

Fatal *Herpes simplex virus 1* (HSV-1) Infection in a Group of Zoo-Kept White-Faced Saki Monkeys (*Pithecia pithecia*) in Israel

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ABSTRACT

On November 2016, a family of three white-faced saki monkeys (*Pithecia pithecia*) died 72–96 hours after the onset of signs of apathy, anorexia and oral ulceration. Despite the palliative treatment all the monkeys died and post-mortem examinations showed multifocal erosive stomatitis with intralesional inclusion bodies, hepatic necrotic foci and random neuronal necrosis in the brain cortex. Herpes simplex virus 1 HSV-1 was identified by PCR testing. HSV-1 is widely dispersed among the human population and infection is often asymptomatic but nonhuman primates commonly develop a severe and often fatal infection. These findings demonstrate the vulnerability of white-faced saki monkeys to HSV-1 similar to other new-world primate species. This is the first report, to the best knowledge of the authors of a fatal HSV-1 infection in zoo-kept white-faced saki monkeys and also of new world monkeys in Israel.

Keywords: Herpes Simplex Virus 1; White-Faced Saki; *Pithecia pithecia*; Nonhuman Primates; Stomatitis.

INTRODUCTION

Nonhuman primates are primary hosts to a number of herpes viruses (1). Herpes viruses usually cause mild or unnoticeable infections in their natural host, but some are associated with severe disease when transmitted to other species. Spontaneous cross-species transmissibility of herpes viruses is of a high zoonotic risk but can also result in fatal diseases of either humans or animals (2).

Herpes simplex virus 1 (HSV-1; human herpes virus type 1) belongs to the alpha herpes virus group. HSV-1 is one of the best-characterized viruses transmissible from human to nonhuman primates, where spontaneous infections in monkeys appear to be rare. Humans are the primary host and the reservoir for HSV-1; they exhibit typical clinical symptoms such as acute oral ulcers and gingivitis during the primary infection, which usually persists to a latent infection in the

nervous system with no or intermittent manifestation and virus shedding (3-7).

In Old-World primates, natural HSV-1 infection has been described in gorillas (*Gorilla gorilla*) (8) chimpanzees (*Pan troglodytes*), bonobos (*Pan paniscus*) (9), Orangutan (*Pongo pygmaeus pygmaeus*) (10) and white-handed gibbons (*Hylobates lar*) (11-13). In these species, the disease remains localized to mucocutaneous tissues and appears to be comparable with the human disease, as it usually does not progress to a systemic course (7).

In New-World monkeys, HSV-1 commonly leads to a fatal disease and was reported in the common marmoset (*Callithrix jacchus*) (7, 14-16), black-tufted marmoset (*Callithrix penicillata*) (17, 18), night monkey (*Aotus trivirgatus*) (19) and white-faced saki (*Pithecia pithecia pithecia*) (20). It is suggested that infection with HSV-1 occurs through

direct contact with mucosal surfaces, wounds, saliva, and maternal milk and via fomites contaminated with infectious saliva (2, 5). Therefore, cross-transmission between an HSV-1-infected human and new world monkeys is of particular concern in veterinary clinical practice (7).

This report describes spontaneous fatal infection of HSV-1 in a family of three white-faced saki monkeys (*Pithecia pithecia*) in the Hai-Park zoological collection in Israel.

CASE SUMMARY

The saki enclosure in the zoo included a family-group of 4 white-faced saki (*Pithecia pithecia*), including one adult male (10 years old), one adult female (8 years old) and their two female siblings (3 and 1 year old). The enclosure was also occupied by 5 male white-lipped tamarins (*Saguinus labiatus*) and one lowland paca (*Cuniculus paca*).

In November 2016, the 1-year-old female was found dead without showing any obvious signs of disease. Her remains were found in poor state and the post mortem examination could not suggest the cause of death. On the following day (Day 1), the zookeeper found an adult female sitting at the bottom of her enclosure. Due to her extreme lethargy, the monkey was handled without any restraint. Following the health issues in this group, all remaining animals were admitted to the zoo clinic for closer monitoring.

