Title

Simian homologues of human herpesviruses and implications for novel viral introduction to free-living mountain gorillas

Journal

American Journal of Primatology, 85(1)

ISSN

0275-2565

Authors

Evans, Tierra Smiley

Lowenstine, Linda J

Ssebide, Benard

et al.

Publication Date

2023

DOI

10.1002/ajp.23439

Peer reviewed

HHS Public Access

Author manuscript

Am J Primatol. Author manuscript; available in PMC 2024 April 15.

Published in final edited form as:

Am J Primatol. 2023 January ; 85(1): e23439. doi:10.1002/ajp.23439.

Simian homologues of human herpesviruses and implications for novel viral introduction to free-living mountain gorillas

Tierra Smiley Evans[1], **Linda J. Lowenstine**[2], **Benard Ssebide**[1], **Peter A. Barry**[3], **Jean Felix Kinani**[4], **Fred Nizeyimana**[1], **Jean Bosco Noheli**[1], **Ricky Okello**[1], **Antoine Mudakikwa**[5], **Michael R. Cranfield**[1], **Jonna A.K. Mazet**[1], **Christine Kreuder Johnson**[1], **Kirsten V. Gilardi**[1]

[1]Gorilla Doctors, Karen C. Drayer Wildlife Health Center, One Health Institute, University of California Davis, School of Veterinary Medicine, One Shields Ave, Davis, CA 95616

[2]Department of Pathology, Microbiology and Immunology, University of California Davis, School of Veterinary Medicine, One Shields Ave, Davis, CA 95616

[3]Center for Immunology and Infectious Diseases; California National Primate Research Center; Department of Pathology and Laboratory Medicine, University of California Davis, One Shields Ave, Davis, CA 95616

[4]One Health Approach for Conservation (OHAC), Gorilla Health, Kigali, Rwanda

[5]Rwanda Development Board, Kigali, Rwanda

Abstract

The endangered mountain gorilla (*Gorilla beringei beringei*) in Rwanda, Uganda, and the Democratic Republic of Congo is frequently in contact with humans through tourism, research activities, and illegal entry of people into protected gorilla habitat. Herpesviruses, which are ubiquitous in primates, have the potential to be shared in any setting where humans and gorillas share habitat. Based on serological findings and clinical observations of orofacial ulcerated lesions resembling herpetic lesions, an alpha-herpesvirus resembling human herpes simplex virus 1 (HSV-1) has long been suspected to be present in human-habituated mountain gorillas in the wild. While the etiology of orofacial lesions in the wild has not been confirmed, HSV-1 has been suspected in captively-housed mountain gorillas and confirmed in a co-housed confiscated Grauer's gorilla (*Gorilla beringei graueri*). To better characterize herpesviruses infecting mountain gorillas and to determine the presence/absence of HSV-1 in the free-living population, we conducted a population-wide survey to test for the presence of orally shed herpesviruses. DNA was extracted from discarded chewed plants collected from 294 individuals from 26 groups, and samples were screened by PCR using pan-herpesvirus and HSV-1-specific assays. We found no evidence that human herpesviruses had infected free-ranging mountain gorillas. However, we found gorilla-specific homologs to human herpesviruses, including cytomegaloviruses (GbbCMV-1 and 2), lymphocryptovirus (GbbLCV-1) and a new rhadinovirus (GbbRHV-1) with similar characteristics (i.e., timing of primary infection, shedding in multiple age groups and potential modes of transmission) to their human counterparts, human cytomegalovirus (HCMV), Epstein-Barr virus and Kaposi's sarcoma-associated herpesvirus, respectively.

Keywords

herpesvirus; cytomegalovirus; rhadinovirus; lymphocryptovirus; HSV-1; reintroduction; mountain gorilla; conservation

Introduction:

Human-introduced infectious pathogens are a major threat to the survival of wild great apes (Leendertz et al., 2006). The endangered mountain gorilla (*Gorilla beringei beringei*) is at especially high risk for contracting human pathogens due to the fact that more than half of its population is human-habituated for tourism and research (Granjon et al., 2020; Hickey et al., 2019). Habituation of mountain gorillas began in the late 1960s, and today the majority of gorilla groups are in close proximity with people daily, including local community members employed as trackers and guides, as well as international tourists and researchers entering the parks. Mountain gorilla habitat is surrounded by some of the densest human populations in continental Africa, providing opportunities for widespread human contact, when people illegally enter the forest for utilization of park resources and when gorillas leave the parks to raid local crops and gardens (Sandbrook and Semple, 2006; Williamson and Fawcett, 2008).

Pathogen spillover from people has been documented in several great ape populations where contact with humans is close and common. Pathogen surveillance and research at this human-wildlife interface has often focused on acute diseases, particularly respiratory and neuroparalytic infections. An outbreak of suspected poliovirus occurred in the Kasakela community of the Gombe (Tanzania) chimpanzee population during the 1960s during which six chimpanzees died and at least six others were paralyzed (Goodall, 1983; Goodall, 1986). It is likely that chimpanzees acquired poliovirus by eating discarded food from nearby human villages. Transmission of respiratory viruses from humans to wild apes was first reported at a chimpanzee research site in Taï Forest, Cote d'Ivoire, where human paramyxoviruses, human respiratory syncytial virus (HRSV) and human metapneumovirus (HMPV) were detected in tissues from dead chimpanzees (Kondgen et al., 2008). Research personnel were the most plausible sources of infection. HRSV, HMPV and other respiratory viruses including human respirovirus 3 and human coronavirus OC43 have since been detected frequently in different great ape species and populations (Kaur et al., 2008; Kondgen et al., 2010; Negrey et al, 2019; Patrono et al, 2018; Patrono et al, 2022). Human pathogens identified to specifically infect mountain gorillas have included gastrointestinal bacteria, HMPV, and HRSV and a suspected outbreak of measles virus (Hastings et al., 1991; Mazet et al., 2020; Palacios et al., 2011; Rwego et al., 2008).

While acute diseases can be more easily observed in wild ape populations, pathogens causing persistent infections, with less evident or subclinical infections, can have dramatic long-term effects on small, isolated primate populations (Wallis and Lee, 1999). Viruses in the family *Herpesviridae*, comprising the alpha, beta and gammaherpesvirinae subfamilies (Gatherer et al., 2021), are ubiquitous in primates, and while some human herpesviruses are considered to have low pathogenic potential in the preponderance of their immune-

competent hosts of origin, cross-species infections or infection of an immune-compromised individual can have adverse effects (Voevodin and Preston A. Marx, 2009b). In humans, herpes simplex virus 1 (HSV-1; an alpha herpesvirus) infections are typically asymptomatic or only associated with mild, recurrent oral-mucosal lesions (Brack, 1977). HSV-1 infections in captive primates have, however, resulted in fatalities, including in a western lowland gorilla (*Gorilla gorilla gorilla*), an orangutan (*Pongo pygmaeus pygmaeus*), patas monkeys (*Erythrocebus patas*), colobus monkeys (*Colobus spp.*), marmosets (*Callithrix jaccbus*), owl monkeys (*Aotus trivirgatus*), gibbons (*Hylobates lar*), and white-faced saki monkeys (*Pithecia pithecia pithecia*) (Heldstab et al., 1981; Kik et al., 2005; Loomis et al., 1981; Matz-Rensing et al., 2003; Melendez et al., 1969; Ramsay et al., 1982; Schrenzel et al., 2003; Smith et al., 1969). HSV-1 has infected captive western and eastern lowland (*Gorilla beringei graueri*) gorillas, causing fever, oral lesions, and malaise (Eberle and Hilliard, 1989; Gilardi et al., 2014). Captive chimpanzees (*Pan troglodytes* and *Pan paniscus*) have also had suspected infections with human simplex virus type 2 (HSV-2; human alphaherpesvirus 2), or an as yet identified chimpanzee simplexvirus, causing genital lesions (McClure et al., 1980). *Varicella zoster*, also an alpha herpesvirus has infected western lowland gorillas in captivity and can lead to debilitating disease (Masters et al., 2010; Myers et al., 1987).

To date, the only molecularly characterized herpesviruses in wild mountain gorillas are: gorilla lymphocryptovirus (GbbLCV-1), which is suspected to be associated with pulmonary reactive lymphoid hyperplasia in infants with immature or compromised immune systems (Smiley Evans et al., 2017); a cytomegalovirus (GbbCMV), which has unknown clinical etiology (Smiley Evans et al, 2016; Murthy et al, 2019) and a simplexvirus which most closely resembles human simplexvirus – 2 (HSV-2), which also has unknown clinical etiology (Wertheim et al, 2021). Mountain gorillas in the wild have shown serological positivity to HSV-1 / HSV-2, although seroassays could have been detecting antibodies to the now known gorilla simplexvirus or were cross-reacting with another closely related virus (Eberle, 1992; Whittier et al., 2005a). Further, raised orofacial, frequently ulcerated lesions observed around the mouths of mountain gorillas were first reported in 1989 and have been observed intermittently in individual gorillas ever since (personal communication, Michael R. Cranfield). It had been suggested that these herpetic-like lesions may be the result of a persistent HSV-1 in these animals.

