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Human Herpesvirus 1 in a Saki Monkey

Abstract

A 20-year-old male intact white-faced saki monkey (*Pithecia pithecia*) presented with acute ocular disease of the right eye. Clinical signs included periocular swelling, conjunctivitis, and anisocoria with a miotic right pupil. Conjunctival swabs were positive for *Human herpesvirus 1* (HHV1) by PCR with sequencing. Initial clinical signs resolved with supportive treatment, and the animal was managed chronically with intermittent acyclovir (5 mg/kg PO twice daily) during flare-ups.

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After more than 2 years, progression of clinical disease led to enucleation of the right eye. Two months later, an acute presentation of severe neurologic signs, including ataxia and blindness, resulted in euthanasia. Histopathology, PCR, and sequencing results were consistent with viral encephalitis from HHV1, with coinfection with Pithecia pithecia lymphocryptovirus 1 also identified. This is the first case of managed HHV1 infection in a platyrrhine primate and the first case of HHV1 in a white-faced saki monkey that was not rapidly fatal.

Abbreviations and acronyms:

Human herpesvirus 1 (HHV1)

Introduction

Human herpesvirus 1 (HHV1) is an alphaherpesvirus in the genus ~~*Simplexirus*~~*Simplexvirus* with high pathogenicity in non-human primates and other Euarchontoglires. In the endemic host, humans (*Homo sapiens*), HHV1 infection is lifelong and usually causes subclinical infection, with a prevalence over 98% in normal adults [~~13~~14]. However, cold sores may occur in some individuals, and severe fatal encephalitis is possible in otherwise healthy neonates [~~18~~19]. The virus host range is widespread across the Euarchontoglires, the mammalian superorder containing primates, rabbits, and rodents [~~21~~22]. A diverse array of nonhuman Euarchontoglires are susceptible to systemic HHV1 disease (typically encephalitis), especially in the case of young or immunocompromised animals [~~11, 14~~12, 15, 16, ~~29, 30~~17, 31, 32]. The parvorder Catarrhini~~,~~ ~~containing~~ contains humans~~, are often large~~ and ~~aggressive, and humans are unlikely to~~ ~~deliberately come into direct contact with~~ other ~~catarrhines~~primates native to the old

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world. The other clade in the infraorder Simiiformes, the parvorder Platyrrhini, contains the primates native to the new world. Platyrrhini are heavily represented in the pet trade and in literature regarding nonhuman HHV1 disease. Rapidly fatal disease associated with acute encephalitis has been reported in black howler monkeys (*Alouatta caraya*), owl monkeys (*Aotus* spp.), marmosets (*Callithrix* spp.), and white-faced saki monkeys (*Pithecia pithecia*) [2, 9, 17, 19, 22 10, 18, 20, 24]. However, some reports of HHV1 in platyrrhines have indicated differences in susceptibility among species and individuals [7, 27 8, 29]. Experimental corneal infection with HHV1 in capuchin monkeys (*Cebus apella*) and squirrel monkeys (*Saimiri sciureus*) produced corneal disease in both species, but subsequent systemic disease was not reported [7, 27 8, 29]. Interestingly, capuchin monkeys have also been reported to be asymptomatically infected with *Macacine herpesvirus 1*, another *Simplexvirus* endemic in a catarrhine host, the rhesus macaque (*Macaca mulatta*) [5 6]. In a naturally occurring outbreak of HHV1 in owl monkeys, some monkeys developed rapidly fatal encephalitis while others showed only mild signs limited to dyscoria and posterior synechia [9 10]. All monkeys that did not die in the outbreak were euthanized, so the long-term outcome was not determined [9 10]. Longer term management of a case of HHV1 disease in a platyrrhine has not been reported.

