modeling for the case-cohort design was used to calculate relative risks (Hazard ratios [H.R.]) in order to determine whether HHV-8 seropositivity was associated with screen-detected incident prostate cancer, after controlling for possible confounders.

There were 81.1% (n = 382/471) prostate cancer-free men in the sub-cohort, 83.3% incident prostate cancer cases in the sub-cohort (n = 25/30), and 75.6% (n = 65/86) incident prostate cancer cases not in the sub-cohort included in the final analysis. At the baseline, wave 2, and wave 3 visits, HHV-8 seropositivity levels were the following: 16.8%, 19.3%, and 16.9% among prostate cancer-free men, respectively; 12.0%, 9.1%, and 11.1% among incident prostate cancer cases in the sub-cohort, respectively; and, 20.0%, 23.1%, and 19.6% among incident prostate cancer cases not in the sub-cohort, respectively. HHV-8 seropositivity among these two comparison groups did not differ at any study visit (p-values > 0.3). There were inverse associations between baseline HHV-8 seropositivity and screen-detected incident prostate cancer when age and baseline prostate cancer screening results (abnormal DRE or serum PSA \geq 4 ng/mL versus normal/minor abnormal DRE, serum PSA < 4 ng/mL, or no screening results) (H.R. = 0.454, 95% C.I., 0.221 – 0.933) and age only (H.R. = 0.517, 95% C.I., 0.262 – 1.020) were considered.

In this up to 9-year cohort of Tobago men, inverse associations between HHV-8 seropositivity and screen-detected incident prostate cancer suggest that HHV-8 seropositivity may not be related to prostate cancer incidence. Further studies are needed to examine the difference in the relationship with HHV-8 seropositivity between screen-detected incident prostate cancer and screen-detected prevalent prostate cancer among Tobago men of African descent.

6.2 INTRODUCTION

Prostate cancer is a global health issue with 679,023 new cases worldwide in 2002¹⁰. It is the 2nd most common cancer diagnosed in men in the United States and the European Union; and, the 4th most common cancer diagnosed in men worldwide²⁶⁰. In the U.S., African-American men are known to have the highest incidence and mortality rate than their white counterparts. Similarly, high rates of prostate cancer have been reported in men of African descent in Tobago, Jamaica, England, and South America^{260, 269-271}. Race, older age, family history, the presence of high-grade prostatic intraepithelial neoplasia (HGPIN), and possibly diet are prostate cancer risk factors. However, other factors like infectious agents may play a role in prostate carcinogenesis since little is known about the etiology of prostate cancer.

Observational studies have found positive associations between infectious agents and prostate cancer^{9, 331, 338, 339}. In addition, viral DNA such as human papillomavirus (HPV), human herpes simplex virus (HSV-2), cytomegalovirus (CMV), Epstein-Barr Virus (EBV), and human herpesvirus 8 (HHV-8) have been identified in prostatic tissue^{15, 209, 314, 328-332}. Studies have hypothesized that the presence of infectious agents in the prostate may elicit an inflammatory immune response that leads to prostate cancer^{314, 315, 321}. This link between prostate cancer and inflammation has been supported by epidemiological studies that have observed lower prostate cancer risk among users of anti-inflammatory agents, such as aspirin^{314, 317-320}.

Among HHV-8 infected individuals, HHV-8 may contribute to the inflammatory process in the prostate. HHV-8 has been shown to infect prostatic epithelial cells¹⁵. The expression of HHV-8 proteins (LANA-1, vIL-6, and K8.1), as well as evidence of local inflammation (macrophage/monocyte marker and B-cell marker), have been found in the prostate¹⁶. Therefore, the presence of HHV-8 DNA and proteins in the prostate may contribute to the development of prostate cancer by injuring prostatic epithelial cells, which results in proliferative inflammatory atrophy (PIA)^{314, 321} that leads to the prostate carcinogenesis.

In the Caribbean island of Tobago, Hoffman et al. reported an association between HHV-8 and prevalent screen-detected prostate cancer among men of African descent (Odds ratio [OR] = 2.24; 95% confidence interval [CI], 1.29-3.90)⁹. In a population where an 11% prostate cancer prevalence rate at initial screening was estimated from the population-based longitudinal Tobago Prostate Cancer Screening Survey¹⁷, examining possible relationships between HHV-8 and prostate cancer may explain the high rate of prostate cancer observed in this population. Unlike a previous case-control that examined prevalent screen-detected prostate cancer at the baseline visit in the Tobago Prostate Cancer Screening Survey, the present study examined the association between HHV-8 seropositivity and screen-detected incident prostate cancer at two subsequent visits. Studying possible relationships between HHV-8 seropositivity and prostate cancer may shed light on the biological mechanism of HHV-8 as well as a possible risk factor for prostate cancer.

6.3 METHODS

6.3.1 Study Population

The Tobago Prostate Cancer Screening Survey is an ongoing population-based longitudinal study that is designed to estimate the prevalence and incidence of prostate cancer as well as to study possible risk factors for prostate cancer among men ≥ 40 years of age in Tobago¹⁷. This longitudinal study, which was initiated in 1997, had three study visits: baseline visit (October 1997 to August 2003), wave 2 visit (February 1999 to August 2003), and wave 3 visit (May 2004 to March 2007). At each study visit, demographic information, medical history, and possible prostate cancer risk factors were ascertained for each study participant by questionnaire. In addition, serum specimens were collected; and, a digital rectal examination (DRE) was performed by a Tobago physician. Questionnaires were mailed to the University of Pittsburgh, Department of Epidemiology in Pittsburgh, Pennsylvania. Serum specimens were stored in

a -20°C freezer at the Tobago Health Studies office in Scarborough, Tobago and later shipped and stored in a -80°C freezer at the University of Pittsburgh, Department of Epidemiology. Serum specimens were used for serum prostate specific antigen (PSA) testing and detection of HHV-8 antibodies against HHV-8 lytic antigens.

To screen for prostate cancer, men who had a serum PSA level of ≥ 4 ng/mL and/or an abnormal DRE were referred to a Tobago physician for prostate biopsy. According to a standard protocol, sextant random prostate biopsies were obtained using a trans-rectal ultrasound and spring-loaded, disposable, fine bore needles¹⁷. The prostate biopsies were stored in formalin and shipped at room temperature to the University of Pittsburgh, Department of Epidemiology and were analyzed by pathologists at the University of Pittsburgh Medical Center in Pittsburgh, Pennsylvania.

At baseline visit, information on a total of 3,380 men was recorded in the survey's database. Of these men, 97% reported to be of African descent. For the present study, the inclusion criteria were men, ages 40-81 years, who were prostate cancer-free (no prostate cancer diagnosis by biopsy) at baseline visit, had blood collected at baseline visit and at wave 2 and/or wave 3 visits, and signed an informed consent approved by the Institutional Review Boards of the Tobago Ministry of Health and Social Services and the University of Pittsburgh. The controls, known as the sub-cohort (which included men who developed prostate cancer at wave 2 or 3 visit), were prostate cancer-free men at the baseline visit. The cases were men who had a prostate biopsy-confirmed incident prostate cancer diagnosis at wave 2 visit or wave 3 visit. . A simple random sample of 501 out of 2,002 men with an available baseline visit serum specimen and wave 2 and/or wave 3 visits serum specimens from a total cohort of 2,688 men identified at risk for prostate cancer, as described elsewhere (McDonald AC, paper 2), were classified as the sub-cohort (471 prostate cancer-free men and 30 screen-detected incident prostate cancer cases) from the Tobago Prostate Cancer Screening Survey. A total of 116 incident prostate cancer cases was identified in the Tobago Prostate Cancer Screening Survey; of these men, 86 were not part of the sub-cohort. Sixty-six and fifty men were identified as incident prostate cancer cases at wave 2 visit and wave 3 visit, respectively.

6.3.2 Laboratory Methods

A modified monoclonal enhanced HHV-8 monoclonal antibody-enhanced immunofluorescence assay (mIFA) , as described elsewhere¹³, using the BCBL-1 cells that contain a doxycycline-inducible RTA gene³⁶⁸ was used to test serum for antibodies against HHV-8 lytic antigens. A HHV-8 seropositive result was indicated by specific fluorescence at a dilution $\geq 1:100$. For each mIFA run, known HHV-8 positive and negative sera were included. Also, a serum specimen from each study participant's study visits was tested on the same IFA slide. Each serum specimen was tested in duplicate on separate days. The mIFA slide order of the serum specimens was different on these two testing days. If there were disagreements in HHV-8 test results on the two different lab days, a 3rd lab day run was performed to determine the HHV-8 sero-status of the specimen. Case and control serum specimens were included in each batch. All serum specimens that were analyzed by mIFA were assessed microscopically in a blinded fashion by the same reader. Laboratory analysis used only frozen serum samples thawed once and never re-frozen.

