

Lethal infection by herpes simplex virus 1 (HSV-1) in a captive Azara's owl monkey (*Aotus azarae*) in Paraguay

*Infección letal por virus herpes simplex 1 (HSV-1) en un mono Marikín de Azara (*Aotus azarae*) cautivo en Paraguay*

*Infecção letal pelo vírus herpes simplex 1 (HSV-1) em um macaco Marikín de Azara (*Aotus azarae*) em cativeiro no Paraguai*

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Abstract

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Anamnesis: A 2-year-old male *Aotus azarae* was brought to the wild animal clinic. The animal weighed 975 grams and was in poor body condition. The individual had been kept in close contact with the caretakers and was largely fed by the people in the household, sharing their meals. According to the caretaker, the animal had been asthenic and anorexic for approximately two days. **Clinical and laboratory findings:** On clinical inspection, the animal was weakened, with pale oral mucosa, hyperemic pharynx, and a rectal temperature of 34.2°C. Dry crusts were observed in the perinasal region, and mild dyspnea was perceived. The patient died during the inspection. The hemogram was suggestive of normocytic normochromic anemia, leukopenia, hypoproteinemia, and thrombocytopenia. Molecular analysis detected HSV-1. **Conclusion:** Data obtained through anamnesis and clinical history, as well as hematologic findings and PCR results, confirmed the diagnosis of HSV-1 infection. To the best of the authors' knowledge, this is the first report of the disease in a non-human primate in Paraguay.

Keywords: *Anthropozoonosis; Aotus azarae; cross-transmission; Herpes simplex virus; Herpesvirus; HSV-1; New World primate; Owl monkey; PCR; Platyrrhini.*

Resumen

Anamnesis: Un mono macho *Aotus azarae* de 2 años fue ingresado al Consultorio de Animales Silvestres. El animal pesaba 975 gramos y presentaba una baja condición corporal. El individuo se había mantenido en estrecho contacto con los cuidadores y era alimentado en gran medida por las personas de la casa, compartiendo sus comidas. Según informó el cuidador, el animal llevaba aproximadamente dos días asténico y anoréxico. **Hallazgos clínicos y de laboratorio:** En la inspección clínica, el animal estaba débil, con mucosa oral pálida,

faringe hiperémica y temperatura rectal de 34,2°C. Se observaron costras secas en la región perinasal y se evidenció una ligera disnea. El paciente falleció durante la inspección. El hemograma fue sugestivo de anemia normocítica normocrómica, leucopenia, hipoproteinemia y trombocitopenia. El análisis molecular detectó la presencia de VHS-1. **Conclusión:** La información de anamnesis e historia clínica, así como los hallazgos hematológicos y los resultados de PCR, confirmaron el diagnóstico de infección por VHS-1. Según el conocimiento de los autores, este es el primer reporte de la enfermedad en un primate no humano en Paraguay.

Palabras clave: *Antropozoonosis; Aotus azarae; Herpesvirus; Herpes simplex virus; HSV-1; Mono búho; PCR; Platirrino; primate del Nuevo Mundo; transmisión cruzada.*

Resumo

Anamnesis: Um *Aotus azarae* macho de 2 anos de idade foi trazido para a clínica de animais selvagens. O animal pesava 975 gramas e estava em condição corporal baixa. O indivíduo estava em contato próximo com os cuidadores e era alimentado principalmente pelas pessoas da casa, compartilhando suas refeições. De acordo com o cuidador, o animal apresentava astenia e anorexia havia aproximadamente dois dias. **Achados clínicos e laboratoriais:** Na inspeção clínica, o animal estava debilitado, com mucosa oral pálida, faringe hiperêmica e temperatura retal de 34,2°C. Crostas secas foram observadas na região perinasal e notou-se uma leve dispneia. O paciente morreu durante a inspeção. O hemograma foi sugestivo de anemia normocítica normocrômica, leucopenia, hipoproteinemia e trombocitopenia. A análise molecular detectou a presença de HSV-1. **Conclusões:** Os dados obtidos na anamnese e na história clínica, bem como os achados hematológicos e os resultados da PCR, confirmaram o diagnóstico de infecção pelo HSV-1. Até onde se tem conhecimento, este é o primeiro relato da doença em um primata não humano no Paraguai.