Case Report

A 20-year-old male, intact, captive white-faced saki monkey (*Pithecia pithecia*) with an acute history of right periocular swelling and lethargy was anesthetized for a complete physical examination. This animal's prior medical history included chronic proteinuria and diarrhea, which were managed with treatments of benazepril (0.5 mg/kg PO daily, Diamondback Drugs, Scottsdale, AZ), flax seed oil (50 mg/kg PO daily,

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Puritan's Pride, Holbrook, NY), and metronidazole (5 mg/kg PO daily, Wedgewood

Village Pharmacy LLC, Swedesboro, NY).

On initial physical examination under anesthesia, findings included right-sided

periocular lid swelling, conjunctival hyperemia and edema, mucoid discharge, corneal

edema, a 3 mm × 2 mm central corneal ulcer, and anisocoria with a miotic right pupil.

Oral examination revealed erythematous gingiva and two thick, off-white plaques on the

tip of the tongue. A low body condition score (3/9) and 15% weight loss from the

animal's typical weight 19 days prior was noted.

Complete blood count, serum biochemistry, and urine analysis revealed

leukocytosis (19.8 k/µl; reference range, 3.7–19.11 k/µl), neutrophilia (13.9 k/µl;

reference range, 1.5–11.96 k/µl), monocytosis (4.4 k/µl, reference range 0.053–1.211

k/µl), hypoalbuminemia (2.2 g/dL, reference range, 2.4–5.9 g/dL), proteinuria (4+

protein, UPC 9.97), hematuria (0–2 RBC/hpf), pyuria (1–3 WBC/hpf), and mild

bacteriuria [25]-[27]. Liver enzymes including ALT (3 U/L, reference range 3–37 U/L), AST

(45 U/L, reference range 20-123 U/L), and GGT (31 U/L, reference range 7-61 U/L)

were unremarkable [27]. However, total bilirubin was not included in the chemistry panel.

A urine culture was negative. Whole body radiographs, abdominal ultrasound, cytology

of a scraping of the tongue, and lead II electrocardiography were unremarkable.

Treatment administered at the time of the examination included lactated Ringer's solution

(45 ml/kg SC, Hospira, Lake Forest, IL), penicillin G benzathine and penicillin G

procaine (PPG, 22,000 IU/kg SC, Bimeda, La Sueur, MN), ivermectin (0.4 mg/kg SC,

Merial Limited, Duluth GA), meloxicam (0.2 mg/kg SC, Norbrook Laboratories Limited,

Northern Ireland), ceftiofur crystalline free acid (10 mg/kg SC, Zoetis, Kalamazoo, MI),

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and neomycin, polymyxin B sulfates, and bacitracin zinc ophthalmic ointment (topically on right eye, Bausch & Lomb, Tampa FL). Treatment courses of tramadol (1.2 mg/kg PO twice daily for 9 days, Diamondback Drugs) and azithromycin (10 mg/kg PO daily for 7 days, Greenstone LLC, Peapack, NJ) were initiated, and ciprofloxacin (10 mg/kg PO twice daily for 24 days, Bayer HealthCare, Whippany, NJ) was initiated 5 days later.

A recheck examination under anesthesia 10 days after the initial presentation revealed persistent ocular abnormalities, including a 3 mm × 5 mm corneal ulcer of the right eye. Mucoid discharge was also present from the left eye. A complete temporary tarsorrhaphy of the right eye to promote corneal healing was performed using 5-0 PDS in a horizontal mattress pattern. In addition, exudative abrasions and ulcerations were noted on the right arm and the face above each eye, on the chin, and on the right cheek. Cytological evaluation of impression smears of the affected areas revealed severe neutrophilic and histiocytic inflammation. Treatments during the examination included penicillin G benzathine and penicillin G procaine (22,000 IU/kg SC), meloxicam (0.2 mg/kg SC), gentamicin (0.05 ml, 100 mg/ml, subconjunctival right eye, VetOne, Boise, ID), lactated Ringer's solution (30 ml/kg SC), and nystatin-neomycin sulfate-thiostrepton-triamcinolone ointment (Animax, topically on right wrist, Dechra, Overland Park, KS), and the scabs and wounds were clipped and cleaned with chlorhexidine solution (Vétoquinol USA, Fort Worth, TX). Due to concern about an underlying viral infection, serum was submitted for serology to VRL Laboratories (San Antonio, TX); the results were negative for ~~cytomegalovirus~~*Saimiriine herpesvirus 4*, *Saimiriine herpesvirus 2*, *Saimiriine herpesvirus 1*, HHV1, *Human herpesvirus 2*, HHV1 IgM, *Human herpesvirus 2* IgM, and Measles, determined by previously reported methods [~~10~~11].