At the zoo clinic three monkeys were examined under isoflurane anesthesia in oxygen via face masks. The adult male showed signs of dehydration (7%, delayed skin turgor) but the two females showed no abnormalities. Blood samples were collected from two adult monkeys, submitted for complete blood count (CBC) and serum biochemistry and compared to a reference range (21).

The male's blood test results were unremarkable apart from a mild increase in the blood urea nitrogen (BUN) concentration (29 mg/dL, normal range 4-27mg/dL) and hyperkalemia (11.3 mmol/L; normal range 3.1-5.8 mmol/L). The adult female's biochemistry was unremarkable but the CBC showed several changes, including leukocytosis ($27.15 \times 10^3/\mu\text{L}$; range $3.44-19.99 \times 10^3/\mu\text{L}$) with mature neutrophilia ($18.46 \times 10^3/\mu\text{L}$; range $1.62-12.65 \times 10^3/\mu\text{L}$), decreased hemoglobin (10.7g/dL; range 11.6-18.1g/dL) and slightly decreased hematocrit (34.1%; range 35.3-58.7%) and polychromasia was observed on the blood smear. Fecal samples were negative for internal parasites. Before recovery from the anesthesia, all animals were administered subcutaneous fluids (Lactated Ringer's

Injection, Teva Medical Marketing, Petah-Tikva, Israel, 100 ml, SC) with dextrose and multivitamins (Duphalyte solution for injection, Zoetis, London, United kingdom, 20 ml, SC). All the saki monkeys were kept at the zoo clinic for further therapy with azithromycin (Azithromycin Teva, 200 mg/5 ml Suspension, Teva Medical Marketing, Petah-Tikva, Israel, 40 mg/kg, PO, SID) and meloxicam (Novacam 0.5 mg/ml, Veterinary, Produlab Pharma B.V, Raamsdonksveer, Netherlands, 0.2 mg/kg, PO, SID).

On Day 2, the remaining young female was observed convulsing for less than a minute and recovered spontaneously without treatment. At that time, all three monkeys appeared lethargic and anorectic. On the morning of Day 3, the adult female was found dead and the body was submitted for postmortem examination that revealed ulcers on the tongue (Figure 1) and pharynx. Histopathology showed multifocal erosive stomatitis with swelling of acanthocytes and evidence of numerous large eosinophilic intranuclear viral inclusions. Multifocal necrotizing Guttural air sacculitis was also observed with multiple foci of necrosis into the air sac wall and multiple foci of mineralization. Rare eosinophilic inclusion bodies were also observed in the guttural air sac. No other findings were found other than a mid-term fetus in the uterus. Because of the appearance and the location of the lesions, herpesvirus infection was highly suspected, and further molecular viral testing was indicated. As oral lesions were also observed on the adult male saki (Figure 2), swabs from the tongue lesions of both animals and blood samples were submitted to an outside human hospital laboratory for further viral testing.

Nucleic acids (NA) were extracted from 200 μl of the blood and swab samples, by the Easymag Nuclisense instrument (Biomerieux, Marcy l'Etoile, France). The on-board lysis specific B 2.0 protocol was used according to the manufacturer's guidelines. Blood samples (200 μl) were extracted by the MagNA Pure LC 2.0 apparatus (Roche Diagnostic GmbH, Mannheim, Germany) and Magna Pure LC total Nucleic Acid Isolation Kit Reagents (Roche Diagnostics, Mannheim, Germany). Volumes of 10 μl (or 5 μl in case of HSV-1) of the NA samples were used as templates for 25 μl real-time PCR reaction for the detection of Cytomegalovirus (CMV gB), Epstein-Barr virus (EBV), Varicella zoster virus (VZV), HSV-1 or HSV2 viruses. The real time PCR was performed by the rotor gene Q/6000 instruments (Corbett

Recherche, Mortlake, Australia). Both the blood sample and tongue swabs were found positive for HSV-1.

Following all test results, the two surviving saki monkeys were started on a treatment with famciclovir (famciclovir 80 mg/ml 10 ml suspension, Vetmarket, Shoam, Israel, 10 mg/kg, PO, SID) but the male saki was found dead on the following day (Day 4) and the remaining female was humanely euthanized due to her severe clinical appearance. The remains of both monkeys were submitted for a postmortem examination that showed no abnormalities other than the oral and pharyngeal lesions.