Opportunities for pathogen transmission differ greatly between free-ranging and captive settings. While mountain gorillas are not maintained in any zoo collections, young gorillas orphaned by poaching in the Democratic Republic of Congo (DRC) are brought into captivity for triage and transition to long-term management in sanctuaries. These orphans require frequent contact with surrogate human caretakers. While close human contact as a management strategy best addresses orphan mountain gorilla's psychological needs, this activity can inadvertently expose them to human pathogens. Mountain gorillas in captivity, reared by human caretakers, are likely to be exposed to HSV-1, since HSV-1 is one of the most common human pathogens (Kaufman et al., 2005) and is frequently shed in saliva of infected humans (Miller and Danaher, 2008). Indeed, HSV-1 was confirmed in a confiscated eastern lowland gorilla co-housed with captive mountain gorillas, and several mountain gorillas in the group displayed clinical signs of an acute herpesviral outbreak, including fever, malaise, and ulcerated lesions in and around the mouth (Gilardi et al., 2014).

To better understand natural herpesviral infections in the wild population and survey for potential human herpesvirus introductions, we surveyed two mountain gorilla sub-populations (Virunga gorillas in the Virunga Volcanoes Conservation Region (spanning Rwanda, Uganda, and the DRC) and the Bwindi gorillas in the Bwindi Impenetrable Forest, Uganda) for the presence of herpesviruses. By implementing broadly reactive herpesviral PCR assays, we were able to screen for all herpesviruses (both endemic and of potential human origin) circulating in the population.

Methods:

Study population

We surveyed Virunga mountain gorillas living in the 447 km^2 Virunga Volcanoes conservation area, which is comprised of the Volcanoes, Virunga, and Mgahinga Gorilla National Parks spanning the borders of Rwanda, Democratic Republic of Congo, and Uganda (respectively), and Bwindi mountain gorillas living in the 331 km^2 Bwindi Impenetrable National Park in Southwestern Uganda. This study protocol was approved by the Uganda Wildlife Authority, the Rwanda Development Board, and the Institutional Animal Care and Use Committee of the University of California, Davis. All research conducted adhered to the American Society of Primatologists principles for the ethical treatment of non-human primates.

Mountain gorilla sample and data collection

Chewed plant samples (n = 383) were collected from 294 individual, human-habituated mountain gorillas in the contiguous Volcanoes National Park in Rwanda and Mgahinga National Park in Uganda (referred to herein as the Virunga population) and in Bwindi Impenetrable National Park in Uganda as described previously (Smiley Evans et al., 2016). Briefly, samples of dropped plants were collected when a gorilla moved to a new foraging area. The portion of the discarded plant material that was the most masticated was cut using disposable, sterile scalpel blades, and plant pieces were placed in viral transport media for storage in liquid nitrogen. Chewed plant samples were collected between November 2012 and June 2013. In addition, a review of histological findings from mountain gorilla ante-mortem and necropsy cases between 1989 and 2013 was conducted to identify potential cases of herpesviral infections. Archived tissue samples from suspect herpesvirus cases were identified from necropsies of mountain gorillas that died in the Volcanoes National Park between 2006 and 2011 (n = 65 tissue samples from 14 individuals).

Sample processing

Chewed plant samples were processed according to previously described techniques (Smiley Evans et al., 2016). Briefly, discarded plant samples stored in viral transport media were thawed, vortexed, and centrifuged to pellet plant material. Sample supernatant was then removed for total nucleic acid extraction. A subset of tissue samples, collected during routine necropsies, were frozen and stored at −80°C. Frozen tissue samples were subsequently cut into small pieces with scissors disinfected with 10% sodium hypochlorite after handling each sample, to prevent DNA cross-contamination between tissues and between samples from different individuals. Total nucleic acid was extracted

from approximately 600 μl of chewed plant supernatant and 100 mg of frozen tissue. Total nucleic acid was extracted using the NucliSENS® MiniMAG® system (bioMérieux, Inc.) according to the manufacturer's protocols. Total nucleic acids were stored at −80° Celsius.

Molecular diagnostics

DNA was analyzed by polymerase chain reaction (PCR) to detect herpesviral DNA utilizing degenerative primers amplifying a 450 bp region of the terminase gene (TERM) and a 225 bp region of the DNA polymerase gene (DPOL) (Smiley Evans et al., 2016). In addition, to generate larger sequences for phylogenetic analysis, a representative subset of samples was analyzed by PCR to detect a fragment of the glycoprotein B (gB) gene (Ehlers et al., 2010). Nested long-distance PCR was performed on a subset of samples to connect the DPOL and gB sequences using the TaKaRa-Ex PCR system, according to manufacturer's instructions (Kakara Bio Inc., Japan) by using virus-specific primers (Supplemental Table 1).

Because degenerate herpesvirus assays can sometimes favor one herpesvirus over another (Prepens et al., 2007), all samples were also screened using an HSV-1 specific PCR assay. Primers were designed from an HSV-1 sequence detected in an orphan Grauer's gorilla (Supplemental Table 1) (Gilardi et al., 2014). Parallel reactions were run amplifying a 544 bp fragment of the mammalian beta-actin gene to test sample quality (Hammond et al., 2005). PCR products of appropriate size were cloned using TOPO® TA and TOPO® XL cloning kits (Invitrogen, Carlsbad, CA, USA) depending on the sequence size, and sequencing was performed using Sanger sequencing at the University of California, Davis DNA sequencing laboratory. Sequences were compared to other published herpesviral sequences in the GenBank Database (National Center for Biotechnology Information, National Library of Medicine, Bethesda, Maryland, USA). Sequences were edited using Geneious version 7.0.6. Alignments were constructed using CLUSTAL W, executed through Geneious. Neighbor-joining trees were built in Geneious and bootstrapping using 1000 repetitions. Trees were constructed with outgroups provided by EBV (Human gammaherpesvirus 4, HHV-4).

Statistical analysis

Wild mountain gorillas were divided into age classes: infants were defined as gorillas less than three years of age, juveniles as three to five years, subadults as six to seven years, adult females as eight years and older, black backs as eight to 11 year-old males, and silverbacks as males 12 years and older (Robbins et al., 2009; Williamson and Gerald-Steklis, 2001). Period prevalence and 95% binomial exact confidence intervals (CI) for oral shedding of herpesviruses were calculated across age class and sex. Bivariate analysis using a 2-sided chi-square test and the odds ratio were used to evaluate distribution among age, sex, and group classifications. Because gorillas within the same family group were likely to be similar to each other with respect to similar stresses imposed on the group at sampling time and herpesvirus transmission through close contact, evaluation of the effects of conservation area, family group, age and sex on gorilla cytomegalovirus oral shedding was further examined by multivariable analysis using a mixed-effects logistic regression model (Demidenko, 2004; Pearson et al., 2008). Furthermore, it was not known at the time of sample collection whether previous samples collected from individual gorillas were

of sufficient quality for virus detection, and an opportunistic approach was taken wherein samples were collected whenever a “good chewed” sample was serendipitously available. Opportunistic sampling presented the risk that repeated sampling of individuals might occur. For prevalence estimates, all samples were assumed to reflect an independent sample of a single individual due to the likelihood of intermittent shedding. For our multivariable analyses, family group and repeated measures were incorporated as random effect variables in the mixed effect model. Multilevel mixed effects modeling implemented a likelihood-based estimation method that allowed for all available data to be used in the analysis while accounting for correlation among groups and repeated samples from individuals with similar risk. An unstructured covariance matrix was chosen for the random effects. Fixed effects variables included age and sex. The decision whether to include a variable in the model was evaluated using the likelihood-ratio test to determine whether each significantly improved model fit ($P<0.05$), compared to a model without that variable. Variables were retained in the model if they improved fit, while minimizing Akaike Information Criterion (AIC), or were determined to be important confounders based on bivariate analyses.

To measure confidence in detection of oral shedding of a herpesvirus, an approximation of the hypergeometric distribution was used to calculate the sample size needed to demonstrate absence of viral shedding (Cameron and Baldock, 1998). The difference between the proportion of live wild mountain gorilla infants orally shedding a specific herpesvirus and dead infants examined with concurrent specific herpesviral infections was evaluated using the Fisher’s exact test (Fisher, 1935). The proportion of mother-infant pairs concordantly shedding a herpesvirus was evaluated using the McNemar’s test (McNemar, 1947). All statistical analyses were performed using STATA version 13.1 (StataCorp, 2013).

Results:

A total of 332 samples were collected and included in analyses. Of the 383 total collected samples, 51 were excluded from statistical calculations because they tested negative for both herpesviruses and mammalian beta-actin; therefore, inadequate collection of sample material could not be ruled out. Based on population sizes at the time of sample collection, we screened approximately 76% of the Bwindi and 48% of the Virunga habituated gorilla populations, and 32% of the Bwindi and 35% of the Virunga total gorilla population (both habituated and non-habituated gorillas) for herpesviruses (representing n=294 individuals from 26 family groups, as determined by visual identification of known individuals). We detected herpesviruses in the beta- and gamma herpesvirinae subfamilies including mountain gorilla-specific strains of cytomegalovirus 1 (GbbCMV-1), cytomegalovirus 2 (GbbCMV-2), rhadinovirus 1 (GbbRHV-1) and lymphocryptovirus 1 (GbbLCV-1) (GbbLCV-1 detailed findings described in Smiley Evans et al., 2017) (Table 1). We did not detect human HSV-1 in wild-habituated mountain gorillas using any of our diagnostic methods, including infants with active raised orofacial lesions, previously suspected to be of herpetic origin.