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A swab collected from the corneal and subconjunctival surfaces of the right eye was

positive for HHV1 by PCR with sequencing at the University of Tennessee utilizing

previously reported methods [2628]. Treatment courses of Animax (topically on right

carpus, twice daily for 5 days), cephalexin (22 mg/kg PO twice daily for 17 days,

Hospira), meloxicam (0.1 mg/kg PO once daily for 10 days), and acyclovir (5 mg/kg PO

twice daily for 24 days, Diamondback Drugs) were initiated. Additional treatments at a

recheck examination 8 days later included removal of the temporary tarsorrhaphy sutures,

gentamicin (0.1 ml subconjunctival in right eye with 0.05 ml in lower lid and 0.05 ml in

upper lid), meloxicam (0.2 mg/kg IM), ceftiofur sodium crystalline free acid (7.5 mg/kg

SC), and lactated Ringer's solution (30 ml, kg SC). Additional medications included l-

lysine (12.5 mg/kg PO daily, Carlson, Arlington Heights, IL) and continued acyclovir,

cephalexin, ciprofloxacin, and meloxicam.

A recheck examination 25 days after the initial presentation showed no evidence

of corneal ulceration. At that time, the right eye was considered non-visual, and there was

evidence of mild corneal degeneration, a narrow anterior chamber, and an active

pupillary light response. Oral administration of l-lysine was continued. The skin lesions

had also resolved at that time.

There were no additional concerns until 6 months after the initial presentation

when a recheck exam showed moderate conjunctival hyperemia of the left eye. A swab of

this eye was positive for HHV1 on PCR, indicating viral shedding at this time. Acyclovir

treatment was then reinitiated. Three additional flare-ups of periocular pruritus and

conjunctivitis were treated with courses of acyclovir over the 2 years and 3 months

following the initial presentation.

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Approximately 2 years and 4 months after initial presentation, enucleation of the

right eye was performed due to concerns of recurring ocular disease. L-lysine was

discontinued approximately 1 week later, because of removal of the affected eye and

worsening patient compliance. Histology of the affected eye revealed chronic, erosive

keratoconjunctivitis; mild to moderate, chronic, lymphohistiocytic iridocyclitis with

multiple synechia; and chronic retinal degeneration and detachment. No viral inclusions

were found.

Two months later, this animal became acutely ataxic, obtunded, and blind.

Euthanasia was elected owing to suspected progression of HHV1 associated disease.

Histopathology confirmed disseminated disease consistent with herpesvirus, including

severe, multifocal to coalescing necrohemorrhagic meningoencephalitis with neuronal

intranuclear inclusion bodies (Figure 1, Figure 2). The optic and trigeminal nerves and

the left eye were infiltrated by lymphocytes and plasma cells. Other findings included

mild lymphohistiocytic hepatitis, mild lymphoplasmacytic interstitial nephritis, mild

lymphocytic gastroenteritis, moderate mucopurulent to lymphocytic laryngitis, mild

lymphocytic conjunctivitis of the left eye, and granulomatous inflammation of the

enucleation site of the right eye.

Brain tissue was submitted to the University of Florida for confirmation of HHV1

via PCR and sequencing. DNA was extracted from brain tissue (DNeasy, Qiagen,

Valencia, CA). Blank extractions were used as negative controls. A nested PCR

amplification was performed using previously described consensus primers for the

herpesviral DNA-dependent DNA polymerase gene [2628]. The PCR amplicon was resolved

in a 1.5% agarose gel, excised, and purified using a QIAquick gel extraction kit (Qiagen),

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then sequenced via the Sanger method, using a Big-Dye™ Terminator Kit (Applied

Biosystems, Foster City, CA) and analyzed on ABI 3130 automated DNA sequencers.

