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Molecular Detection and Characterization of *Herpesvirus Papio 2* (HVP2) in Wild-Caught Olive Baboons from Selected Regions in Kenya

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ABSTRACT

Backgrounds: There is a remarkable similarity between *Herpesvirus* papio 2 (HVP2) infecting baboons and human simplex virus (HSV) in terms of molecular biology, protein functions, and resulting infections. However, no definitive therapy exists, and the available drugs only improve the clinical signs of recurrent or asymptomatic infections. This research results may be useful for studies on the quest for HVP2 curative and preventive drugs in baboon models. Later, a similar study could be done on HSV in humans.

Materials & Methods: A total of 60 baboons were sampled from six different counties in Kenya. Of these, 51 cases were wild caught from five counties, and nine cases were from the Institute of Primate Research (IPR) colonies designated as captive baboons. Oral and genital swabs were collected for analysis. The trigeminal ganglia of three study subjects were also aseptically sampled. Polymerase chain reaction test was used to determine the prevalence of HVP2. HVP2-positive samples were sequenced and aligned to GenBank sequences using BLAST to identify specific circulating strains and generate phylogenetic relationships. DnaSP6 was used for genetic diversity analysis.

Findings: Among 60 baboons studied, 65% were positive for the virus. One strain, A951, was identified as the prevalent strain. Extremely low fixation index values (Fst) were recorded, showing low genetic diversity within and between subpopulations.

Conclusion: The identified strain was non-pathogenic but could be clinically manifested as painful sores on the host's mucosal membranes and cause stillbirths. The virus prevalence was 75.86% in genital samples and 54.86% in oral samples, indicating that oral transmission is less common than genital transmission.

Keywords: *Herpesvirus 2*, Prevalence, Strains, Asymptomatic, Genetic, Variability.

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Introduction

Non-human primates (NHPs) are also affected by herpes viruses just like humans. So far, several of these viruses, closely related to human viruses, have been isolated from NHP species [1]. Some of these NHP neuro-tropic viruses that are similar to human simplex virus (HSV) include: *Herpesvirus papio* 2 (HVP2) (also called *Cercopithecine herpesvirus 16*) in baboons, *Herpesvirus saimiri* 1 (commonly called *Saimirine herpesvirus* 1) in squirrel monkeys, monkey B virus (BV) (also called *Cercopithecine herpesvirus* 1) in macaque species of monkeys, and simian agent 8 (SA8) (or *Cercopithecine herpesvirus* 2) that infect African green monkeys [2]. All these viruses are characterized by a short life cycle and a linear DNA genome of about 155 kbp. This genome is surrounded by a 125 nm icosahedral-shaped capsid. This capsid's primary function is to host and protect the viral genome [3]. They are also similar in structure, protein functions, and viral replication processes. The hallmark of alpha herpesviruses is that they create latent infections in the trigeminal or sensory ganglia [4]. They could be reactivated by viral shedding in body secretions, resulting in recurrent clinical manifestations (lesions) or asymptomatic infections [5]. Primary infections caused by these viruses affect mucosal membranes, especially in the genital area in mature animals, and the mouth in infants and juveniles. Alpha herpesviruses cause fewer fatal diseases in their natural hosts, while high fatality is more likely in cross-species infections [6].

HVP2 in baboons has been reported to have remarkable biological similarities to HSV in humans. They are both transmitted through body secretions and direct contact. The papiine virus is known to cause hydrocephaly in infants and stillbirths and painful herpetic lesions in adult baboons [7]. For an extended period, HVP2 was considered the same as simian agent 8 (SA8), but with more studies, it was found that they are homologous but very distinct [8]. Monkey B virus is exceptionally virulent and considered a biosafety level 4 pathogen when transmitted to other species.

On the contrary, HVP2 and SA8 infections have not been reported so far in humans or other NHPs and are therefore considered zoonotically unimportant [9]. However, there are both neuro-virulent and apathogenic strains of HVP2 based on studies on mice [10]. Serological evidence indicates that about 90% of mature baboons are infected by the virus, and these infections could symptomatically range from vesicles to ulcers in the genitals or mouth [11]. However, molecular statistics indicate that specific circulating strains in some regions are unavailable in some other regions.