The histopathology of the male's lesions showed focal areas of oral mucosal erosion and ulceration with marked swelling of squamous epithelial cells and the presence of intranuclear inclusion bodies (Figure 3). The areas of erosion were infiltrated by neutrophils. The hepatocellular parenchyma had focal areas of necrosis with macrophages present, but no intranuclear inclusion bodies were observed. The brain cortex of the 3-years-old female had a few random neuronal necrotic foci which could have explained her severe



Figure 1: Picture of the adult female saki's tongue in this report, showing HSV lesions of the ulcerative glossitis.



Figure 2: Picture of the adult male saki in this report, showing the severe clinical presentation of the HSV lesions with the oral ulceration.

neurological symptoms prior to death. The overall diagnosis in all three saki monkeys was multifocal erosive stomatitis with intralesional intranuclear inclusion bodies, typical for HSV-1 infection.

DISCUSSION

The clinical course of disease in this group of zoo—kept white-faced saki monkeys together with the histopathological findings and the PCR test results are in line with a diagnosis of HSV-1 infection and demonstrate the vulnerability of this species to HSV-1 similar to other new-world primate species.

Erosive oral stomatitis was observed in all affected monkeys in this group. It has been reported that New World monkeys are highly susceptible to herpes simplex virus-1 infection (HSV-1) and the most common pathology described is vesicular or ulcerative stomatitis (2, 5). Involvement of other organs, such as the adrenal glands, liver and central nervous system may occur in cases of systemic HSV-1 infection (2, 5). In this case liver involvement showed in one monkey and the brain in another saki monkey that also showed neurological signs (convulsions) prior to her death. The guttural air sacculitis observed in the adult female was likely a progression of the oropharyngeal pathology. The younger female saki found dead initially could have also been part of this fatal group HSV-1 infection. However, in the absence of supporting pathological findings this remains speculative.

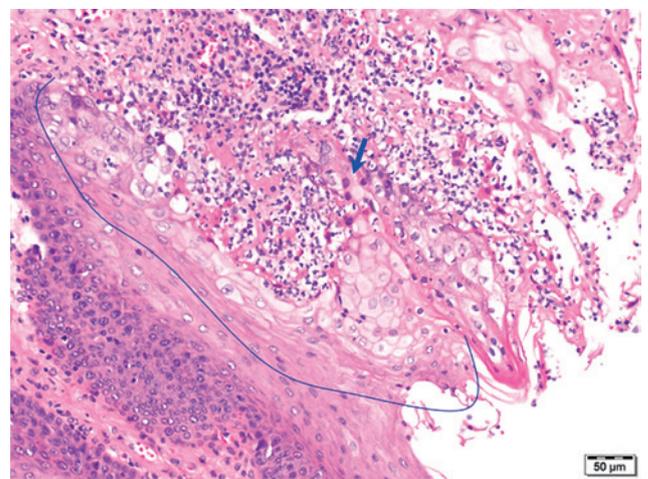


Figure 3: Histopathological image of the ulcerative oral mucosa of the adult male saki in this report. The arrow indicates the intranuclear inclusion bodies of the HSV. Within the thin blue line there is swelling of the squamous epithelial cells (Hematoxylin Eosin).

Clinical pathology results were mostly unremarkable in the tested monkeys in this case despite the liver pathology found in the male saki monkey and the changes on the hemogram in the adult female saki were likely due to the active infection. Dehydration and mild leukocytosis were also reported in HSV-1 infected marmosets (7), however leukopenia was also observed in the previously reported group of sick white-faced saki monkeys (20).

The HSV-1 diagnosis in this case included the typical clinical and histological findings that were further confirmed by specific HSV-1 PCR test results. Immunohistochemical staining could have also been done for confirmation of HSV-1 in the affected organs. However, cercpithecine herpesvirus 1 shows extensive antigenic cross-reactivity with HSV, and definitive diagnosis requires genetic analysis and/or epidemiological evidences as was also performed in this case (7).