Our sample sizes were sufficient for demonstrating absence of viral shedding for a shedding prevalence of 10% (the estimated prevalence of orofacial lesions seen in mountain gorillas) and would have been sufficient for a true shedding prevalence as low as 2.5% (Sergeant, 2016). Herpesvirus-like inclusion bodies in tissues from three mountain gorilla necropsy

cases (n = 1 buccal lesion, n = 1 tongue, n = 1 esophagus) were identified and included in analyses. Two antemortem cases of mountain gorilla infants displaying clinical signs of multiple, diffuse, raised orofacial lesions (not observed elsewhere on the body) that progressed to vesicles consistent with a herpes-like infection were also identified (Whittier et al., 2005b). Furthermore, during our collection of chewed plant oral samples from wild mountain gorillas, two infants were identified to have herpesvirus-like orofacial lesions at the time of sample collection. All identified necropsy and antemortem tissue and oral samples from individuals with associated herpesvirus-like lesions were tested using the same pan-herpesvirus and HSV-1-specific PCR assays described above. HSV-1 was not detected in any of these cases.

Gorilla specific strains of cytomegalovirus (GbbCMVs) were detected in 9.26% and 14.7% of the Bwindi and Virunga gorillas, respectively. Sequences detected were divided into two major clades (Figure 1), similar to western lowland gorilla CMV 1.1 and 2.1 (GgorCMV-1.1, GgorCMV-2.1) described previously (Leendertz et al., 2009) (Figure 2). Alignment of a 605 bp fragment of the gB gene of GbbCMV-1 (Genbank Accession # KX839482) showed 96.9% nucleotide similarity and 96.6% amino acid similarity to GgorCMV-1.1, and 70.5% nucleotide and 68.0% amino acid similarity to HCMV. Alignment of a 1,280 bp fragment of the gB gene of GbbCMV-2 (Genbank Accession # KX839483) showed 96.02% nucleotide and 96.24% amino acid similarity to GgorCMV-2.1, and 80.16% nucleotide and 79.34% amino acid similarity to HCMV.

The highest prevalence of GbbCMV oral shedding (both GbbCMV-1 and GbbCMV-2) was detected in infants between the ages of 2.5 and 3 years (Table 2). Based on multivariable analysis, infants were 2.5 times as likely to be shedding GbbCMV compared to other ages (P=0.04). While GbbCMV oral shedding was highest in the infant age group, we also detected GbbCMV oral shedding in juveniles, subadults and adults (Table 2). We did not detect any significant differences between sex, other age groups, park or family group and GbbCMV oral shedding. Infant GbbCMV oral shedding was not correlated with maternal oral shedding. We tested oral samples from 19 Bwindi and Virunga mother-infant pairs including one mother-twin infant pair. Three mother-infant pairs were both shedding GbbCMV in saliva, 12 mother-infant pairs were both not shedding, in one pair only the mother was shedding, and in three pairs only the infant was shedding. All three pairs in which both mother and infant were shedding CMV on the same day of sample collection were shedding the same CMV type (either CMV type 1 or type 2).

We tested postmortem tissues for herpesviruses from fourteen infants that died of natural causes and had suspected herpetic-like lesions on histological examination (Figure 3). GbbCMV was detected in tissues from three infants (27%) including the lung tissue of two infants and the pancreas of one infant. Two of the deaths were related to maternal abandonment, and the infants demonstrated thymic atrophy at the time of death (thymus was not available for histology from one infant). All three infants had pulmonary lymphoid reactive hyperplasia (PRLH) incidental to their primary cause of death and were co-infected with GbbLCV-1, which is thought to be associated with PRLH (Smiley Evans et al., 2017). The proportion of infant deaths with concurrent GbbCMV infection was not significantly different from the proportion of live infants orally shedding GbbCMV. It is unclear whether

GbbCMV was potentially associated with PRLH in any of these cases. Two antemortem cases with perioral herpes-like lesions which were negative for all other herpesviruses were positive for GbbLCV-1.

We also detected a mountain gorilla-specific strain of Rhadinovirus 1 (GbbRHV-1) using pan herpesvirus assays in a total of six mountain gorillas, including two juveniles and one adult Bwindi gorilla and one juvenile and two adult Virunga gorillas. To further characterize the detected rhadinovirus sequences, all positive samples were analyzed by PCR to detect a fragment of the gB gene. Long-distance-PCR was then performed to connect DPOL and gB gene sequences. Alignment of a 3,326 bp sequence, encompassing fragments of the DPOL and gB genes, showed 98.96% nucleotide and 100% amino acid similarity to Gorilla gorilla rhadinovirus 1 (Genbank Accession # AY177144.2), and 86.59% nucleotide and 91.04% amino acid similarity to human KSHV. Alignment of a 510 bp fragment of the DPOL gene showed that GbbRHV-1 clustered with GgorRHV-1 (Figure 4). GbbRHV-1 was not detected in infants, including infants of infected mothers. We did not detect any significant differences between sex, other age groups, park or family group and GbbRHV-1 oral shedding. GbbRHV-1 was also not detected in any mountain gorilla post-mortem tissues tested.

Discussion:

Our results indicate that wild mountain gorilla populations were not infected with human HSV-1 at the time of sample collection, despite known frequent direct contact between mountain gorillas and humans. We expected to identify HSV-1 viral shedding if it were present in an individual gorilla given the frequency and duration of shedding in infected humans. In humans, epidemiological studies have confirmed HSV-1 oral shedding in approximately 70% of healthy adults (Miller and Danaher, 2008), and among positive people, virus is shed in greater than 98% of individuals over a 30-day period (Kaufman et al., 2005). Furthermore, we know that eastern gorillas can be infected with HSV-1 and that virus is detectable in oral swabs from eastern gorillas using the pan-herpes PCR assays utilized in this study (Gilardi et al., 2014). In addition, our HSV-1 specific assay had a detection limit of two viral copies. Further evidence from a small sample size of mountain gorillas (n = 8 individuals) that demonstrated herpetic like orofacial lesions also failed to detect the presence of a simplexvirus in fecal samples (Wertheim et al, 2021). Given that we did not detect HSV-1 in oral samples collected from a significant proportion of the total mountain gorilla population or in suspected herpetic-like lesions from opportunistically collected antemortem and pathology cases over the course of 24 years, we conclude that HSV-1 had not entered the wild population at the time of this study.

While we did not amplify any alpha herpesvirus DNA using pan-herpevirus primers, serological positivity to a virus antigenically similar but not identical to HSV-2 has been reported in mountain gorillas, and serological positivity to HSV-1 but not HSV-2 has also been reported (Eberle, 1992; Whittier et al., 2005a). These contradictory findings may represent exposure to the simplexvirus most closely resembling HSV-2 that was genetically characterized in mountain gorillas in 2022 (Wertheimer et al, 2022). Alternatively, the earlier reported serological results may provide additional evidence for the cross-reactivity

associated with human serological assays when applied to gorillas (Smiley Evans et al., 2017). We did not detect HSV-1 in infants with active herpetic-like orofacial lesions present at the time of sampling, providing evidence that these lesions, previously theorized to be caused by an alpha herpesvirus, were not caused by HSV-1 and are likely caused by an etiological agent that is not a herpesvirus, or at least, a herpesvirus-like agent whose genome cannot be amplified with these primer sets and conditions.

By applying broadly reactive PCR screening assays in our search for HSV-1, we documented several gorilla-specific herpesviruses, including two beta herpesviruses (GbbCMV-1 and GbbCMV-2) and two gamma herpesviruses (GbbRHV-1 and GbbLCV-1) that demonstrated epidemiologic similarities to their counterparts in humans. For example, infant mountain gorillas were significantly more likely to be shedding GbbCMV compared to other age groups, similar to people, where oral shedding of human CMV (HCMV) peaks between 1 and 2 years of age (Cannon et al., 2011). Human infants and young children often acquire primary HCMV infection through breastfeeding (Schleiss, 2006) or through contact with other children who are actively shedding virus (Adler, 1985). We did not find a significant correlation between mother and infant oral GbbCMV shedding in mountain gorillas, indicating that infection may be occurring through contact with other gorillas of similar age within family groups. Mountain gorilla infants spend a large portion of their day playing with and grooming other infants, during which time they are in contact with other gorillas' saliva and sometimes urine, providing potential routes for transmission of GbbCMV. Mother to offspring transmission of GbbCMV through breast milk or saliva is also likely a potential mode of transmission, as three mother / infant pairs were documented to be shedding the same type of CMV simultaneously (CMV-1 vs CMV-2). While these inferences are made on a small cross-sectional sample size, they are consistent with what we know about human CMV transmission commonly occurring among young human children and rhesus macaque CMV transmission occurring horizontally through exposure to urine, saliva or feces of other animals (Cannon et al, 2011). We recommend longitudinal sampling and/or further viral sequence analyses to prove a specific transmission route.