Human simplex viruses, both serotypes 1 and 2 responsible for neonatal and genital herpetic infections in humans, are classified as double-stranded DNA. Neonatal herpes is associated with more than 80% mortality in infected individuals [12]. HSV2 is not a deadly disease but could lead to psychological diseases, social wreckages due to vesicles and ulcers, encephalitis in severe cases, and death in some cases [13]. In pregnant women, HSV2 accelerates the contraction of hepatitis, perinatal transmission leads to spontaneous abortion. This antigenic variant also increases the risk of acquisition, transmission, and even progression of HIV1 infection, especially in newly infected individuals due to induced lesions [14]. HIV1 along with other factors like female gender, male uncircumcision, and age also increase the probability of contracting simplex virus. This condition has increased the disease burden to >500 million people globally, with Africa having the highest number of cases. About 68% of the adult population in Kenya is affected by this disease [15].

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Genetically and phylogenetically, *Papio* species are closely related to humans more than all other NHPs, and HVP2 and HSV viruses in their corresponding native hosts are similar [16]. This makes baboons the best animal models for understanding the etiology of HSV in humans.
Objectives: This study provided information on molecular epidemiology, available papiine strains, and genetic variation of the virus and its phylogenetic relationship with other alpha herpesviruses in selected counties in Kenya. This information is expected to contribute to HSV management and treatment strategies in humans.

Materials and Methods

Animal sampling: Wild asymptomatic baboons of *Papio anubis* species at different ages, categorized as infants, juveniles, subadults, and adults, were sampled from five selected counties in Kenya. The target counties were Laikipia, Machakos, Nyandarua, Kajiado, and Nyeri. A total of 51 baboons were trapped and then transported to the Institute of Primate Research (IPR) in Kenya, where they were housed for two weeks for acclimatization before taking swab samples. Also, nine captive olive baboons were obtained from IPR in Nairobi County and enrolled in the study. Laboratory analyses were later done from December 2020 to April 2021. The study design employed in this research was cross-sectional with a purposive random sampling method, in which different regions of Kenya that harbored baboons were selected, thus reducing the risk of researcher bias while increasing the credibility of the research. The total number of animals sampled was based on their availability, and further analyses were done in triplicate to ensure consistency.

Sample size determination: This study was conducted on trigeminal ganglia samples and oral and genital swabs collected from baboons. The sample size was determined using the following formula according to Dell and colleagues (2002) [17]:

$n = C[(poqo+peqe)\div d2] + 2/d + 2$

Where n is the minimum sample size, C is a constant that depends on the values chosen as α and β (0.05), po is the observed prevalence (90%) [18], qo is 1-po, pe is the postulated prevalence (85%) [18], qe is 1- pe, and d is | po –pe |.

Thus, a minimum of 47 baboon samples was required in this study. However, a total of 60 baboons were sampled in this study.

Sample collection: The ketamine-medetomidine combination was used at dosages of 3.5 and 0.035 mg/kg, respectively, to provide complete immobilization, excellent muscle relaxation, and adequate analgesia for sampling procedures as described by Murphy et al. (2010) [19]. Adult baboons were swabbed from their genitals, while infants were orally swabbed. This is because juveniles acquire the infection orally from birth, while adults contract it when they reach sexual maturity. For juveniles, swabbing was done on the cheeks and tongue, while in adults, swabbing was done on the vaginal lining and cervix for females and on the urethra for males. The digene® female swab specimen collection kit (Qiagen, Germany) was used for swabbing.

Three baboons were selected randomly from the nine captive olive baboons and sacrificed. During autopsy, three samples of trigeminal ganglia (TG) (TG1, TG2, and TG3) were taken aseptically, and all dura and fat were removed. The samples were then cooled on ice and then transferred to a -80 ^{0}C refrigerator for subsequent analysis.

DNA isolation and polymerase chain reaction: The TG samples and oral and genital swabs were used to isolate viral DNA using Radi DNA extraction kit from KH Medical Company Limited based in Seoul, South Korea, according to the manufacturer's manual. The presence and integrity of the obtained

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viral DNA were confirmed using agarose gel electrophoresis. The concentration and purity of nucleic acid were determined using a Nano-Drop device. DNA quantification was done using a bio-drop quantification machine with standard ratios of 1.5-2.0. To amplify the 1100 bp region of the UL41 VHS gene of Papiine virus, a set of specific primers (GFP1,5' GCGATGATGGAGATGACGTA 3' and GRP1,5' GGACCTGTGGAACGTGATG 3') was designed.