Sequencing completed at Genewiz (South Plainfield, NJ) resulted in dual peaks at most

nucleotides, consistent with mixed sequence (Figure 3). The sequence obtained was

compared with sequences in the databases of GenBank (National Center for

Biotechnology Information, Bethesda, Maryland, USA), EMBL (Cambridge, UK), and

the Data Bank of Japan (Mishima, Shizuoka, Japan) using BLASTN [1]. The BLASTN

search found closest sequence homology with HHV1 (GenBank accession # KX424525).

When peaks matching HHV1 were subtracted, the sequence of the remaining peaks

showed 100% homology with Pithecia pithecia lymphocryptovirus 1 (PpLCV1; GenBank

accession # AY139025), previously reported from the lung of a captive white-faced saki

monkey in Germany [67]. Use of fluorescence peak heights associated with different

nucleotides to assess nucleotide polymorphisms has been validated, and the dominance of

HHV1 suggests it was likely present in greater copy numbers than PpLCV1 [1213]. This

confirmed HHV1 in this saki monkey's brain tissue.

Discussion

This is the first report of the management of a platyrrhine with HHV1, and the

first report of HHV1 in a white-faced saki monkey that was not rapidly fatal. Treatment

for the animal in this case was aimed at reducing shedding and consisted of acyclovir

given during times of suspected increased shedding. L-lysine was also administered, but

has not been found efficacious for control of herpesviral disease and is considered

unlikely to have played a role in this case [3, 45]. Oral acyclovir and oral valacyclovir have

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both been shown to be effective for treatment of HHV1, although the long-term efficacy

of these therapies is uncertain [45]. A survival time of 2.5 years has not been previously

reported in this species or in other platyrrhines; this may be related to lack of antemortem

diagnostics in previous cases. Previous reports have identified infection without rapid

fatality in platyrrhines [7, 9, 278, 10, 29]. Antemortem diagnostic investigation is indicated in

platyrrhines with ocular inflammatory disease.

    Proteinuria detected at the time of the saki monkey's initial presentation was

similar to previous findings in this individual due to suspected glomerular protein loss

first diagnosed three years prior to the ocular findings. An ultrasound with a consulting

radiologist at the time of initial diagnosis of proteinuria revealed mild pyelectasia

bilaterally and prominent vasculature in the left renal pelvis (5 mm diameter). Clinical

response to benazepril and flax seed oil was varied over the clinical course, but ultimately

did not completely control protein loss. Chronic renal disease was further confirmed on

necropsy findings. Other causes of proteinuria such as due to low grade herpes meningitis

cannot be completely ruled out, as no spinal tap was performed.

    At the time of serological testing in this animal, there was no evidence of

seroconversion. Previous cases of HHV1 in white-faced saki monkeys have also been

negative for antibodies to HHV1 [24]. The negative serologic response may be due to lack

of cross-species serologic reactivity when utilizing anti-human secondary antibodies to

detect white-faced saki monkey antibodies. Some other Platyrrhini have shown evidence

of serologic response, but it is unknown if this is due to species differences or due to

variation in serologic testing [8, 10]. Testing in this case utilized a rapid dot immunobinding

assay, while other reported tests that have yielded positive results in Platyrrhini have

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utilized serum neutralization or ELISA [8, 10, 11]. It is also possible that seroconversion occurred later in this case, but additional serological testing was not done.

It is unknown what triggered the final progression of the disease in this case. While it is possible that low-level meningitis was present earlier, the hemorrhage and necrosis observed on histopathology indicated acute progression, and it is consistent with the observed normal mentation and mobility until immediately prior to death. While white-faced saki monkeys have been documented to live up to 36 years of age, a more typical lifespan in the wild is 15 years [2830]. At 22 years of age, this animal was geriatric. Reactivation, and reactivation of HHV1 has been associated with age and coinfection with other herpesviruses [23]. [25].