6.3.3 Data Analysis

Pearson's chi-square, Fisher's exact, or Mann-Whitney tests were used to examine differences in baseline characteristics listed in Table 14 between men included in the sampling scheme in comparison to men excluded from the sampling scheme. Also, these tests were used to examine differences in baseline characteristics listed in Table 14 and additional studied variables (median years of follow-up, family history of prostate cancer, ever diagnosed with cancer, have taken ibuprofen in the past 12 months, have taken aspirin in the past 12 months) in the following groups: 1) men with missing and non-missing baseline and wave 2 visit and/or wave 3 visit HHV-8 test results in prostate cancer-free men and all screen-detected incident prostate cancer cases, 2) screen-detected incident prostate cancer cases in the sub-cohort and not in the sub-cohort, 3) and screen-detected incident prostate cancer cases and prostate cancer-free men in the sub-cohort.

The frequency of HHV-8 seropositivity was examined at each study visit and by the date of laboratory analysis (\leq April 6, 2008 and $>$ April 6, 2008). This stratification was performed in order to examine possible effects from the performance drift manifesting on April 6 as a change in split sample test-retest reproducibility. Pearson's chi-square or Fisher's exact test was used to determine if there was a significant difference in HHV-8 seropositivity between screen-detected incident prostate cancer cases in the sub-cohort and screen-detected incident prostate cancer cases not in the sub-cohort and between screen-detected incident prostate cancer cases and prostate cancer-free men in the sub-cohort. Age-specific HHV-8 seropositivity rates were examined at the baseline visit and compared between screen-detected incident prostate cancer cases in the sub-cohort and screen-detected incident prostate cancer cases not in the sub-cohort and between screen-detected incident prostate cancer cases and prostate cancer-free men in the sub-cohort. Logistic regression, adjusted for age (as a continuous variable) and the date of laboratory analysis (\leq April 6, 2008 and $>$ April 6, 2008), was used to see whether there was a difference in baseline HHV-8 seropositivity among prostate cancer-free men and all screen-detected incident prostate cancer cases who had blood collected at all study visits (baseline, wave 2, and wave 3 visits) in comparison to prostate cancer-free men and all screen-detected incident prostate cancer cases who had blood collected at only two study visits (baseline and wave 2 visits, baseline and wave 3 visits).

Markers of prostate cancer (abnormal DRE and serum PSA level ≥ 4 ng/mL) and HHV-8 seropositivity were examined and compared between screen-detected incident prostate cancer cases not in the sub-cohort and screen-detected incident prostate cancer cases in the sub-cohort and between screen-detected incident prostate cancer cases and prostate cancer-free men in the sub-cohort at each study visit. Fisher's exact test was used to determine whether there was a difference in HHV-8 seropositivity in these two comparison groups.

Cox proportional hazards modeling for the case-cohort design, the un-weighted method³⁶⁹, was used to calculate relative risks (Hazard ratios [H.R.]) in order to examine the following associations: 1) the association between baseline HHV-8 seropositivity and screen-detected incident prostate cancer, adjusted for age (as a continuous variable) and adjusted for age (as a continuous variable) and baseline

screening results (serum PSA \geq 4 ng/mL or abnormal DRE results in comparison to normal serum PSA, normal DRE results, or no screening results); 2) the association between baseline HHV-8 seropositivity and screen-detected incident prostate cancer in baseline screen-negative men, adjusted for age (as a continuous variable); and, 3) the association between baseline HHV-8 seropositivity and screen-detected incident prostate cancer diagnosed at wave 2 visit and wave 3 visit, separately, adjusted for age (as a continuous variable) and baseline screening results.

Stratified analyses were conducted using Cox proportional hazards modeling for the case-cohort design to examine the following associations between baseline HHV-8 seropositivity and screen-detected incident prostate cancer, adjusted for age (as a continuous variable): 1) baseline screening results (serum PSA \geq 4 ng/mL or abnormal DRE results [positive] in comparison to normal serum PSA and/or DRE results or no screening results [negative]); 2) median age ($<$ the median age in comparison to \geq the median age); 3) the date of laboratory analysis (\leq April 6, 2008 in comparison to $>$ April 6, 2008); and, 4) benign prostatic hyperplasia (BPH) or prostatitis (have BPH or prostatitis in comparison to do not have BPH or prostatitis). For each stratified analysis, an interaction term was examined to determine whether there was a significant difference between the two regression models.

The follow-up time for the screen-detected incident prostate cancer cases was from the date of the baseline visit blood collection to the visit blood collection date in which the cancer was diagnosed or detected. The follow-up time for the prostate cancer-free men was the date of the baseline visit blood collection to the time these men were censored, which was either the time of their last study visit or wave 3 visit.

Data analyses were conducted at alpha level 0.05 in SPSS 15.0 for Windows and SAS version 9.1.

6.4 RESULTS

6.4.1 Baseline Characteristics

Between Tobago men included in the sampling scheme ($n = 2002$) in comparison to men excluded from the sampling scheme ($n = 686$), there were significant differences in baseline characteristics (Table 14). Men who were not part of the sampling scheme were significantly older in age, had higher serum PSA levels, had a higher frequency of abnormal DRE, had a lower frequency of married/ever married, and a higher frequency of ever smoking cigarettes > 6 months (p -values < 0.03).

Of the eligible study population, 81.1% ($n = 382/471$) prostate cancer-free men in the sub-cohort, 83.3% screen-detected incident prostate cancer cases in the sub-cohort ($n = 25/30$), and 75.6% ($n = 65/86$) screen-detected incident prostate cancer cases not in the sub-cohort had a baseline HHV-8 test result and wave 2 visit and/or wave 3 visit test results for HHV-8. When baseline characteristics were compared between men with a missing and non-missing baseline and wave 2 visit and/or wave 3 visit HHV-8 test results, there were no significant differences between these two groups for most of the baseline characteristics listed in Table 20. However, among the screen-detected incident prostate cancer cases who had a missing baseline and wave 2 visit and/or wave 3 visit HHV-8 test results, they had more years of education and a higher frequency of benign prostatic hyperplasia (BPH) in comparison to men who did not have missing HHV-8 test results at these visits (p -values < 0.02). Among the prostate cancer-free men who had missing HHV-8 test results at baseline and wave visit 2 and/or wave visit 3, they were older in age, had a higher frequency of ever having gonorrhoea, and a higher frequency of BPH in comparison to their counterparts (p -values < 0.03).

Table 20. Baseline Characteristics among Tobago Men, aged 40-81 years, with and without HHV-8

Test Results

	Not Tested Incident Cases (N=26)	Tested Incident Cases (N=90)	P-value^a	Not Tested Controls (N=89)	Tested Controls (N=382)	P-value^b
Median Years of Follow-up	3.03 years (n=26)	3.58 years (n=90)	.489	5.13 years (n=89)	5.15 years (n=382)	.857
Median Age (in years)	60 years (N=26)	60 years (n=90)	.830	57 years (n=89)	51 years (n=382)	.002
Median Serum PSA (ng/mL)	2.25 ng/mL (n=26)	2.85 ng/mL (n=88)	.769	1.2 ng/mL (n=84)	1.0 ng/mL (n=380)	.289
Abnormal DRE	33.3% (n=8/24)	28.6% (n=24/84)	.652	16.5% (n=13/79)	13.7% (n=46/335)	.533
Married/Ever Married	96.2% (n=25/26)	84.4% (n=76/90)	.185	11.2% (n=10/89)	19.2% (n=73/381)	.078
≤ 11 years of education	61.5% (n=16/26)	83.3% (n=75/90)	.017	74.2% (n=66/89)	77.6% (n=294/379)	.491
Family history of prostate cancer	7.7% (n=2/26)	4.8% (n=4/84)	.625	4.8% (n=4/83)	7.7% (n=27/350)	.480
Ever diagnosed with cancer	0% (n=0/26)	0% (n=0/90)	-----	0% (n=0/86)	0.3% (n=1/380)	1.00
Ever had Gonorrhea	26.9% (n=7/26)	14.6% (n=13/89)	.145	29.9% (n=26/87)	17.9% (n=66/369)	.012
Ever had Syphilis	0% (n=0/25)	3.4% (n=3/87)	1.00	6.0% (n=5/83)	5.2% (n=19/366)	.761
Prostatitis diagnosed	0% (n=0/25)	0% (n=0/87)	-----	1.2% (n=1/86)	1.4% (n=5/359)	1.00
Benign prostatic hyperplasia (BPH) diagnosis	32.0% (n=8/25)	11.4% (n=10/88)	.013	11.5% (n=10/87)	5.1% (n=19/375)	.026
Has taken Ibuprofen (in the past 12 months)	11.5% (n=3/26)	5.7% (n=5/88)	.380	5.6% (n=5/89)	5.1% (n=19/372)	.846
Has taken Aspirin (in the past 12 months)	19.2% (n=5/26)	11.2% (n=10/89)	.287	7.9% (n=7/89)	11.6% (n=44/378)	.304
Smoke cigarettes (> 6 months)	30.8% (n=8/26)	35.6% (n=32/90)	.651	37.1% (n=33/89)	41.6% (n=158/380)	.437

Note: PSA = prostate specific antigen, DRE = digital rectal examination, N and n = number of study participants,

---- = p-value not determined, ^a = the p-value of the comparison between the incident cases groups, ^b = the p-value of the comparison between the controls groups

Among Tobago men who had a baseline HHV-8 test result and wave 2 visit and/or wave 3 visit HHV-8 test results, most of the baseline characteristics listed in Table 21 did not differ between screen-

detected incident prostate cancer cases in the sub-cohort and screen-detected incident prostate cancer cases not in the sub-cohort. Screen-detected incident prostate cancer cases in the sub-cohort were older in age and had a higher frequency of BPH than screen-detected incident prostate cancer cases not in the sub-cohort (p-values < 0.03). When these characteristics were compared between screen-detected incident prostate cancer cases and prostate cancer-free men in the sub-cohort who had baseline and wave 2 visit and/or wave 3 visit HHV-8 test results, screen-detected incident prostate cancer cases had shorter years of follow-up time, older in age, had higher serum PSA levels, and had a higher frequency of abnormal DRE and BPH diagnosis than prostate cancer-free men (p-values < 0.02); however, these observations were expected (Table 21).