The Platinum™ multiplex PCR master mix (Invitrogen ™), obtained from ThermoFisher Scientific Company based in Massachusetts in the USA, was used for DNA amplification. The thermo-cycling conditions provided in the protocol were set as follows: an initial 5-min denaturation at 94 °C, followed by 30 cycles of 1-min denaturation at 94 °C and half-min DNA annealing at 53 °C. This was followed by elongation at 72 °C for 2 min in 30 cycles of amplification. The final elongation was done for 4 min at 72 °C and finally held at 4 °C. Amplified fragments were visualized using 1% agarose gel electrophoresis run in TAE buffer at 70 V for 1 hour. SYBR® Safe DNA Gel Stain was used in the gel and buffer for DNA visualization.

Sequencing and analysis of sequenced data: PCR product clean-up was done at Macrogen Company (Netherlands), where samples were sent for sequencing. AB1 V3.1 Big Dye kit was used for bidirectional sequencing. ABI Prism 3130 automated sequencer (Applied Biosystems) was used to sequence the samples following the manufacturer's protocols. AB1 3500 XL genetic analyzer using a 50 cm array and POP7 was used to complete all analyses.

Raw sequencing reads were qualitatively assessed based on Phred 20 (Q20) scores. Consensus sequences were analyzed through BLAST and matched to GenBank known sequences. BLAST results were analyzed based on E-values. BioEdit sequence alignment editor tool (Ver.7.0.9.0) was used to edit the sequences. Sequence alignment was completed using MAFFT Version 7. The genetic variation of the sequences within and between the populations of the selected counties was analyzed using DnaSP6 software. Phylogenetic analysis of aligned sequences was performed using MEGA 11. The model selection tool was used to model nucleotide substitutions in the dataset. Tamura's 3-parameter model (T92+G model) with five gamma categories had a minor Bayesian information criterion (BIC) score and was therefore preferred as the best fit model. The neighbor-joining method was used to construct the tree, while bootstrapping was used to assess the robustness of the groupings obtained. An out-group sequence was included to determine the root of the tree.

Statistical analysis: The data obtained in percentage were compared in terms of freedom status (captive and wild-caught), age, gender, region, and source of swabs. The obtained data were presented in tables and graphs. A p value of $< .05$ was considered significant in neutrality and Chi-square tests.

Findings

Polymerase chain reaction was used to amplify the 1100 bp region of the UL41 gene of the herpes virus. A total of 51 wild baboons were sampled from five counties as follows: Laikipia (24 cases), Nyeri (8 cases), Machakos (3 cases), Nyandarua (12 cases), and Kajiado (4 cases). In addition, nine captive baboons obtained from IPR (in Nairobi County) were also sampled. Of the 60 animals swabbed, 39 cases were positive for HVP2, representing a 65% positivity level, while 21 cases were negative, accounting for 35%. All three trigeminal ganglia samples were positive for the virus. Among the positive samples, 10 samples were from males, and 29 samples were from females. In addition, there were 16 infants (4 males and 12

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females), three sub-adults (all males), and 20 adults (17 females and three males). Of the nine captive baboons, 55.6% were positive, which was relatively lower than the percentage of positive wild-caught baboons (66.7%).

Confirmation of the presence of viral DNA: Bright bands were observed after running agarose gel electrophoresis. The negative control was molecular grade water, and DNA extracted from a monkey was used as a positive control. The nucleic acid was quantified, and all samples had values within the recommended ratios of 1.5 to 2.0.

Prevalence of HVP2 based on PCR: After PCR amplifications, 39 of the 60 collected samples including all three TG samples were positive for HVP2, accounting for a 65% prevalence, while 21 (35%) samples were negative. Molecular grade water was used as a negative control, archived OPC sample (Oklahoma positive control sometimes obtained from Veterinary Health Science Center, Oklahoma State University, USA) as a positive control, and 1000 bp GeneRuler as a molecular ladder. After running gel electrophoresis, an image of the gel was taken to visualize the bands under a UV spectrophotometer (Figure 1).

In this study, 51 wild baboons caught from the selected counties and nine baboons obtained from the captive colonies at IPR were sampled. A prevalence of 66.7% was recorded for the virus among wild baboons, as 34 out of 51 cases were positive. Also, five of the nine captive animals were positive, accounting for a 55.6% prevalence. This indicates that the prevalence of the virus is relatively higher in the wild than in captive colonies.

Based on the sex of the animals, there were 16 males, of which ten were positive, and six were negative, and there were 44 females, of which 29 were positive, and 15 were negative. A comparatively higher virus prevalence was reported in females (65.9%) than in their male counterparts (62.5%), indicating that the virus is more prevalent in females than in males.