Palavras-chave: *Antropozoonose; Aotus azarae; Herpesvírus; HSV-1; Macaco-coruja; PCR; Platirrino; Primata do Novo Mundo; transmissão cruzada; Vírus herpes simplex.*

Introduction

Herpesviruses are DNA-enveloped viruses with complex genomes that infect a wide variety of vertebrate species, with many primates and humans being natural hosts (Ludlage and Mansfield, 2003; Casagrande, 2014). Formerly classified within the family *Herpesviridae*, these viruses are now grouped into three families within the order *Herpesvirales*: the family *Herpesviridae*, which retains all mammalian, avian, and reptile herpesviruses; the new family *Alloherpesviridae*, comprising fish and frog viruses; and the new family *Malacoherpesviridae*, containing the viruses of bivalves. The family *Herpesviridae* is further divided into three subfamilies, among which are the 39 known species of human and non-human primate herpesviruses (Casagrande, 2014).

The genus *Simplexvirus*, within the subfamily *Alphaherpesvirinae*, contains five species of New World Primate herpesviruses (NWP), as well

as two species of human herpesviruses: *Herpes simplex virus 1* (HSV-1) and *Herpes simplex virus 2* (HSV-2). Many human and non-human primates carry their own species of herpesviruses belonging to this genus, which normally do not cause clinical disease in immunocompetent natural hosts (Casagrande, 2014). Humans are the only primate species infected by two distinct Herpes simplex viruses: HSV-1 and HSV-2 (Wertheim et al., 2014). Herpesviruses have been infecting and coevolving with their vertebrate hosts for hundreds of millions of years (Wertheim et al., 2014). However, when these viruses infect different primate species, they can cause significant and often fatal clinical disease (Casagrande, 2014).

Unlike Old World Primates (OWP), which generally develop a self-limiting infection similar to that in humans, NWPs are more susceptible to HSV-1 infection, typically presenting a generalized and fatal disease characterized

by anorexia, dermatitis, pruritus, depression, and ulcerative lesions in the oral cavity and gastrointestinal tract (Casagrande, 2014; Fortman et al., 2018). The disease caused by these viruses has been reported in captive NWP of the genus *Cebus* (Zinsser, 1929; Souvignet, 2019), *Aotus* (Katzin, 1967; Barahona et al., 1976; Meignier et al., 1990; Gozalo et al., 2008; Kreutzer et al., 2011), *Callithrix* (Huemer et al., 2002; Mätz-Rensing et al., 2003; Hatt et al., 2004; Casagrande, 2007; Sekulin et al., 2010; Imura et al., 2014; Araújo et al., 2016), and *Pithecia* (Schrenzel et al., 2003; Lapid and Eshar, 2017). Primates of the genus *Aotus* are known for their high susceptibility to HSV infection, in which the virus appears to have a predilection for the cerebral cortex, causing encephalitis (Calle and Joslin, 2015). For this reason, these primates are also used as a model for the study of the pathogen (Katzin et al., 1967; Todo et al., 2000; Roth et al., 2014).

Because of the frequent but unapparent spread of herpesviruses, careful handling of these animals should be recommended, and appropriate hygiene measures should be applied for the sake of both owners and pet monkeys. Standard veterinary practice considers whether diseases of primates that have been in close contact with humans may have been caused by human viruses (Huemer et al., 2002). In addition to considering the transmission of NWP herpesviruses, such as CeHV-1, to a human host, which can result in potentially fatal encephalitis (Casagrande, 2007), the potential link between a wild animal and an infection acquired from a human host should be highlighted, as humans in contact with the animal may not necessarily show signs of disease (Huemer et al., 2002). This occurs because herpesviruses typically do not cause severe infections in immunocompetent individuals of their natural host species, as most infections remain asymptomatic (Eberle and Jones-Engel, 2017). A distinctive feature of herpesviruses is their ability to establish latent infections that persist throughout the life of the host without clinically apparent signs of infection (Eberle and Jones-Engel, 2017).

The objective of the present work is to report a case of lethal infection by *Herpes simplex virus 1* (HSV-1) in a captive Azara's owl monkey (*Aotus azarae*) in Paraguay.

Patient Examination

Anamnesis

A 2-year-old male pet *Aotus azarae* was brought to the Wild Animal Clinic at the Faculty of Veterinary Sciences, National University of Asunción. The animal weighed 975 grams and was in poor body condition. The individual was kept in close contact with the caretakers, either freely inside the house or tied at the waist with a nylon leash while being carried on the caretakers' shoulders. It was fed primarily by household members, sharing their meals and even engaging in mouth-to-mouth feeding. As reported by the caretaker, the animal had been asthenic and anorexic for approximately two days.

Clinical findings

On clinical inspection, the animal appeared weakened, with pale oral mucosa, a hyperemic pharynx, and a rectal temperature of 34.2°C (hypothermia). Dry crusts were observed in the perinasal region, and slight dyspnea was perceived. The patient was placed in an oxygenation chamber and died one hour later.

