SERO-PREVALENCE AND IDENTIFICATION OF HERPESVIRUS PAPIO 2 STRAINS IN WILD-CAUGHT OLIVE BABOONS FROM SELECTED REGIONS IN KENYA

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DECLARATION

I Sharon Cherotich Chepkwony hereby declare that this thesis is my original work and has not been presented for degree or other awards in any other university.

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DEDICATION

This thesis is dedicated to my dad Julius Chepkwony and my mum Christine Chepkwony who laid foundation for my education, and to my siblings, Joselyne Chepkwemoi, Linet Chepkwony, Dorine Chepkorir, Paul Koech, Mercy Chepkwony, David Kiprop, Emma Chepchumba, Ruth Chebet and Elsie Cherono for their support during the entire duration of my studies. You have always been a source of my inspiration.

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ACRONYMS AND ABBREVIATIONS

AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care
BSA	Bovine Serum Albumin
BV	Monkey B Virus
CDC	Center for Disease Control
ChHV	Chimpanzee Herpes Virus
CNS	Central Nervous System
CPE	Complete cytopathologic effect
DNA	Deoxyribonucleic Acid
dT	Deoxythymidine
dTMP	Deoxythymidine-5'-monophosphate
DPI	Days Post Infection
EBSCO	Elton B Stephens Company
EDTA	Ethylene DiamineTetraacetate
ELISA	Enzyme Linked ImmunosorbentAssay
HIV	Human Immunodeficiency Virus
HSV	Herpes Simplex Virus
HVA	Herpesvirus ateles
HVP2	HerpesvirusPapio 2
HVS	Herpesvirus saimiri
IFN	Interferon
IPR	Institute of Primate Research

IRC	Institutional Review Committee
KAIS	Kenya AIDS Indicator Survey
NHPs	Non-Human Primates
NMP	Nucleoside monophosphate
OPC	Oklahoma positive control
PBS	Phosphate Buffer Saline
PBST	Phosphate Buffer Saline + Tween
PCR	Polymerase Chain Reaction
SA8	Simian Agent 8
SDS	Sodium Dodecyl Sulphate
TAE	Tris Acetate EDTA buffer
TG	Trigeminal Ganglia
TK	Thymidine Kinase
TMB	Tetra Methyl Benzidine
UL	Unique Long region
US	Unique Short region
USA	United States of America

ABSTRACT

Herpes simplex virus infection is caused by two Herpes simplex virus (HSV) serotypes, HSV-1 and HSV-2. HSV has been associated with the risk of miscarriage, premature labor, low fetal growth rate, meningitis, chronic skin infection and sometimes physical disability. The prevalence of HSV has been found to be higher in females than in males, thus the detrimental effects seen in pregnant women and their offspring. Herpes simplex virus has no cure and once one is infected, the virus remains in the trigeminal ganglia (TG) and dorsal root ganglia in latent form, and causes the development of lesions upon reactivation. The available drugs for HSV do not cure but only lengthen the period of recurrence. Finding drugs and vaccines to cure and thus completely eradicate HSV has been a great challenge. Infection of baboons (*Papio* species) with herpesvirus papio 2 (HVP 2) produces a disease that is clinically similar to HSV. Baboons have been identified as animal models for studying HSV. However, the prevalence and the circulating strains of HVP-2 in baboons from different regions of Kenya, is not known. In this study, the sero-prevalence of HVP 2 in baboons from selected geographical regions in Kenya was determined. The infection rates of HVP 2 in different sexes and age groups were also determined. The baboons were trapped using rectangular traps made of iron bars, wire mesh and weighted trap doors. The baboons were baited for a week using carrots, maize cobs and bananas. The technique used gave an equal chance for all the animals regardless of their gender or age to be trapped. The infected animals were identified by detection of anti-HVP 2 antibodies in sera from 189 baboons captured from different geographical regions using enzyme linked immunosorbent assay (ELISA). Pearson Chi- x^2square was used to compare the infection rates of baboons of different sexes and age groups. Graph pad prism 5 software was used for data processing. A P value of less than 0.05 was considered significant. To identify the HVP 2 strains circulating in these baboons, DNA was extracted from trigeminal ganglia, oral and genital swabs from sero-positive baboons. Polymerase chain reaction (PCR) was used to amplify the unique long (UL23) region that code for thymidine kinase. Positive amplicons from PCR were sequenced. Sequences were generated with the MEGA version 5 program package and blasted at the NCBI database using a blast algorithm. This study showed that 87% of the baboons in Kenya have been exposed to HVP-2 infection. Over 70% of baboons from the sampled regions, that is, Laikipia, Nyandarua, Kirinyaga, Machakos, Kajiado and Nairobi had been exposed to HVP-2. About 90% of the female and 83% of the male baboons were found to be sero-positive for HVP-2 antibodies. However, the difference between the sexes was statistically not significant (X^2=1.6; df=1; P>0.05). The sero-prevalence of HVP 2 in adult (94%) and sub adult baboons (90%) was found to be higher than in the juveniles (52%). Contrary to the difference in sexes, the difference in the age group sero-prevalence was statistically significant (X^2 =40; df=2; P<0.05). A herpes virus with 99% identity to HVP 2 strain A951 was identified. The virus was from a swab sample of a baboon trapped from Laikipia. The sero-prevalence of HVP 2 in baboons is universal and not depended on the geographical regions. It is recommended that virus isolation be done in order to identify more strains of HVP 2. Information on prevalence of HVP-2 and the circulating strains is essential in developing a baboon model to study the pathogenesis of HSV and for testing vaccines as well as development of effective drugs.

CHAPTER ONE: INTRODUCTION

1.1 Background Information

Herpes simplex virus (HSV) is an alphaherpesvirus that infects humans (Finnen and Banfield, 2010). There are two serotypes; HSV-1 and HSV-2. HSV-1 causes oral ulcers while HSV-2 is the leading cause of genital ulcers, a co-factor in HIV-1 acquisition and transmission as well as a cause for neonatal herpes infection (Joyce *et al.*, 2011). Reactivated HSV-2 infections are estimated to cause 45% of neonatal herpes transmission (Ades *et al.*, 1989; Brown *et al.*, 1997). Neonatal herpes infection can also be caused by HSV-1 infection in about 20% of the instances (Ades *et al.*, 1989; Brown *et al.*, 1997). The acquisition of genital herpes during pregnancy has been associated with spontaneous abortion, prematurity, and congenital and neonatal herpes (Zane *et al.*, 1997). Infection at the time of delivery may result in infection of the newborn infant during passage through the infected birth canal, or by ascending infection after rupture of the membranes. Neonatal herpes is a serious condition with about 60% mortality (Ades *et al.*, 1989). Studies in the African continent have shown variation in HSV-2 prevalence across diverse populations of women, ranging from 22% among adults in Tanzania and 68% among adults in Kenya to 90% among sexual workers in Zaire (Weiss *et al.*, 2001). In Western Kenya, a HIV survey among women aged 13-34 years conducted in 2003-4 revealed a HSV-2 prevalence of 53% (Amornkul *et al.*, 2009). A more recent National AIDS Indicator Survey among general women aged 16-64 years estimated HSV-2 prevalence to be 42% (KAIS, 2007).

A large proportion of viral pathogens that have emerged in humans have originated from various non-human primates including chimpanzees, gorilla orangutans, baboons, cynomogus marmosets and rhesus monkeys. Non- human primates are thus the best models for investigating the biological roots of human diseases (Gessain and Calattin, 2008). After initial interspecies transmission, these viruses have evolved and disseminated in the human population through various distinct mechanism. However, understanding of the initial step of emergency of some viruses and associated diseases remains poor. Baboons shares 91% of human genes (Karin, 2004). For this reason, they play a critical role to biomedical research in the advancement of human and animal health by targeting the cause, progression, prevention and treatment of a wide variety of diseases.

Herpes viruses that infect Non human primates (NHPs) include; *Cercopithecine herpesvirus 16* (Herpesvirus papio 2, HVP-2) of baboons, *Cercopithecine herpesvirus 1* (monkey B virus, BV) of macaques, *Cercopithecine herpesvirus 2* (Simian Agent 8, SA8) of African green monkeys, and *Saimirine herpesvirus 1* (*Herpesvirus saimiri 1*) of squirrel monkeys. Of great concern in this study is HVP 2 since the baboon is closer in terms of phylogenetic relationship to man than the other mentioned NHPs (Rogers and Hixson, 1997). Herpesvirus papio 2 (HVP-2) formally, is a simian alphaherpesvirus belonging to the family Herpesviridae and subfamily Herpesvirinae.

Baboons have been identified as animal models to study HSV. This study therefore, sort to find out if baboons in Kenya have been exposed to HVP 2 infection and determine

the exposure rates in baboons from different geographical regions, as well as identify the circulating HVP 2 strains.

1.2 Problem statement

Herpes simplex virus infection in pregnant women has been associated with risk of miscarriage, premature labor, low fetal growth rate, or transmission of the herpes infection to the infant during vaginal delivery leading to meningitis, chronic skin infection and sometimes physical disability. It has also been associated with HIV-1 infection. No cure for HSV has been identified and once one is infected, the virus remains in the body system. Viral reactivation always occur following sunburn, upper-respiratory infections, gastrointestinal tract infections, fevers, emotional stress, or anxiety (Whitley and Gnann, 2002). Available antiviral medicines help shorten the length of a herpes outbreak and cut down on recurring outbreaks, but do not cure the disease. Baboons are phylogenetically related to man and HVP-2 that affects baboons produces a disease that is clinically similar to HSV (Bigger and Martin, 2003), yet much consideration has not been given to studying HVP-2 infection in baboons in Kenya. Hence, serocreening and molecular characterization of HVP-2 is required to give some light in fully understanding the pathogenecity of HVP-2 in baboons which could serve as a model for studying HSV in humans.

1.3 Study Justification

According to a national representative household study (The Kenya AIDS Indicator Survey, KAIS, 2007), more than a third of Kenyan men and women are infected with herpes simplex virus type 2 (HSV-2). Women have a greater risk of being infected than men (Christie *et al.*, 1997). Preventing and treating herpes simplex viruses will significantly reduce the cases of miscarriages and fetal disability in affected women as well as reducing the cases of HIV-1 acquisition and transmission. Baboons are infected with HVP-2 which produces a disease that is clinically similar to HSV. There is however no scientific data on seroprevalence and circulating strains of HVP-2 in Kenyan baboons. In order to come up with the best preventive and curative methods, molecular biology of HSV must be well understood. One way to achieve this is by studying the molecular characterization of HVP-2, which produces a disease that is clinically similar to HSV. The results obtained from this study will help in identifying and targeting specific genes for future study and also provide additional insight into the evolutionary biology of herpes viruses. Establishing levels of HVP-2 infection of baboons in Kenya therefore, will provide a model to study the pathogenesis of simplex virus in the context of a natural, primate host and possibly help scientists come up with ways of managing HSV infections. This baboon model would also permit studies of the biology of HSV that could not be conducted in humans.

1.4 Research questions

i. What is the difference in HVP-2 sero-prevalence in baboons trapped from different geographical regions of Kenya?

ii. What is the difference in HVP-2 sero-prevalence between male and female baboons from selected regions of Kenya?

iii. What are the circulating strains of HVP-2 in baboons?

1.5 Null hypotheses

i. There is no difference in HVP-2 prevalence in baboons from different geographical regions in Kenya.

ii. There is no difference in HVP-2 infection prevalence between female and male baboons.

1.6 Objectives

1.6.1 General Objective

To determine the sero-prevalence and identify HVP-2 strains in wild caught olive baboons (*Papio anubis*) from different geographical regions in Kenya.

1.6.2 Specific objectives

i. To determine the sero-prevalence of HVP-2 in baboons from different geographical regions in Kenya.

ii. To determine the difference in HVP-2 infection rates between male and female baboons.

iii. To identify HVP-2 strains in baboons trapped from different geographical regions in Kenya.

1.7 Significance of the study

Since there is no available scientific data on HVP 2 surveillance in baboons in Kenya, this study will shade some light on whether baboons in Kenya get infected by HVP 2 or not. It will provide information on HVP 2 exposure rates in baboons from different geographical regions. Also establishing the infection rates of HVP 2 between male and female baboons is important given that the rate of HSV infection is higher in women than in men. The development of a model requires that there is a high degree of similarity in pathogenesis between the host in question and the animal model to be. The study will also provide information on the circulating strains of HVP 2 which will be important in characterization of a baboon as an infection model for HSV. Information on the circulating strains of HVP-2 and its prevalence will immensely influence the use of baboons as models to study the pathogenesis of HSV and in designing vaccines and drugs for HSV.

CHAPTER TWO: LITERATURE REVIEW

2.1 Herpes Simplex Virus

Herpes simplex virus is a member of the herpes virus family, *Herpesviridae,* which infects humans (Ryan and Ray, 2004). Herpes simplex virus (HSV) exists as two types, 1 and 2 (HSV-1 and HSV-2), and causes a lasting infection with recurrent lesions. Herpes simplex virus-1 has been associated with oro-labial disease, with most infections occurring during childhood, and HSV-2 with genital disease with infection following sexual debut (Nahmias *et al.*, 1990). However, it is possible for HSV-2 to cause oro-labial herpes and HSV-1 to cause genital herpes (Lafferty *et al.*, 1987). Genital infection with HSV-1 is associated with less severe disease and fewer recurrences than genital infection with HSV-2 (Wald *et al.*, 2002), although it is more likely to result in neonatal infection (Brown *et al.*, 2003). In addition, the severity of clinically apparent first episodes and reactivation with HSV-2 infection are lower in those with prior HSV-1 (Corey *et al.*, 2004).

During an infection, the virus passes through broken skin or the lining of the mouth, vagina, or anus and goes to the nuclei of the cells and tries to reproduce itself (CDC, 2006). The virus's replication process destroys the cells it has invaded causing blisters or ulcers to form on the skin. The blisters or ulcers crust over and heal without scarring. The virus is then transported back through the nerve to important nerve branching points called ganglia deep in the body. The virus is mainly found in the trigeminal ganglia (TG). It stays in the ganglia in an inactive, or latent, form. During this time, the virus does not replicate. It stays in this latent form for varying amounts of time

(Heather, 2014). Certain triggers may cause the virus to travel back down the nerve to the skin and cause symptoms again. The first episode of genital herpes includes symptoms such as; fever, headache, muscle aches, urinary pain or difficulty and swollen glands in the groin area (Beauman, 2005). A few days following the initial symptoms, sores or lesions erupt at the site of the infection. These sores can occur inside the vagina or on the cervix in women, as well as in the urinary passage in both men and women. Genital herpes lesions may first appear as small red bumps that develop into blisters which become painful, open sores (Figure 2.1, 2.2 and 2.3) (Whitley and Roizman, 2001). After several days these sores become crusted and then heal without scarring (American Academy of Pediatrics, 2003).

Infection with HSV-1 occurs worldwide, equally between the sexes. There are no seasonal variations for the infection with this particular virus. In the United States, it is estimated that there are approximately 500,000 primary infections annually (Scott *et al.*, 1997). Herpes simplex virus type 2 is a common infection in many countries, with prevalence in some regions, such as sub-Saharan Africa, higher than in the USA (Weiss, 2001). According to the U.S. Centers for Disease Control and Prevention, 45 million people in the United States aged 12 years and older, or 1 out of 5 of the total adolescent and adult population, are infected with HSV-2. Most data from Central and South America are from women, in whom HSV-2 prevalence ranges from about 20% to 40%. Prevalence in the general population in developing Asian countries appears to be lower (10-30%). Prevalence in adult general populations in sub-Saharan Africa ranges from 30% to 80% in women, and from 10% to 50% in men (Weiss, 2001). Just like in the

developed world, HSV-2 seropositivity is uniformly higher in women than in men and increases with age (Weiss, 2004). In Kenya, since the late 1970s, the number of people with genital herpes infection has increased to 30 percent (KAIS, 2007).