Table 21. Comparison of Baseline Characteristics among Tobago Men, aged 40-81 years, with Baseline and Wave 2 and/or Wave 3 HHV-8 Test Results

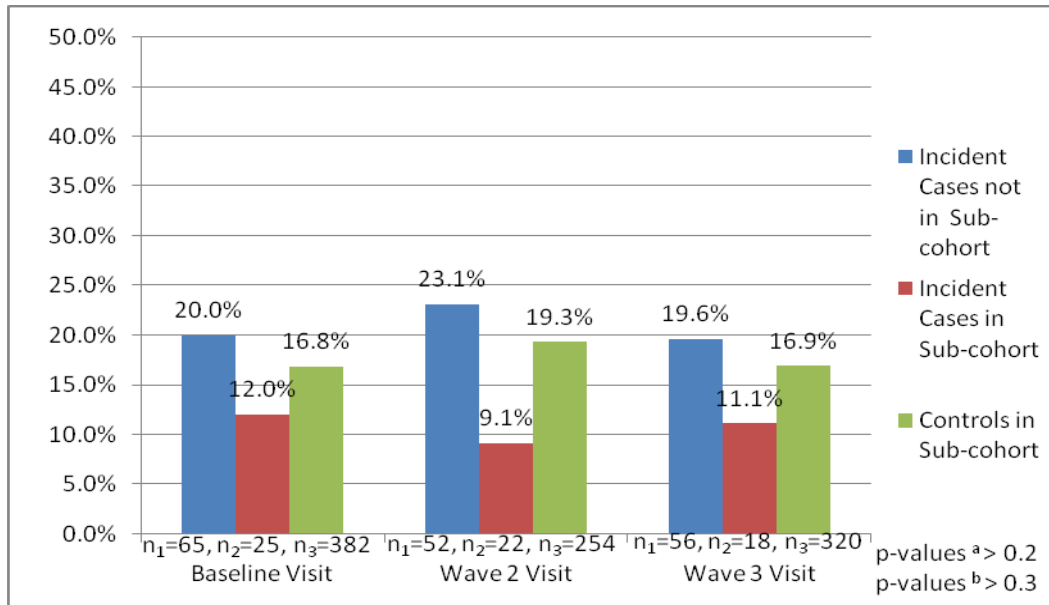
	Incident Cases not in Sub-Cohort (N=65)	Incident Cases in the Sub-Cohort (N=25)	Controls in the Sub-Cohort (N=382)	P-value^a	P-value^b
Median Years of Follow-up	3.61 years (n=65)	3.57 years (n=25)	5.15 years (n=382)	.358	.000
Median Age (in years)	60 years (n=65)	64 years (n=25)	51 years (n=382)	.039	.000
Median Serum PSA (ng/mL)	3.15 ng/mL (n=64)	2.8 ng/mL (n=24)	1.0 ng/mL (n=380)	.694	.000
Abnormal DRE	27.4% (n=17/62)	31.8% (n=7/22)	13.7% (n=46/335)	.695	.021
Married/Ever Married	81.5% (n=53/65)	92.0% (n=23/25)	80.8% (n=308/381)	.334	.283
≤ 11 years of education	81.5% (n=53/65)	88.0% (n=22/25)	77.6% (n=294/379)	.545	.317
Family history of prostate cancer	5.1% (n=3/59)	4.0% (n=1/25)	7.7% (n=27/350)	1.00	1.00
Ever diagnosed with cancer	0% (n=0/65)	0% (n=0/25)	0.3% (n=1/380)	-----	1.00
Ever had Gonorrhea	14.1% (n=9/64)	16.0% (n=4/25)	17.9% (n=66/369)	1.00	1.00
Ever had Syphilis	3.1% (n=2/64)	4.3% (n=1/23)	5.2% (n=19/366)	1.00	1.00
Prostatitis diagnosed	0% (n=0/63)	0% (n=0/24)	1.4% (n=5/359)	-----	1.00
Benign prostatic hyperplasia (BPH) diagnosis	6.2% (n=4/64)	25.0% (n=6/24)	5.1% (n=19/375)	.022	.000
Has taken Ibuprofen (in the past 12 months)	6.3% (n=4/63)	4.0% (n=1/25)	5.1% (n=19/372)	1.00	1.00
Has taken Aspirin (in the past 12 months)	14.1% (n=9/64)	4.0% (n=1/25)	11.6% (n=44/378)	.271	.338
Smoke cigarettes (> 6 months)	33.8% (n=22/65)	40% (n=10/25)	41.6% (n=158/380)	.585	.877

Note: PSA = prostate specific antigen, DRE = digital rectal examination, N and n = number of study participants,

----- = p-value not determined, ^a = the p-value of the comparison between the incident cases groups, ^b = the p-value of the comparison between the incident cases and the controls in the sub-cohort

6.4.2 HHV-8 Seroprevalence

HHV-8 seroprevalence was 16.8% (95% confidence interval [C.I.], 13.2% - 20.9%), 19.3% (95% C.I., 14.6% - 24.7%), and 16.9% (95% C.I., 12.9% - 21.4%) among prostate cancer-free men in the sub-cohort at the baseline visit, wave 2 visit, and wave 3 visit, respectively (Figure 19). For screen-detected incident prostate cancer cases in the sub-cohort, HHV-8 seroprevalence was 12.0% (95% C.I., 2.5% - 31.2%), 9.1% (95% C.I., 1.0% - 29.2%), and 11.1% (95% C.I., 1.3% - 34.7%) at the baseline visit, wave 2 visit, and wave 3 visit, respectively (Figure 19). HHV-8 seroprevalence was 20% (95% C.I., 11.1% - 31.8%), 23.1% (95% C.I., 12.5% - 36.8%), 19.6% (95% C.I., 10.2% - 32.4%) in the screen-detected incident prostate cancer cases not in the sub-cohort at baseline visit, wave 2 visit, and wave 3 visit, respectively (Figure 19). HHV-8 seropositivity among these groups did not differ at any study visit (p -values > 0.3).



Note: n_1 = the number of incident prostate cancer cases not in the sub-cohort, n_2 = the number of incident prostate cancer cases in the sub-cohort, n_3 = the number of prostate cancer-free men (controls) in the sub-cohort, ^a = the p -value of the comparison between the incident cases groups, and ^b = the p -value of the comparison between groups in the sub-cohort

Figure 19. Overall HHV-8 Seropositivity Rates by Prostate Cancer Status for all Study Visits

As for age, baseline HHV-8 seropositivity was the following for the prostate cancer-free men in the sub-cohort: 6.9% (n = 12/173) in men 40-49 years, 17.0% (n = 18/106) in men 50-59 years, 30.1% (n = 22/73) in men 60-69 years, and 40% (n = 12/30) in men 70-81 years (p-value_{trend} = 0.000). For screen-detected incident prostate cancer men in the sub-cohort, baseline HHV-8 seropositivity by age was the following: 0% (n = 0/2) in men 40-49 years, 0% (n = 0/6) in men 50-59 years, 11.1% (n = 1/9) in men 60-69 years, and 25.0% (n = 2/8) in men 70-81 years (p-value_{trend} = 0.498). Among screen-detected incident prostate cancer men not in the sub-cohort, baseline HHV-8 seropositivity by age was the following: 0% (n = 0/8) in men 40-49 years, 8.3% (n = 2/24) in men 50-59 years, 40.7% (n = 11/27) in men 60-69 years, and 0% (n = 0/6) in men 70-81 years (p-value_{trend} = 0.005). There were no age-specific differences in HHV-8 seropositivity between screen-detected incident prostate cancer cases and prostate cancer-free men in the sub-cohort (p-values > 0.4) and between screen-detected incident prostate cancer cases in the sub-cohort and screen-detected incident prostate cancer cases not in the sub-cohort (p-values > 0.2).