The prevalence rate of the virus in focus was shown to increase with age. The lowest prevalence rate (57.1%) was recorded for infants and juveniles, followed by sub-adults (60%),

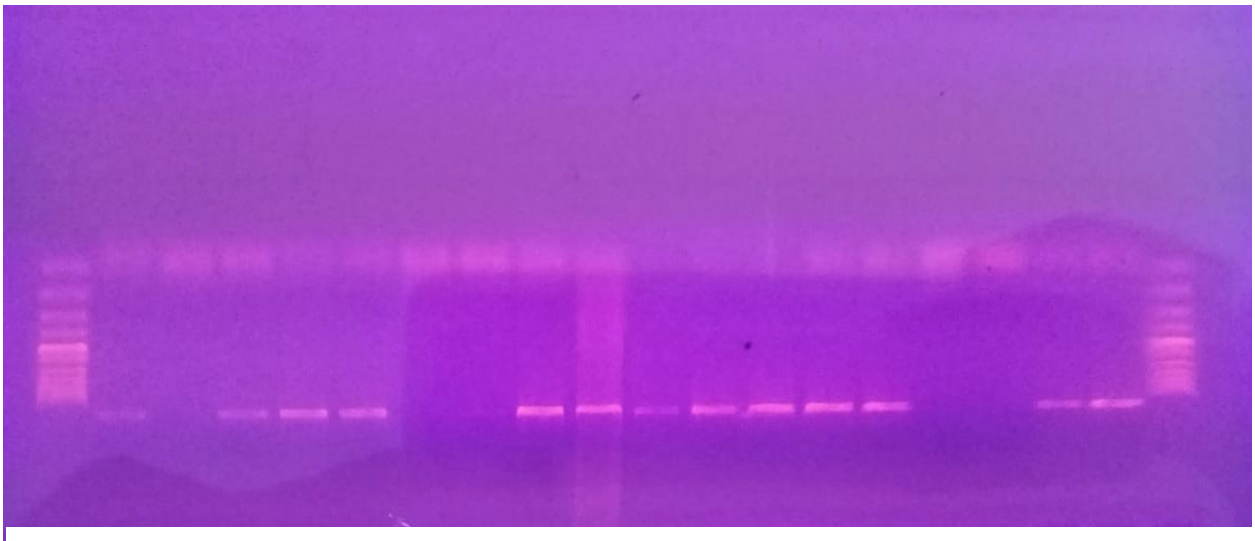

Figure 1) A sample gel image of PCR amplicons after amplification of a 1100 bp region of the VHS gene: (L→ molecular ladder, OPC→ positive control, NTC→ negative control, 00n where n is the baboon number)

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while the highest prevalence rate (74.1%) was recorded for fully matured adults, which was relatively higher than the general prevalence (65%). Baboons between 0 to 3 years are classified as infants and Juveniles and between 3-5 years as sub-adults, while adults are those above five years.

Of the 39 positive cases, 12 cases were related to female infants (75%, 12 of 16 infants), and 17 cases were related to female adults (85%, 17 of 20 adults). Therefore, adults (animals above five years) were infected more than infants and sub-adults. The prevalence of HVP2 was relatively higher in female infants and adults compared to their male counterparts.

HVP2 is relatively more transmitted through genitals (75.9%) compared to the oral transmission route (54.8%). In this study, 22 swab samples taken from genitals were positive, while only 17 oral swab samples taken from animals were positive.

Based on the counties where the baboons were caught and sampled, Nyandarua County (with a cold climate) had the highest number of positive cases (83.3%), while Kajiado (with a hot climate) had the lowest number of positive cases (25%). The prevalence rate was 66.7% in Machakos and Laikipia and 62.5 and 55.6% in Nyeri and Nairobi, respectively (Figure 2).

Genetic diversity: Genetic variations are caused by evolutionary forces like mutations, natural selection, migration, and drift. DnaSP6 software was used to estimate nucleotide diversity, haplotype diversity, and fixation index (F_{st}) values.

Gene flow and genetic differentiation: The genetic differentiation estimated by F_{st} value for HVP2 virus isolated from baboons in the selected counties was very low (F_{st}= -0.01953). The genetic differentiation estimates were as follows: Chi-square was 12.733, and the *p*-value of Chi-square was 0.4687. All these figures were insignificant since a *p*-value of < .05 was considered significant; therefore, a low divergence was inferred.