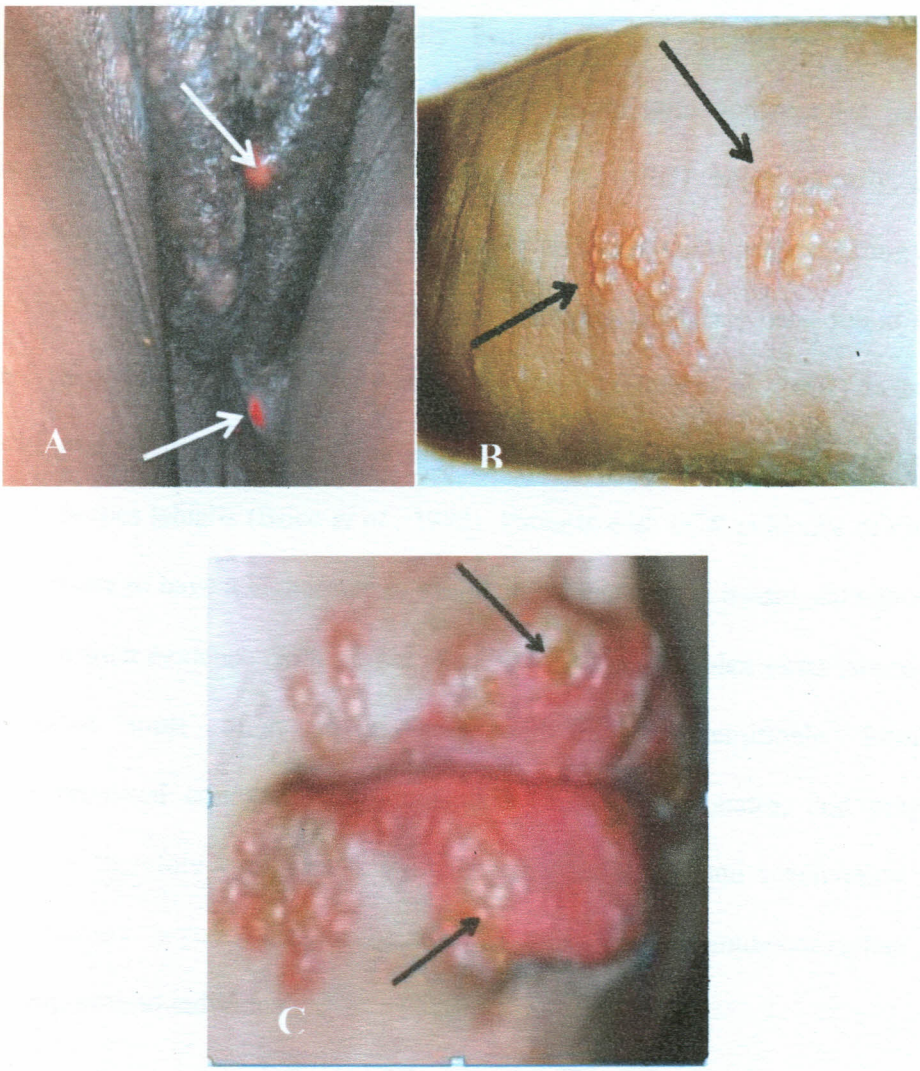


Figure 2.1: (A) Lesions caused by HSV-2 appearing on the vagina in humans. (B) Blisters caused by HSV-2 appearing on the penis in humans and (C) Lesions caused by HSV-1 appearing on the lips and mouth area in human (Borrowed from CDC, 2006)

2.1.1 Viremia in Herpes Simplex Virus Infection

Herpes simplex virus was isolated from the blood of an immunocompetent child with herpetic rhinitis in the mid 1900 (Ruchman *et al.*, 1950), signaling the possibility of more widespread infection in clinically benign disease. Sensitive HSV culture techniques were however unable to identify viremia in immunocompetent pediatric patients with clinical HSV infection (Halperin *et al.*, 1983). More recently, highly sensitive polymerase chain reaction (PCR) assays have altered the understanding of the natural history of HSV infection. Herpes simplex virus DNA has been found in the blood in 34% of children with primary gingivostomatitis (Harel *et al.*, 2004), in 24% of adults with primary genital herpes (Johnston *et al.*, 2008), and in 20% of persons with reactivation herpes labialis (Brice *et al.*, 1992). Patients with PCR evidence of viremia have been shown to have a higher rate of systemic symptoms and meningeal signs than those without such evidence (Johnston *et al.*, 2008). Herpes simplex virus viremia has been reported most often in patients with some identifiable form of immunocompromised conditions (eg, transplant recipients, neonates, and pregnant women). It is therefore evident that primary HSV infection and reactivation may frequently expand beyond the mucocutaneous surface in immunocompetent and immunocompromised individuals.

A study by Richard (2009), indicated that HSV viremia can occur with primary disease and with reactivation, as suggested by serologic testing that was performed for a subset of these patients. The study also found high mortality rates in patients with detectable HSV DNA in the blood.

2.1.2 Role of thymidine kinase in the virulence and latency of HSV

Thymidine kinase is an enzyme, a phosphotransferase, present in two forms in mammalian cells, TK1 and TK2 (Kit, 1985; Wintersberger, 1997). Certain viruses including the alphaherpesviruses, also have genetic information for expression of viral thymidine kinases. The enzyme plays a role in DNA synthesis and inhibition of this enzyme by antiviral drugs (acyclovir, IDU) inhibits virus multiplication. The drugs are effective only against viruses from the herpes group with their specific thymidine kinase (Mar *et al.*, 1985).

In a study to investigate the role of thymidine kinase in promoting virulence and latency in mice, it was shown that there was a correlation between TK expression and virulence and latency of HSV-1 in mice (Gordon *et al.*, 1983). Thymidine Kinase-positive (KT^+) strain of HSV 1 proved to be the most neurovirulent as compared to Thymidine Kinase-positive/negative ($TK^{+/-}$) and the Thymidine Kinase-negative (TK^-) strains used in the study. The results of the study thus indicated that TK gene activity is essential for HSV-1 murine neurovirulence that is, efficient CNS invasion, replication and establishment of latency. However, another study conducted using thymidine kinase deficient HSV 2 to induce genital infection in guinea pigs suggested that virus coded thymidine kinase may influence virulence but not latency (Stanberry, 1985). Thymidine kinase negative mutants established latent infections thus implying that thymidine kinase activity is not necessary for latency, but in reactivation from latency. This is according to a study by Donald *et al.* (1989). The presence of thymidine kinase in herpesvirus-infected cells is

used to activate a range of antivirals against herpes infection, and thus specifically target the therapy towards infected cells only.

The structure of TK is complexed with ADP at the ATP-site and deoxythymidine-5'-monophosphate (dTMP), deoxythymidine (dT), or idoxuridine-5'-phosphate (5-iodo-dUMP) at the substrate-site and exhibits important structural similarities to the nucleoside monophosphate (NMP) kinase family, which are known to show large conformational changes upon binding of substrates. TK catalyzes the phosphorylation of dT resulting in an ester, and the phosphorylation of dTMP giving rise to an anhydride. Numerous anti-Herpes drugs have been developed; they have been reviewed by De Clercq (1993). The initial target of these drugs is nearly in all cases the viral thymidine kinase (TK). This enzyme is part of the thymine salvage pathway, catalyzing the transfer of the y-phosphate from ATP to the 5'-hydroxyl of deoxythymidine (dT) to form deoxythymidine-5'-monophosphate (dTMP). In contrast to the corresponding host cell enzyme, TK from HSV-1 is not very specific and phosphorylates its primary product, dTMP, as well as a variety of substrate analogues such as the uridine derivatives idoxuridine and brivudin or guanosine derivatives aciclovir and ganciclovir. Figure 2.2 shows the structure of Thymidine Kinase.

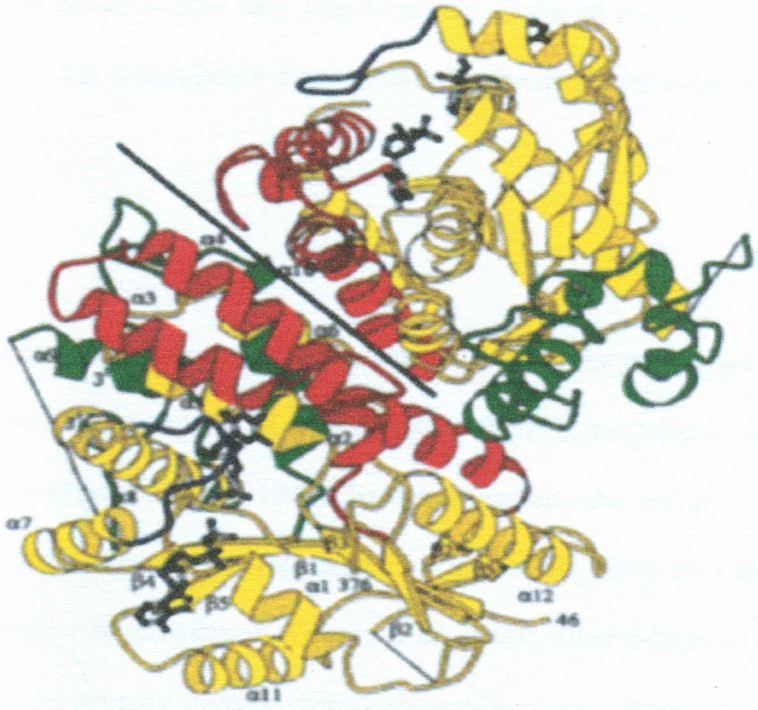


Figure 2.2: Structure of thymide kinase from herpesvirus. Stereoview of the symmetric TK dimer with bound ADP and dTMP. The domains are defined according to the NMP-kinases (residues 46-81, 143-218, 227-250, 323-376, yellow), NMP $_{bind}$ (82-142, red). 72 additional residues around position 290 are green (Klemens *et al.*, 1997)

2.1.3 Transmission of Herpes Simplex Virus

Transmission of HSV is through direct contact with a lesion or body fluid of an infected individual (David, 2012). People often get genital herpes by having sexual contact with others who don't know they are infected or who are having outbreaks of herpes without any sores. A person with genital herpes also can infect a sexual partner during oral sex (Wald, 2006). The virus is spread only rarely, if at all, by touching objects such as a toilet seat or hot tub according to National Institute of Allergy and Infectious Diseases

(1999). Infection of the mother may lead to severe illness in pregnancy and may be associated with virus transmission from mother to foetus/newborn (Anzivino *et al.*, 2009).

2.1.4 Treatment

Antiviral medications such as acyclovir, famciclovir, and valacyclovir are among the drugs used to treat the symptoms of herpes. Valacyclovir is available as a generic as well as acyclovir (Agrawal *et al.*, 1990). Evidence supports the use of acyclovir and valacyclovir in the treatment of herpes labialis (Chon *et al.*, 2007) as well as herpes infections in people who have cancer (Glenny *et al.*, 2009). The evidence to support the use of acyclovir in primary herpetic gingivostomatitis is less strong (Nasser *et al.*, 2008). As much as these antiviral medicines are used, they do not cure the disease but only help shorten the length of a herpes outbreak. Certain dietary supplements and alternative remedies are also claimed to be beneficial in the treatment of herpes (EBSCO, 2012). There is, however, insufficient evidence to support use of many of these compounds including euchinecea, eleuthero, L-lysine, zinc, monolaurin bee products and *Aloe vera* (Perfect *et al.*, 2005). While there are a number of small studies showing possible benefits from monolaurin, L-lysine, aspirin, lemon balm, topical zinc or licorice root cream in treatment, these are preliminary studies that have not been confirmed by higher quality randomized controlled studies (Beauman, 2005). It has been difficult to develop effective vaccines against herpes viruses, many of which have complex life cycles and can lie dormant in the body for long periods of time. The only vaccine that can successfully prevent infection by a member of the herpes virus family

is the chickenpox vaccine, which uses a live, weakened virus. (Ledford, 2012). There is need therefore, to study effectively the biology of HSV in order to come up effective drugs and vaccines.

2.2 Herpes virus in Non-Human Primates

There are several neurotropic alpha-herpesviruses that are related to human herpes simplex viruses (HSV-1 and HSV-2), that infect non-human primates (Eberle and Hilliard, 1995; Wertheim *et al.*, 2014). All these viruses produce a disease that is similar to HSV in their hosts. *Cercopithecine herpesvirus 1* (Monkey B virus [BV]) is common in macaques (*Macaca spp.*). B Virus is of major zoonotic concern. When transmitted to humans via bites or scratches, BV replicates in the peripheral epithelial tissue where it invades the sensory ganglia. The virus then progresses into the central nervous system (CNS) where it continues to replicate and spread resulting in extensive tissue damage leading to death in most cases (Huff and Barry, 2003). *Cercopithecine herpesvirus 2* or Simian Agent 8 (SA8) infects the African Green Monkeys (*Cercopithecus aethiops*). Simian Agent 8 was originally isolated from the African Green Monkeys but it has also been found to be common in both wild and captive baboons (Hull, 1973; Eberle *et al.*, 1995). *Herpesvirus saimiri* and *Herpesvirus ateles* are common in squirrel monkeys and spider monkeys respectively.

The baboons' *Cercopithecine herpesvirus 16* or Herpesvirus Papio 2 (HVP-2) had earlier been misidentified as SA8, but recent molecular analyses has shown it to be distinct from SA8 (Eberle *et al.*, 1995). A Part from the Monkey B Virus, no cases of

human infection have been reported in the other simian viruses (Rogers *et al.*, 2003). A recent study by Wertheim *et al.* (2014) has linked HSV 2 infection to cross-species transmission. Herpes simplex virus 2 is thought to have originated from chimpanzee, the closest relative of man. Table 2.1 gives a summary of herpes viruses and their hosts,

Table 2.1: Summary of herpes viruses and their hosts (Wertheim *et al.*, 2014)

Virus	Virus Abbreviation	Host Latin Name	Host Common Name
Herpesvirus Papio 2	HVP-2	*Papio spp*	Baboon
Simian Agent 8	SA8	*Cercopithecus aethiops*	African green monkey
Chimpanzee herpes virus	ChHV	*Pan troglodytes*	Chimpanzee
Herpes simplex virus 1	HSV-1	*Homo sapiens*	Human
Herpes simplex virus 2	HSV-2	*Homo sapiens*	Human
Monkey B Virus	BV	*Macaca spp.*	Macaques
Saimiriine herpes virus	HVS-1	*Saimiri sciureus*	Squirrel monkey
Spider monkey herpes virus	HVA-1	*Ateles geoffroyi*	Spider monkey

The phylogenetic tree below (Figure 2.3) shows the relationship between the chimpanzee herpes virus (ChHV), the baboon herpes virus (HVP 2) and the human herpes virus with other simian herpes viruses.

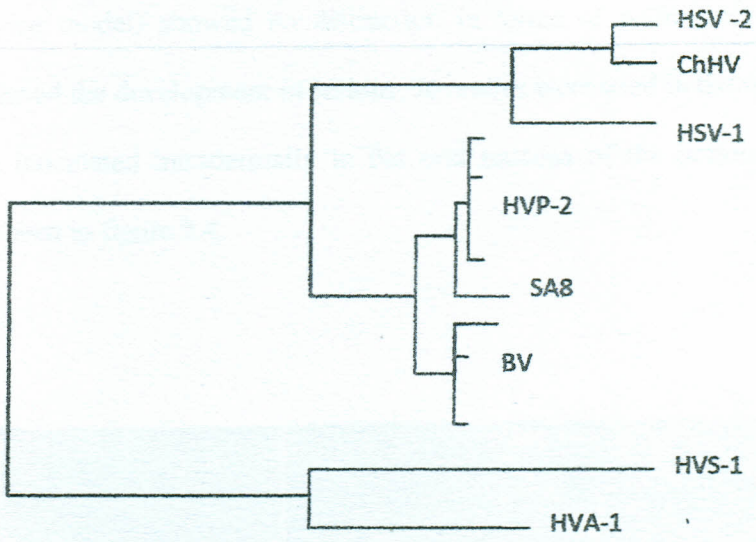


Figure 2.3: Phylogenetic tree showing the relationship between human and simian herpes viruses (HSV-2-Herpes Simplex Virus type 2; ChHV-Chimpanzee Herpes Virus; HVP-2-Herpesvirus Papio 2; SA8-Simian Agent 8; BV-Monkey B Virus; HVS-1-Saimiriine herpes virus; HVA-1-Spider Monkey Herpes Virus)(Wertheim *et al.*, 2014)

2.2.1 Herpesvirus papio 2

Herpesvirus papio 2 (HVP-2) is an indigenous B-lymphotropic virus of baboons (*Papio sp.*) present in latent form in baboon lymphoblastoid cell lines (Lawrence, 1976). Herpesvirus papio 2 exists as two major serotypes, HVP apathogenic (HVP*ap*) and HVP neurovirulent (HVP *nv*) (Ritchey *et al.*, 2002). This difference in pathogenesis however, is only seen in the mouse model. For instance, HVP 2 strain A951 has been identified as apathogenic in the mouse model. However, an experimental study in which

baboons were infected with different strains of HVP-2 (both apathogenic and neurovirulent in a mice model) showed no distinction in terms of pathogenesis in baboons as they all caused the development of lesions. Juveniles were used in the study, where the virus was inoculated intradermally in the oral mucosa of the bottom lip. Lesions developed as seen in figure 2.4.