Treatment with famciclovir was attempted in this case following the laboratory confirmation of HSV-1 involvement. This antiviral drug is considered effective in treatment of feline herpesvirus infection and was tried in this case as it can be administered once daily only (22). However, due to the rapid progression course of the disease in these monkeys, only a single treatment was administered and given the fatal outcome, was ineffective. In one study, common marmosets treated with acyclovir lived a while longer than did untreated animals, but this antiviral treatment did not prevent death (23). In another report, acyclovir was used in addition to antiemetic and anti-inflammatory agents to treat a common marmoset infected with HSV-1 but this animal also eventually died (14). Further studies on anti-herpesvirus treatments in nonhuman primates are indicated.

The exact source of the HSV-1 infection in the saki monkeys in this report is unknown. The monkeys were kept in an open zoo exhibit, and although limited were without an absolute isolation from public exposure. Infection of HSV-1

occurs through direct contact with virus shedding lesions, commonly being the saliva. Therefore, it is likely that these saki monkeys probably acquired the HSV-1 from partially consumed food items that were thrown into their enclosure by a HSV-positive zoo visitor (10). Although also potentially susceptible to HSV-1 infection, the group of white-lipped tamarins co-inhabiting the sakis' enclosure, were not infected (or at least did not show any signs of disease). It is possible that the larger-sized saki monkeys ate the HSV-infected food item exclusively or that one of the saki monkeys had ate that food item and the infection was passed within the saki group through their common mutual grooming behavior. This initial phase of the disease could have gone unnoticed.

This potential route of infection from a human zoo-visitor to a HSV-susceptible New-world primate is of utmost importance in regards to cage design and human-animal exposure. Public education is also important as infected humans can excrete herpes virus even in the absence of visible lesions (4). Zookeepers working directly with nonhuman primates should be properly informed and need to be provided with appropriate clothing and trained in proper handling and hygiene practices (7, 10).

HSV-1 infection in new-world primates is often fatal. This is the first report, to the best knowledge of the authors of a fatal HSV-1 infection in zoo-kept white-faced saki monkeys and also of new world monkeys in Israel. For prevention of further cases it important to avoid interspecies transmission, and humans (symptomatic or subclinical HSV-1) should not feed animals and in general feeding nonhuman primates should be discouraged.