There was also no difference in HHV-8 seropositivity by the date of laboratory analysis (< = April 6, 2008 vs > April 6, 2008) for the prostate cancer-free men (p-values > 0.1) and screen-detected incident prostate cancer cases in the sub-cohort (p-values > 0.1) and screen-detected incident prostate cancer cases not in the sub-cohort at each study visit (p-values > 0.1) (Data not shown). When serum collected by study visits (baseline visit and wave 2 visit, baseline visit and wave 3 visit, and all study visit) was examined among prostate cancer-free individuals (N = 382), men who had blood collected at all study visits had a higher frequency of baseline HHV-8 seropositivity (21.9%) than men who had blood collected at baseline and wave 2 visit only (14.5%) and baseline and wave 3 visit only (10.2%) (p-value = 0.020) (Table 22). As for screen-detected incident prostate cancer cases (N = 90), men who had blood collected at all study visits had a lower baseline HHV-8 seropositivity (15.5%) in comparison to men who had blood collected at only two study visits (18.8% and 25%); however, there was no significant difference among these groups (p-value = 0.676). After adjusting for age, the date of laboratory analysis, and prostate cancer status, there was no significant difference between baseline HHV-8 seropositivity and

the number of study visits blood was collected; however, this relationship was borderline non-significant (p-value = 0.088) (Table 22).

Table 22. The Association between the Number of Study Visits Blood Collected and Baseline HHV-8 Seropositivity among Tobago Men

	⁴ Percentage of HHV-8 Seropositivity in Controls	⁵ Percentage of HHV-8 Seropositivity in Cases	¹ Unadjusted Odds Ratio (95% confidence interval)	² Age-adjusted Odds Ratio (95% confidence interval)	³ Age-, Lab-, Prostate cancer - adjusted Odds Ratio (95% confidence interval)
Baseline and Visit 2 Only	14.5% (n=9/62)	25% (n=4/16)	.780 (.399 – 1.525)	.611 (.302 – 1.234)	.621 (.307 – 1.257)
Baseline and Visit 3 Only	10.2% (n=12/128)	18.8% (n=3/16)	.488 (.267 - .892)	.523 (.281 - .972)	.527 (.283 - .981)
All Study Visits	21.9% (n=42/192)	15.5% (n=9/58)	Reference	Reference	Reference

Note: ¹Wald's p-value = 0.065, ²Wald's p-value = 0.080, ³Wald's p-value = 0.088, ⁴Pearson chi-square p-value = .020, ⁵Pearson chi-square p-value = 0.676, n = the number of study participants, age is continuous, and lab-adjusted (<= April 6, 2008 in comparison > April 6, 2008)

As for markers of prostate cancer and HHV-8 seropositivity, there were no significant differences in baseline HHV-8 seropositivity with abnormal DRE and serum PSA levels ≥ 4 ng/mL between screen-detected incident prostate cancer cases not in the sub-cohort and screen-detected incident prostate cancer cases in the sub-cohort at each study visit (p-values > 0.6) (Table 23). There were also no significant differences in HHV-8 seropositivity with these prostate cancer markers between screen-detected incident prostate cancer cases and prostate cancer-free men in the sub-cohort, except for serum PSA level ≥ 4 ng/mL at wave 2 visit. Prostate cancer-free men in the sub-cohort had a significantly higher frequency of serum PSA level of ≥ 4 ng/ mL at wave 2 visit (47.6%) in comparison to screen-detected incident prostate cancer cases in the sub-cohort (8.3%) (p-value = 0.027) (Table 23).

Table 23. The Comparison between Baseline HHV-8 Seropositivity and Markers of Prostate Cancer at Baseline, Wave 2, and Wave 3 Visits among Tobago Men

	Incident Cases not in the Sub-Cohort (N=65)	Incident Cases in the Sub-Cohort (N=25)	Controls in the Sub-Cohort (N=382)	P-value^a	P-value^b
Serum PSA >= 4 ng/mL at Baseline Visit	18.2% (n=4/22)	14.3% (n=1/7)	37.5% (n=12/32)	1.000	0.388
Serum PSA >= 4 ng/mL at Wave 2 Visit	19.2% (n=5/26)	8.3% (n=1/12)	47.6% (n=10/21)	0.643	0.027
Serum PSA >= 4 ng/mL at Wave 3 Visit	28.6% (n=6/21)	25.0% (n=2/8)	21.4% (n=6/28)	1.000	1.000
Abnormal DRE at Baseline Visit	23.5% (n=4/17)	14.3% (n=1/7)	23.9% (n=11/46)	1.000	1.000
Abnormal DRE at Wave 2 Visit	30.8% (n=4/13)	22.2% (n=2/9)	38.7% (n=12/31)	1.000	0.453
Abnormal DRE at Wave 3 Visit	18.5% (n=5/27)	16.7% (n=1/6)	35.5% (n=11/31)	1.000	0.641

Note: PSA = prostate specific antigen, DRE = digital rectal examination, N and n = number of study participants,

----- = p-value not determined, ^a = the p-value of the comparison between the incident cases groups, and ^b = the p-value of the comparison between the incident cases and the controls in the sub-cohort

6.4.3 The Association between Baseline HHV-8 Seropositivity and Screen-Detected

Incident Prostate Cancer

There was an inverse but not significant association between baseline HHV-8 seropositivity and screen-detected incident prostate cancer, after adjusting for age (as a continuous variable) (H.R. = 0.517, 95% C.I., 0.262 – 1.020) (Table 24). When the regression model was adjusted for age (as a continuous variable) and baseline prostate cancer screening results (serum PSA >= 4 ng/mL or abnormal DRE results in comparison to normal serum PSA, normal DRE results, or no screening results), there was a significant

inverse association between baseline HHV-8 seropositivity and screen-detected incident prostate cancer (H.R. = 0.454, 95% C.I., 0.221 – 0.933) (Table 24). However, when the regression model (HHV-8 seropositivity and screen-detected incident prostate cancer) was restricted to baseline screen-negative men (comparing 46 incident prostate cancer cases [13 sub-cohort members] and 288 controls), adjusted for age (as a continuous variable), a non-significant inverse association was found between baseline HHV-8 seropositivity and screen-detected incident prostate cancer (H.R. = 0.547, 95% C.I., 0.213 – 1.403) (Table 24). Inverse but not significant associations were also found between baseline HHV-8 seropositivity and screen-detected incident prostate cancer diagnosed at wave 2 visit (H.R. = 0.585, 95% C.I., 0.254 – 1.348) and wave 3 visit (H.R. = 0.373, 95% C.I., 0.131 – 1.062), after adjusting for age (as a continuous variable) (Table 24).

Table 24. The Association between Baseline HHV-8 Seropositivity and Incident Prostate Cancer

			Yes No	Yes	Yes All	No Yes	Case Sub- cohort
	H.R.	95% C.I.	N	N	N	N	
Age-adjusted ^a	0.517	0.262 – 1.020	65	25	90	407	
Baseline screening results ^{b,c}	0.454	0.221 – 0.933	65	25	90	407	
Incident at Wave 2 Visit ^c	0.585	0.254 – 1.348	65	25	90	407	
Incident at Wave 3 Visit ^c	0.373	0.131 – 1.062	65	25	90	407	
Baseline screen-negative men ^c	0.547	0.213 – 1.403	65	25	90	407	

Note: H.R. = hazard ratio, C.I. = confidence interval, N= # of study participants, ^a = age is continuous, ^b = serum prostate specific antigen (PSA) > = 4 ng/mL or abnormal digital rectal examination (DRE) in comparison to normal serum PSA, normal DRE results, or no screening results, ^c = age (continuous)-adjusted

In stratified analyses, there were inverse associations between baseline HHV-8 seropositivity and screen-detected incident prostate cancer with baseline screening results (positive and negative), age (< 55 years and \geq 55 years), the date of laboratory analysis (\leq April 6, 2008 and $>$ April 6, 2008), and BPH or prostatitis diagnosis (yes and no) (Table 25). Significant associations between baseline HHV-8 seropositivity and screen-detected incident prostate cancer, controlling for age (as a continuous variable), were found among Tobago men \geq 55 years of age (H.R. = 0.503, 95% C.I., 0.256 – 0.986) and the date of laboratory analysis after April 6, 2008 (H.R. = 0.281, 95% C.I., 0.097 – 0.810). There were no significant differences between the regression models in each stratified analysis (p-values_{interaction} $>$ 0.1) (Table 25).