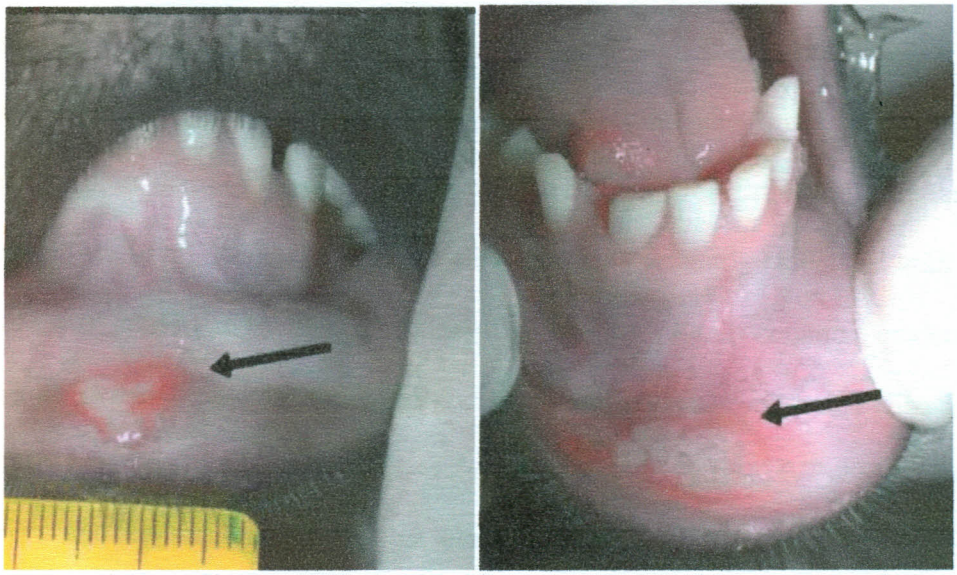


Figure 2.4: Increasing size of oral lesion in the baboon mouth following inoculation with HVP 2 (Rogers *et al.*, 2005)

The lesions continued increasing up to 21 days post infection (DPI), and then they disappeared. The study was conducted by Rogers *et al.* (2005). The study proves that mice are good models for studying cross-species transmission of herpes viruses. The fact that HVP-2 infection in the baboon mimics HSV infection in humans in terms of

lesions development regardless of the strain shows that baboon is a good model for HSV study.

Infection of baboons (*Papio* species) with HVP-2 produces a disease that is clinically similar to herpes simplex virus (HSV-1 and HSV-2) that affects human (Bigger and Martin, 2003). Infected adult baboons develop genital lesions whereas oral lesions represent the predominant form of disease in younger animals (Levin *et al.*, 1988). The lesions range from vesicles to ulcers (Figure 2.5). Spontaneous reactivation from latency has been demonstrated, a pattern consistent with HSV infection of humans (Martino *et al.*, 1998). Acquisition of genital infection is primarily associated with the onset of sexual activity in baboons. Secondary bacterial infection is common, epidermal necrosis with central erosions and peripheral parakeratosis is also observed (Eberle *et al.*, 1998). Like as with other members of the genus Simplex virus, HVP-2 also causes Urogenital and neuronal infections that closely resemble those caused by HSV in humans (Martino *et al.*, 1998).

HVP-2 outbreaks in baboon breeding colonies have been associated with genital infections of adults and an increased incidence of stillbirths and hydrocephaly in newborns (Levin *et al.*, 1988).

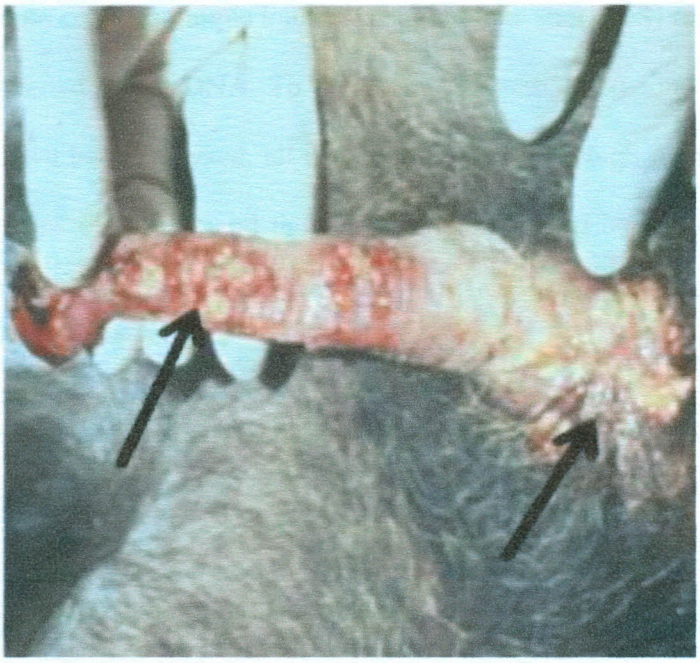


Figure 2.5: Ulcers appearing on the baboon penis following infection by HVP-2 (Borrowed from Dr. Richard Eberle from Department of veterinary pathobiology, Oklahoma State University, USA)

The prevalence of HVP-2 in several groups of captive and wild-caught baboons was determined by detection of anti-HVP-2 antibodies in 133 sera of adult baboons. Over 90% of newly imported (wild-caught) adult olive baboons (*Papio anubis*) from Kenya and chacma baboons (*P. ursinus*) from South Africa were found to have anti-HVP-2 antibodies. Similarly, approximately 85% of captive breeding colony baboons (*P. anubis* and *P. cynocephalus*) were seropositive for HVP-2 (Eberle *et al.*, 1997). Baboons are social animals and move in large groups called troops (Figure 2.6) and this could be the cause of high rates of infection by HVP-2, especially for the sexually mature animals in the troop (Berman *et al*., 2003).

The HVP-2 genome contains unique long (UL) and unique short (US) regions separated by regions of inverted repeat DNA. To date some 27 partial and four complete open reading frames have been analyzed, all of which demonstrate high degrees of identity between the proteins of HVP 2 and their HSV homologues (Eberle and Black *et al.*, 1995; 1997). The similarity between HVP 2 and HSV at both the clinical and genetic levels indicates that the HVP 2 system has the potential to be a powerful surrogate model to study the biology of herpes simplex virus replication in the context of a natural primate host.

Because of lack of any reported human infections by HVP2, the virus has largely been considered unimportant from a zoonotic standpoint. Studies have however shown that intraperitoneal inoculation of mice with HVP 2 causes rapid death (Eberle *et al.*, 1995), suggesting that HVP 2 could affect other mammalian species.

Figure 2.6: A troop of baboons in the wild (Berman *et al.*, 2003)

2.2.2 Herpesvirus papio 2 in non primates

In a study comparing SA8 and HVP 2 to HSV 1 in a mouse model, two strains of HVP 2, HVP 2 apathogenic (HVP*ap)* and HVP 2 neurovirulent, (HVP2*nv*) were found to readily invade the central nervous system (CNS) following peripheral intramuscular inoculation (which most closely resembles a bite or scratch), producing paralysis and death (Ritchey *et al*., 2002). Herpesvirus papio 2 *ap* is not competent for spread within the brain. Herpesvirus papio 2 *nv* on the other hand readily invades the CNS and produces a fulminant ascending encephalomyelitis which proves fatal once the virus reaches the brainstem (Rogers *et al*., 2003). Previous studies implicated the type I interferon (IFN) response as being a major factor in controlling infection by apathogenic isolates.

2.2.3 Transmission of human herpes to Monkeys

Infection by herpes 1 from human to monkeys can cause fatal herpes 1 disease in monkeys (Herpes simplex, cold sores, fever and blisters). Just as the human immune system is not equipped to deal with the Monkey B virus, the immune system of monkeys is not equipped to deal with herpes 1 virus from humans. If the monkey does not die immediately, the same treatment used in humans can be given. A case of transmission of herpes from human to monkeys was observed by Matz-Rensing *et al.* (2003) in a group of common marmosets that were infected by fatal herpes simplex.

An outbreak occurred in a group of *Callithrix jacchus* housed as a family group after contact with a person who had recrudescent HSV-1 disease. On the first day of the outbreak, a male marmoset showed apathy, anorexia, weakness, and marked salivation and died the same afternoon. At that time, no other animals in the group showed clinical signs (Mat-Rensing *et al.*, 2003). The following morning, one young adult and a female developed excessive salivation and apathy. Closer examination showed ulceration of the lips and the oral cavity. In total, eight animals were infected and all had a 1–2 day history of vesicular to ulcerative gingivitis and stomatitis accompanied by severe salivation, serous nasal discharges, anorexia, and depression. Three of the animals developed lesions covered with fibronecrotic exudate at the mucocutaneous junctions. At necropsy, all animals showed an acute gingivitis and stomatitis. The tongues had multifocal single to coalescing lingual erosions with rough borders up to 0.5–1.0 cm in diameter. Some lesions appeared to be lingual or gingival ulcers covered by a fibrinopurulent exudates. Mild splenomegaly and severe lymphadenopathy of the regional lymph nodes was also observed. These animals had had contact with a caretaker suffering from a herpes labialis–like illness a few days before the outbreak occurred (Mat-Rensing *et al.*, 2003).

Naturally occurring herpes simplex was also reported to be fatal in a family of white faced saki monkeys. From the study, a family of three white-faced saki monkeys (*Pithecia pithecia pithecia*) died 48-96 hours after the onset of anorexia, nasal discharge, pyrexia and oral ulceration. One animal also had clonic seizures. Lesions found post-mortem consisted of oral and esophageal ulcers, hepatic and intestinal

necrosis, meningoencephalitis and sporadic neuronal necrosis. Intranuclear inclusion bodies and syncytial cells were present in oral lesions and affected areas of liver. Herpes simplex virus 1 (HSV-1) was identified as the etiology of disease by virus isolation, polymerase chain reaction, or in situ hybridization in all three animals. Immunohistochemistry for detection of apoptotic DNA and activated caspase-3 showed significant levels of apoptosis in oral and liver lesions and occasional apoptotic neurons in the brain. The finding of the study demonstrated the vulnerability of white-faced saki monkeys to HSV-1 (Schrenzel *et al.*, 2003). In most of the reported infections in various non human primates species, it was indicated that contact with humans was the source of herpes virus infection. Visitors, students, or caretakers might be able to infect the animals, for example, by passing partly eaten food into the cage.

2.3 Role of interferons in herpes virus infections

Interferons (IFNs) are proteins made and released by host cells in response to the presence of pathogens such as viruses, bacteria, parasites or tumor cells. More than twenty distinct IFN genes and proteins have been identified in animals, including humans. They are typically divided among three classes: Type I IFN, Type II IFN, and Type III IFN, based on the type of receptor through which they signal. IFNs belonging to all three classes are important for fighting viral infections and for the regulation of the immune system. The type I interferons present in humans are IFN-α, IFN-β, IFN-ε, IFN-κ and IFN-ω (Liu, 2005). Type II interferon is only IFN γ while type III interferons consist of Interleukin 10 Receptor 2 (IL10R2) and Interferon Lamda Receptor 1 (IFNLR1) (Vilcek, 2003; Hermant *et al.*, 2014)

2.3.1 Role of interferons in Replication of HSV

A study that was performed to analyze the effect of IFNs on HSV replication in human primary cells, showed that Human macrophages were sensitive to the antiviral activity of exogenous IFN α and IFN β, whereas IFN ɣ exhibited only very little antiviral activity (Ingrid *et al.*, 1986). The effect was macrophage specific, because inhibition of HSV replication on HEp-2 cells by IFN-ɣ was comparable to virus inhibition by other IFN types. The results of the study also indicated that IFN blocks a step before HSV DNA synthesis. The main block during the viral replication cycle occurs either during or before the synthesis of immediate early α-proteins (Ingrid *et al.*, 1986).

Another study describes differences in biological activities of IFN-ɣ on human monocytes and WISH cells; a comparison of the IFN-ɣ receptor on both cell types revealed different binding characteristics (Orchansky *et al.*, 1986). Thus, the lack of antiviral activity of IFN- Y against HSV on macrophages could be related to a different receptor on these cells. This is in agreement with the finding that IFN- ɣ is ineffective in potentiating the antiviral activities of IFN-α or IFN-β (Ingrid *et al.*, 1986).

2.3.2 Role of type 1 interferon on HVP-2 infection

A critical component of the innate anti-viral response is production of the type I interferons α and β (IFN-α, IFN-β). IFN-α is rapidly produced by infected plasmacytoid dendritic cells (pDCs) or when they are stimulated by recognition of viral components

via toll-like receptors (TLRs) either through endocytosis of viral particles or autophagy of infected cells (Lee *et al.*, 2007).

IFN-β is rapidly induced in many cell types (including those infected by herpesviruses such as fibroblasts and epithelial cells) due to their constitutive expression of IRF-3. IFN-α and –β produced by infected cells binds to the IFN-α/β receptor (IFNAR) initiating a signal transduction cascade via the Janus associated kinase – signal transducer and activator of transcription (Jak-Stat) pathway (Darnell *et al.*, 1994; Goodbourn *et al.*, 2000).

Following inoculation of mice with HVP 2, two subtypes (HVP*nv* and HVP*ap*) of the virus were identified. These subtypes vary in their pathogenesis in mice. Since the pathogenesis of HVP2nv and HVP2ap infection in mice diverges well before an adaptive immune response develops, innate immunity likely plays a major role in effecting the divergent pathogenicity of the two HVP2 subtypes. IFN-β appears to be involved in controlling HVP2ap infections since HVP2nv isolates do not induce and/or overcome an IFN-β response, and are thus not as susceptible to IFN-β in vitro as HVP2ap isolates are (Rogers *et al.*, 2007). The experiments presented by Rogers *et al.* (2009), extend the above observations and confirm a critical role for the type I IFN response in controlling HVP2ap infections *in vivo*.