Cytomegaloviruses are of unknown pathogenicity in mountain gorillas or other non-human great apes. In humans, HCMV is usually asymptomatic in immune-competent people, or it sometimes presents as transient flu-like illness and self-resolving clinical outcomes. However, HCMV is a significant cause of morbidity and mortality in immunologically immature or immune-compromised individuals, such as transplant recipients, human immunodeficiency virus-infected patients, and congenitally infected fetuses/newborns (Alford and Britt, 1993; Stagno, 1990). HCMV is the most common congenital infection globally, occurring in 0.5 % up to 6% of all pregnancies (average ~1%) (Zuhair et al, 2019). Intrauterine HCMV is a known teratogen for fetal growth and development with the fetal central nervous system a prominent target of sequelae resulting in lifelong neurological deficits, including microcephaly, hearing loss, developmental and motor impairments (Voevodin and Preston A. Marx, 2009a; Yue and Barry, 2008; Coppola et al, 2019). In rhesus macaques, rhesus cytomegalovirus (RhCMV) is endemic with near 100% seroprevalence in both wild populations of macaques as well as in breeding facilities (Feeroz et al, 2013; Vogel et al, 1994). RhCMV elicits asymptomatic infections in immune-competent individuals but can result in congenital disease in experimentally infected animals *in utero* and disseminated

disease in immune-compromised animals (Barry et al., 2006; Bialas et al., 2015; Tarantal et al., 1998; Voevodin and Preston A. Marx, 2009a; Yue and Barry, 2008). In breeding facility-reared macaques, RhCMV is asymptomatically shed in bodily fluids, such as saliva and urine, for years and even decades following primary infection of an uninfected animal (Oxford et al, 2015; Eberhardt et al, 2016). Seroconversion within newborn animals is rapid (~1 year) and appears coincident with post weaning from the dam (Vogel et al, 1994). Human and non-human primates suffering from other bacterial and viral diseases may experience disease exacerbation by fulminant reactivation of CMV. In the mountain gorilla cases reported here, we found thymic atrophy associated with GbbCMV infection in two infant mountain gorillas that were necropsied, which is consistent with the theory of immune dysregulation leading to reactivation of endemic CMV in these infants (Figure 3).

We detected two gamma herpesviruses in this study, a rhadinovirus (GbbRHV-1) and a lymphocryptovirus (GbbLCV-1) using a pan-herpesvirus assays. Only a small number of individual mountain gorillas were shedding GbbRHV-1 (6 / 332 individuals), indicating that GbbRHV-1 infection is not widespread in the mountain gorilla population, or it is not frequently shed in saliva. In humans, in endemic regions such as sub-Saharan Africa, transmission of Kaposi's sarcoma associated herpesvirus (KSHV; a rhadinovirus) typically occurs during childhood, likely through close contact with infected individual's saliva or shared household utensils (Mayama et al., 1998; Mbulaiteye et al., 2006). However, in non-endemic countries, transmission typically occurs at older ages through sexual contact (Martin et al., 1998). Mountain gorillas are likely infected as juveniles (GbbRHV-1 was not detected in any infants) through close contact with other infected gorillas' saliva or after sexual maturity through sexual contact. Our findings suggest a scenario more similar to the non-endemic epidemiologic pattern in humans although larger longitudinal samples sizes are needed to definitively determine transmission patterns.

Little is known about the pathogenic potential of gamma herpesviruses in non-human great apes, but parallels can be considered based on research performed in humans. Previously, we described the detection of GbbLCV-1 in mountain gorilla lung tissue in association with lesions consistent with pulmonary lymphoid reactive hyperplasia (Smiley Evans et al., 2017), a condition noted in immune-deficient humans infected with Epstein-Barr virus. In humans, KSHV can lead to Kaposi's sarcoma and plays a role in the pathogenesis of two rare B-cell lymphoproliferative disorders, primary effusion lymphoma and multi-centric Castleman's disease (Chang et al., 1994; Nador et al., 1996; Soulier et al., 1995). KSHV is also associated with HIV-related solid immunoblastic/plasmablastic diffuse large B-cell lymphoma (Deloose et al., 2005). In SIV infected macaques, rhesus rhadinovirus 1 is associated with hyperplastic B-lymphoproliferative disease (Wong et al., 1999) and retroperitoneal fibromatosis-associated herpesvirus; rhesus rhadinovirus 2 is associated with SAIDS-related Kaposi's sarcoma-like tumors (Cai et al., 2005). We do not know if the mountain gorilla rhadinovirus detected here is associated with clinical features or pathological outcomes. All mountain gorillas sampled in this study were closely observed for visible oral or skin lesions prior to collecting each sample, and all gorillas in which GbbRHV-1 was detected appeared healthy at the time of sampling. GbbRHV-1 was not detected in any post-mortem tissue samples tested in this study. Furthermore, lesions similar to those seen with KSHV infections in people have not been observed in mountain gorilla

tissues. Future examinations of gorillas should be conducted with a heightened awareness that mountain gorilla CMVs, a rhadinovirus and a lymphocryptovirus naturally circulate within the population, so that if present, pathological conditions similar to those associated with their human herpesvirus counterparts can be documented. Particular attention should be paid to individuals suspected to be immune compromised, such as infants exhibiting signs of failure to thrive and animals with poor condition, as well as known immunocompromised individuals as identified on post-mortem.

Through prospective and retrospective analysis of herpesviruses circulating in the wild mountain gorilla population, we demonstrate potential similar epidemiologic patterns for herpesviruses of gorilla origin (GbbCMV-1, GbbCMV-2, GbbRHV-1, and GbbLCV-1) with those identified in humans, as well as the absence of human introduced HSV-1 with implications for conservation management. For example, if conservation strategies for mountain gorillas were to include reintroduction of formerly captive individuals, the potential for introducing novel human pathogens to wild populations will require careful consideration. We conclude that as eastern gorillas continue to be brought into captivity, they will be exposed to and likely infected with HSV-1. Evidence that wild mountain gorillas are not currently infected with human HSV-1, while captive eastern gorillas are, suggests that reintroducing an HSV-1 infected orphan gorilla could introduce a human virus that would be novel to the wild gorilla population. While infection of adult western lowland gorillas with HSV-1 has resulted in clinically observable mild symptoms compared to other primate species, it is important to consider that HSV infection has been associated with decreased fertility in human males and systemic disease leading to mortality in human and western lowland gorilla infants (Heldstab et al., 1981; Monavari et al., 2013; Whitley et al., 1980). A mountain gorilla has never successfully been reintroduced to the wild after living in a sanctuary; however, this conservation practice could hypothetically be considered in the future if larger numbers of mountain gorillas require captive care. With such a small remaining wild population of mountain gorillas, every individual is potentially of genetic importance. Because the potential health and reproductive effects of HSV-1 infection in mountain gorillas are unknown, reintroduction of formerly captive mountain gorillas infected with HSV-1 could pose an unacceptable risk to this isolated and endangered species. We recommend continuing to screen wild mountain gorillas and other great ape populations for all potential human introduced infectious diseases in advance of decision-making regarding reintroductions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

This research was supported by the William J. Fulbright Program, the United States Agency for International Development (USAID) Emerging Pandemic Threats PREDICT program, and the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under award #U01AI151814. The authors would like to thank the Rwanda Development Board, the Uganda Wildlife Authority, and the Karisoke Research Center for facilitating this project; Brett Smith, Jasmine Pante, Alex Tremeau-Bravard, Cheyenne Coxon, Kim Schmidt, and Julie Rushmore for project advice and laboratory assistance; and Dawn Zimmerman, Rachael Mbabazi, Abdulhameed Kateregga, Ricky Okello, and Jean-Paul Lukusa for field support.

References:

Adler SP (1985). The molecular epidemiology of cytomegalovirus transmission among children attending a day care center. Journal of Infectious Diseases, 152(4),760–8. [PubMed: 2995502]

Alford CA, Britt WJ (1993). Cytomegalovirus. In: Roizman B, Whitley RJ, & Lopez C (Eds.), The Human Herpesviruses (pp. 227–255). New York, USA: Raven Press.

Barry PA, Lockridge KM, Salamat S, Tinling SP, Yue Y, Zhou SS, Gospe SM Jr., Britt WJ, Tarantal AF (2006). Nonhuman primate models of intrauterine cytomegalovirus infection. Institute of Laboratory Animal Resources Journal, 47(1),49–64. 10.1093/ilar.47.1.49

Bialas KM, Tanaka T, Tran D, Varner V, De La Rosa E. Cisneros, Chiuppesi F, Wussow F, Kattenhorn L, Macri S, Kunz EL, Estroff JA, Kirchherr J, Yue Y, Fan Q, Lauck M, O'Connor DH, Hall AH, Xavier A, Diamond DJ,… Permar SR (2015). Maternal CD4+ T cells protect against severe congenital cytomegalovirus disease in a novel nonhuman primate model of placental cytomegalovirus transmission. Proceedings of the National Academies of Science, 112(44), 13645–50. 10.1073/pnas.1511526112

Brack M (1977). Morphological and Epidemiological Aspects of Simian Herpesvirus Infections: Berlin and Hamburg: VerlagPaulParey, Inc.