DNA polymorphisms and indel mutations:

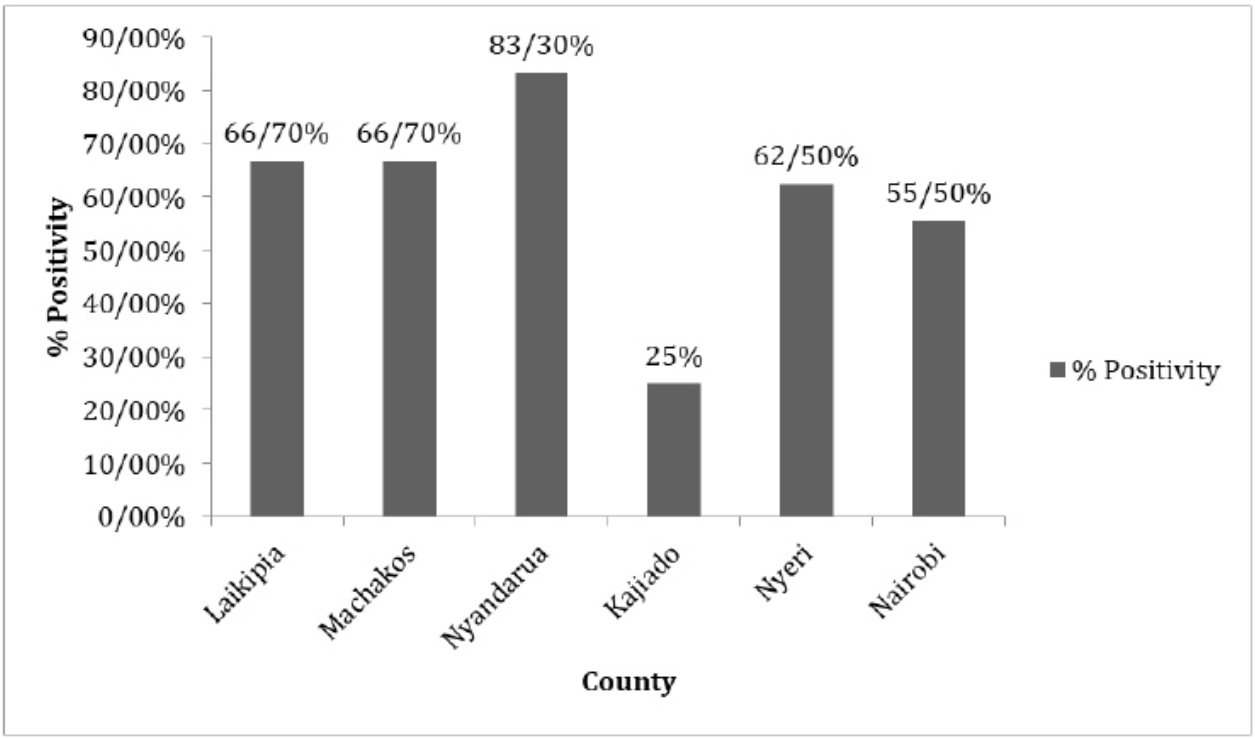

Figure 2) Prevalence of HVP2 based on PCR comparison by county

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The samples were used for insertion-deletion polymorphism analysis. According to the results, 14 indel haplotypes were generated from 11 indel sites, giving indel haplotype diversity of 0.959 and indel diversity of k (i): 2.327. These figures were insignificant leading to the inference of silent mutations. The mutations were considered protein function conservative.

The average inter-county synonymous nucleotide diversity was 0.01070, ranging from 0.0000 to 0.0412 (Table 1). The total number of singleton mutations (Etas) was 30, and the average number of pairwise nucleotide differences (k) was 6.942. The general haplotype (gene) diversity (hd) was 0.912. Little