The role of *Pithecia pithecia* lymphocryptovirus 1 in this case is unknown;. Lymphocryptoviruses are gammaherpesviruses, and, unique lymphocryptoviruses have been identified in at least 42 species of non-human primates. These viruses are generally considered to be host-adapted; however, fatal lymphoproliferative disease was associated with the lymphocryptovirus *Callitrichine herpesvirus 3* in a group of common marmosets (*Callithrix jacchus*) [21, 23]. In addition, intranuclear inclusions similar to those seen in this case have been identified in rhesus macaques infected with *Macacine herpesvirus 4*, another lymphocryptovirus [4]. However, there has been no investigation of the role of this virus in disease [20] *Pithecia Pithecia* lymphocryptovirus 1 in disease [21]. The repeated positive PCR tests for HHV1 during the course of this animal's disease and the similarities in final clinical course to other cases of white-faced saki monkeys also suggest that HHV1 was the cause of ocular disease and ultimate meningoencephalitis in this case [18, 24].

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Reactivation of HHV1 has also been associated with coinfection with other herpesviruses, so it is possible that the presence of the lymphocryptovirus indirectly contributed to worsening of clinical disease [25]. Central nervous system coinfection with HHV1 and the human lymphocryptovirus, *Human herpesvirus 4*, has been reported [2426]. Coinfection with HHV1 and *Human herpesvirus 4* results in significantly altered production of cytokines by cultured lymphocytes in comparison to single infection with either virus [89]. Cytokines have a significant effect on the course of infectious disease.

At the time of the initial presentation, the saki monkey in this case report shared an enclosure with a female white-faced saki monkey and two golden lion tamarins (*Leontopithecus rosalia*). Because of concern about their exposure to HHV1, these animals were subsequently housed separately from the male saki monkey and underwent examinations for diagnostic testing. All three animals were negative for HHV1 by serology and PCR and never exhibited clinical signs of disease consistent with HHV1. While it is assumed that these exhibit-mates are susceptible to the disease, it is possible that the close contact required for transmission did not occur. In another case, a family of white-faced saki monkeys with HHV1 resulted in the death of all three saki monkeys, but five white-lipped tamarins (*Saguinus labiatus*) in the same enclosure never developed clinical illness [1718].

When deciding to treat a captive platyrrhine for HHV1, it is important to consider the health of other animals in the collection because the disease has been reported to affect multiple animals [9, 17, 19, 22][10, 18, 20, 24]. In this case, the decision was made to house this saki monkey separately to reduce risk to other animals. However, housing a social primate alone has long-term implications for quality of life. Such concerns may be

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partially mitigated by allowing visual contact with other primates or providing additional

enrichment. This decision should be made on a case by case basis for each individual.