Table 25. The Association between Baseline HHV-8 Seropositivity and Incident Prostate Cancer based on Stratified Analysis

			Yes No	Yes Yes	Yes All	No Yes	Case Sub- cohort	
	H.R.	95% C.I.	N	N	N	N		P-value (interaction)
Baseline Screening Results^e								0.5192
Positive^a	0.449	0.172 – 1.175	31	11	42	81		
Negative^b	0.524	0.204 – 1.348	34	14	48	326		
Age^{c,e}								0.3737
<55 years	0.660	0.128 – 3.400	17	4	21	244		
\geq 55 years	0.503	0.256 – 0.986	48	21	69	163		
Lab date^{d,e}								0.1319
\leq April 6	0.866	0.348 – 2.156	36	13	49	210		
$>$ April 6	0.281	0.097 – 0.810	29	12	41	197		
BPH or prostatitis diagnosis^e								0.3722
Yes	0.226	0.028 – 1.848	4	6	10	25		
No	0.564	0.274 – 1.165	61	19	80	382		

Note: H.R. = hazard ratio, C.I. = confidence interval, N= # of study participants, BPH= benign prostatic hyperplasia, ^a = serum prostate specific antigen (PSA) \geq 4 ng/mL or abnormal digital rectal examination (DRE), ^b = normal serum PSA, normal DRE results, or no screening results, ^c = categorized by median age, and ^d = the date of laboratory analysis, ^e = age-adjusted (as a continuous variable)

6.5 DISCUSSION

In the present study, inverse but not significant associations were found between baseline HHV-8 seropositivity and screen-detected incident prostate cancer, after adjusting for age (as a continuous variable). However, there was a significant inverse association between baseline HHV-8 seropositivity and screen-detected incident prostate cancer when age (as a continuous variable) and prostate cancer baseline screening results were considered. Also, significant inverse associations were found among Tobago men age ≥ 55 years (adjusted for continuous age) and the date of laboratory analysis conducted after April 6, 2008 (adjusted for continuous age). These inverse associations between HHV-8 seropositivity and prostate cancer are consistent with other studies that have examined the relationship between prostate cancer and HHV-8 infection in U.S. populations, which included African-American men^{345, 347}.

At each study visit, there was no significant difference in HHV-8 seropositivity between screen-detected incident prostate cancer cases not in the sub-cohort and screen-detected incident prostate cancer cases in the sub-cohort and between screen-detected incident prostate cancer cases and prostate cancer-free men in the sub-cohort. Studies that examined the relationship between prostate cancer and HHV-8 seropositivity also found no significant difference in HHV-8 seropositivity between men with and without prostate cancer³⁴⁵⁻³⁴⁷. In fact, lower HHV-8 seropositivity rates have been reported for the prostate cancer cases rather than for their controls. For example, 17.1% and 19.0% HHV-8 seropositivity were found among African-American men with benign prostatic hyperplasia and prostate cancer, respectively, in the Washington, D.C. area³⁴⁵. Among these men, there was no significant association between HHV-8 seropositivity and prostate cancer (O.R. = 0.88, 95% C.I., 0.35 – 2.24)³⁴⁵. A similar pattern of HHV-8 seropositivity was observed in African-American and White American men with and without prostate

cancer that were from four U.S. areas (Georgia, New Jersey, Detroit, and Washington D.C): 17.5% (cases) and 27.5% (controls) seropositivity in African-American men, and 18.7% (cases) and 24.4% (controls) seropositivity in White American men³⁴⁵. In this U.S. population-based case-control study, inverse but not significant associations between prostate cancer and HHV-8 seropositivity were found for both African-American (O.R. = 0.56, 95% C.I., 0.28 – 1.14) and White American men (O.R. = 0.71, 95% C.I., 0.36 – 1.43)³⁴⁵. However, among men in the Pittsburgh, Pennsylvania area, a positive but not significant association was found between HHV-8 seropositivity and prostate cancer (O.R. = 1.65; 95% C.I., 0.77 – 3.54), with a seropositivity of 20% in cases and 13% in controls⁹. In addition, a positive but not significant association between HHV-8 seropositivity and prostate cancer was found among Italian men in the Bologna region (O.R. = 1.08, 95% C.I., 0.27 – 4.33), with a seropositivity rate of 40% and 38.2% in prostate cancer men and benign prostatic hyperplasia men, respectively³⁴⁵. The reason for the difference in the positive but not significant association among Pittsburgh and Italian men from the Bologna region may be due to regional differences in HHV-8 seropositivity³⁴⁵. All of these prostate cancer/HHV-8 seropositivity studies used the same serological assay, mIFA, used in the present study¹³. Therefore, the reason for the patterns of lower seropositivity among prostate cancer cases compared to the controls in U.S. populations remains unclear.

Despite the non-significant associations found in several study populations, a nested case-control study conducted among men in the U.S. Health Professional Follow-up Study found a significant inverse association between prostate cancer and HHV-8 seropositivity (O.R. = 0.70, 95% C.I., 0.52 – 0.95), with seropositivity of 13.5% and 18% for the cases and controls, respectively³⁴⁷. Sutcliffe et al. stated that their cases were composed of early stage disease³⁴⁷. For the present study, stages of prostate cancer were not collected unless radical prostatectomy was performed; however, majority of the screen-detected incident prostate cancers had a Gleason score of 6 and 7 (94.4%) which was the majority in Hoffman et al. study. Therefore, there may be differences between incident and prevalent prostate cancer that affect HHV-8 seropositivity; however, these differences are unknown.

In the present study, screen-detected incident prostate cancer cases and prostate-cancer free men had lower HHV-8 seropositivity rates at each study visit in comparison to Hoffman et al. cases and age-matched controls, respectively, at baseline visit. Hoffman et al. study found HHV-8 seropositivity rates of 39.9% and 22.9% in the cases and controls, respectively⁹. In the present study, HHV-8 seropositivity was < 24% for the cases and < 20% for the controls at each study visit. One possible reason for these seropositivity differences may be that cases and controls in Hoffman et al. study were older men, mean age of 67 years, opposed to the cases and controls in the present study, mean age of 61 and 53 years, respectively. Studies have shown a relationship between HHV-8 seropositivity and age, in which seropositivity increases with age^{3, 5, 138, 145, 146, 153, 157}. Even though the present study population was younger than Hoffman et. al cases and controls, HHV-8 seropositivity was shown to increase from 6.9% in 40-49 year olds to 40% in 70-81 year old prostate cancer-free men at baseline visit; this pattern of increase was also seen in screen-detected incident prostate cancer men. This increase in HHV-8 seropositivity may be due to factors related to cumulative exposure to the virus, sexually or non-sexually; however, in Tobago, these factors are not yet known. As for other studies that have investigated the relationship between HHV-8 seropositivity and prostate cancer, seropositivity by increasing age was not examined.

The inverse associations between screen-detected incident prostate cancer and HHV-8 seropositivity in this present study were different from the results Hoffman et al. found with screen-detected prevalent prostate cancer men and HHV-8 seropositivity (O.R. = 2.24; 95% C.I., 1.29-3.90) in Tobago⁹. A possible reason for this difference may be due to the selection of the study participants. The cases in Hoffman et al. study were screen-detected prevalent prostate cancer men at baseline visit. In the present study, the cases were men who had a screen-detected prostate cancer at a subsequent study visit from the baseline visit. Therefore, the cases in the present study were considered prostate cancer-free at baseline visit opposed to the cases in Hoffman et al. study. In addition, there was no significant difference in HHV-8 seropositivity between the incident and prostate cancer-free men at any study visit in the present study. A possible explanation for the lack of difference in HHV-8 seropositivity between incident

prostate cancer cases and prostate cancer-free men could be due to the difference in how the BCBL-1 cells were prepared in the present study in comparison to Hoffman et al study. In the present study, the BCBL-1 cells contained a doxycycline-inducible RTA gene³⁶⁸ which was used for the purpose of reducing background on the mIFA slides when they were read. However, in Hoffman et al. study, this inducible gene was not used. Therefore, the introduction of this gene may have affected the sensitivity of the assay. Another possible reason for the lack of difference in HHV-8 seropositivity between the incident and prostate cancer-free men could be that the incident prostate cancer men were different from the prevalent prostate cancer men; and therefore, HHV-8 seropositivity may not be related to prostate cancer incidence. For the prevalent prostate cancer cases, chronic inflammation induced by the cancer may reactivate HHV-8, therefore, increasing their likelihood of being HHV-8 seropositive in contrast to the incident prostate cancer cases. Therefore, HHV-8 may not be the factor that causes chronic inflammation that leads to the development of prostate cancer in Tobago men of African descent. Also, HHV-8 may cause an increase in serum PSA levels in a subject of men, therefore, improving the detection of prostate cancer at baseline screening. This improvement may explain why there was a positive association between screen-detected prevalent prostate cancer men and HHV-8 seropositivity in Hoffman et al. study opposed to the findings in the present study among screen-detected incident prostate cancer men.