2.4 Importance of using a baboon as a model to study HSV

There are several advantages of using captive baboons for studies on herpesvirus associated diseases. First, the animals are subjected to the same environmental conditions, which naturally trigger reactivation of the endogenous herpesviruses; the animals are genetically diverse (Sidebottom *et al.,* 2001), yet most are related, and familial associations within the breeding cohort are easily established. The animals are also available for intervention as well as extensive observational studies on a long-term basis. Female baboons have a prominent sex skin, which swells visibly and can be used to follow the menstrual cycle. Since herpesviruses are transmitted *in utero* or during birth, and since in particular the beta herpesviruses are a leading cause of congenital infections and associated diseases, this unique feature in the natural history of baboons lends itself for detailed studies of theses pathologies (Sidebottom *et al.,* 2001). At the Institute of Primate Research, the baboons (*Papio anubis*) are housed in special facilities (Figure 2.7). The animals are housed according to the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

Figure 2.7: Wild caught baboons in the animal facility of the Institute of Primate Research

Vaccine research is one of the applications for baboon models of infectious disease. Since baboons carry a full complement of the gamma isotype of immunoglubulins, human reagents can be used to measure immune response and the animals can be challenged to assess vaccine efficacy. Therefore, baboons are an outstanding model for vaccine studies. There is already an existing extensive history of testing vaccines in baboons (Jeong *et al.*, 2004). The vaccines are for various infectious agents including viruses, bacteria, and parasites. Recent vaccine studies in baboons have demonstrated

that Hepatitis C virus-like particles, produced using a recombinant baculovirus containing the cDNA of HCV structural proteins, can induce both cellular and humoral immunity in baboons (Jeong *et al.*, 2004). Other viral vaccine studies have demonstrated the ability of baboons to produce an antibody response to a commercially available encephalomyocarditis virus vaccine, and baboons were the chosen model for analyzing the safety of using *Escherichia coli* heat-labile toxin as an adjuvant for the nasal influenza vaccine (Huneke *et al.*, 1998; Zurbriggen *et al.*, 2003). Other studies have proven the baboon to be an effective model for elucidating the necessity of an antibody response when vaccinating against the parasite, *Schistosoma mansoni*. In the studies, IgG levels directly correlated with the level of protection (Soisson *et al.*, 1993; Kariuki *et al.*, 2004). Capitalizing upon the high similarity between baboon and human reproductive biology, studies have demonstrated the ability of the fetus to mount a fetal specific antibody response and the neonates go on to develop immunological memory against hepatitis B surface antigen (Watts *et al.*, 1999; Bot *et al.*, 2001).

A survey on the prevalence of HSV-2 in the world was done by Katherine *et al.* (2008). According to the survey, the estimated total number of people aged 15–49 years who were living with HSV-2 worldwide in 2003 was 536 million. More women than men were infected, with an estimated 315 million infected women compared to 221 million infected men. The number infected increased with age, most markedly in the younger ages, until it peaked in the age stratum 35–39 years of age, after which it declined slightly. According to the same survey, analysis by region reveals similar trends. The patterns in the numbers infected within each region predictably mirror the trends in

prevalence, with more women than men infected, and higher number of cases among those at older age. Many studies that have been conducted on prevalence of HSV-2 in Kenya have shown similar results. According to Mugo *et al.* (2011), the total prevalence of HSV-2 in Kenya is 35%, of which 42% were women and 26% were men.

The prevalence of HVP-2 in baboons is 90%. This prevalence was obtained after sampling baboons from Southwest Foundation for Research in San Antonio in Texas, New England Regional Primate Center and also baboons that had been captured in Kenya and South Africa (Eberle *et al.,* 1997). There is however, no data showing either the comparative prevalence of HVP-2 in baboons of different sexes or age groups. The prevalence of HVP-2 in Kenyan baboons alone is also not known.Several strains of HVP-2 in baboons have been identified. These strains have been classified as the neurovirulent subtype, and the apathogenic subtype. The neurovirulent strains include; A189164, OU1–76, OU3–1, OU3-18, X313 and 960. The apathogenic strains on the other hand include; A951, OU2-5, OU2-9, OU2-12, OU4-2, OU4-8 and OU4-15. This classification however was determined based on HVP-2 infection in mice (Rogers *et al.,* 2003). In baboons, there is no distinction in terms of pathogenecity of the strains as they all cause development of lesions (Rogers *et al.,* 2005)

Currently in Kenya, there is no available scientific data on circulating strains of HVP-2 in non human primates which include the olive baboons (*Papio anubis*) and the African green monkeys (vervets). Such data would provide an insight into the evolutionary biology of herpes viruses.

The similarity between HVP-2 and HSV at both the clinical and genetic levels indicates that the HVP 2 system has the potential to be a powerful surrogate model to study the biology of herpes simplex virus replication in the context of a natural primate host.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study site

This study was conducted at the Reproductive health and Biology Laboratories of the Institute of Primate Research (IPR) in Karen, Nairobi County, Kenya. The Institute of Primate Research is a biomedical research facility mandated to undertake research using non-human primates (NHPs) as models with a mission to improve human health. In this study, samples were collected from the captive baboons at the IPR animal facility and also from IPR blood bank. The baboons were captured from six geographical regions of Kenya. These regions include; Laikipia, Nyandarua, Kirinyaga, Machakos, Kajiado and Nairobi.

3.2 Study Design

This was a cross sectional study where 5 ml of blood was drawn from wild caught olive baboons. These baboons were captured from the following regions; Laikipia, Nyandarua, Kirinyaga, Machakos, Kajiado and Nairobi.

3.3 Animal capture

The baboons were trapped using rectangular traps (1.5m high and 0.9m wide) made of iron bars, wire mesh and weighted trap doors designed by Institute of Primate research (IPR) staff. The NHPs were baited for a week using maize cobs, carrots and bananas. The monkeys then became habituated. The traps were set in the evening after the animals had scampered to their sleeping site with the aim of capturing them the next

day (Jeneby *et al.*, 2002; Eley *et al.*, 2005). This technique gave an equal chance for all the animals regardless of their gender or age to have an equal opportunity to feed and thus trapped as they came for the bait. The animals were trapped then transferred to their holding cages and transported to Institute of Primate Research quarantine area.

3.4 Sample size determination

This study involved serum samples, trigeminal ganglia and swabs collected from baboons. The sample size was determined using the following formula according to Dell *et al.* (2002).

$$n = C\frac{p_c q_c + p_e q_e}{d^2} + \frac{2}{d} + 2$$

Where;

n = Minimum sample size required

p_c = Observed prevalence (90%), (Eberle *et al.*, 1997)

p_e = Postulated prevalence (85%), (Eberle *et al.*, 1997)

$qc = 1 - p_c$

$qe = 1 - p_e$

$d = | Pc - Pe |$

C = Constant that depend on chosen values of α and β; 10.519

$$n = 10.519\left(\frac{0.9 \times 0.1 + 0.85 \times 0.4}{0.3^2}\right) + \frac{2}{0.3} + 2 = 59$$

Thus, a minimum of 59 baboon samples was required in this study. However, a total of 189 baboons were sampled in the study.

3.5 Sample collection

Five milliliters of blood was collected from the baboons. Genital and oral swabs were collected from 100 out of the 189 baboons. Trigeminal Ganglia (TG) were collected from 9 asymptomatic baboons at autopsy. Blood was used to screen for anti-HVP2 IgG. Deoxyribonucleic Acid (DNA) extraction was done for swabs and TG from asymptomatic animals. Thymidine kinase coding region of HVP-2 was amplified using TK1/TK2 primer set. Positive sample amplicons from PCR were purified before sequencing. During sampling, baboons quarantined and those housed at the Institute of Primate Research cages were injected with anaesthesia; a mixture of xylazine (2%) and Ketamine hydrochloride (10%) in the ratio of 3:7 respectively. The dosage given was 0.1ml/kg body weight (Suleiman *et al.*, 2008).

3.5.1 Blood sample

Blood samples were collected from 105 freshly captured baboons from the wild. Five milliliters of venous blood were collected in sterile 9 ml tubes (Greiner bione, France) for serum separation. Serum was stored at -20°C before ELISA was done. Eighty four serum samples were acquired from the IPR serum bank. These sera were from baboons that had been at IPR for more than three months.

3.5.2 Swabs

Swabs were collected from asymptomatic baboons. Since baboons acquire virus as an oral infection while young or as a genital infection after reaching sexual maturity, Oral swabs were collected from 31 juveniles while genital swabs were collected from 69 adult baboons. For the juveniles, swabbing was done in the chicks and the tongue. As for the adults, swabbing was done on the vaginal lining and the cervix for the females, and in the urethra for the males. Cotton-tipped applicators were used as swabs and placed in 1-ml transport medium (*digene*® Female Swab Specimen Collection Kit, QIAGEN). A total of 100 genital and oral swabs from the baboons were collected.

3.5.3 Trigeminal ganglia

Nine TG tissues were collected. At the time of autopsy, all TG were aseptically removed and trimmed of dura and fat. All TG were aseptically removed after opening the skull, and trimmed of dura and fat. The three branches of the TG were dissected away prior to immediate snap-freezing and storage at -80°C

Clinical swabs and the brain tissues were then submitted to the Reproductive Health laboratory at IPR Nairobi, where analysis was done.

3.6 Viral antigen preparation

The HVP 2 antigens, positive and negative controls were generously provided by Professor Richard Eberle of Veterinary Pathology Department of Oklahoma States University, USA. Herpesvirus Papio 2 antigens were prepared by Dr. Richard Eberle in his laboratory (Eberle *et al.*, 1997). During the preparation, HVP2 was isolated from an adult baboon. The virus was propagated in African green monkey (vero) cells. Substrate antigen for enzyme-linked immunosorbent assays (ELISA) was propagated by inducing an infection to a 150 cm^2 flask of vero cells with HVP2. When complete cytopathologic effect (CPE) had taken place, cells were scraped into a medium and pelleted by centrifugation at 400xg for 5 min and then, the medium removed. The infected cells were frozen for 30 mins in ice, thawed and then resuspended in 0.5% Triton x-100 Tx-100 (in water) at a concentration of 2 x 10^7 cells/ml. Two microfuge tubes containing 500 µl of infected cells in suspension were prepared. The negative control constituted uninfected vero cells. The cells, positive and negative controls were then lyophilized before being shipped to IPR from Oklahoma State University.

3.6.1 Lyophilized HVP-2 antigen resuspension

The lyophilized antigens were resuspended in 500 µl/tube of sterile water containing 0.5% triton x-100 and 0.01% of Sodium Dodecyl Sulphate (SDS). Up and down

pipeting was done to resuspend the cells. Vortexing was done every few minutes for a period of 10 min. Centrifugation was done at full speed (14,000 rpm) for 30 seconds. The supernatant (antigen) was aliquoted and stored at -80°C while the pellet was discarded.

3.7 Screening for HVP-2 antibodies using Enzyme Linked Immunosorbent Assay (ELISA)

For ELISA, 96 well flat-bottomed plates were coated with HVP 2 antigen (100µl/well) diluted at 1:600 in 1x Phosphate Buffered Saline (1x PBS), and incubated overnight at 4°C. The plate was washed 3 times in the ELISA washer. Excess binding site was blocked by addition of 100 µl of PBS containing 3% Bovine serum albumin BSA and 5 µl Tween 20 (PBS/TW/BSA) for 1 hour at 37°C. The excess blocking buffer was then aspirated and the plate washed 5 times. Fifty microlitres of sample sera diluted in PBS/TW containing 0.3% of BSA at 1:200 was added to each well and incubated at room temperature for 2 hours. Wells were washed 5 times with PBS/TW. Fifty microlitres per well of goat anti-human IgG peroxidase conjugate diluted in 1:2000 was added and incubated for 1hour at room temperature. Wells were washed again in PBS/TW and 50 µl of substrate solution (TMB) per well was added, then incubated in dark for 10 min (when +control was blue and –control clear). Optical density OD at a wavelength of 630 nm was determined using a microplate reader (DYNATECH Laboratories, Germany).

3.8 Extraction of Deoxyribonucleic Acid (DNA)

Deoxyribonucleic acid was extracted from 76 swabs (both oral and genital) from animals that were seropositive by ELISA. A total of 24 swabs were left out since the animals from which the swabs were collected tested negative for HVP 2 when ELISA was done. DNA was also extracted from the 9 TG samples that were collected.

3.8.1 Oral and genital swabs

Viral DNA was extracted from swabs of seropositive animals, using the ZYMO RESEARCH ZR -DNA extraction Minikit (Inqaba, South Africa) according to the manufacturer's protocol. These swabs had been stored at -80^{o}C. Swabs were picked from the transport media. The stick part of the swab was cut off and the cotton swab inserted into an eppendorf tube. Five hundred microlitres of genomic lysis buffer was added to each eppendorf tube. Vortexing was done for 10 sec and the latter was left to stand for 10 min at room temperature. The mixture was transferred to a zymospin column in a collection tube. Centrifugation was done at 11,000 rpm for 1 min. The zymospin column was transferred to a new collection tube and 200 µl of DNA pre-wash buffer added. Centrifugation was done at 11,000 rpm for 1 min. Five hundred microlitres of g-DNA wash buffer was added to the spin column. Centrifugation was done at 11,000 rpm for 1 min. The spin column was then transferred to a clean eppendorf tube. Thirty microlitres of DNA elution buffer was added to the spin column. Incubation was done at room temperature for 10 min. Centrifugation at top speed

(14,000 rpm) was done for 30 sec. The eluted DNA was stored at -20°C before being used for PCR.

3.8.2 Trigeminal ganglia

Deoxyribonucleic acid extraction was done using the Qiagen kit according to the manufacturer's protocol. Twenty five milligrams of the trigeminal ganglia tissue was cut up into small pieces and put in a 1.5 microfuge tube. A hundred microlitres of the ATL buffer was added. Twenty microlitres of proteinase K was added and mixed by vortexing thereafter incubation at 56°C overnight followed. Occasional vortexing during incubation was done to disperse the sample. Centrifugation was done to remove drops from the inside of the lid. Two hundred microlitres of AL buffer was added to the sample and pulse-vortexing done for 15 sec. Incubation followed at 72°C for 10 min. Two hundred microlitres of ethanol (99%) was added to the sample and mixed by pulse-vortexing done for 15 min. The mixture was carefully applied to a QIAmp spin column in a collection tube and centrifugation done for 1 min at 8,000 rpm. The QIAmp spin column was carefully opened and 500 µl of AW1 buffer added. Centrifugation was done at 8,000 rpm for 1 min. The QIAmp spin column was transferred to a new collection tube. Five hundred microlitres of AW2 buffer was added and centrifugation done at full speed (14,000 rpm) for 3 min. The QIAmp spin column was transferred to another collection tube. Two hundred microlitres of buffer (AE) was added to the spin column. Incubation at room temperature for was done for 5 min. The eluted DNA was stored at -20°C before PCR was done.

3.8.3 Confirmation of the extracted Deoxyribonucleic Acid

Gel electrophoresis was done to confirm whether the DNA had been extracted. 1% agarose gel was prepared. Ten microlitres per well of the extracted DNA was added in each well. It was then allowed to run for 40 min. The presence of DNA was confirmed by viewing the gel in a UV trans-illuminator.

3.9 Amplification of deoxyribonucleic Acid

For PCR assay, the unique long region (UL 23) of herpes virus that code for thymidine kinase was amplified using primer set TK1 (5'TTCACCGTGGGCTGGACTGG 3') and TK2 (5'GCGGTTCTGGAGCTCGGACCA 3'). For virus characterization, PCR products were purified before being sent for direct sequencing.

3.10 Polymerase Chain Reaction conditions

A ready mix PCR master mix from zymogen was used. The PCR components consisted of 9.5 µl of PCR master mix, 1 µl of the forward primer, 1 µl of the reverse primer, 1 µl of the DNA template and 9.5 µl of nuclease free water to bring the final Volume to 25 µl. PCR was run using 30 cycles of initial denaturation at 94°C for 5 min, denaturation at 94°C for 60 sec, annealing at 60°C for 60 sec, and elongation at 72°C for 120 sec in a thermo cycler. A final extension of products was carried out at 72°C for 4 min.

3.11 Gel electrophoresis

Agarose gel electrophoresis was used to determine and visualize the presence of PCR products. Specific-DNA amplification was confirmed by electrophoresis using 1.5% agarose gels prepared by dissolving 1.5 gm of agarose into 100 ml of Tris acetate EDTA (TAE) buffer and then adding 5 µl of ethydium bromide. The gel wells were loaded with 9 µl of the amplicons. The gel was then visualized using a UV transiluminator.

3.12 Purification of PCR products

Polymerase chain reaction products were purified prior to sequencing using Gene JET^{TM} PCR Purification kit. Binding buffer was added to the PCR mixture at a ratio of 1:1 and mixing done vigorously. The solution was transferred to the Gene JET^{TM} purification column. Centrifugation was then done for 1 min at 14,000 rpm. The flow-through was then discarded. Seven hundred microlitres of the wash buffer was added to the Gene JET purification column. Centrifugation was done at 14,000 rpm for 1 min. The flow-through was discarded and the purification column put back into the collection tube. The Gene JET purification column was centrifuged for an additional 3 min to completely remove any residual wash. The Gene JET purification column was transferred to a clean microfuge tube. Twenty five microlitres of the DNA elution buffer was added to the centre of the column. Incubation was done for 1 min at room temperature. Centrifugation was done for 1 min at 14,000 rpm. The purified PCR products were then stored at 4^{o}C before being sent for sequencing at the Inqaba biotech laboratories in South Africa.