Cai X, Lu S, Zhang Z, Gonzalez CM, Damania B, Cullen BR (2005). Kaposi's sarcoma-associated herpesvirus expresses an array of viral microRNAs in latently infected cells. Proceedings of the National Academies of Science, 102(15),5570–5. 10.1073/pnas.0408192102

Cameron AR, Baldock FC (1998). A new probability formula for surveys to substantiate freedom from disease. Preventive Veterinary Medicine, 34(1),1–17. [PubMed: 9541947]

Cannon MJ, Hyde TB, Schmid DS (2011). Review of cytomegalovirus shedding in bodily fluids and relevance to congenital cytomegalovirus infection. Review of Medical Virology, 21(4),240–55. 10.1002/rmv.695

Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, Moore PS (1994). Identification of Herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science, 266, 1865–1869. [PubMed: 7997879]

Coppola T, Mangold JF, Cantrell S, Permar SR (2019). Impact of maternal immunity on congenital cytomegalovirus birth prevalence and infant outcomes: a systematic review. Vaccines, 7(4):129. [PubMed: 31561584]

Deloose ST, Smit LA, Pals FT, Kersten MJ, van Noesel CJ, Pals ST (2005). High incidence of Kaposi sarcoma-associated herpesvirus infection in HIV-related solid immunoblastic/plasmablastic diffuse large B-cell lymphoma. Leukemia, 19(5),851–5. 10.1038/sj.leu.2403709 [PubMed: 15744337]

Demidenko E (2004). Mixed Models: Theory and Applications. Hoboken, NJ: Wiley.

Eberhardt MK, Deshpande A, Fike J, Short R, Schmidt KA, Blozis SA, Walter MR, Barry PA (2016). Exploitation of interleukin-10 (IL-10) signaling pathways: Alternate roles of viral and cellular IL-10 in rhesus cytomegalovirus infection. Journal of virology, 90(21):9920–9930. [PubMed: 27558431]

Eberle R (1992). Evidence for an alpha-herpesvirus indigenous to mountain gorillas. Journal of Medical Primatology, 21(5),246–51. [PubMed: 1328641]

Eberle R, Hilliard JK (1989). Serological evidence for variation in the incidence of herpesvirus infections in different species of apes. Journal of Clinical Microbiology, 27(6), 1357–66. [PubMed: 2546978]

Ehlers B, Spiess K, Leendertz F, Peeters M, Boesch C, Gatherer D, McGeoch DJ (2010). Lymphocryptovirus phylogeny and the origins of Epstein-Barr virus. Journal of General Virology 91(3), 630–42. 10.1099/vir.0.017251-0 [PubMed: 19923263]

Feeroz MM, Soliven K, Small CT, Engel GA, Andreina Pacheco M, Yee JL, Wang X, Kamrul Hasan M, Oh G, Levine KL, Rabiul Alam SM, Craig KL, Jackson DL, Lee EG, Barry PA, Lerche NW, Escalante AA, Matsen Iv FA, Linial ML, Jones-Engel L (2013). Population dynamics of rhesus macaques and associated foamy virus in Bangladesh. Emerging Microbes and Infection, 2(5):e29.

Fisher R (1935). The logic of inductive inference. Journal of the Royal Statistical Society Series A 98:39–54.

Gatherer D, Depledge DP, Hartley CA, Szpara ML, Vaz PK, Benkő M, Brandt CR, Bryant NA, Dastjerdi A, Doszpoly A, Gompels UA, Inoue N, Jarosinski KW, Kaul R, Lacoste V, Norberg P, Origgi FC, Orton RJ, Pellett PE, Schmid DS, Spatz SJ, Stewart JP, Trimpert J, Waltzek TB, Davison AJ (2021). ICTV Virus Taxonomy Profile: Herpesviridae 2021. Journal of General Virology, 102(10):001673. [PubMed: 34704922]

Gilardi KV, Oxford KL, Gardner-Roberts D, Kinani JF, Spelman L, Barry PA, Cranfield MR, Lowenstine LJ (2014). Human herpes simplex virus type 1 in confiscated gorilla. Emerging Infectious Diseases, 20(11). 1883–6. 10.3201/eid2011.140075 [PubMed: 25341185]

Goodall J. (1983). Population dynamics during a fifteen-year period in one community of free-living chimpanzees in the Gombe National Park, Tanzania. Zeitschrift fur Tierpsychologie 61(1),1–60.

Goodall J (1986). The Chimpanzees of Gombe: Patterns of Behavior. Cambridge, Massachusetts: Harvard University Press.

Granjon AC, Robbins MM, Arinaitwe J, Cranfield MR, Eckardt W, Mburanumwe I, Musana A, Robbins AM, Roy J, Sollmann R, Vigilant L, Hickey JR (2020). Estimating abundance and growth rates in a wild mountain gorilla population. Animal Conservation, 23. 455–465.

Hammond JA, Pomeroy PP, Hall AJ, Smith VJ (2005). Identification and real-time PCR quantification of Phocine distemper virus from two colonies of Scottish grey seals in 2002. Journal of General Virology, 86(9). 2563–7. 10.1099/vir.0.80962-0 [PubMed: 16099915]

Hastings B, Kenny D, Lowenstine L, Foster J (1991). Mountain gorillas and measles: ontogeny of a wildlife vaccination program. American Association of Zoo Veterinarians, p198–205.

Heldstab A, Ruedi D, Sonnabend W, Deinhardt F (1981). Spontaneous generalized herpesvirus hominis infection of a lowland gorilla (Gorilla gorilla gorilla). Journal of Medical Primatology, 10,129–135. [PubMed: 6279853]

Hickey J, Uzabaho E, Akantorana M, Arinaitwe J, Bakebwa I, Bitariho R, Eckardt W, Gilardi K, Katutu J, Kayijamahe C et al. 2019b. Bwindi-Sarambwe 2018 Surveys: monitoring mountain gorillas, other select mammals, and human activities. Kampala, Uganda: GVTC, IGCP and partners.

Kaufman HE, Azcuy AM, Varnell ED, Sloop GD, Thompson HW,, Hill J,M 2005. HSV-1 DNA in tears and saliva of normal adults. Investigative Ophthalmology and Visual Science, 46(1),241–7. 10.1167/iovs.04-0614 [PubMed: 15623779]

Kaur T, Singh J, Tong S, Humphrey C, Clevenger D, Tan W, Szekely B, Wang Y, Li Y, Muse EA, Kiyono M, Hanamura S, Inoue E, Nakamura S, Huffman MA, Jiang B, Nishida T (2008). Descriptive epidemiology of fatal respiratory outbreaks and detection of a human-related metapneumovirus in wild chimpanzees (Pan troglodytes) at Mahale Mountains National Park, Western Tanzania. American Journal of Primatology, 70,755–765. [PubMed: 18548512]

Kik MJ, Bos JH, Groen J, Dorrestein GM (2005). Herpes simplex infection in a juvenile orangutan (Pongo pygmaeus pygmaeus). Journal of Zoo and Wildlife Medicine, 36(1),131–4. 10.1638/03-004 [PubMed: 17315472]

Kondgen S, Kuhl H, N'Goran PK, Walsh PD, Schenk S, Ernst N, Biek R, Formenty P, Matz-Rensing K, Schweiger B, Junglen S, Ellerbrok H Nitsche A, Briese T, Lipkin WI, Pauli G, Boesch C, Leendertz FH (2008). Pandemic human viruses cause decline of endangered great apes. Current Biology, 18(4):260–4. 10.1016/j.cub.2008.01.012 [PubMed: 18222690]

Kondgen S, Schenk S, Pauli G, Boesch C, Leendertz FH (2010). Noninvasive monitoring of respiratory viruses in wild chimpanzees. EcoHealth, 7(3):332–41. 10.1007/s10393-010-0340-z [PubMed: 20865440]

Leendertz FH, Deckers M, Schempp W, Lankester F, Boesch C, Mugisha L, Dolan A, Gatherer D, McGeoch DJ, Ehlers B (2009). Novel cytomegaloviruses in free-ranging and captive great apes: phylogenetic evidence for bidirectional horizontal transmission. Journal of General Virology, 90(10),2386–94. 10.1099/vir.0.011866-0 [PubMed: 19553394]

Leendertz FH, Pauli G, Maetz-Rensing K, Boardman W, Nunn C, Ellerbrok H, Jensen SA, Junglen S, Boesch C (2006). Pathogens as drivers of population declines: The importance of systematic monitoring in great apes and other threatened mammals. Biological Conservation, 131,325–337.

Loomis MR, O'Neill T, Bush M, Montali RJ (1981). Fatal herpesvirus infection in patas monkeys and a black and white colobus monkey. Journal of the Americal Veterinary Medical Association, 179(11),1236–9.