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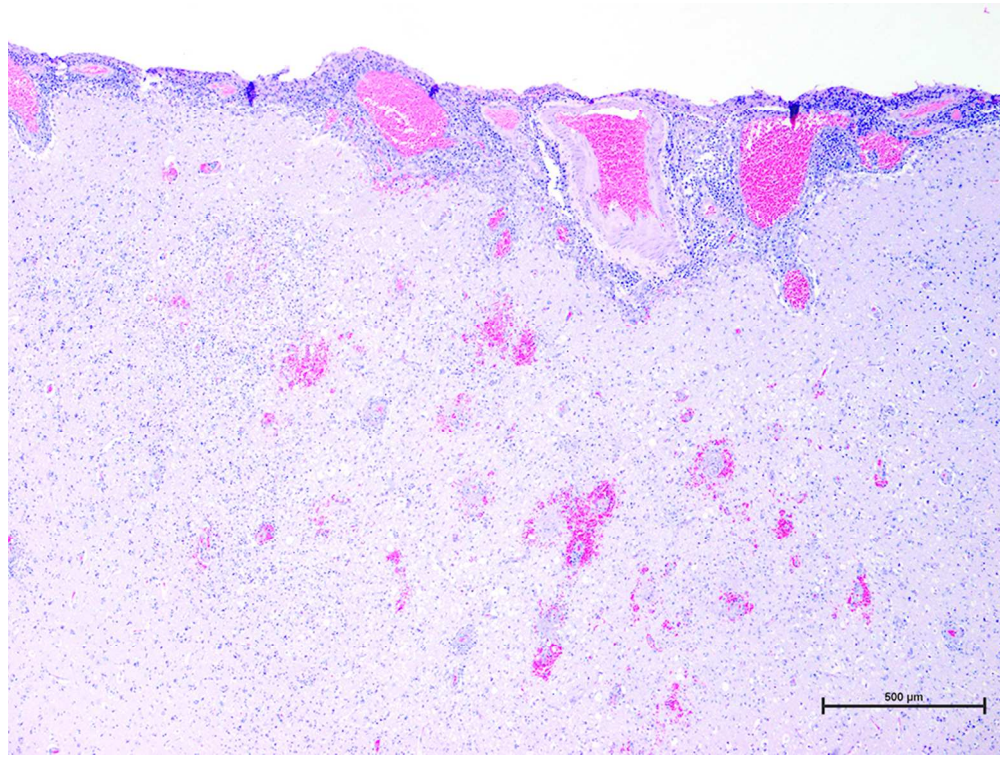**Figure Legends**

Figure 1. Photomicrograph of the frontal cerebrum of a 22-y-old saki monkey, showing marked expansion of the leptomeninges by mixed inflammatory cells, with lymphocytes predominating. Inflammatory infiltrates extend into the cerebrum forming perivascular cuffs. Vasculature is congested, and there is frequent perivascular hemorrhage into the neuropil with attendant necrosis, vacuolation, and inflammation. Hematoxylin and eosin stain; magnification 40x.

Figure 2. Photomicrograph of the frontal cerebrum of a 22-y-old saki monkey, showing degenerate neutrophils and karyorrhectic debris surrounding vessels and infiltrating the neuropil. Neuronal nuclei contain indistinct basophilic (open arrows) or distinct eosinophilic (solid arrow) inclusions with
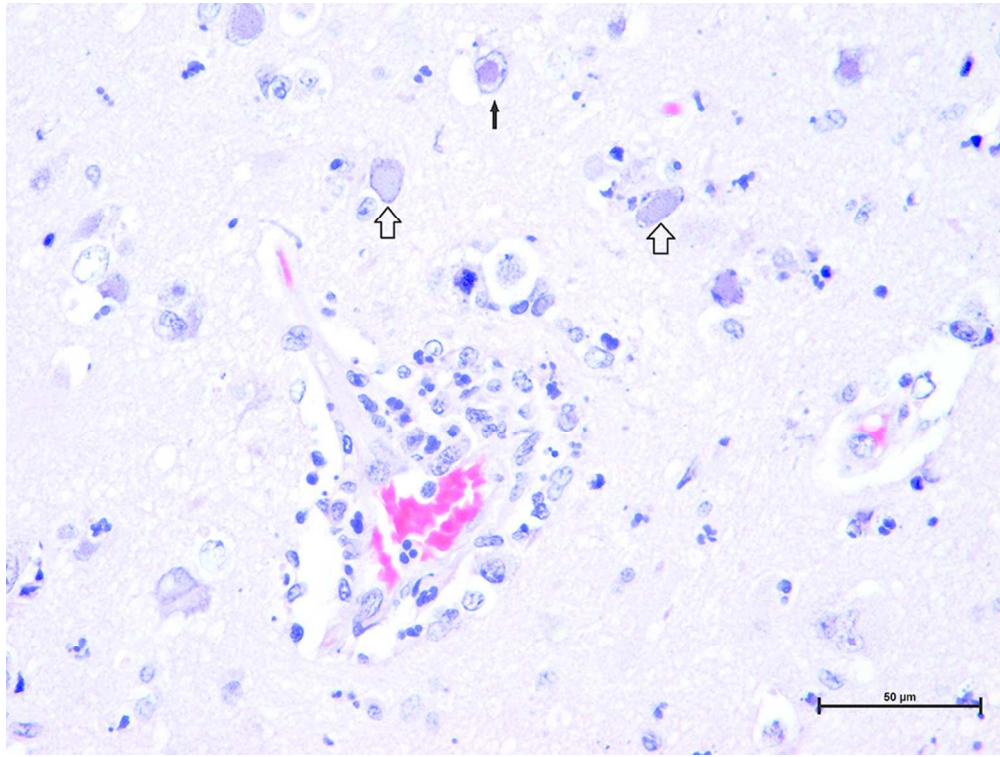
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margination of chromatin. Morphology of the inclusions is characteristic of herpesvirus. Hematoxylin and