3.13 Clean up of DNA before sequencing

Deoxyribonucleic Acid clean up was done at the Inqaba biotech laboratories in South Africa where samples were sent for sequencing. The clean up was done using the ZR-96 DNA sequencing clean up kit ™. During clean up, 240 µl of sequencing binding buffer was added to 5 µl of the sequencing reaction. The mixture was transferred to a zymo-spin ™ IB – 96 plate mounted onto a collection plate. Centrifugation was done at 5000 xg for 2 min. Three hundred microlitres of sequencing wash was added to each well of the plate. Centrifugation was done at 3000 xg for 5 min. Twenty microlitres of water was added directly to the matrix of the filter plate. The Zymo – Spin ™ IB – 96 was placed on top of the supplied 96 – well PCR plate and the assembly mounted on the collection plate. Centrifugation was done at 3000 xg for 2 min to elude the DNA. The ultra-pure DNA was ready to be loaded onto a sequencer. The primers that were used during PCR amplification, that is, TK1 and TK2 were used for sequencing.

3.14 Sequence analysis

ABI V3.1 Big Dye kit was used for sequencing. The samples were sequenced on an ABI Prism 3130 automated sequencer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Analysis was done on the ABI 3500 XL genetic analyzer using a 50 cm array and POP7. Sequences were generated with the MEGA version 5 program package and blasted at the NCBI database using a blast algorithm. The purpose of blast analysis is to find regions of sequence similarity, which will yield functional and evolutionary clues about the structure and function of the generated sequence.

3.15 Phylogenetic analysis

Nucleotide sequences for HVP 2(samples S55 and OPC) were edited and analysed using BioEdit Sequence Alignment Editor (V.7.0.9.0) [Hall, 1999]. A Basic Local Alignment Search Tool (BLAST) search (*http://blast.ncbi.nlm.nih.gov*) was performed to compare the identity of the sequenced with the reference strains from GenBank by pairwise comparison. The nucleotide sequences were aligned with human herpes virus and other HVP 2 virus strains reference sequences from GenBank using MAFFT version 7 (*http://mafft.cbrc.jp/alignment/server/*). After manual adjustment of the alignment, phylogenetic analysis was performed with MEGA 6 using the neighbour-joining methods (Tamura *et al.*, 2013). The HSV strains were assigned based on the clustering in the phylogenetic tree (>70% bootstrap support).

3.16 Data Analysis

Pearson Chi- x^2 square (two by two table) was used to compare the number of positive samples to negative samples (proportions) for both female and male baboons in both groups that is, captive and wild caught. Graph pad prism was used for data analysis (Motulsky, 1999). Percentages of exposed animals were determined where Age, sex and location were compared. A P value of less than 0.05 was considered significant.

3.17 Ethical Considerations

Ethical approval before conducting the study was obtained from the Institute of Primate Research, Scientific Review Committee (Appendix I). Animal acquisition care and

maintenance were carried out as according to the guidelines of Institutional Review Committee (IRC) of the Institute of Primate Research, Kenya.

CHAPTER FOUR: RESULTS

4.1 Anti- HVP-2 antibodies

The prevalence of HVP-2 was compared among baboons from different regions, sexes and ages by detection of anti-HVP 2 antibodies. Some of the baboons had been in the IPR colony for more than 3 months therefore the study also compared the prevalence of HVP-2 in freshly captured baboons from the wild with those that had been in the colony for over 3 months.

4.1.1 Seroprevalence of HVP-2 in baboons by region

A total of 189 baboons from six counties in Kenya were sampled. The counties include Laikipia (65), Nyandarua (33), Kirinyaga (31), Machakos (24), Kajiado (16) and Nairobi (20) Counties. These counties experience different climatic conditions, with Nyandarua County being the coldest of the six counties and Machakos and Kajiado counties, the hottest. Findings from this study showed a high prevalence of HVP-2 infection in baboons from all the regions ranging from 71% in Kirinyaga county to 100% in Machakos, Kajiado and Nairobi counties. (Figure 4.1).

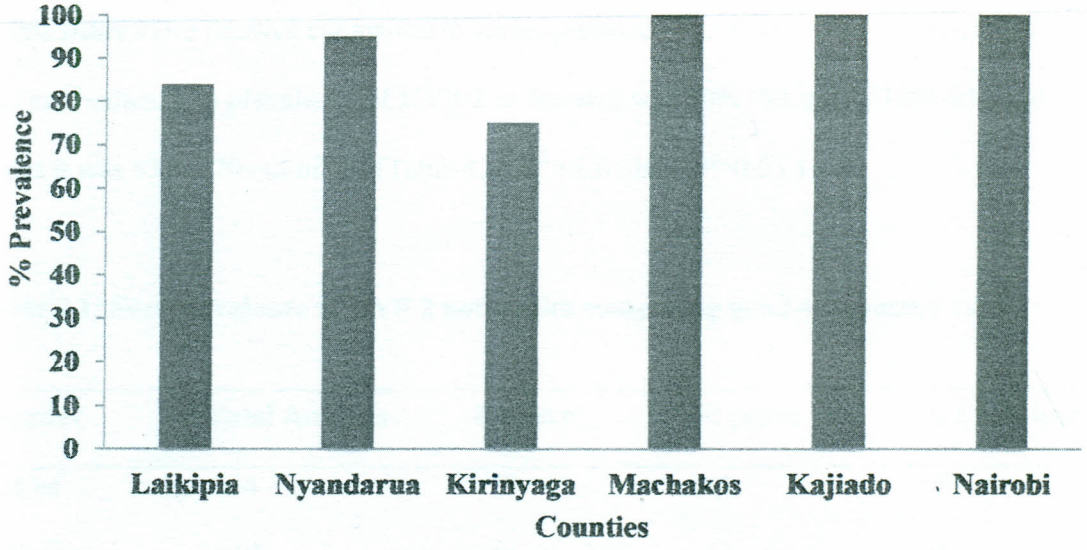


Figure 4.1: Percentage prevalence of HVP 2 in baboons from selected regions of Kenya

4.1.2 Seroprevalence of HVP-2 in baboons by sex

In this study more females compaired to males sampled were found to have anti-HVP-2 IgG antibodies. The prevalence of HVP-2 in females was 90% (94 out of 105) while in males it was 83% (70 out of 84) (Table 4.2; X^2 =1.6; df=1; P>0.05) .

Table 4.1: Seroprevalence of HVP 2 antibodies comparing gender exposure rates

Gender	Total Animals	Positive	Negative	% Prevalence
Males	84	70	14	83
Females	105	94	11	90
Total	**189**	**164**	**25**	**87**

There was no statistical significance in gender prevalence.

4.1.3 Seroprevalence of HVP-2 in baboons by Age

In relation to the three age groups sampled in the study (Adults, Sub-adults and Juveniles), more adults (94%) and sub-adults (90%) were found to be sero-positive as compared to the juveniles (52%) (Table 4.3; X^2=40; df =2; P<0.05).

Table 4.2: Sero-prevalence of HVP-2 antibodies comparing age exposure rates

Age	Total Animals	Positive	Negative	% Prevalence
Adults	148	139	9	94
Sub-Adults	10	9	1	90
Juveniles	31	16	15	52
Total	**189**	**164**	**25**	**87**

The difference in age prevalence was statistically significant.

4.1.4 Exposure rates in freshly-caught and wild captive colony baboons

A comparison in exposure frequencies of HVP 2 in freshly caught and captive colony baboons was made. The prevalence of HVP 2 in captive colony baboons (89%) was found to be slightly higher than in wild-caught baboons (85%) (Table 4.3; X^2=0.83; df=1; P>0.05).

Table 4.3: Sero-prevalence of HVP-2 antibodies comparing exposure rates in freshly-caught and captive colony baboons

	Total Animals	Positive	Negative	% Prevalence
Freshly caught	105	89	16	85
Colony	84	75	9	89
Total	**189**	**164**	**25**	**87**

The difference in prevalence between the freshly caught and captive colony baboons was not statistically significant.

4.2 Confirmation of the presence of extracted DNA

There were visible bands seen on the gel indicating that DNA was successfully extracted. However, the bands seen were more faint as compared to the positive control which was DNA extracted from whole goat blood (Figure 4.2). DNA quantification was however not done. Since the gel was only for confirmation of DNA, a molecular marker was not used.

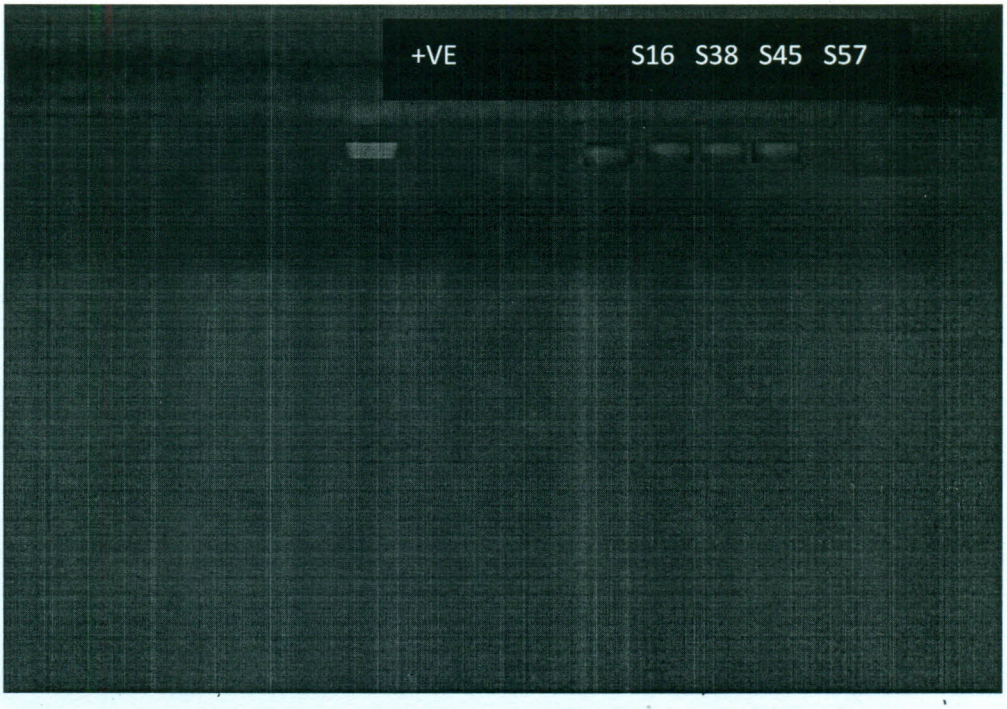


Figure 4.2: Image of gel electrophoresis run to confirm the presence of DNAin four randomly selected samples (+ve- positive control; Sn- Sample number, where n-baboon number)

4.3 Polymerase Chain Reaction

Polymerase Chain Reaction was used to amplify a 500bp region of the UL23 region of the herpes virus that code for thymidine kinase. Out of 100 baboons swabbed, 76 were seropositive for HVP2. Deoxyribonucleic acid from these 76 swab samples were subjected to PCR. Thirty two of these amplified DNA samples showed visible bands on agarose gel. Three of the brain tissues out of nine also had visible bands. Polymerase Chain Reaction confirmed the presence of herpesvirus papio 2 circulating in wild-caught olive baboons.Forty two percent of the samples that were positive for ELISA were also positive for PCR (Figure 4.3).

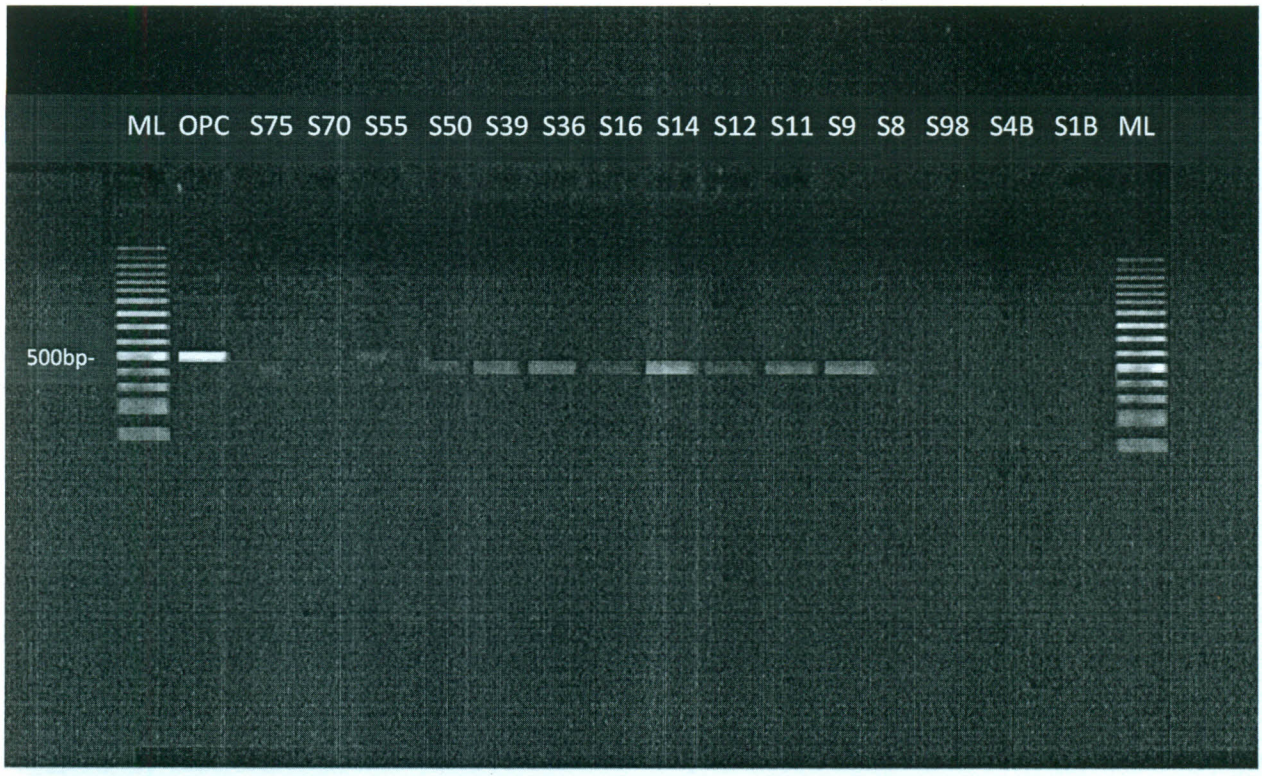


Figure 4.3: Gel image forPCRamplicons after amplification of a 500bp region of the thymidine kinasegene.Polymerase Chain Reaction was run using 30 cycles of initial denaturation at 94^{0}C for 5 min, denaturation at 94^{0}C for 60 sec, annealing at 60^{0}C for 60 sec, and elongation at 72^{0}C for 120 sec in a thermo cycler. A final extension of products was carried out at 72^{0}C for 4 min. (ML→Molecular ladder, PC→Positive control, Sn→Sample numbers, where n is the baboon number, B→ Brain sample)

4.3 Sequencing analysis

Of the 35 samples that had visible bands in agarose gel, 10 of them that had clearer bands including the positive control from Oklahoma state university were sent to South Africa for sequencing.From the analysis, the positive control had 98% identityto virion shut off protein gene of HVP 2. One of the remaining nine samples had 99% identityto HVP 2 strain A951from BLAST results. The rest of the samples had noisy sequences and were therefore not identified.Noisy sequences are sequences with weak DNA

signals. They are caused by partially failed sequencing reaction, too much or too little DNA or partial loss of sample during clean-up. The bases on such a sequence contain letters that represent ambiguity which are used when more than one kind of nucleotide occurs at that position (Figure 4.4). Good sequences have defined and sharp peaks, with even spacing between them.