Diagnostic aids

Immediately after confirming the animal's death, 3 ml of blood was extracted via cardiac puncture. Of the extracted blood, 1 ml was placed in a vial with EDTA for a hemogram, and 2 ml were placed in a vial without anticoagulant for molecular diagnostics. Both samples were refrigerated at 4°C until processing. The caretaker refused to send the patient's body for routine post-mortem studies.

The blood sample for the hemogram was processed using the following methods:

(a) Manual counting of blood cells and hemoglobin levels; (b) Differential leukocyte counting in blood smears stained with Giemsa;

(c) Morphological evaluation of blood cells in blood smears stained with Giemsa; (d) Determination of total plasma protein levels by refractometry.

The results, shown in Table 1, were suggestive of normocytic normochromic anemia, leukopenia, hypoproteinemia, and thrombocytopenia, indicative of viremia.

Table 1. Hematological results from a sample taken from an *Aotus azarae* infected with HSV-1.

Analyte (unit)	Breed	Reference*
Hematocrit (%)	24.0	34.1 – 53.6
Hemoglobin (g/100 ml)	8.6	12.0 – 19.4
Red blood cells ($10^6/\text{mm}^3$)	3.2	4.56 – 7.06
Mean corpuscular volume (fl)	75	71 – 83
Mean corpuscular hemoglobin (pg)	26	23 – 30
Mean corpuscular hemoglobin concentration (gr/dl)	35	32 – 39
White blood cells ($10^6/\text{mm}^3$)	4300	4900 – 21000
Segmented (μl)	989	910 – 7190
Lymphocytes (μl)	3311	3140 – 10600
Monocytes (μl)	--	0 – 302
Eosinophils (μl)	--	94 – 4062
Basophils (μl)	--	0 – 411
Platelets ($10^3/\mu\text{l}$)	2.0	3.7 – 24.5
Total proteins (g/L)	4.2	6.9 – 8.1

*Takeshita et al. (2011)

From the blood sample, RNA extraction was performed using the commercial kit Ribospin vRD (GeneAll), following the manufacturer's instructions.

The primers used for HSV-1 detection were:

HSV-1 Forward: 5' GCAGTTTACGTACAACCACATACAGC 3'

HSV-1 Reverse: 5' AGCTTGCGGGCCTCGTT 3'

HSV-1 Probe: CGGCCCAACATATCGTTGACATGGC

The primers used for HSV-2 detection were:

HSV-2 Forward: 5' TGCAGTTTACGTATAACCACATACAGC 3'

HSV-2 Reverse: 5' AGCTTGCGGGCCTCGTT 3'

HSV-2 Probe: CGCCCCAGCATGTCGTTTACGT

The conditions set in the BioRad CFX96 thermal cycler were:

Activation: 95°C for 2 minutes

40 cycles of denaturation: 95°C for 5 seconds

Extension: 60°C for 20 seconds

The observed result was positive for HSV-1 and negative for HSV-2.

Discussion

Given that humans and nonhuman primates (NHPs) are genetically and physiologically similar, it is not surprising that some herpesviruses from NHPs can infect humans, and vice versa. Although most of these cross-species infections are probably abortive (i.e., the virus cannot complete its replicative cycle to produce an active or latent infection or cause clinically apparent disease), some herpesviruses produce

severe or lethal infections when transmitted to a non-natural host species (Eberle and Jones-Engel, 2017).

The clinical signs reported in captive primates of the genus *Aotus* infected with HSV-1 partially coincide with those observed in the present case, including severe dyspnea, apathy, hypothermia, and lethargy that gradually worsened until the animal's death within a period of 4 to 7 days (Melendez et al., 1969; Kreutzer et al., 2011). Reports also mention lesions in the oral cavity, including vesicles, mucous exudate, necrotic plaques, moderate multifocal gingival defects, and ulcers in the oral mucosa and tongue, which may extend to the pharynx, esophagus, and trachea (Melendez et al., 1969; Gozalo et al., 2008; Kreutzer et al., 2011). All cases report infections with high case fatality rates (Melendez et al., 1969; Meignier et al., 1990; Gozalo et al., 2008; Kreutzer et al., 2011).