eosin stain; magnification 400x.

Figure 3. Representative region of DNA sequence chromatogram of pan-herpesviral DNA polymerase

gene PCR product from the brain tissue of a 22-y-old saki monkey Guanine is in black, adenine is in green,

cytosine is in blue, and thymine is in red. Sequences for *Human herpesvirus 1* (GenBank accession #

KX424525) and Pithecia pithecia lymphocryptovirus 1 (GenBank accession # AY139025) are below the

chromatogram. It can be seen that when one sequence is subtracted from the chromatogram, the other is
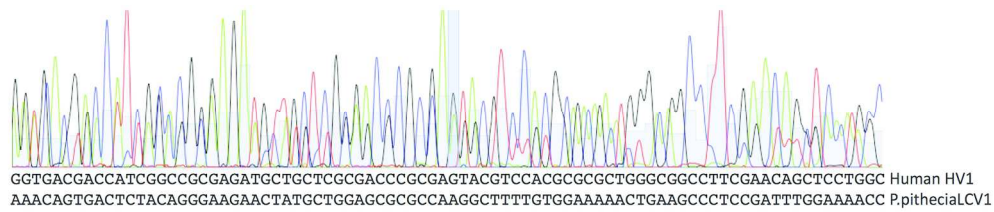
present.

Photomicrograph of the frontal cerebrum of a 22-y-old saki monkey, showing marked expansion of the leptomeninges by mixed inflammatory cells, with lymphocytes predominating. Inflammatory infiltrates extend into the cerebrum forming perivascular cuffs. Vasculature is congested, and there is frequent perivascular hemorrhage into the neuropil with attendant necrosis, vacuolation, and inflammation. Hematoxylin and eosin stain; magnification 40x.

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Photomicrograph of the frontal cerebrum of a 22-y-old saki monkey, showing degenerate neutrophils and karyorrhectic debris surrounding vessels and infiltrating the neuropil. Neuronal nuclei contain indistinct basophilic (open arrows) or distinct eosinophilic (solid arrow) inclusions with margination of chromatin. Morphology of the inclusions is characteristic of herpesvirus. Hematoxylin and eosin stain; magnification 400x.

88x66mm (300 x 300 DPI)

GGTGACGACCATCGGCCGCGAGATGCTGCTCGCGACCCGCGAGTACGTCCACGCGCGCTGGGCGGCCTTCGAACAGCTCCTGGC Human HV1
AAACAGTGACTCTACAGGGAAGAACTATGCTGGAGCGCGCCAAGGCTTTTGTGGAAAAACTGAAGCCCTCCGATTTGGAAAACC P.pitheciaLCV1

Representative region of DNA sequence chromatogram of pan-herpesviral DNA polymerase gene PCR product from the brain tissue of a 22-y-old saki monkey Guanine is in black, adenine is in green, cytosine is in blue, and thymine is in red. Sequences for Human herpesvirus 1 (GenBank accession # KX424525) and Pithecia pithecia lymphocryptovirus 1 (GenBank accession # AY139025) are below the chromatogram. It can be seen that when one sequence is subtracted from the chromatogram, the other is present.

187x40mm (300 x 300 DPI)