The examination of HHV-8 seropositivity up to a 9-year period provided important information on the relationship between screen-detected incident prostate cancer and HHV-8 infection in Tobago. However, there were some limitations in the present study. First, the incident prostate cancer men were screen-detected based on a serum PSA level of ≥ 4 ng/mL and/or an abnormal DRE. A study conducted among men in the Prostate Cancer Prevention Trial discovered that 15% of the men who had a serum PSA ≤ 4 ng/mL had a screen-detected prostate cancer²⁵². In the present study, men who had a serum PSA < 4 ng/mL did not receive a prostate biopsy unless they had an abnormal DRE; therefore, prostate cancers identified at serum PSA < 4 ng/mL were missed in this group of men. A second limitation was that one study participant did not have a prostate cancer screening at baseline visit. This individual,

diagnosed with incident prostate cancer at wave 2 visit, may have had prostate cancer at baseline visit; therefore, misclassification of incident prostate status may have occurred for this individual.

The variability of the serological assay used to test the participants' serum for HHV-8 seropositivity may be another limitation to the study. To examine this variability, participants' serum specimens were tested on 2 different days in a blinded fashion. In the present study, stratified analysis by the laboratory date was used to compare days in which a consistent kappa statistic of > 0.85 was observed (before and on April 6, 2008) in comparison to days in which the kappa statistic ranged from 0.54 to 1.00 (after April 6, 2008). Based on the stratified analysis, there was no significant difference in HHV-8 seropositivity by the laboratory date. A fourth limitation was that levels of HHV-8 antibody titers were not examined on HHV-8 seropositive men. HHV-8 antibody titers would have provided information on whether HHV-8 reactivation had occurred among Tobago men and whether this activity was responsible for the persistent seropositivity levels observed in this population.

Another limitation in the present study was that the study participants in the Tobago Prostate Cancer Screening Study did not participate in all study visits and some men were excluded due to missing HHV-8 test results. Even though baseline and wave 2 visit and/or wave 3 visit serum specimens were available for 81.1%, 83.3%, and 75.6% of the prostate cancer-free men, screen-detected incident prostate cancer men in the sub-cohort, and screen-detected incident prostate cancer men not in the sub-cohort, respectively, men who were not part of the study due to missing HHV-8 test results were different. The prostate cancer-free men had significantly higher frequency of gonorrhea diagnosis and BPH diagnosis as well as a higher median age. The screen-detected incident prostate cancer men (all incident cases in the study) had a higher frequency of BPH diagnosis and they were more educated. As a result of these differences between men with missing and non-missing serum specimens, selection bias may have been introduced. And finally, based on a 2-sample binomial proportion (2-sided, $\alpha = 0.05$), the present study did not have adequate power (2.66%) to observe a true difference in baseline HHV-8 seropositivity between prostate cancer-free men (16.8%, $n = 382$) and incident prostate cancer men (17.8%, $n = 90$).

The small sample size in the present study may explain the lack of significant seropositivity difference between screen-detected incident prostate cancer and prostate cancer-free men in Tobago.

In this up to 9-year, population-based study among Tobago men of African descent, there were significant and not significant inverse associations between HHV-8 seropositivity and screen-detected incident prostate cancer, after adjusting for continuous age and baseline screening results and continuous age only, respectively. These inverse associations may suggest that HHV-8 seropositivity may not be related to prostate cancer incidence. Further studies are needed to investigate the difference in the relationship with HHV-8 seropositivity between screen-detected incident prostate cancer and screen-detected prevalent prostate cancer among men of African descent in Tobago.

7.0 SUMMARY/CONCLUSION

Human herpesvirus 8 (HHV-8) is widespread, geographically, with seroprevalence ranging from 0.2% in Japan¹⁷² to 87% in Botswana, Sub-Saharan Africa². HHV-8 has sexual and non-sexual modes of transmission in which all ages are affected by this virus. Because of its tropism to B cells, HHV-8 is the causal agent of B cell lymphomas like Kaposi's sarcoma and primary effusion lymphoma and is associated with multicentric Castleman's disease, in which these lymphoproliferative disorders usually develop in immunocompromised individuals. In addition, HHV-8 has been identified in prostatic epithelial cells; and, its DNA and protein expressions have also been identified in prostatic tissue^{15, 16, 332}. In the Caribbean island of Tobago, a case-control study conducted among African-Caribbean men found prostate cancer to be associated with HHV-8 seropositivity, 39.9% and 22.9% in men with and without prostate cancer, respectively (O.R. = 2.24; 95% C.I,1.29-3.90)¹⁷. In a population where 11% prostate cancer prevalence was detected at initial screening⁹, this significant finding prompted an investigation to understand the relationship between HHV-8 and prostate cancer in Tobago. Therefore, examining HHV-8 seropositivity among Tobago women, identifying possible modes of sexual transmission of HHV-8, studying the natural history of HHV-8 seropositivity, and investigating the relationship between HHV-8 and prostate cancer risk were methods used to evaluate HHV-8 infection and its possible relationship with prostate cancer in Tobago.

Three studies were conducted to understand the epidemiology of HHV-8 infection and the relationship between HHV-8 seropositivity and prostate cancer. Study 1 "Human Herpesvirus 8 Seroprevalence among Tobago Women and the Role of Sexual Lifestyle Behavior" was designed to provide information on things not known in relation to HHV-8 seropositivity in Tobago, such as HHV-8

seropositivity among presumably healthy Tobago women, possible sexual modes of HHV-8 transmission among the women, and HHV-8 seropositivity differences between Tobago men and women of similar ages. Study 2 “The Natural History of Human Herpesvirus 8 Seropositivity in a Cohort of Tobago Men of African Descent, 1997-2007” was designed to study the patterns of change in HHV-8 sero-status over a 9-year period among Tobago men at risk for prostate cancer, and to see whether HHV-8 seropositivity is persistent among HHV-8 seropositive men over this time period. Finally, Study 3 “Human Herpesvirus 8 Seropositivity in Relation to Incident Prostate Cancer among Men of African Descent in Tobago” was designed to determine whether baseline HHV-8 seropositivity among men who were not screened-positive for prostate cancer at baseline visit was associated with incident prostate cancer. These three studies allowed the evaluation of HHV-8 infection and its relationship to prostate cancer risk in Tobago.

7.1 THE SEROPREVALENCE OF HHV-8 IN TOBAGO

From these three studies, HHV-8 seroprevalence was the following at baseline: 14.1% among 213 Tobago women, ages 18-65 years; 14.8% among 122 Tobago women, ages 40-65 years; 20.8% among 159 Tobago men (controls in Hoffman et al.), ages 40-65 years; 16.5% among 407 Tobago men, ages 40-81 years, at risk for incident prostate cancer; 16.8% among 383 Tobago men, ages 40-81 years, who were prostate cancer-free in the 9-year cohort; and, 17.8% among 90 men, ages 40-81 years, who developed prostate cancer (incident prostate cancer) in the 9-year cohort. These HHV-8 seropositivity rates were similar between genders and across study groups. However, the women had a lower HHV-8 seropositivity rate than the men. A possible reason for this gender difference could be that men have different sexual behaviors that increase their risk for acquiring HHV-8 infection. As shown in Study 1, this gender difference was not significant which this lack of difference in HHV-8 seropositivity is consistent to studies conducted in Sub-Saharan Africa³, Italy¹⁴⁴, and the United States¹⁶⁶.

The frequencies of HHV-8 seropositivity observed in Study 1, 2, and 3 were comparable to HHV-8 seropositivity reported in the Mediterranean^{6, 7, 143}, a region where KS, in particular classic KS, is common. As for Tobago, no cases of KS have been reported since the establishment of the cancer registry in 1994^{10, 11}. The lack of KS in a population that has moderate levels of HHV-8 seropositivity is puzzling. However, factors such as genetic make-up that prevents disease development/progression to clinical stage, diet, immune status, under-reporting of cases to health officials, or small sample size may explain the lack of KS cases reported in Tobago.

In Study 3, screened-detected incident and prostate-cancer free men had lower HHV-8 seropositivity levels at all study visits in comparison to Hoffman et al. cases and age-matched controls at baseline visit, respectively. In Hoffman et al. study, HHV-8 seropositivity of 39.9% and 22.9% was found among the cases and controls, respectively⁹. In Study 3, HHV-8 seropositivity was < 24% for the cases and < 20% for the controls at each study visit. One possible reason for these seropositivity differences is that the cases and controls in Hoffman et al. study were older men, mean age of 67 years, opposed to the cases and controls in the present study, mean age of 61 and 53 years, respectively.

Studies have shown that HHV-8 seropositivity increases with age^{3, 5, 138, 145, 146, 153, 157}. In all three studies, HHV-8 seropositivity increased with age. For example, in Study 1, HHV-8 seropositivity increased from 10.6% in women ages 30-39 years to 20.0% in women ages 60-65 years. As for women ages 18-29 years, a higher frequency of seropositivity (16.3%) was observed in comparison to women ages 30-49 years; this difference in seropositivity could be due to a cohort effect in which the younger women may have participated in activities (sexually or non-sexually) that increased their risk of acquiring HHV-8 infection. However, women ages 40-65 years had a higher antibody titer than the women < 40 years of age. This higher antibody titer in the older women may be due to cumulative exposure to the virus or higher rates of HHV-8 reactivation.