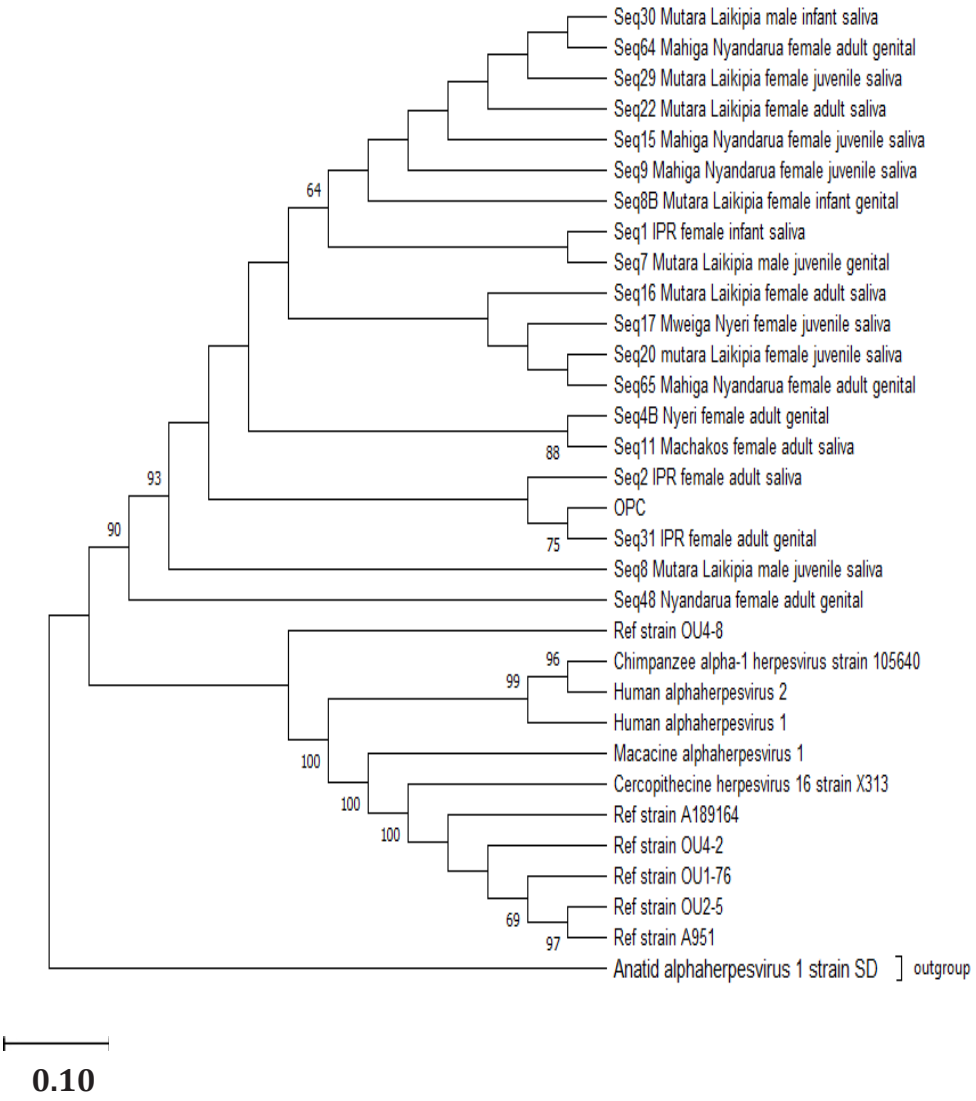

Figure 3) Evolutionary relationships of taxa**.** Neighbor-joining was the preferred method for evolutionary history inferences [29] as shown by the optimal tree. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches [30]. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Tamura 3-parameter method [31] in units of the number of base substitutions per site. This analysis involved 32 nucleotide sequences. All positions containing gaps and missing data were eliminated (complete deletion option). Evolutionary analyses were conducted in MEGA11 [32].

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Table 1) Indel mutations and neutrality tests

Parameter	Estimated Value
Indel haplotype	14
Insert-deletion haplotype diversity	0.959
Insert-deletion diversity, k (i)	2.327
Total number of singleton mutations (Etas)	30
Mean number of pairwise nucleotide differences, (k)	6.942
General haplotype (gene) diversity, (hd)	0.912
Tajima's D	-1.91635
Fu and Li's D test	-1.97379
Fu and Li's F test	-2.27632

*__Reference (HD):__ 0.1-0.4 low, 0.5-0.7 medium, and 0.8-1.0 high

divergence was observed in the populations; therefore, a set combining all sequences was pooled for Tajima's D test, yielding -1.91635. Fu and Li's D test was -1.97379, while Fu and Li's F test was -2.27632. Statistically, a *p* value less than 0.10 and greater than 0.05 was recorded, which was not significant. This neutrality test predicts that the nucleotide diversity measured against polymorphic site proportions should be equal under selective neutrality. Fu & Li's D statistic test measures the difference between total mutations and the number of singletons, while Fu & Li's F statistic test considers the variance between singletons and the average number of nucleotide differences in sequence pairs.