In *Callithrix* spp., another group of NHPs, an acute course of infection has also been reported, with an evolution ranging from 1 to 8 days (Juan-Sallés et al., 1997; Huemer et al., 2002; Mätz-Rensing et al., 2003; Hatt et al., 2004; Casagrande, 2007). Clinical signs are similar, including prostration, paresis, hyporexia, hypersalivation, vomiting, diarrhea, aggressiveness, seizures, nystagmus, anisocoria, and dyspnea (Huemer et al., 2002; Hatt et al., 2004; Casagrande, 2007; Imura et al., 2014). In association with the neurological signs, most animals develop small ulcers covered by crusts on the facial skin, extensive ulcers covered by whitish fibrinous material on the oral mucosa and tongue, and, in some cases, conjunctivitis (Mätz-Rensing et al., 2003; Hatt et al., 2004; Casagrande, 2007; Sekulin et al., 2010; Araújo et al., 2016). Some animals exhibit neurological alterations without skin and mucosal lesions (Juan-Sallés et al., 1997; Casagrande, 2007; Imura et al., 2014), whereas others show no clinical manifestations and are found dead (Mätz-Rensing et al., 2003; Hatt et al., 2004; Casagrande, 2007). In *Pithecia pithecia*, outbreaks of acute and fatal infection have also been reported, with animals dying within 48 to

96 hours after the onset of signs, similar to cases in *Aotus* spp. and *Callithrix* spp. (Schrenzel et al., 2003; Lapid and Eshar, 2017).

Regarding diagnostic methods, the use of serological testing for herpesvirus diagnosis should be approached with caution since many primates are asymptomatic carriers of various herpesvirus species. When histopathological examinations are performed, non-suppurative meningoencephalitis with necrotizing vasculitis and the presence of typical intranuclear inclusions are observed; however, this does not allow for the determination of the specific herpesvirus species. In contrast, immunohistochemistry using monoclonal antibodies enables differentiation of several herpesvirus species, although anti-HSV-1 and anti-HSV-2 antibodies are polyclonal and cross-react (Casagrande, 2014).

Currently, PCR is the technique of choice for the definitive diagnosis of herpesvirus species involved in diseases of humans and NHPs, although its use must be correlated with the clinical presentation and lesions of the patient. Swabs from lesions or blood samples from diseased animals can be used, as well as organs collected during necropsy (Casagrande, 2007; Casagrande, 2014). The reports by Schrenzel et al. (2003), Casagrande (2007), and Sekulin et al. (2010) detail the primers used for the detection of HSV-1 in *Pithecia pithecia* and *Callithrix jacchus* specimens, respectively.

Treatment with acyclovir does not prevent death from encephalitis in HSV-1-infected animals, although it remains the antiherpetic drug of choice in humans (Casagrande, 2014; Kukhanova et al., 2014). Other drugs used in humans include valacyclovir and ganciclovir, which could be used in NHPs (Casagrande, 2014). There is a single report of a *Callithrix jacchus* surviving a natural HSV-1 infection and recovering spontaneously without treatment (Hatt et al., 2004).

It should be noted that HSV-1 infection in NHPs is an anthroponozoonotic disease, and the

present report coincides with acute spontaneous HSV-1 infections described as fatal in *Aotus* spp. and *Callithrix* spp. following contact with a person carrying HSV-1 (Mätz-Rensing et al., 2003), being kept as a pet in close contact with its owners (Juan-Sallés et al., 1997; Huemer et al., 2002; Hatt et al., 2004; Kreutzer et al., 2011; Imura et al., 2014), or even sharing food with the animal (Araújo et al., 2016). HSV-1 infection in NWP has already been widely reported in Brazil, affecting animals from zoos, conservation and breeding centers, and research facilities, as well as pet primates, particularly those of the genus *Callithrix* (Casagrande, 2007). A study conducted in Peru on primates kept as pets found that 50.4% of individuals sought veterinary consultation for infectious diseases, while 11.4% were presented with both infectious and non-infectious conditions simultaneously (Nolasco, 2017), highlighting the potential impact of household primate ownership on public health.

Conclusion

Data obtained through anamnesis and clinical history, as well as observable signs during the physical examination, hematological findings, and PCR results, confirmed the diagnosis of HSV-1 infection as the cause of death in a captive Azara's owl monkey. To the authors' best knowledge, this is the first report of the disease in a non-human primate in Paraguay.

Declarations

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Conflicts of interest

The authors declare they have no conflicts of interest regarding the work presented in this report.

Author contributions

J. Richard Vetter: study conception, data collection, wrote the paper. Marlene Florentín-Morel: data collection, critical review of the paper. MaríaGraciela Riera-Domínguez: data collection, wrote the paper. Ricardo G. Cañiza: data collection, critical review of the paper.

Use of artificial intelligence (AI)

No AI or AI-assisted technologies were used during the preparation of this work.

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