Among Tobago men, a similar increase in HHV-8 seropositivity was observed. In Study 1, HHV-8 seropositivity increased in men who were the prostate cancer controls in Hoffman et al. study, from 17.6% in men ages 40-49 years to 23% in men ages 60-65 years. In Study 2, among Tobago men at risk

for incident prostate cancer, HHV-8 seropositivity increased from 6.9% in men ages 40-49 years to 36.8% in men ages 70-81 years. As for Study 3, a similar pattern was seen when the men in Study 2 were examined by prostate cancer status: 6.9% to 40% in prostate cancer-free men ages 40-49 years and 70-81 years, respectively, and 0% to 33.3% in screen-detected incident prostate cancer men (all incident cases) ages 40-49 years and 60-69 years, respectively. As for screen-detected incident prostate cancer men ages 70-81 years, HHV-8 seropositivity of 14.3% was found; the reason for the lower seropositivity in comparison to men ages 60-69 years was probably due to the small sample size ($n = 2/12$) in this age group. In summary, higher HHV-8 seropositivity with increasing age again suggests that cumulative exposure to the virus, sexually or non-sexually, is occurring in the Tobago population.

Study 2 demonstrated that HHV-8 seropositivity was persistent over time. In this study, HHV-8 seropositivity was 16.5%, 18.5%, and 16.6% at baseline, wave 2, and wave 3 visits, respectively, among Tobago men at risk for incident prostate cancer. Among HHV-8 seropositive men, 88.1% remained seropositive from baseline visit to wave 2 and/or wave 3 visits. However, HHV-8 seroreversion (11.9%) did occur in this population. These seroreversions were probably attributed to low antibody titers at baseline visit that became undetectable by the serological assay, mIFA, at subsequent study visits. These moderate levels of HHV-8 seroreversion indicate that a more sensitive assay is needed for the detection of antibodies against HHV-8 antigens in populations.

7.2 TRANSMISSION OF HHV-8 IN TOBAGO

In Study 1, a minimal significant association between HHV-8 sero-status and age at first sexual intercourse was found among Tobago women. Tobago women who reported to have their first sexual intercourse at ≤ 17 years of age were 2.51-folds more likely (95% C.I., 1.09-5.78) to be HHV-8 seropositive than women who reported to have their first sexual intercourse at ≥ 18 years of age. The minimal significant association between HHV-8 seropositivity and age at first sexual intercourse reported

suggests that sexual activity may not be a major contributor in acquiring HHV-8 infection. Plus, in Study 1, several variables that assess sexual lifestyle behaviors were not associated with HHV-8 sero-status among the women. In addition, the detection of HPV DNA in oral or cervical cavity was not associated with HHV-8 seropositivity. The lack of associations between seropositivity and several sexual behavior variables may indicate that non-sexual transmission of HHV-8 may be a possible mode in acquiring this viral infection; however, evidence of this transmission remains not known.

As for Tobago men, sexual lifestyle behaviors were not collected in the Tobago Prostate Cancer Screening Study. This lack of information was a limitation in understanding routes of sexual transmission of HHV-8 among Tobago men. Information on whether one has ever been diagnosed with gonorrhea or syphilis was collected in the Tobago Prostate Cancer Screening Study. In Study 2, the frequencies of gonorrhea and syphilis diagnosis at baseline visit were examined and compared between HHV-8 seropositive and seronegative men at risk for incident prostate cancer. There were no differences in the frequencies of these STDs between these 2 groups. Examining STDs in HHV-8 seropositive men in comparison to HHV-8 seronegative men was one way Study 2 tried to assess whether sexual behavior was related to HHV-8 seropositivity. However, the lack of difference in the diagnosis of these STDs between HHV-8 seropositive and seronegative men did not provide information on whether sexual activity was one way of acquiring this viral infection in Tobago.

In Study 2, low HHV-8 seroconversion (0.50 per 100 person-years) was observed among Tobago men, ages 40-81 years, at risk for incident prostate cancer. There was no significant difference in STDs diagnosis such as syphilis and gonorrhea between HHV-8 seroconverters and persistent seronegative men; manner of fact, none of the seroconverters reported that they were ever diagnosed with these diseases. Therefore, sexual transmission may not be the primary mode of acquiring HHV-8 infection for “HHV-8 seroconverters” in this study population.

In summary, evidence on whether sexual or non-sexual activity is the primary mode of HHV-8 transmission among Tobago men remains to be seen. However, evidence that low seroconversion

occurred after the age of 40 years suggests that HHV-8 infection is probably acquired at younger ages, \leq 39 years, among Tobago men.

7.3 HHV-8 AND PROSTATE CANCER IN TOBAGO

In Study 3, there were inverse associations between HHV-8 seropositivity and screen-detected incident prostate cancer when continuous age and baseline screening results (H.R. = 0.454, 95% C.I., 0.221 – 0.933) and continuous age (H.R. = 0.517, 95% C.I., 0.262–1.020) were considered. These inverse associations were consistent with other studies that examined the relationship between prostate cancer and HHV-8 infection in U.S. populations^{345, 347}. However, the inverse associations in Study 3 were different from the positive association Hoffman et al. found between HHV-8 seropositivity and screen-detected prevalent prostate cancer among Tobago men (O.R. = 2.24; 95% C.I., 1.29 - 3.90)⁹. One reason for this difference in association in Tobago may be that screen-detected incident prostate cancer men and screen-detected prevalent prostate cancer men are different. Therefore, one may hypothesize that HHV-8 seropositivity does not cause an inflammatory immune response that leads to the development of prostate cancer. However, an inflammatory response induced by prevalent prostate cancer may activate HHV-8, therefore, increasing men with prevalent prostate cancer likelihood of being HHV-8 seropositive. Also, HHV-8 may cause an increase in serum PSA levels in a subject of men, therefore, improving the detection of prostate cancer at baseline screening. In summary, HHV-8 may not be the factor that causes chronic inflammation that leads to the development of prostate cancer in Tobago men of African descent.

7.4 CONCLUSION

HHV-8 infection is present among women and men of African descent in Tobago, with seroprevalence ranging from 14.1% to 20.8% in a presumably healthy population. There was no significant difference in HHV-8 seropositivity between Tobago women and men and between prostate cancer-free men and screen-detected incident prostate cancer men. Among women, age at 1st sexual intercourse was the only sexual lifestyle behavior associated with HHV-8 seropositivity. Therefore, sexual transmission may not be the predominate mode of HHV-8 transmission, and that non-sexual modes may be a major contributor in HHV-8 transmission among Tobago women. Among Tobago men, ages 40-81 years, a low seroconversion rate (incidence of 0.50 per 100 person-years, 95% C.I., 0.22 – 0.99) was found; this low rate suggests that, HHV-8 is probably acquired before the age of 40 years. A moderate seroreversion rate (incidence of 2.52 per 100 person-years, 95% C.I., 1.09 – 4.96) was observed among Tobago men, which this rate was probably due to the sensitivity of the serological assay. However, a high frequency of persistent HHV-8 seropositivity was observed among HHV-8 seropositive men. And finally, there were significant and non-significant associations between HHV-8 seropositivity and screen-detected incident prostate cancer after controlling for continuous age and baseline prostate cancer screening results and continuous age only, respectively. These inverse associations between HHV-8 seropositivity and screen-detected incident prostate cancer suggest that HHV-8 seropositivity may not be related to prostate cancer incidence.

In conclusion, further studies are needed 1) to examine possible sexual and non-sexual modes of HHV-8 transmission in Tobago, 2) to examine the seroprevalence of HHV-8 among pre-pubertal children in Tobago, and 3) to study the relationship between HHV-8 seropositivity and prostate cancer (incident and prevalent cases) in Tobago.

**APPENDIX A: THE PROTOCOL FOR ARRANGEMENT OF TOBAGO SAMPLES ON
IFA SLIDES FOR HHV-8 AIM 2 (COHORT) AND AIM 3 (SUB-COHORT AND
INCIDENT CASES)**

1. Alicia needs a list of Tobago serum samples that she will test by IFA in a day. This list will include 1) Tobago men at risk for incident prostate cancer, the cohort and sub-cohort (n = 501) from wave visit 1 to 3, and 2) men who developed incident prostate cancer, the cases that are not a part of the cohort (n = 86) from wave visits 1 to 3. The reason for testing these two study groups in an assay run is to reduce possible IFA slide variability between the groups. Since Alicia is limited on the number of samples she can test in a day, she will test an equal proportion of these two groups per lab run. Therefore, 50 out of 501 Tobago men from the cohort/sub-cohort list (list #1) and 8 out of 86 Tobago men from the case list (list #2) will be tested in a lab run.
2. In order to test the reliability of the IFA results, serum samples will be tested on 2 different days, “lab day 1” and “lab day 2”. However, the order of the serum specimens will be different for both days so that the slide reader is blinded to the serum specimen’s results (HHV-8 positive or negative). Therefore, Alicia will design two different maps indicating the location of the 58 sample sets (one set equals all of 1 man’s wave samples) on the IFA slides for “lab day 1” and “lab day 2”. “Lab day 2” samples will be placed in a random order on the IFA slides by the statistical computer package SAS. An abbreviated study id number and its wave visits will be written on these maps for Alicia to have a record on what serum samples are being tested per lab run.