Martin JN, Ganem DE, Osmond DH, Page-Shafer KA, Macrae D, Kedes DH (1998). Sexual transmission and the natural history of human herpesvirus 8 infection. New England Journal of Medicine, 338(14),948–54. [PubMed: 9521982]

Masters N, Niphuis H, Verschoor E, Breuer J, Quinlivan M, Wawrzynczyk T, Stidworthy M (2010). Debilitating clinical disease in a wild-born captive western lowland gorilla (Gorilla gorilla gorilla) co-infected with varicella zoster virus (VZV) and simian T-lymphotropic virus (STLV). Journal of Zoo and Wildlife Medicine, 41(4),713–6. 10.1638/2009-0122.1 [PubMed: 21370655]

Matz-Rensing K, Jentsch KD, Rensing S, Langenhuyzen S, Verschoor E, Niphuis H, Kaup FJ (2003). Fatal Herpes simplex infection in a group of common marmosets (Callithrix jacchus). Veterinary Pathology, 40(4),405–11. 10.1354/vp.40-4-405 [PubMed: 12824512]

Mayama S, Cuevas LE, Sheldon J, Omar OH, Smith DH, Okong P, Silvel B, Hart CA, Schulz TF (1998). Prevalence and transmission of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) in Ugandan children and adolescents. International Journal of Cancer, 77(6),817–20. [PubMed: 9714046]

Mazet JAK, Genovese BN, Harris LA, Cranfield M, Noheri JB, Kinani JF, Zimmerman D, Bahizi M, Mudakikwa A, Goldstein T Gilard KV (2020). Human Respiratory Syncytial Virus Detected in Mountain Gorilla Respiratory Outbreaks. Ecohealth, 17(4):449–460. [PubMed: 33345293]

Mbulaiteye S, Marshall V, Bagni RK, Wang CD, Mbisa G, Bakaki PM, Owor AM, Ndugwa CM, Engels EA, Katongole-Mbidde, Biggar RJ, Whitby DE (2006). Molecular evidence for mother-to-child transmission of Kaposi sarcoma-associated herpesvirus in Uganda and K1 gene evolution within the host. Journal of Infectious Diseases, 193(9),1250–1257. [PubMed: 16586362]

McClure HM, Swenson RB, Kalter SS, Lester TL (1980). Natural genital herpesvirus hominis infection in chimpanzees (Pan troglodytes and Pan paniscus). Laboratory Animal Science, 30(5),895–901. [PubMed: 6253747]

McNemar Q (1947). Note on the sampling error of the difference between correlated proportions or percentages. Psychometrika, 12(2),153–157. [PubMed: 20254758]

Melendez LV, Espana C, Hunt RD, Daniel MD, Garcia FG (1969). Natural herpes simplex infection in the owl monkey (Aotus trivirgatus). Laboratory Animal Care, 19(1),38–45. [PubMed: 4304237]

Miller CS, Danaher RJ (2008). Asymptomatic shedding of herpes simplex virus (HSV) in the oral cavity. Oral Surgery, Oral Medicine, Oral Pathology, and Oral Radiology, 105(1),43–50. 10.1016/j.tripleo.2007.06.011

Monavari SH, Vaziri MS, Khalili M, Shamsi-Shahrabadi M, Keyvani H, Mollaei H, Fazlalipour M (2013). Asymptomatic seminal infection of herpes simplex virus: impact on male infertility. Journal of Biomedical Research, 27(1),56–61. [PubMed: 23554795]

Myers MG, Kramer LW, Stanberry LR (1987). Varicella in a gorilla. Journal of Medical Virology, 23(4):317–22. [PubMed: 2826674]

Nador RG, Cesarman E, Chadburn A, Dawson DB, Ansari MQ, Sald J, Knowles DM (1996). Primary effusion lymphoma: a distinct clinicopathologic entity associated with the Kaposi's sarcoma-associated herpes virus. Blood, 88(2):645–56. [PubMed: 8695812]

Negrey JD, Reddy RB, Scully EJ, Phillips-Garcia S, Owens LA, Laangergraber KE, Mitani JC, Thompson ME, Wrangham RW, Muller MN, Otali E, Machanda Z, Heroba D, Grindle KA, Pappas TE, Palmenberg AC, Gern JE, Goldberg TL (2019). Simultaneous outbreaks of respiratory disease in wild chimpanzees caused by distinct viruses of human origin. Emerging Microbes & Infections, 8(1):139–149. [PubMed: 30866768]

Oxford KL, Dela Pena-Ponce MGA, Jensen K, Eberhardt MK, Spinner A, Van Rompay KK, Rigdon J, Mollan KR, Krishnan VV, Hudgens MG, Barry PA, De Paris K (2015). The interplay between immune maturation, age, chronic viral infection and environment. Immunity and Aging, 9(12):3.

Palacios G, Lowenstine LJ, Cranfield MR, Gilardi KV, Spelman L, Lukasik-Braum M, Kinani JF, Mudakikwa A, Nyirakaragire E, Bussetti AV, Savyi N, Hutchison S, Egholm M, Lipkin WI (2011). Human metapneumovirus infection in wild mountain gorillas, Rwanda. Emerging Infectious Diseases, 17(4):711–3. 10.3201/eid1704.100883 [PubMed: 21470468]

Patrono LV, Samuni L, Corman VM, Nourifar L, Rothemeier C, Wittig RM, Drosten C, Calvignac-Spencer S, Leendertz FH (2018). Human coronavirus OC43 outbreak in wild chimpanzees, Cot d'Ivoire, 2016. Emerging Microbes & Infections, 7:118. [PubMed: 29950583]

Patrono LV, Rothemeier C, Kouadio L, Couacy-Hymann E, Wittig RM, Calvignac-Spencer S, Leendertz FH (2022). Non-invasive genomics of respiratory pathogens infecting wild great apes using hybridization capture. Influenza and Other Respiratory Viruses, 10.1111/irv.12984.

Pearson BL, Judge PG, Reeder DM (2008). Effectiveness of saliva collection and enzyme-immunoassay for the quantification of cortisol in socially housed baboons. American Journal of Primatology, 70(12):1145–51. 10.1002/ajp.20613 [PubMed: 18785637]

Prepens S, Kreuzer KA, Leendertz F, Nitsche A, Ehlers B (2007). Discovery of herpesviruses in multi-infected primates using locked nucleic acids (LNA) and a bigenic PCR approach. Virology Journal, 4. 10.1186/1743-422x-4-84

Ramsay E, Stair EL, Castro AE, Marks MI (1982). Fatal Herpesvirus hominis encephalitis in a white-handed gibbon. Journal of the American Veterinary Medical Association, 181(11),1429–30. [PubMed: 6294039]

Robbins MM, Gray M, Kagoda E, Robbins AM (2009). Population dynamics of the Bwindi mountain gorillas. Biological Conservation, 142:2886–2895.

Rwego IB, Isabirye-Basuta B, Gillespie TR, Goldberg TL (2008). Gastrointestinal bacterial transmission among humans, mountain gorillas, and livestock in Bwindi Impenetrable National Park, Uganda. Conservation Biology, 22(6),1600–7. [PubMed: 18717695]

Sandbrook C, Semple S (2006). The rules and the reality of mountain gorilla (Gorilla beringei beringei) tracking: how close do tourists get? Oryx, 40(4),428–433.

Schleiss MR (2006). Role of breast milk in acquisition of cytomegalovirus infection: recent advances. Current Opinion in Pediatrics, 18(1),48–52. [PubMed: 16470162]

Schrenzel MD, Osborn KG, Shima A, Klieforth RB, Maalouf GA (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(1),7–14. [PubMed: 12733597]

Sergeant E (2016). Epitools epidemiological calculators. AusVet Animal Health Services and Australian Biosecurity Cooperative Research Centre for Emerging Infectious Disease.

Smiley Evans TS, Gilardi K, Barry P, Ssebide B, Kinani JF, Nizeyimana F, Noheri JB, Byarugaba D, Mudkikiwa A, Cranfield M Mazet J, Johnson C (2016). Detection of viruses using discarded plants from wild mountain gorillas and golden monkeys. American Journal of Primatology, 78(11),1222–1234. 10.1002/ajp.22576 [PubMed: 27331804]

Smiley Evans T, Lowenstine L, Gilardi K, Barry P, Ssebide B, Kinani JF, Nizeyimana F, Noheri JB, Cranfield M, Mudakikwa A, Goldsten T, Mazet J, Johnson C (2017). Mountain gorilla lymphocryptovirus has Epstein-Barr Virus-like epidemiology and pathology in infants. Scientific Reports, 7, e5352. 10.1038/s41598-017-04877-1

Smith PC, Yuill TM, Buchanan RD, Stanton JS, Chaicumpa V (1969). The gibbon (Hylobates lar); a new primate host for herpesvirus hominis 1. A natural epizootic in a laboratory colony. Journal of Infectious Disease, 120:292–297.

Soulier J, Grollet L, Oksenhendler E, Cacoub P, Cazals-Hatem D, Babinet P, d'Agay MF, Clauvel JP, Raphael M, Degos L (1995). Kaposi's sarcoma-associated herpesvirus-like DNA sequences in multicentric Castleman's disease. Blood, 86(4):1276–80. [PubMed: 7632932]

Stagno S (1990). Animal cytomegaloviruses In: Remington JS, Klein JP, editors. Infectious Diseases of the Fetus and Newborn Infant. Philadelphia: W.B. Saunders. p 241–281.

StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP.

Tarantal AF, Salamat MS, Britt WJ, Luciw PA, Hendrickx AG, Barry PA (1998). Neuropathogenesis induced by rhesus cytomegalovirus in fetal rhesus monkeys (Macaca mulatta). Journal of Infectious Diseases, 177(2),446–50. [PubMed: 9466534]

Voevodin AF, Preston A, Marx J (2009a). Cytomegaloviruses. In: Voevodin AF, Preston A. Marx J, editors. Simian Virology Ames, Iowa: Blackwell Publishing

Voevodin AF, Preston A Marx J (2009b). Simian Herpesviruses. Simian Virology. Ames, Iowa: John Wildey & Sons

Vogel P, Weigler BJ, Kerr H, Hendrickx AG, Barry PA (1994). Seroepidemiologic studies of cytomegalovirus infection in a breeding population of rhesus macaques. Laboratory Animal Science, 44(1):25–30. [PubMed: 8007656]

Wallis J, Lee DR (1999). Primate Conservation: The Prevention of Disease Transmission. International Journal of Primatology, 20(6),803–826.

Whitley RJ, Nahmias AJ, Visintine AM, Fleming CL, Alford CA (1980). The natural history of herpes simplex virus infection of mother and newborn. Pediatrics, 66(4),489–94. [PubMed: 6253866]

Whittier C, Nutter F, Cranfield M (2005a). Seroprevalence of infectious agents in free-living mountain gorillas (Gorilla beringei ssp). Wildlife Disease Association.

Whittier C, Nutter F, Lowenstine L, Cranfield M (2005b). An outbreak of apparent poxvirus infection in two groups of mountain gorillas (Gorilla beringei beringei). Wildlife Disease Association Australia, p 202.

Williamson EA, Fawcett KA (2008). Long-term research and conservation of the Virunga mountain gorillas. In: Wrangham R, Ross E, editors. Science and Conservation in African Forests The Benefits of Longterm Research Cambridge University Press. p 213–229.

Williamson EA, Gerald-Steklis N (2001). Composition of gorilla gorilla beringei groups monitored by Karisoke Research Center, 2001. African Primates, 5(1&2),48–51.

Wong SW, Bergquam EP, Swanson RM, Lee FW, Shiigi SM, Avery NA, Fanton JW, Axthelm MK (1999). Induction of B cell hyperplasia in simian immunodeficiency virus-infected rhesus macaques with the simian homologue of Kaposi's sarcoma-associated herpesvirus. Journal of Experimental Medicine, 190(6),827–40. [PubMed: 10499921]

Yue Y, Barry PA (2008). Rhesus cytomegalovirus a nonhuman primate model for the study of human cytomegalovirus. Advanced Virus Research, 72,207–26.

Zuhair M, Smit GSA, Wallis G, Jabbar F, Smith C, Devleesschauwer B, Griffiths P (2019). Estimation of the worldwide seroprevalence of cytomegalovirus: A systematic review and meta-analysis. Reviews in Medical Virology, 29(3):e2034. [PubMed: 30706584]

Research Highlights

- Discovery of mountain gorilla-specific homologs to human herpesviruses, including cytomegaloviruses, lymphocryptovirus and a rhadinovirus with similar characteristics to their human counterparts.
- Human herpes simplex virus 1 (HSV-1) has not entered the wild mountain gorilla population despite frequent contact with humans.
- Consideration of reintroduction of captive mountain gorillas should carefully consider the potential of introducing HSV-1 and other human pathogens to the wild population.

Figure 1.
Suspected viral lesions with potential herpetic etiology prompting investigation of infectious cause in free-ranging mountain gorilla populations (Photo credit: Gorilla Doctors).

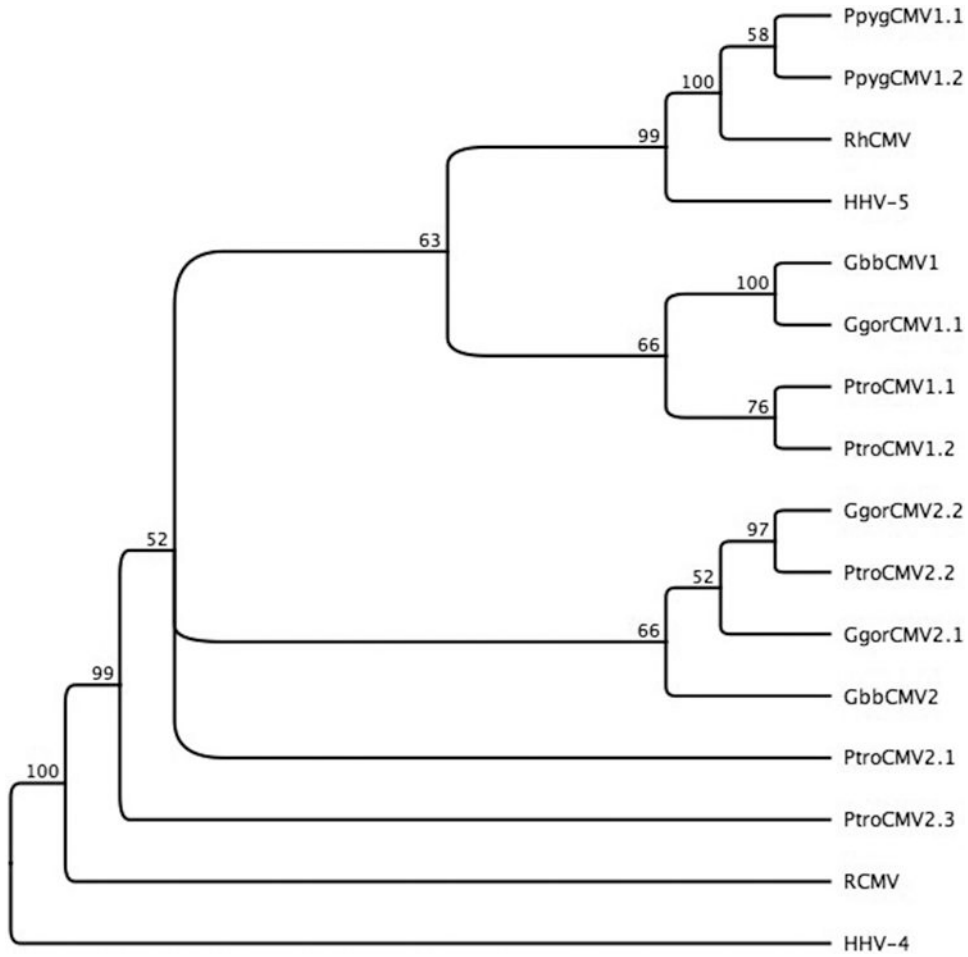

Figure 2.

Phylogenetic tree for primate CMVs based on partial UL55 (gB) sequences. The tree was derived from a 605 bp alignment using Neighbor-joining in Genious v.7.0.6 with outgroup provided by EBV (Human gammaherpesvirus 4, HHV-4). Numbers at each node indicate the percentage of bootstrap samples (out of 100) in which the cluster to the right is supported. Sequences published in Genbank used to construct this tree included: *Gorilla beringei beringei cytomegalovirus 1* (GbbCMV1, KX839482), *Gorilla beringei beringei cytomegalovirus 2* (GbbCMV2, KX839483), *Gorilla gorilla cytomegalovirus 1.1* (GgorCMV-1.1, FJ538492), *Gorilla gorilla cytomegalovirus 2.1* (GgorCMV-2.1, FJ538490), *Gorilla gorilla cytomegalovirus 2.2* (GgorCMV-2.2, FJ538491), *Pan troglodytes cytomegalovirus 1.1* (PtroCMV-1.1, FJ38485), *Pan troglodytes cytomegalovirus 2.1* (PtroCMV-2.1, FJ538487), *Pan troglodytes cytomegalovirus 2.2* (PtroCMV-2.2, FJ538488), *Pan troglodytes cytomegalovirus 2.3* (PtroCMV-2.3, FJ538489), *Chimpanzee cytomegalovirus (Panine herpesvirus 5)* (CCMV, NC_003521), *Human herpesvirus 5* (HHV-5-Merlin, AY446894), *Pongo pygmaeus cytomegalovirus 1.1* (PpygCMV-1.1, AY129396), *Pongo pygmaeus cytomegalovirus 1.2* (PpygCMV-1.2, FJ538493), *Rhesus cytomegalvoirus (cercopithecine herpesvirus 8)* (RhCMV, AY186194), *Rat cytomegalovirus* (RCMV, AF232689), *Human herpesvirus 4* (HHV-4, LN827558).