DNA divergence: The divergence between HVP2 strains isolated from wild and captive baboons was analyzed. There were 12 shared mutations with no fixed differences. Mutations polymorphic in captive population and monomorphic in wild-caught population were seven, while those polymorphic in wild-caught population and monomorphic in captive population were 27. The average number of nucleotide substitutions for each site between populations (D_{xy}) was 0.01156, while the average number of nucleotide differences between populations was 7.500, indicating low levels of divergence.

Phylogenetic relationship: Of the 39 positive samples, 24 samples were sent out for sequencing. Nucleotides were compared to those registered in GenBank and found to have 99% identity to *Papiine herpesvirus 2* strain A951 complete genome with accession number KF908242.1. To construct a phylogenetic tree with bootstrapping of 500 (Figure 3), 20 sample sequences were selected. *Anatid herpesvirus 1* with accession number NC 013036.1 was used as an outgroup. The samples were compared to other Papiine strains (strain OU4-8, strain OU1-76, strain A189164, strain A951, strain OU4-2, strain OU2-5, and strain X313), non-human primate alpha herpesvirus (Chimpanzee alpha-1 herpesvirus strain 105640 and Macacine alphaherpesvirus 1), and human simplex virus-1 (HSV-1/0116209) and simplex virus-2 (isolate 2020-3450AC).

Discussion

Papio animals live in tropical forests, savannahs, woodlands, and agricultural lands [8]. The animals were sampled from six Kenyan counties representing different climatic re-

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gions to investigate climate effects on viral transmission. Mahiga and Mweiga of Nyandarua district and Nyeri region of Aberdare Forest represent the coldest and most forested regions with high numbers of baboons. Machakos and Kajiado (Yatta and Ngomongo, respectively) are the warmest places with few baboons due to the shortage of food and shade and very high temperatures [15]. This explains the small or large number of animals sampled from each location since they were captured based on availability. In this research, the average prevalence of HVP2 virus in all locations was about 65%. This indicates that the climate does not affect the viral spread but rather the number of animals available.

PCR-based identification was used to detect the presence and prevalence of HVP2, yielding a prevalence of 65%. This result is relatively lower than the global prevalence of *Herpesvirus papio 2*, which ranges from 70 to 80% [20]. This study finding is lower than that of another study by Chepkwony et al. (2016) [16], reporting a sero-prevalence of 87%, a relatively higher prevalence because the use of antigens assumes shedding of viruses. According to their study results [16], only 42% of the baboons were positive in PCR test. HVP2 mainly causes latent infections in the host's central nervous system and could be detected by amplification only when animals are shedding viruses [6].

Characteristically, HVP2 very selectively invades tissues, which are oral and genital mucosal surfaces. The virus then moves through the sensory ganglia to the lumbosacral or cervical ganglia and sometimes to the trigeminal ganglia, where it establishes a chronic latent infection [21]. This infection is more dangerous than skin infections. This explains why all the brain samples showed positive bands on gel electrophoresis in this study. In this research, the samples collected from the genitals showed a prevalence of 75.86%, while those collected from saliva showed a prevalence of 54.86%. These results are consistent with the results of other studies indicating that the virus is spread from one host to another through direct contact with saliva or genital secretions and occasionally during organ transplantation [22]. Most of the saliva samples were taken from infants and juveniles (oral sores are common in juveniles), while the genital samples were taken from sub-adults and adults due to maturity differences. However, this difference was not statistically significant (Table 4).

Socially, olive baboons live in troops consisting of one-third mature males, two-thirds mature females, and many juveniles. A single troop could include 200 to 250 animals [23]. It should be noted that females mature faster than males, and sexual success depends on solid nonsexual bonds between animals. This is true for those living in the wild and those in captive colonies [24]. According to a study of HVP2 in adult baboons at the University of Oklahoma Health Sciences Center, the virus is more prevalent among wild-caught baboons than among indoor baboons [25]. Consistent with this result, the viral load in captive animals in the current study was 55.56%, which was relatively lower than the viral load among those newly caught from the wild (66.67%). The critical value obtained was not statistically significant. Once a single animal is infected, the virus is easily transmitted to other baboons due to the very close intimate relationships between baboons, whether in cages or in the wild [3]. This well explains the high mean prevalence (60%) obtained in this study.