Looking at the sequences of the ten samples, the identified samples had two or less ambiguous bases following each other consecutively. The resulting sequences had less number of base pairs compared to the target (500bp), after trimming of primers and noisy ends that didn't have clear peaks. These sequences were easily edited and identified. The sequences were then aligned pair wise using the BLAST algorithm. Sequence alignments of the two positive samples are demonstrated in the figures 4.5 and 4.6. The sample labeled OPC was the positive control. Sequencing for this sample was successful it was 98% identical to virion shut off protein gene of herpesvirus papio 2.

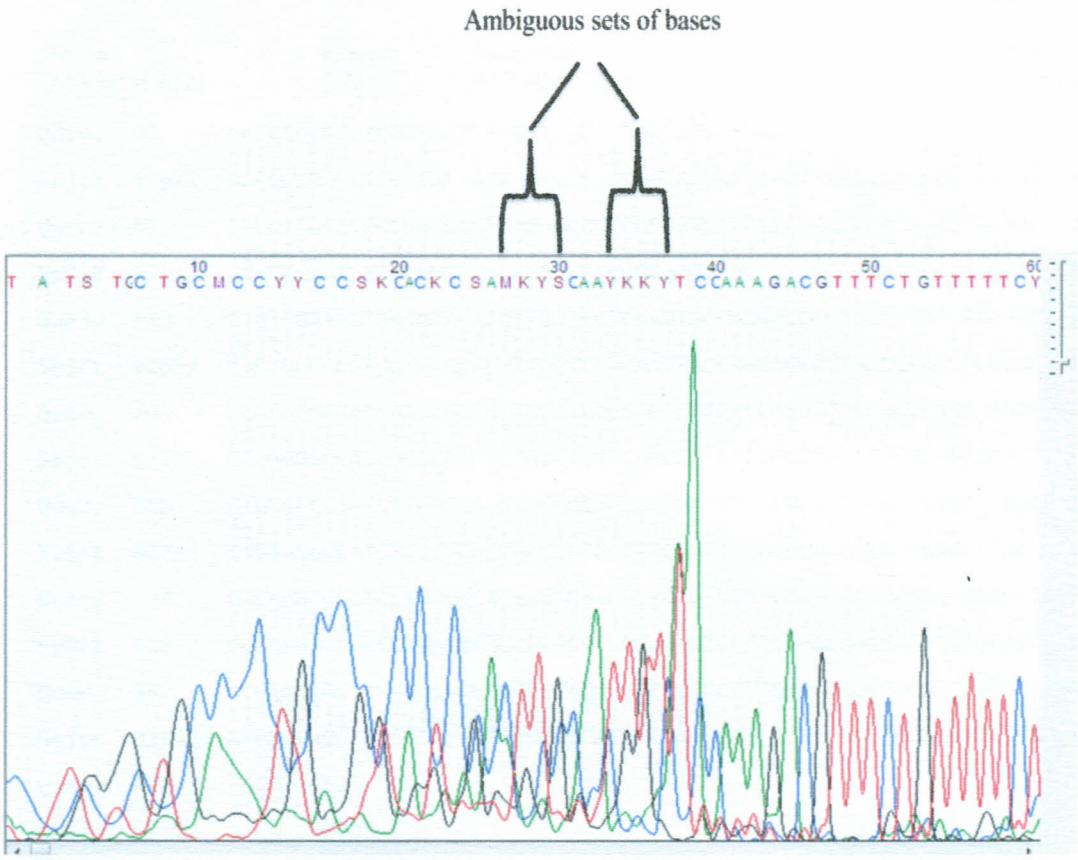


Figure 4.4: A chromatogram for sample S11. Many ambiguous bases can be seen. This could explain why the sequence could not be identified.

Figure 4.5:Pair wise Alignments of the DNA sequences of sample OPC obtained from Oklahoma University.Target region was 500bp. The aligned region is 424bp Regions of sequence inconsistencies are highlighted in red. (Sbjct→Subject).

Sample S55 was obtained from one of the baboons from Laikipia County. Sequencing results were positive for this sample.Results from NCBI blast showed 99% identity to Herpesvirus papio 2 strain A951. Figure 4.6shows the sequence alignments.

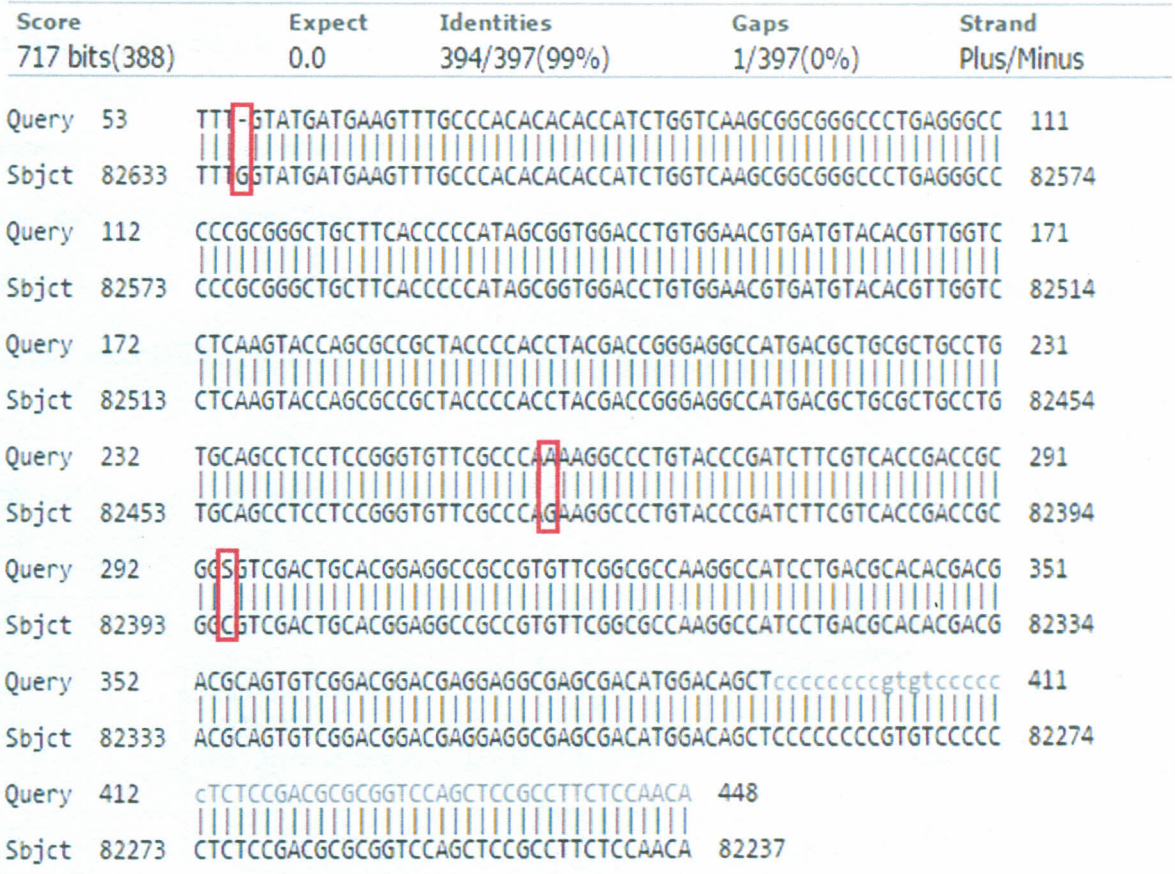


Figure 4.6:Pair wise alignments of the DNA sequences of sample S55 obtained from a baboon captured in Laikipia.Target region was 500bp. The aligned region is 397bp Regions of sequence inconsistencies are highlighted in red. (Sbjct→ Subject)

4.4 Phylogenetic analysis

The nucleotide sequences were aligned with human herpes virus and other HVP 2 virus strains reference sequences from GenBank (Table 4.4). The results from phylogenetic analysis showed a close relationship between the two identified virus strains (S55 and OPC) and other HVP 2 strains and two human HSV 1 strains (Figure 4.7).

Table 4.4: Herpes virus strains from the GenBank used for phylogenetic analysis

Source of herpes virus	Type of herpes virus	Strain	Accession number
Baboon	*Cercopithecine herpesvirus 16* (Herpevirus papio 2; HVP 2)	A951	AB232124.1
		OU1-76	KF908240
		OU2-5	KF908241
		OU2-5 (UL41)	AB232124.2
		A189164	KF908239
		Shut off protein gene	AF294581
Human	Herpes simplex virus 1 (HSV 1) also known as human herpes virus 1 (HHV 1)	HSZP (UL41)	Z72337
		CJ311	JN420338

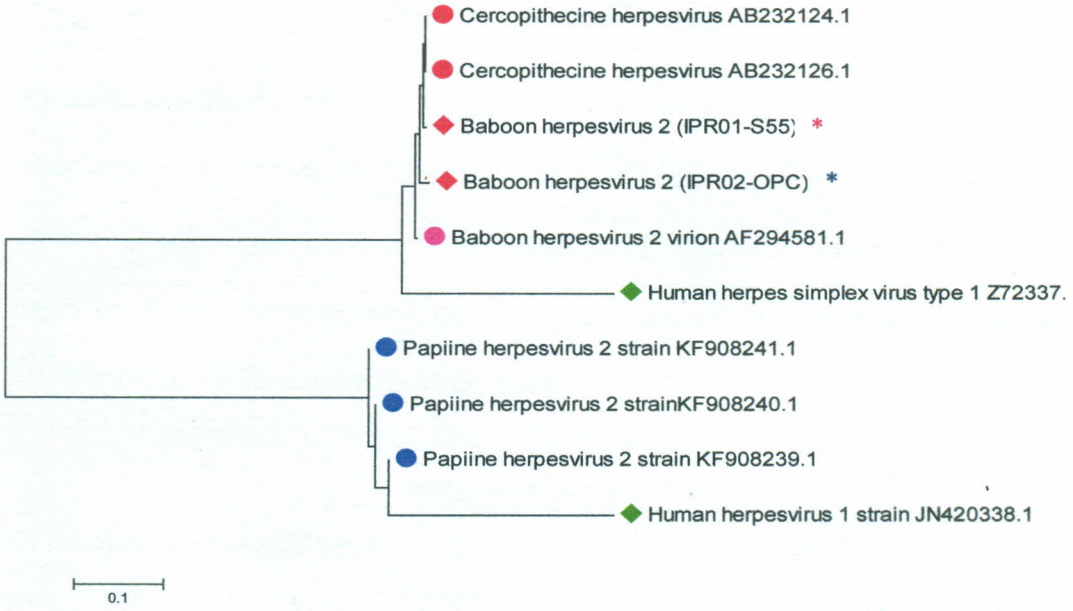


Figure 4.7: Phylogenetic tree showing the relationship between the baboon herpesvirus papio2 and the human herpes simplex virus. The branch length is proportional to the number of substitutions per site that is, the number of changes per 100 nucleotides.The HSV strains were assigned based on the clustering in the phylogenetic tree (>70% bootstrap support).(HSV →Herpes Simplex virus; HVP2 →Herpesvirus papio 2; HHV →Human Herpes virus, which is another name for herpes simplex virus; the sample S55 is shown by the asterisk* , while the positive control sample OPC is shown by the asterisk*).

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Study overview

The current study has provided strong evidence of natural exposure of wild caught olive baboons (*Papio anubis*) from selected geographical regions in Kenya to Herpesvirus papio 2. It has also confirmed the high prevalence of 87% in these baboons and identified some of the circulating virus strains.

5.2 Seroprevalence of HVP-2

5.2.1 Seroprevalence of HVP-2 in baboons from different regions in Kenya

The regions in which the baboons were captured experience different climatic conditions. Nyandarua county and particularly Aberdares where the baboons were captured from is one of the sampled regions. It is part of the Kenyan highlands that experience the coldest weather conditions. Machakos and Kajiado Counties on the other hand, are the hottest of the six counties from which the baboons were sampled. Despite the different climatic conditions, the prevalence of HVP-2 was high in all these regions, with over 70% of animals from each region having been exposed. This shows that climatic changes have got no effect on the infection rates of HVP-2 in baboons. This can be attributed to the fact that baboons are known to migrate from one region to another. Thus the distribution of herpesvirus papio 2 infections in baboons is universal.

5.2.2 Sero-prevalence of HVP-2 by age

The prevalence HVP-2 antibodies, was found to be high in adult baboons (94%) and sub- adults (90%) as compared to juveniles (52%). High antibody presence in adult human population infected with HSV-2 has also been reported (Smith *et al.,* 2002). This difference in prevalence can be explained by the fact that adult baboons are sexually active while most of the sub-adults are on the onset of sexual activities. Infection of juveniles by HVP-2 can be attributed to mother to infant infection. A study that was carried out to investigate the shedding and transmission of HVP 2 in baboons (Eberle *et al.,* 1998), showed a likelihood of transmission of HVP-2 from mother to infant. The virus that was isolated from the mother, who was shedding the virus, was the same as the one that was isolated from the oropharynx of the infant. Thus the virus was transmitted during shedding. The study further showed a high possibility of the infant passing the virus to its play mates during the shedding period. This explains the prevalence of HVP 2 in juveniles that had HVP-2 antibodies, yet they were not sexually mature.

5.2.3 Seroprevalence of HVP-2 by sex

The rate of HVP-2 infection between male and female baboons was compared. Results showed that the rate of infection was slightly higher in females (90%) as compared to males (83%) although this difference was not statistically significant. This is consistent with a study that was done in human population comparing HSV-2 infection rates between men and women (Gregory *et al.,* 2001). Biologically, females are more

vulnerable to sexually transmitted infections as compared to males. During heterosexual sex, the exposed surface area of the vagina and labia is larger in females than the vulnerable surface area in males (Simmons *et al.,* 1996) and these increases chances of transmission in females.

5.2.4 Sero-prevalence comparing captive colony baboons with freshly caught wild baboons

There was insignificant difference in HVP-2 prevalence in captive colony baboons that had been in the facility for more than 3 months (85%) and the freshly caught wild baboons (89%). This can be attributed to the fact that baboons live in troops in the wild. The troop sizes vary between 5 and 250 animals (often about 50 or so), depending on specific circumstances, especially species and time of year (Berman *et al.,* 2003). The same behavior is observed in caged baboons. In case of an infection by a single baboon, the virus can easily be transmitted to other baboons that are sexually mature in the cage. This explains why there is a small difference in HVP 2 infection prevalence between the captive colony baboons and the wild-caught baboons.

5.3 Amplification of the thymidine kinase coding region of HVP-2

Lesser animals were PCR positive as compared to those that were seropositive by ELISA (42% of the ELISA positive). These results can be attributed to the fact that HVP-2 is usually in latent form in the trigeminal ganglia (TG) and it is only during reactivation, that the virus moves down the nerves to the epithelial lining where it is then shed. Therefore, the virus can only then be found in the swab if the animal is

shedding. Shedding of HVP 2 on the other hand, is a rare occurence among the baboons. This is according to a study by Eberle *et al.* (1998). They demonstrated that out of 342 swabs collected from 128 animals that were tested over a period of one and a half years, the infectious virus was only detected in 13 swabs. This was 3.8 % of the total number of swabs collected. The study also showed that shedding of the virus was high among infants as compared to adults and the shedding was in the oral cavity. Most of the swabs collected during this study were from the genital region of the adult baboons and this could explain the lack of virus in the swab samples.

Thymidine Kinase (TK) is a conserved region in alpha herpes virus. However, some mutant viruses may lack this region (Lawrence *et al.,* 1985). It is possible that some of the samples that were seropositive had viruses that lacked the TK region. Since the primers used in this study targeted the TK region, it is therefore not possible to detect mutant viruses by amplifying the TK region. It would require that another primer set that code for a different conserved region be used to identify these viruses using PCR. Acquisition of a second primer set was however not possible due to financial constrains experienced during this study. The existence of mutant strains may partly explain the large disparity between the seropositive samples and the PCR results.