3. For each map, Tobago men's samples for the wave visits (Wave 1, 2, and/or 3) will be plated on the same slide in order to reduce IFA slide variability among the wave visits; in addition, these samples will be placed in random order so that the slide reader is blinded to the order of the wave visits on the slides.
4. For each lab run, this slide order of the specimens will be duplicated (slides A1 and A2 are duplicates) so that the slide reader can determine the sero-status of each sample. Also, each slide will be given an alphabet label (A1/A2 to ZZ1/ZZ2) so that Alicia will have a record of what samples were placed on what slide in a lab run.
5. Six samples can be tested per slide. Therefore, the number of men per slide (a slide equals 6 rows which include 1:50 and 1:100 dilutions) will be the following ones in random order: (A positive HHV-8 result is a serum specimen that has a fluorescent at dilutions at 1:50 and 1:100)
 - a. 3 men (only have 2 wave samples) per slide
 - b. 2 men (have 3 wave samples) per slide
 - c. 1 positive control, 1 negative control, and 2 men (only have 2 wave samples) per slide
 - d. 1 positive control, 1 negative control, 1 man (have 3 wave samples), and 1 man (only have 1 wave, the other wave sample was not found) per slide
 - e. 2 men (only have 2 wave samples), 1 man (have 3 wave samples), and 1 man (only have 1 wave, the other wave sample was not found) per slide

These sets of random order per slide are aimed at reducing possible IFA slide variability. Since a lot of serum specimens will be tested per lab run, at least 3 sets of positive and negative controls will be plated per lab run for quality control purposes.

A	1	2	3	4	5	6
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

6. In order for the slider reader to be blinded to the serum samples' study id, the sample order from the map for each lab run will be given an unique lab id; and, this order will be written on a lab result sheet for the slide reader to record the results (HHV-8 positive or negative). The samples that were run on "lab day 1" will have a different lab id than the samples run on "lab day 2" for blinding purposes. The lab result sheet looks like the following:

Date:

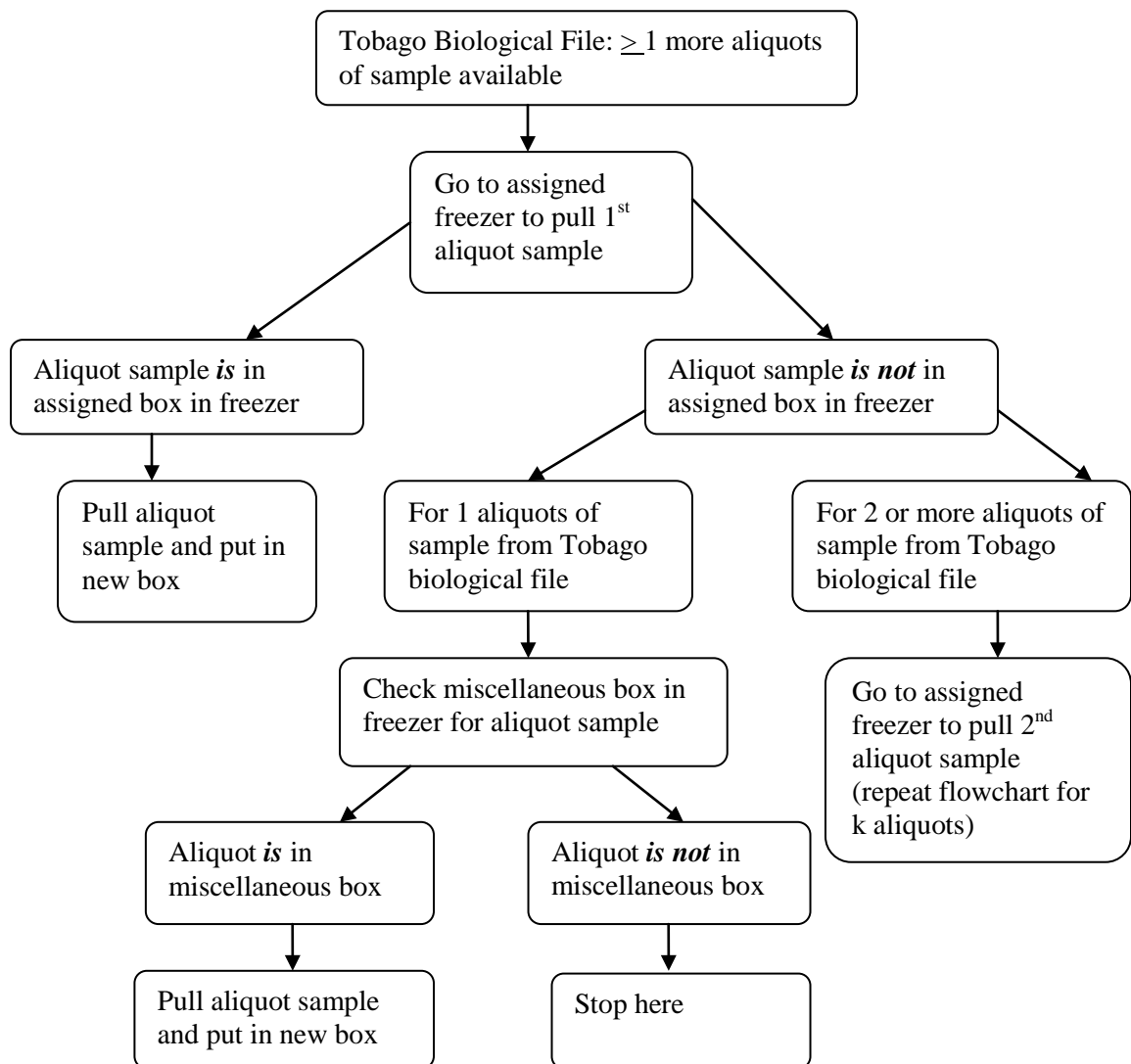
A1	1:50	1:100	A2
1. Positive			
2. Negative			
3. 0001			
4. 0002			
5. 0003			
6. 0004			

7. Results from the lab result sheet (HHV-8 positive, negative, or repeat) and its lab run date for "lab day 1" and "lab day 2" will be recorded in the HHV-8 Aims 2 and 3 database for data analysis. The final HHV-8 sero-status result will be based on 2 lab day runs' agreement (this is the standard protocol requirement for IFA).

- a. If sample results from both lab day runs agree (day 1: positive, day 2: positive), the final HHV-8 sero-status result (positive) will be recorded in the database.
 - b. If sample results from both lab day runs disagree (day 1: positive, day 2: negative), that sample (all its wave visits) will be repeated after all the samples on the list 1 and list 2 have been tested. That sample will be recorded as “lab day 3”. The “lab day 3” result will determine whether the sample will be recorded positive or negative for the final HHV-8 sero-status in the database (the majority rules; this action is based on the standard protocol of IFA).
 - c. If Tobago samples have an R (repeat) written on the slide reader’s result sheet for only 1 lab day run, that sample (all its wave visits) will be repeated after all the samples on the list 1 and list 2 have been tested. That sample will be recorded as “lab day 3”. The “lab day 3” result will determine whether the sample will be recorded positive or negative (the majority rules; this action is based on the standard protocol of IFA).
 - d. For Tobago samples that have discrepant results, Alicia will continue to test these samples until 2 lab day runs agree in HHV-8 sero-status (“lab day 4”, “lab day 5”, etc.).
8. After all Tobago samples from lists 1 and 2 have been tested, antibody titers will be performed on Tobago samples that have a final HHV-8 seropositive result in the database. Only positive samples will have antibody titers that are detectable by the IFA.
- e. Antibody titers (1:50 to 1:1600) will be performed (lab day 1). Lab results will be recorded by 2-folds on samples that are 1:100 to 1:800 dilutions. Samples that are marked as 1:1600 dilution may have a higher antibody titer; therefore, these samples will be tested again at higher antibody titers on another day. If a sample is not \geq 1:100 dilution, that sample will be marked as HHV-8 negative in database. Samples 1:50 or less are marked as HHV-8 negative.

- f.** If samples that have an antibody titer of dilution 1:1600 on lab day 1, antibody titers (1:50 to 1:102,400) will be performed on those samples at lab day 2. Lab results will be recorded in the database for data analysis.
- 9.** To ensure reliable data, lab results will be double-entered in Access and then transferred to SPSS or SAS; Alicia and another person will enter the data. If the data disagrees, Alicia will examine the lab result form and decide the final result in the database.

**APPENDIX B: THE PROTOCOL FOR PULLING TOBAGO SAMPLES FROM
FREEZER**



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