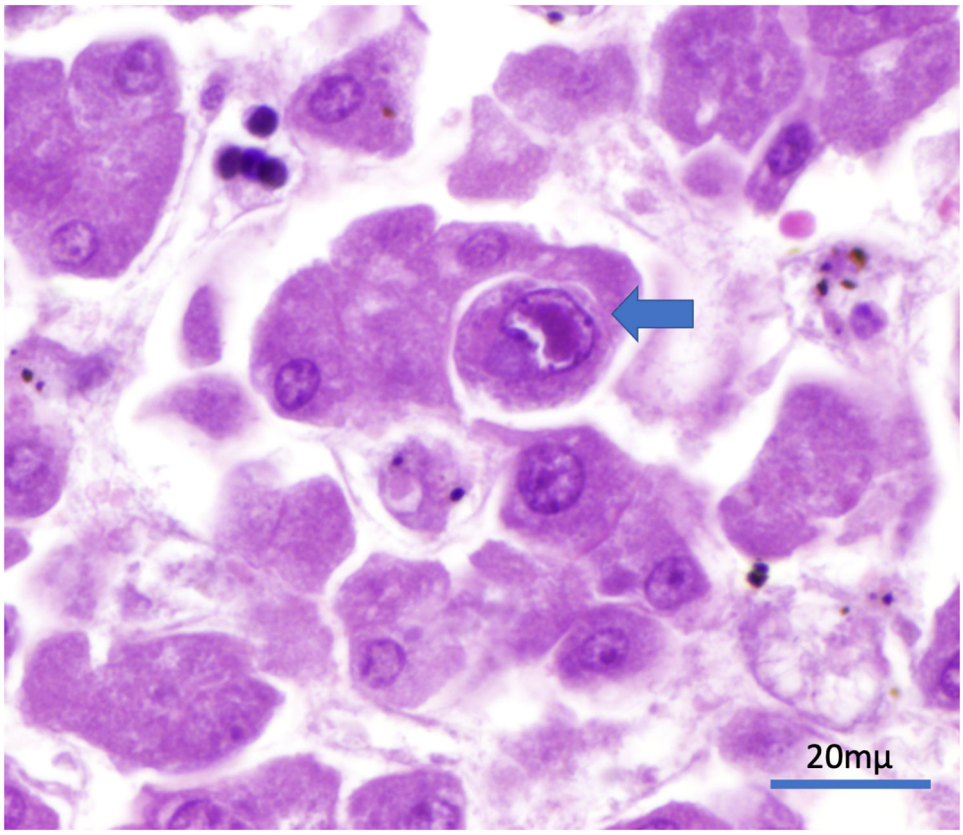

Figure 3.
Liver from an infant mountain gorilla that died from septicemia secondary to gangrenous necrosis of the left leg. An enlarged (cytomegalic) hepatocyte (arrow) with enlarged nucleus (karyomegaly) containing an intranuclear inclusion body and a small cytoplasmic inclusion body all of which are characteristic of cytomegalovirus infection.

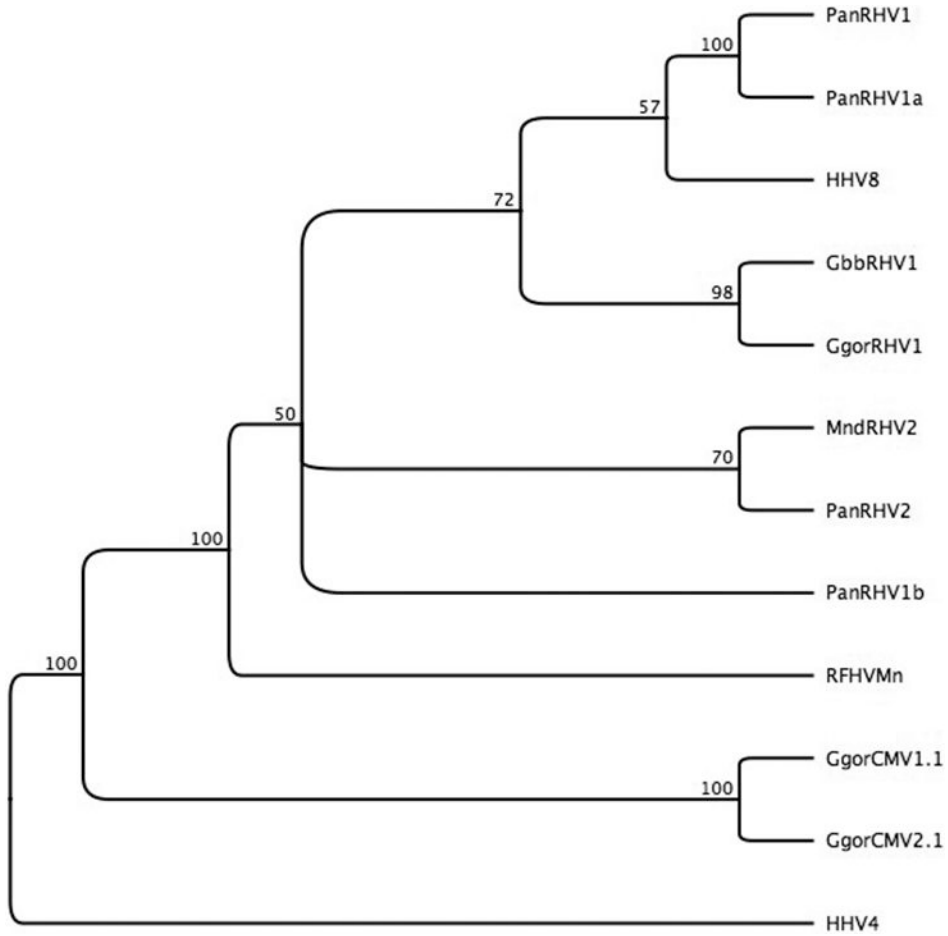

Figure 4.

Phylogenetic tree of primate rhadinoviruses based on partial DNA polymerase (DPOL) sequences. The tree was derived from a 510 bp alignment using Neighbor-joining in Genious v.7.0.6 with outgroup provided by EBV (Human gammaherpesvirus 4, HHV-4). Numbers at each node indicate the percentage of bootstrap samples (out of 100) in which the cluster to the right is supported. Sequences published in Genbank used to construct this tree included: *Gorilla beringei beringei rhadinovirus 1* (GbbRV1, sequence submitted), *Gorilla gorilla cytomegalovirus 1.1* (GgorCMV-1.1, FJ538492), *Gorilla gorilla cytomegalovirus 2.1* (GgorCMV-2.1, FJ538490), *Human herpesvirus 4* (HHV-4, LN827558), *Retroperitoneal fibromatosis-associated herpesvirus Macaca nemestrina* (RFHVMn, AF005478), *Chimpanzee rhadinovirus 1b* (PanRHV1b, AF250881), *Gorilla gorilla rhadinovirus 1* (GgorRHV-1, AY177144), *Kaposi sarcoma associated herpesvirus* (HHV-8, AF148805), *Chimpanzee rhadinovirus 1* (PanRHV1, AY138585), *Chimpanzee rhadinovirus 1a* (PanRHV1a, AF250879), *Mandrill rhadinovirus 2 (*MndRHV2, AF282937), *Chimpanzee rhadinovirus 2* (PanRHV2, AF246488).

Table 1.

Herpesviruses detected from mountain gorilla (*Gorilla beringei beringei*) chewed plant samples in the Bwindi Impenetrable Forest and Virunga Conservation Region between November 2012 and June 2013.

Herpesvirus subfamily	Genus	Virus	Prevalence	95 %Binomial Exact CI
Beta herpesvirinae	*Cytomegalovirus*	*Gorilla beringei beringei strain of cytomegalovirus 1 (GbbCMV-1)*	18 / 332 (0.05)	0.03 - 0.08
		Gorilla beringei beringei strain of cytomegalovirus 2 (GbbCMV-2)	24 / 332 (0.07)	0.04 – 0.10
Gamma herpesvirinae	*Lymphocryptovirus*	*Gorilla beringei beringei strain of lymphocryptovirus 1 (GbbLCV-1)*	139 / 332 (0.42)	0.37 – 0.47
	Rhadinovirus	*Gorilla beringei beringei strain of rhadinovirus 1 (GbbRHV-1)*	6 / 332 (0.02)	0.01 – 0.04

Table 2.

Mountain gorillas (*Gorilla beringei beringei*) from the Bwindi Impenetrable Forest and Virunga Conservation Region orally shedding GbbCMV between November 2012 and June 2013 summarized by age and sex.

Age Group†	Sex	Period Prevalence	95% Binomial Exact CI
Infant	Male	0.35	0.15 - 0.59
	Female	0.13	0.02 - 0.38
	Unknown	0.00	0.00 - 0.26
Juvenile	Male	0.21	0.09 - 0.39
	Female	0.08	0.00 - 0.38
	Unknown	0.18	0.02 - 0.52
Subadult	Male	0.27	0.06 - 0.61
	Female	0.00	0.00 - 0.52
Adult	Male	0.05	0.02 - 0.12
	Female	0.11	0.06 - 0.19

†Infants were defined as gorillas less than three years of age, juveniles as three to five, subadults as six to seven, adult females as eight years and older, black backs as eight to 11 years, and silverbacks as 12 years and older [Robbins et al., 2009; Williamson and Gerald-Steklis, 2001].