5.4 HVP 2 strain

There are thirteen known strains of HVP-2. These are; A189164, OU1–76, OU3–1, OU3-18, X313, 960, A951, OU2-5, OU2-9, OU2-12, OU4-2, OU4-8 and OU4-15 strains (Rogers *et al.,* 2003). The strain identified in this study showed 99% nucleotide

identity to HVP 2 strain A951 that was identified in colony baboons in the USA. Herpesvirus papio 2 strain A951 is one of the strains considered to be apathogenic in the mouse model. The strain however causes the development of a disease in its host, the baboon. This raises concerns about the zoonotic potential of the strain given that humans are phylogenetically close to baboons. Phylogenetic analysis of this baboon virus also showed its close relationship to the human herpes simplex virus. This confirms that a baboon is a good model to be used to study the pathogenesis of HSV.

In this study, only one of the virus strains was identified from the sequencing. The amount of HVP 2 DNA extracted from swabs is usually minimal (Unless the animal is actively shedding) as compared to the amount extracted from other tissues such as the whole blood, spleen and many others. Shedding is a rare occurrence (Eberle *et al.*, 1998) and this could be the reason why many samples had noisy sequences. The issue of non specific binding, which occurs when the unintended DNA binds to the target primers due to unoptimized PCR conditions (Kouhei *et al.*, 1998), could not be ruled out as this could be another reason why some of the amplicons were not identified.

Identification of HVP 2 A951 strain in this study is a step forward towards the development of a baboon model to study HSV. Successful detection and amplification of the thymidine kinase coding region of HVP 2 which is associated with virulence in herpesviruses, also raises concerns regarding the potential HVP2 zoonotic infections. No cases of human infection with HVP 2 have been reported, but there is also no evidence that humans cannot be infected with the virus. This data provides baseline

information important for characterization of the baboon as an infection model to study the pathogenesis and evaluate the new interventions for HSV.

5.5 Study limitations

The baboons used in this study were trapped by a group of animal science technicians of IPR. This study took advantage of the ongoing project therefore the prevalence of HVP 2 was determined based on the available animals. Also this project was self-sponsored therefore funding was a limitation. Only 10 out of 35 PCR positive samples were sequenced and unfortunately, only 2 samples gave sequences, thus characterization was done based on these 2 samples.

5.6 Conclusion

i. The seroprevalence of Herpesvirus Papio 2 infection in baboons in Kenya is 87%. The prevalence based on PCR was 42%. The prevalence was high in baboons from all the selected regions (over 70%). Therefore, HVP 2 infection in baboons is not dependent on geographical region.

ii. Herpesvirus Papio2 infection rates in females and males are about the same although the females had a slightly higher seroprevalence rates as compared to males. Infection rates of HVP-2 are higher in adult and sub-adult baboons as compared to the juveniles. This suggests that HVP 2 infection and transmission is associated with the onset of sexual activities in olive baboons (*Papio anubis*).

iii. Herpesvirus papio 2 strain A951 was identified in baboons in Kenya. This strain is apathogenic in the mouse model but in earlier studies, it was found to causes the development of lesions in its host, the baboon.

5.7 Recommendations

i. It is recommended that sampling be done in other regions of Kenya that are not included in this study in order to determine the prevalence of HVP 2 in those regions.

ii. The prevalence of HVP-2 in female and male baboons is almost the same. In humans however, the prevalence of HSV in females is higher than in males. It is recommended that the study be extended to other NHPs to see if the results will be consistent with those of this study.

iii. In this study, only one strain of HVP 2 was identified. This is due to very little amount of DNA that was obtained from the swabs. It is recommended that virus isolation be done, where animals are swabbed, and the swabs put in culture media and later virus grown in vero cells before DNA extraction is done. This will increase the amount of DNA required for sequencing. Culturing was not done in this study due to financial constraints.

iv. It is recommended that screening of HVP-2 be done using antibody ELISA rather than PCR. This is because HVP-2 usually goes to the nerves after infection and remains latent there. Shedding of this virus is rare and therefore, there is a high chance that PCR may fail to detect the virus in baboons that are not shedding. Antibody ELISA on the other hand detects anti-HVP-2 antibodies in all the exposed animals.

REFERENCES

Ades A. E., Peckham C. S., Dale G. E., Best J. M., Jeansson S. (1989). Prevalence of antibodies to herpes simplex virus types 1 and 2 in pregnant women, and estimated rates of infection. *Journal of Epidemiology and Community Health,* **43** (1): 53-60

Agrawal, Caroline A. Hastings, Joseph Torkildson, AnuragKisho (1990). Handbook of pediatric hematology and oncology: Children's Hospital & Research Center Oakland (2nd ed. ed.). Chichester, West Sussex: *Wiley-Blackwell.* p. 360.

American Academy of Pediatrics (2003): Herpes simplex. In Red Book: Report of the Committee on Infectious Diseases.344-353.

Amornkul mail P.N., Vandenhoudt H., Nasokho P., Odhiambo F., Mwaengo D., Hightower A., Buvé A., Misore A., Vulule J., Vitek C., Glynn J., Greenberg A., Slutsker L., Kevin M. De Cock.(2009). HIV Prevalence and Associated Risk Factors among Individuals Aged 13-34 Years in Rural Western Kenya. *Journal PLoS ONE.0006470* **4**(7): e6470.

Anzivino E., Fioriti D., Mischitelli M., Bellizzi A., Barucca V., Chiarini F., Pietropaolo V. (2009). Herpes simplex virus infection in pregnancy and in neonate: status of art of epidemiology, diagnosis, therapy and prevention. *Virology Journal* , **6**:40.

Beauman J. G. (2005). Genital herpes; a review. *American family physician*, **72** (8): 1527–1534.

Berman T. J., Beehner J. C., Cheney D. L., Seyfarth R. M., (2003). Hierarchical classification by rank and kinship in baboons. *Science,* **302** (14): 1234–1236

Bigger J.E. and Martin D.W. (2003).The genome of herpesvirus papio 2 is closely related to the genomes of human herpes simplex viruses. *Journal of General Virology.* **84**: 1411–1414.

Black D. and Eberle R. (1997). Detection and differentiation of primate α-herpesviruses by PCR. *Journal of Veterinary Diagnostic Investigation,* (9):225-231.

Bot, A., Shearer M., Bot S., Avriette M., Garcia-Sastre A., White G., Woods C., Kennedy R., Bona C. (2001). Induction of immunological memory in baboons primed with DNA vaccine as neonates. *Vaccine,* **19**:1960-1967.

Brice S. L., Stockert S .S., Jester J. D., Huff J. C., Bunker J. D., Weston W. L. (1992). Detection of herpes simplex virus DNA in the peripheral blood during acute recurrent herpes labialis. *Journal of the American Academy of Dermatology*, **26**:594–598.

Brown Z. A., Wald A., Morrow R. A., Selke S., Zeh J., Corey L. (2003). Effect of serologic status and cesarean delivery on transmission rates of herpes simplex virus from mother to infant. *Journal of the American Medical Association,* **289:**203-209.

Brown Z. A., Selke S., Zeh J., Kopelman J., Maslow A., Ashley R. L., Watts DH., Berry S., Herd M., Corey L. (1997). The acquisition of herpes simplex during pregnancy. *New England Journal of Medicine,* **337:**509-515.

Centers for Disease Control and Prevention Website (2006)**:** Sexually transmitted disease guidelines. *[http://www.cdc.gov/std/treatment/2006/rr5511.pdf] webcite.*

Corey L., Wald A., Patel R., Sacks S. L., Tyring S. K., Warren T., Douglas J. M. Jr., Paavonen J., Morrow R. A., Beutner K. R., Stratchounsky L. S., Mertz G., Keene O. N., Watson H. A. (2004). Once-daily valacyclovir to reduce the risk of transmission of genital herpes. *New England Journal of Medicine,* **350**:11–20.

Chon T., Nguyen L., Elliott T. C. (2007). Clinical inquiries. What are the best treatments for herpes labialis? *Journal of Family Practice,* **56** (7): 576–578.

Christie S. N., McCaughey C., McBride M., Desai A., Wyatt D., McCaughey C. (1997). Herpes simplex type 1 and genital herpes in Northern Ireland., *International Journal of STD & AIDS,* **8**:68–69.

Darnell Jr., I.M. Kerr, G.R. (1994). Stark Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science,* **264**:1415–1421

David Rakel. (2012). Herpes Simplex Virus. *Integrative Medicine*. Elsevier Health Sciences, p. 165.

De Clercq E. (1993). Antivirals for the treatment of Herpesvirus infections. *Journal of Antimicrobial Chemotherapy Suppl A,* **32**:121-132.

Dell R. B., Holleran S., Ramakrishnan R.(2002). Sample Size Determination . *Institute for Laboratory Animal Research,* **43** (4):207-213

Donald M. C., Magdalena K., Jennie G. J., David A. L., Connie L. B., Priscilla A. S., Kenneth L. T., David M. K. (1989). Thymidine Kinase-negative herpes simplex virus mutants establish latency in mouse trigeminal ganglia but do not reactivate, *Proceedings of the National Academy of Sciences*, **86**:4736 – 4740.

Eberle R., Black D., Lehenbauer T., White G. (1998). Shedding and transmission of baboon Herpesviruspapio 2 (HVP2) in a breeding colony. *Journal of laboratory animal science*, **48:**23-28.

Eberle R., Black D., Lipper S. L., Hilliard J. K. (1995). *Herpesviruspapio 2*, an SA8-like α-herpesvirus of baboons. *Archives of Virology,* **140**:529-545.

Eberle R. and Hilliard J. K. (1995). The simian α-herpesviruses, *Infectious agents and disease,* 4:55-70.

Eberle, R., Black D., Blewett E., White G. (1997). Prevalence of *Herpesvirus papio 2* in baboons and identification of immunogenic viral polypeptides. *Laboratory Animal Science*, **47**:256–262.

EBSCO DynaMed (2012). Herpes genitalis. *http://www.ebscohost.com/dynamed/what.*

Eley, R. M., Strum S. C., Muchemi G., Reid G. D. F. (2005). "Nutrition, Body Condition, Activity Patterns, and Parasitism of Free-ranging Troops of Olive Baboons (Papio Anubis) in Kenya." *American Journal of Primatology,* **18** (3): 209–219.

Gessain A.. and Calattin S. (2008). Emergence of simian foamy viruses in humans; facts and unanswered questions. *Future virology*, **3**(1):71-81.

Glenny A. M., Fernandez Mauleffinch L. M., Pavitt S., Walsh T. (2009). Interventions for the prevention and treatment of herpes simplex virus in patients being treated for cancer, *Cochrane database of systematic review,***1**

Goodbourn S., Didcock L., Randall R.E. (2000). Interferons: cell signalling, immune modulation, antiviral response and virus countermeasures. *Journal of General Virology,* **81**: 2341–2364

Gordon Y. J., Gilden D. M., Becker Y. (1983). HSV-1 Thymidine Kinase promotes virulence and latency in mice. *Investigative Ophthalmology & Visual Science*, **24**:599-602.

Gregory L. A., Julie S., Lauri M., Andrew J. N., Robert E. J., Geraldine M. M and Michael E. St. Louis. (2001). Incidence of Herpes Simplex Virus Type 2 Infection in the United States. *American Journal of Epidemiology*, **153**:912–20.

Hall T. A. (1999). Bio Edit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acid Symposium Series*, 41: 95–98.

Halperin S. A., Shehab Z., Thacker D., Hendley J. O. (1983). Absence of viremia in primary herpetic gingivostomatitis. *Pediatric Infectious Disease Journal*, **2**: 452–3.

Harel L., Smetana Z., Prais D., et al. (2004) Presence of viremia in patients with primary herpetic gingivostomatitis. *Clinical Infectious Diseases,* **39**: 636–40.

Heather B., Markowitz L. E., Gibson T., McQuillan G. M. (2014). Seroprevalence of Herpes Simplex Virus Types 1 and 2—United States, 1999–2010. *Journal of Infectious Diseases,* **209** (3): 315-317

Hermant P., Demarez C., Mahlakõiv T., Staeheli P., Meuleman P., Michiels T. (2014). Human but not mouse hepatocytes respond to interferon-lambda *in vivo. Public Library of Science*, 9 (1).

Hull R.N. (1973). The simian herpesviruses. In: The herpesviruses, ed. Kaplan AS, pp. 389-426. Academic Press, New York, NY

Huff J. L., Barry P. A. (2003). B-virus (Cercopithecine herpesvirus 1) infection in humans and macaques: potential for zoonotic disease. *Emerging Infectious Diseases,* **9**(2):246-250.

Huneke, R. B., Michaels M. G., Kaufman C. L., Ildstad S. T. (1998). Antibody response in baboons (Papio cynocephalus anubis) to a commercially available encephalomyocarditis virus vaccine. *Laboratory Animal Science,* **48:**526-528.

Ingrid D., Petra S., Holger K. (1986). Effect of Interferon on Replication of Herpes Simplex Virus Types 1 and 2 in Human Macrophages, *American Society for Microbiology,* **1**:37-42.

Jeneby M. M., Suleman M. A., Gichuki C. (2002). Sero-Epizootiologic Survey of Trypanosoma Brucei in Kenyan Nonhuman Primates. *Journal of Zoo and Wildlife Medicine:* **33** (4): 337–341.

Jeong, S. H., Qiao M., Nascimbeni M., Hu Z, Rehermann B., Murthy K., Liang T. J. (2004). Immunization with hepatitis C virus-like particles induces humoral and cellular immune responses in nonhuman primates. *Journal of Virology*, **78:**6995-7003.

Johnston C., Magaret A., Selke S., Remington M., Corey L., Wald A. (2008). Herpes simplex virus viremia during primary genital infection. *Journal of Infectious Diseases*, **198**:31–34.

Joyce U. N., Eduard J. S., Caroline N., Steve W., Ken A., Elizabeth B., Matthew A. P., Pauli N. A., Nokes D. J. (2011). Seroprevalence, predictors and estimated incidence of maternal and neonatal Herpes Simplex Virus Type 2 infection in semi-urban women in Kilifi, Kenya. *Biomedical Central Infectious Diseases,***11**:155

Kariuki, T. M., I. O. Farah, D. S. Yole, J. M. Mwenda, G. J. Van Dam, A. M. Deelder, R. A. Wilson, and P. S. Coulson. (2004). Parameters of the attenuated chistosome vaccine evaluated in the olive baboon. *Infection and Immunity*. **72:**5526-5529.

Katherine J. L., Geoffrey P. G., George P. S. (2008). An estimation of the global prevalence and incidence of herpes simplex virus type 2 infection. *Bulletin of the World Health Organization,* **86** (10): 737-816

Kenya AIDS Indicator Survey (KAIS) data sheet. (2007).

Kit S. (1985). Thymidine Kinase. *Journal of Microbiology*, **2**(12):369-375

Klemens W., Thomas B., Gerd F., Georg E. S. (1997). The structures of thymidine kinase from Herpes simplex virus type 1 in complex with substrates and a substrate analogue. *Protein Science,* **6**:2097-2106.

Kouhei S., Takeshi K. and Michiteru Y. (1998). The mechanism of sequence non-specific DNA binding of HMG1/2-box B in HMG1 with DNA. *Oxford Journals*, **12**(3):235-242

Lawrence A. F. Jr. (1976). A review of *Herpesvirus papio*, a B-lymphotropic virus of baboons related to EBV. *Comparative Immunology, Microbiology and Infectious Diseases*, **2**(2-3)257-264

Ledford H. (2012). Failed herpes vaccine puzzles virologists. *Nature,* 9739

Levin J. L., Hilliard J. K., Lipper L., Butler T. M., and Goodwin W. J. (1988). A naturally occurring epizootic of simian agent 8 in the baboon. *Journal of laboratory animal science,* **38**:394-397.

Lee H.K., Lund J.M., Ramanathan B., Mizushima N., Iwasaki A. (2007). Autophagy-dependent viral recognition by plasmacytoid dendritic cells. *Science,* 315(5817):1398–1401.

Lafferty W. E., Coombs R. W., Benedetti J., Critclow C., Corey L. (1987). Recurrences after oral and genital herpes simplex virus infection. Influence of site of infection and viral type. *New England Journal of Medicine,* **316**:1444–1449.