On average, 30% of adults show clinical signs of HSV1 in the form of cold sores, which are more common in children. In adolescents up to about 30 years, HSV2 burden is about 30%, but this prevalence increases with age to about 60% in people between 30 and 60 years of age [14]. In the current study, the prev-

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alence of HVP2 virus was 57.14% in juvenile and infant baboons (0-3 years), 60% in sub-adults aged 3-5 years, and 74.07% in adults aged five years and above. This indicates an increase in viral burden with increasing age, similar to HSV in humans. Genital ulcers or lesions in sub-adults and adults could be attributed to sexual maturity and the onset of sexual activities in animals [26]. Also, female baboons begin sexual activities earlier than their male counterparts [8], which is probably the reason for the relatively higher prevalence of the virus in female adult baboons. Oral sores are common in juveniles, and this could be due to intimate mother-child relationships with infected mothers or mother-to-child transmission during birth. The latter is due to the mother shedding the virus, which could sometimes result in neonatal fatality [5].

One of the factors influencing the transmission and acquisition of herpes simplex virus 2 in humans is gender. Studies conducted in Kenya, Mexico, and the USA have shown that females due to their morphological biology are more susceptible to this virus and other sexual risk patterns than men [12]. Unlike males, a large surface area of women's vagina and labia is exposed to infection during sex. This is regardless of age, although the prevalence of infection increases with age [14]. In the case of baboons, the results are pretty similar. In a study conducted on Kenyan baboons in 2016 [16], the seroprevalence of the virus in males and females was confirmed to be 62.50 and 65.90%, respectively, and this slight difference was insignificant.

For human simplex viruses, shallow genetic variations have been recorded in recent studies, suggesting that recombination plays a role in genomic evolution [21]. Consistent with this result, in the current research, very high haplotype diversities, low nucleotide diversities, and shallow fixation index (Fst) figures ($p< .05$) were reported, resulting in statistically non-significant variations within and between subpopulations. This explains a significant expansion but low genetic differentiation among subpopulations [27]. A significant negative Tajima's D test suggests net selection or recent population expansion [28], explaining the negative test obtained in the current study. Net selection also maintains the viral genome functional integrity. Also, low genetic variability observed in this study could result from the positive selection of more adaptive species. All the 46 indel mutations recorded caused no changes in protein function and were therefore considered silent.

Phylogenetically, HVP2 in baboons is closely related to other alpha herpes viruses in their natural hosts [1]. Wild-caught baboons from Laikipia, Nyandarua, and Nyeri were more closely related to each other based on the UL41 gene, which is a virion host shutoff protein (VHS) responsible for evading the natural host's immune response. Those from captive colonies in IPR had close homology to those from Machakos. This research confirms the findings of other studies showing that HVP2 strains share a common ancestor, compared to other primate alpha herpes viruses, [21]. The *Anatid herpesvirus* 1(Duck plague virus) used as an out-group was distantly related to the study animals.

Conclusion

In conclusion, the PCR test results in this study demonstrated that the prevalence of *Herpesvirus papio 2* was 65%, and the prevalent strain circulating in the counties included in this study was the strain A951. This strain is one of the non-virulent strains in mice since it does not invade the mice's central nervous system. However, this strain causes ulcers similar to ulcers in humans infected with HSV, which could be extremely painful in some cases. Furthermore, low and non-significant genetic variations ob-

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served in HVP2 virus in intra-county and inter-county subpopulations eliminated host adaptability as a cause of genetic variation. Future studies should consider other possible causes of low variability.

Limitations of the study

In this study, only five counties were selected, and the animals were compared to captive animals at the Institute of Primate Research (Nairobi County). However, these areas are not the only geographical locations that harbor wild olive baboons in the country. Other wild baboon species like yellow baboons, which are also found in parts of Kenya, were not considered in this study. Therefore, it is recommended to sample all baboon species in other regions of Kenya not included in this study to determine HVP2 prevalence, available strains, and genetic variability.

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Ethical permissions: The necessary scientific approvals were obtained in 2019 from the Institute of Primate Research Ethics Committee called the Institutional Review Committee (IRC) under the Reference number IRC/01/14 before proceeding with the study procedures. Any discomfort and pain to the baboons were reduced by all efforts during these experiments.

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Novelty statement: This project determined HVP2 regional molecular epidemiology, prevalent strains, and genetic variability in wild and captive olive baboon populations in Kenya.

Authors' contributions: GK developed the research concept, executed the actual research work, and took part in writing the manuscript. KM developed the study design and took part in data analysis and manuscript writing. As the veterinary officer, JM collected the swabs and trigeminal ganglia samples, obtained the consumables, and supervised the execution, while AN obtained the ethical approvals and working bench, supervised the execution, and took part in manuscript writing.

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