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REFERENCES

1. Brack, M.: Morphological and epidemiological aspects of simian herpesvirus infections, vol. 5, pp. 1–60. Paul Parey, Berlin, 1977.
2. Lerche, N.W.: Common viral infections of laboratory primates. In Wolfe-Coote S, editor: *The Laboratory Primate*, London, U.K., 2005, Elsevir.
3. McGeoch, D.J. and Cook, S.: Molecular phylogeny of the alphaherpesvirinae subfamily and a proposed evolutionary timescale. *J. Mol. Biol.* 238:9–22, 1994.
4. Matz-Rensing, K., Jentsch, K. D., Rensing, S., Langenhuyzen, S., Verschoor, E., Niphuis, H. and Kaup, F. J.: Fatal herpes simplex infection in a group of common marmosets (*Callithrix jacchus*). *Vet. Pathol.* 40: 405–411, 2003.
5. Hunt, R.D.: *Herpesvirus simplex* infection. In: *Nonhuman Primates*, ed. Jones, T.C., Mohr, U. and Hunt, R.D. pp. 82–86. Springer Verlag, Berlin, Germany, 1993.
6. Hunt, R.D. and Melendez, L.V.: Herpesvirus infections of nonhuman primates: a review. *Lab. Anim. Care.* 19:221–234, 1969
7. Imura, K., Chambers, J.K., Uchida, K., Nomura, S., Suzuki, S., Nakayama, H. and Miwa, Y.: Herpes Simplex Virus Type 1 Infection in Two Pet Marmosets in Japan. *J. Vet. Med. Sci.* 76: 1667–1670, 2014.
8. Heldstab, A., Ruëdi, D., Sonnabend, W. and Deinhardt, F.: Spontaneous generalized *Herpesvirus hominis* infection of a lowland gorilla (*Gorilla gorilla gorilla*). *J. Med. Primatol.* 10:129–135, 1981.
9. McClure, H.M., Swenson, R.B., Kalter, S.S. and Lester, T.L.: Natural genital *Herpesvirus hominis* infection in chimpanzees (*Pan troglodytes* and *Pan paniscus*). *Lab. Anim. Sci.* 30:895–900, 1980.
10. Kik, M.J., Bos, J. H., Groen, J. and Dorrestein, G.M.: Herpes simplex infection in a juvenile orangutan (*Pongo pygmaeus pygmaeus*). *J. Zoo Wildlife Med.* 36: 131–134, 2005.
11. Ramsay, E., Stair, E.L., Castro, A.E. and Marks, M.I.: Fatal *Herpesvirus hominis* encephalitis in a white-handed gibbon. *J. Am. Vet. Med. Assoc.* 181:1429–1430, 1982.
12. Smith, P.C., Yuill, T.M., Buchanan, R.D., Stanton, J.S. and Chai-cumpa, V.: The gibbon (*Hylobates lar*): a new primate host for *Herpesvirus hominis* I. A natural epizootic in a laboratory colony. *J. Infect. Dis.* 120:292–297, 1969.
13. Landolfi, J.A., Wellehan, J.F.X. Johnson, A.J. and Kinsel, M.J.: Fatal human herpesvirus type 1 infection in a white-handed gibbon (*Hylobates lar*). *J. Vet. Diagn. Investig.* 17: 369–371, 2005.
14. Huemer, H.P., Larcher, C., Czedik-Eysenberg, T., Nowotny, N. and Reifinger, M.: Fatal infection of a pet monkey with Human herpesvirus. *Emerg. Infect. Dis.* 8: 639–642, 2002.
15. Juan-Sallés, C., Ramos-Vara, J.A., Prats, N., Solé-Nicolás, J., Segalés, J. and Marco, A.J.: Spontaneous herpes simplex virus infection in common marmosets (*Callithrix jacchus*). *J. Vet. Diagn. Invest.* 9: 341–345, 1997.
16. Hatt, J. M., Grest P., and Bossart, W.: Serologic survey in a colony of captive common marmosets (*Callithrix jacchus*) after infection with herpes simplex type 1-like virus. *J. Zoo Wildlife Med.* 35: 387–390, 2004.
17. Bruno, S.F., Liebhold, M.M., Mätz-Rensing, K., Romao, M.A., Didier, A., Brandes, F., Bressan, A.C. and Kaup, F. J.: Herpesvirus infections in free living black tufted ear marmosets (*Callithrix penicillata*, E. Geoffroyi 1812) at the State Park of Serra da Tiririca, Niterói, Rio de Janeiro, Brazil]. *Berl. Munch. Tierarztl. Wochenschr.* 110: 427–430, 1997.
18. Costa, E.A., Luppi, M.M., Malta, M.C., Luiz, A.P., de Araujo, M.R., Coelho, F. M., da Fonseca, F.G., Ecco, R. and Resende, M.: Outbreak of human herpes virus type 1 infection in nonhuman primates (*Callithrix penicillata*). *J. Wildl. Dis.* 47: 690–693, 2011.
19. Melendez, L.V., España, C., Hunt, R.D., Daniel, M.D. and Garcia, F.G.: Natural herpes simplex infection in the owl monkey (*Aotus trivirgatus*). *Lab. Anim. Care* 19: 38–45. 1969.
20. Schrenzel, M.D., Osborn, K.G., and Shima, A.: Naturally occurring fatal herpes simplex virus 1 infection in a family of white-faced saki monkeys (*Pithecia pithecia pithecia*). *J. Med. Primatol.* 32: 7–14, 2003.
21. ISIS homepage. [ISIS] International Species Identification System Physiological Reference Intervals for Captive Wildlife—2013, white-faced saki (*Pithecia pithecia*). [Internet]. [cited 2017 February 21]. Available from: <http://www.isis.org>
22. Thomasy, S.M., Lim, C.C., Reilly, C.M., Kass, P.H., Lappin, M.R. and Maggs, D.J.: Evaluation of orally administered famciclovir in cats experimentally infected with feline herpesvirus type-1. *Am. J. Vet. Res.* 72:85–95, 2011.
23. Casagrande, R.A.: Herpes simplex tipo 1 em saguis (*Callithrix jacchus e Callithrix penicillat*) - Caracterizaao anatomopatologica e molecular. MS Dissertation, Universidade de Sao Paulo, Sao Paulo, Brazil, pp. 110, 2007.