Lawrence R. S, Saul K., Martin G. M., (1985). Thymidine Kinase- deficient HSV 2 genital infection in guinea pigs. *Journal of virology* , **55**(2):322-328

Mar EC, Chiou JF, Cheng YC, Huang ES (1985). "Inhibition of cellular DNA polymerase alpha and human cytomegalovirus-induced DNA polymerase by the triphosphates of 9-(2-hydroxyethoxymethyl)guanine and 9-(1,3-dihydroxy-2-propoxymethyl)guanine". *Journal of Virology.* **53** (3): 776–780.

Martino M. A., GB Hubbard., Butler T. M., Hilliard J. K. (1988). Clinical disease associated with simian agent 8 infection in the baboon. *Journal of laboratory animal science*, **48**:18-22

Matz-Rensing K, Jentsch K. D., Rensing S., Langenhuyzen S., Verschoor E., Niphuis H., Kaup F. J. (2003)Fatal Herpes simplex infection in a group of common marmosets (Callithrix jacchus),*Veterinary Pathology*. **40**:405–411.

Motulsky H. J. (1999) .Analyzing Data with GraphPad. *GraphPad Software Inc., San Diego CA, www.graphpad.com.*

Mugo N., Dadabhai S. S., Bunnel R., Williamson J., Benett E., Baya I., Akinyi N., Mohamed I., Kaiser R. (2011). Prevalence of herpes simplex virus type 2 infection, human immunodeficiency virus/herpes simplex virus type 2 co-infection, and associated risk factors in a national population-based survey in Kenya. *Sexually Transmitted Diseases,* **38**(11):1059-1066

Nahmias A. J., Lee F. K., Beckman-Nahmias S. (1990). Sero-epidemiological and - sociological patterns of herpes simplex virus infection in the world. *Scandinavian Journal of Infectious Diseases,* **69**:19–36.

Nasser M., Fedorowicz Z., Khoshnevisan M. H., ShahiriTabarestani M. (2008). Acyclovir for treating primary herpetic gingivostomatitis. In Nasser, Mona. *Cochrane Database of Systematic Reviews,* (4): CD006700.

National Institute of Allergy & Infectious Diseases, "An Introduction to Sexually Transmitted Diseases" July 1999.

Orchansky, P., Rubinstein, M., and Fischer, D.G. (1986). The interferon-γ receptor in human monocytes is different from the one in non-hematopoetic cells. Journal of Immunology, **136**:169–173

Perfect M. M., Bourne N., Ebel C., Rosenthal S. L. (2005). Use of complementary and alternative medicine for the treatment of genital herpes. *Herpes,* **12** (2): 38–41.

Richard A. Z. (2009). The Clinical Spectrum of Herpes Simplex Viremia, *Clinical Infectious Diseases,* **49** (9):1302-1304.

Ritchey J. W., Ealey K. A., Payton M., Eberle R. (2002). Comparative pathology of infections with baboon and African green monkey alpha-herpesviruses in mice. *Journal of Comparative Pathology* ,**127**:150-161.

Rogers K. M., Wolf R. F., White G. L., Eberle R. (2005). Experimental infection of baboons (*Papio cynocephalus anubis*) with apathogenic and neurovirulent subtypes of *Herpesvirus papio* 2. *Comparative Medicine,* 55:425–430.

Rogers K.M., Deatheridge M., Breshears M.A., Chapman S., Black D., Ritchey J.W., Payton M., Eberle R. (2009). Type I IFN response to Papiine herpesvirus 2 (Herpesvirus papio 2; HVP2) determines neuropathogenicity in mice. *Virology,* 386:280–289.

Rogers K. M., Black D. H., Eberle R. (2007). Primary mouse dermal fibroblast cell cultures as an in vitro model system for the differential pathogenicity of cross-species herpesvirus papio 2 infections. *Archives of Virology*, 152:543–552.

Rogers, K. M., Ealey, K. A., Ritchey, J. W., Black, D. H., Eberle, R. (2003). Pathogenicity of different baboon Herpesvirus papio 2 isolates is characterized by either extreme neurovirulence or complete apathogenicity. *Journal of Virology,* 77: 10731–10739.

Rogers J, Hixson JE (1997). Baboons as an animal model for genetic studies of common human disease. *American Journal of Human Genetics*, **61**:489–493

Ryan K. J., Ray C. G. (2004). Sherris Medical Microbiology (4th ed.). McGraw Hill, pp. 555–562.

Ruchman I, Dodd K.(1950). Recovery of herpes simplex virus from the blood of a patient with herpetic rhinitis. *Journal of Laboratory and Clinical Medicine*, **35**: 434–439.

Schrenzel M. D., Osborn K. G., Shima A., Klieforth R. B., Maalouf G. A. (2003). Naturally occurring fatal herpes simplex virus 1 infection in a family of white-faced saki monkeys (Pithecia pithecia pithecia). *Journal of Medical Primatology*, **32**:7–14.

Scott, D. A., Coulter, W. A. & Lamey, P. J. (1997). Oral shedding of herpes simplex virus type 1: a review. *Journal* of *Oral Pathology & Medicine*, **26**:441–447.

Sidebottom D. A., Kennedy R., Hildebrand W. H. (2001). Class I MHC expression in the yellow baboon. *Journal of Immunology,* **166**:3983-3993.

Smith J. S., Robinson N. J. (2002). Age-specific prevalence of infection with herpes simplex virus types 2 and 1: a global review. *Journal of Infectious Diseases*, **186**: 3-28

Simmons G., Wilkinson D., Reeves J. D., Dittmar M. T., Beddows S., Weber J., Carnegie G., Desselberger U., Gray P. W., Weiss R. A., Clapham P. R. (1996). Primary, syncytium-inducing human immunodeficiency virus type 1 isolates are dual-tropic and most can use either Lestr or CCR5 as coreceptors for virus entry. *Journal of Virology*. **70**(12):8355-8360.

Soisson L. A., Reid D. G., Farah I. O, Nyindo M., Strand M. (1993). Protective immunity in baboons vaccinated with a recombinant antigen or radiation-attenuated cercariae of Schistosoma mansoni is antibody-dependent. *Journal of Immunology*, **151**:4782-4789.

Stanberry L. R., Kit S., Myers M. G. (1985). Thymidine kinase-deficient herpes simplex virus type 2 genital infection in guinea pigs. *Journal of Virology*. **55**(2):322.

Suleiman M. S., Zacharowski K., Angelini G. D. (2008). Inflammatory response and cardioprotection during open-heart surgery: The importance of anaesthetics. *British Journal of Pharmacology*, 153(1):21–33.

Tamura K, Stecher G, Peterson D, Filipski A, and Kumar S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution*, **30**: 2725-2729.

Vilcek J. (2003). Novel interferons. *Nat Immunology*, 4(1):8–9.

Wald A. (2006). Genital HSV-1 infections. *Sexually Transmitted Infections*, **82**(3): 189–190.

Wald A., Zeh J., Selke S., Ashley R. L., Corey L. (2002). Genital shedding of herpes simplex virus among men. *Journal of Infectious Diseases*, **186** (Suppl 1):S34–39.

Watts A. M., Stanley J. R., Shearer M. H., Hefty P. S., Kennedy R. C. (1999). Fetal immunization of baboons induces a fetal-specific antibody response. *Nature Medicine*, **5**:427-430.

Weiss H. A., Buve A., Robinson N. J., Van Dyck E., Kahindo M., Anagonou S., Musonda R., Zekeng L., Morison L., Carael M., Laga M., Hayes R. J. (2001). The epidemiology of HSV-2 infection and its association with HIV infection in four urban African populations. *Aids*, **15**(Suppl 4):S97-108.

Weiss H. (2004). Epidemiology of herpes simplex virus type 2 infection in the developing world. *Herpes*. Apr; 11Suppl1:24A-35A.

Wertheim J. O., Smith M. D., Smith D. M., Scheffler K., Kosakovsky Pond S. L. (2014) Evolutionary Origins of Human Herpes Simplex Viruses 1 and 2. *Molecular Biology and Evolution*, **31**(9):2356-2364

Whitley R. J., Roizman B. (2001). Herpes Simplex Virus infections. *Lancet*, **357**:1513

Whitley RJ, Gnann JW Jr (2002): Herpes simplex virus. In Mucocutaneous Manifestations of Viral Diseases. *Informa Healthcare*, pp 69-117.

Wintersberger E. (1997). Regulation and biological function of Thymidine Kinase. *Biochemical Society Transactions*, **25**(1): 303-308.

Zane A. B., Stacy S., Judy Z., Jerome K., Arthur M., Rhoda L. A., Heather W., Sylvia B., Millie H., Lawrence C. (1997). The acquisition of herpes simplex virus during pregnancy. *Journal of Medicine* **337**(8):509-515

Zurbriggen, R., Metcalfe I. C., Gluck R., Viret J. F., Moser C. (2003). Nonclinical safety evaluation of Escherichia coli heat-labile toxin mucosal adjuvant as a component of a nasal influenza vaccine. *Expert Review* of *Vaccines*, **2:**295-304.

APPENDICES

Appendix I

Ethical Clearance

Institute of Primate Research
Address: P. O. Box 24481-00502 Karen Nairobi Kenya | Tel: +254 02 2606235 | Fax: +254 02 2606231
URL: www.primateresearch.org | Email: directoripr@primateresearch.org

NATIONAL MUSEUMS OF KENYA
WHERE HERITAGE LIVES ON

P.O BOX 24481, KAREN, NAIROBI
TELEPHONE 254-20-882571/4
FAX: 254-20-882546
E-Mail: ozwara@primateresearch.org

INSTITUTIONAL REVIEW COMMITTEE (IRC)

FINAL PROPOSAL APPROVAL FORM

Our ref: **IRC/01/14**

Dear **Dr Atunga Nyachieo,**

It is my pleasure to inform you that your proposal entitled ***"Serological survey and molecular characterization of herpes virus papio 2 in olive baboons from selected regions in Kenya"***, in collaboration with **Dr. Michael Gicheru** and **Ms. Sharon Chepkwony,** Department of Zoological Sciences, Kenyatta University, P.O. Box 43844-00100, Nairobi, Kenya has been reviewed by the Institutional Review Committee (IRC) at a meeting of 21st February 2014. The proposal was reviewed on the scientific merit and ethical considerations on the use of animals for research purposes. The committee is guided by the Institutional guidelines as well as International regulations, including those of WHO, NIH, PVEN and Helsinki Convention on the humane treatment of animals for scientific purposes and GLP.

This proposal has been approved and you are bound by the IPR Intellectual Property Policy.

Signed.....Chairman IRC: Dr. Hastings Ozwara

Signed.....Secretary IRC: Dr. Ngalla Jillani

Date 27/02/2014

INSTITUTE OF PRIMATE RESEARCH
INSTITUTIONAL REVIEW COMMITTEE
P. O. Box 24481-00502 KAREN
NAIROBI - KENYA
APPROVED

Appendix II

Reagents for IgG Antibody ELISA

1. Coating Buffer (1X PBS)

NaCl 8g

KCl 0.2g

KH_2PO_2 0.14g

Na_2HPO_2 0.91g

Top up with distilled water to 1L. pH to 7.4

2. Blocking Buffer

3gm Bovine Serum Albumin (BSA)

10 ml of 1x Phosphate Buffered Saline (PBS)

3. Wash buffer

1000 ml of 1x PBS

500 µl of Tween 20

4. Substrate Solution

Tetra methyl benzidine (TMB) 50 µl/well

Appendix III

ELISA plates showing results obtained (Blue colour –positives, no colour – negatives). ELISA was run in triplicates in rows.

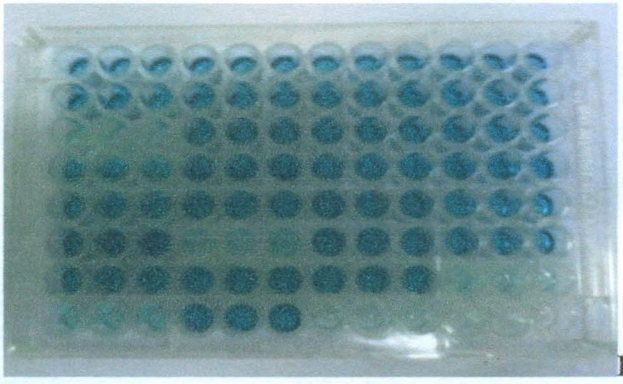


Plate 1

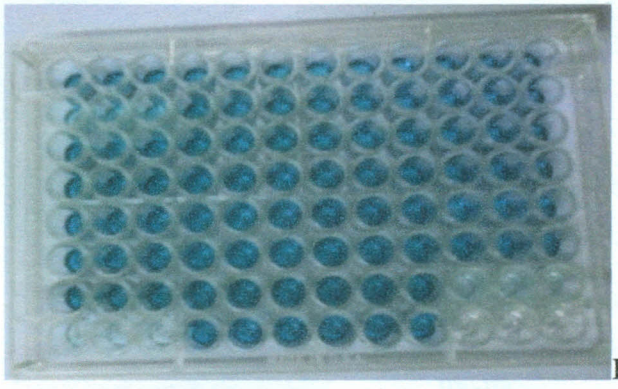


Plate 2

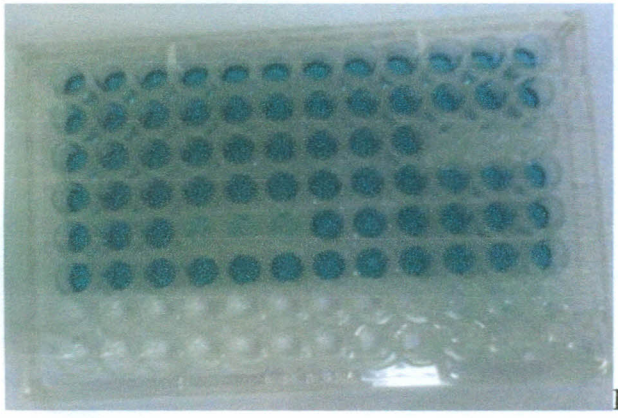
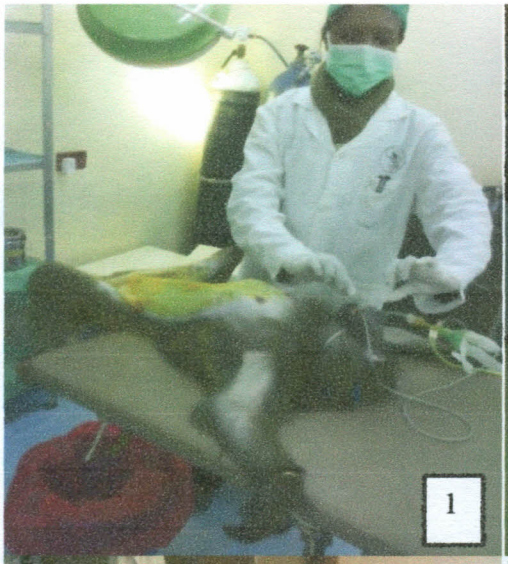
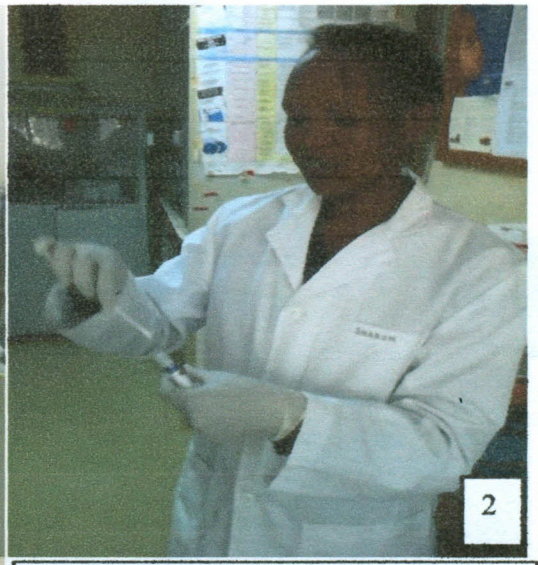


Plate 3

Appendix IV

Collection of genital swabs and running of PCR





1. Sedation of the baboon before swabbing.
2. Insertion of the collected swabs into tubes containing transport media
3. Arranging of samples on the q-Tower PCR machine.

Appendix V

Map indicating different counties from which the baboons were captured (Edited from Google)