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Herpes simplex virus type 1 infection in pet marmoset – case report

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ABSTRACT

A 19-month-old female common marmoset (*Callithrix jacchus*) was presented with a 4-day history of anorexia and behavioral changes. At presentation, a combination of neurological signs, erosions, and ulcerations of the mucous membranes of the oral mucosa and mucocutaneous junction of the lips were observed, suggesting herpesvirus infection. Although etiological and symptomatic therapy was initiated immediately, the animal died the next day. A necropsy was performed, and gross pathology and pathohistology findings indicated a systemic viral infection. Definitive diagnosis was based on the results of molecular testing that demonstrated the presence of human herpes simplex virus type 1. In conclusion, marmosets are a highly susceptible host for human herpes simplex virus. To reduce the risk of infection and prevent this highly lethal disease in monkeys, contact with humans who have symptomatic or asymptomatic forms of HSV-1 should be limited and accompanied by appropriate hygiene measures.

Key words: common marmoset; *Callithrix jacchus*; herpes simplex virus type 1

Introduction

Several campaigns have been launched in recent years to advocate a ban on keeping primates as pets. Among the reasons on which this proposal is based is the fact that primates are animals with complex social, nutritional, and welfare needs that cannot be met in a home environment (NORCONK et al., 2020; SEABOCH and CAHOON, 2021). In addition, owners' knowledge of their special husbandry requirements and disease susceptibility is often inadequate. Marmoset monkeys are one of the most commonly kept and traded primate species (SEABOCH and CAHOON, 2021). However, diseases related to inadequate and improper nutrition (marmoset wasting syndrome and metabolic bone disease) are the leading cause of morbidity and mortality in captive animals (BAXTER et al., 2019; OTOVIC et al., 2015). There are also several zoonotic diseases of concern. Certain

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pathogens (e.g. *Shigella, Salmonella, Giardia, Cryptosporidium*) can be transmitted to humans from nonhuman New World primates, including marmosets, via the fecal-oral route (KRAMER, 2018). Viruses such as hemorrhagic fever viruses and monkeypox virus can also be transmitted from nonhuman primates, but they are not expected to be present in the pet population. Some other viruses that are well adapted to their primary hosts, in which they cause only very mild or subclinical infections, could pose a more significant threat. Some of them, e.g., certain herpesviruses, have the potential to cross the species barrier and cause a severe, sometimes fatal, disease in other susceptible species (MÄTZ-RENSING and BLEYER, 2018).

This report describes a case of the fatal infection of pet marmoset with herpes simplex virus type 1 (HSV-1).

Case description

A 19-month-old female common marmoset (*Callithrix jacchus*) was brought to the clinic with a history of lethargy, anorexia, and severe neurological signs. The monkey had been acquired from another private owner six months before. Since then, it had been kept in an indoor enclosure with occasional access to living quarters. The animal was mostly fed with fruits and cooked vegetables. The diet was supplemented with live insects, baby porridge, amber raisins, and vitamin D3. The first symptoms appeared 4 days before, when the owners noticed a loss of appetite and behavioral changes. The animal was less active, stopped climbing and kept itself to the floor. The condition progressively worsened. At the time of presentation, the animal was extremely weak and ataxic, with hind leg weakness and severe tremor. Excessive squinting and blinking were observed. Further physical examination revealed hypothermia (the temperature was immeasurably low), dehydration, and erosions on the skin of the left commissure of the mouth and gums (Fig 1.), accompanied by large, coalescing vesicles on the tip of the tongue. Due to the open mouth breathing, a chest radiograph was obtained, but no pathological changes were noted. On the basis of the clinical picture and the data obtained from the medical history, in which the owner reported occasional episodes of herpes labialis, a herpesvirus infection was suspected. Swabs of the erosions and ulcers, and rectal swabs were collected and submitted for herpesvirus PCR testing. Treatment began immediately after sampling and included: rewarming, subcutaneous fluid administration (intravenous access was not tolerated by the animal), acyclovir (20 mg/kg PO every 6 hours), amoxicillin and clavulanic acid (15 mg/kg SC BID), and assisted feeding (EmerAid Intensive Care Omnivore). Despite the initiated treatment, the animal died the next day. Necropsy was performed and additional liver samples were taken for molecular testing.

Fig. 1. Open mouth breathing, erosions on the skin and large, coalescing vesicles on the tip of the tongue in a marmoset with herpes simplex virus type 1 (HSV-1) infection

Pathology findings. Gross examination revealed multifocal to coalescing ulcerative cheilitis and gingivitis covered by fibrinopurulent exudate. The dermis showed discrete infiltration of lymphocytes and plasma cells, as well as single-cell necroses and ballooning of epidermal cells and focal epidermal vesicles. On the ventral side of the body, affecting the neck, thorax, and abdomen, there was extensive edema of the subcutis, measuring up to 2 mm in thickness. All subcutaneous lymph nodes were enlarged and edematous on the cut surface (Fig. 2A), with multifocal necrosis and apoptosis of the lymphocytes in the cortex on histology. The body cavities were filled with red, slightly opaque fluid: 5 ml in the abdominal cavity- , 3 ml in the thoracic cavity, and 1 ml in the pericardial cavity. The tongue exhibited multifocal to coalescing erosions measuring up to 2 mm in diameter (Fig. 2B). The lungs were diffusely enlarged, pale to dark red in color (Fig. 2B), with foamy liquid oozing from the cut surface, corresponding to pulmonary edema. Histology revealed multifocal necrosis of the septa, with leakage of protein-rich, eosinophilic fluid into the alveolar spaces, corresponding to alveolar edema (Fig. 3A). The myocardium showed multifocal, single-cell necroses of myocytes, with a mild histiocytic reaction (Fig. 3B). The liver was moderately enlarged, with accentuated lobular pattern grossly, and with hepatocyte dissociation, multifocal mononuclear hepatitis (Fig. 3C) and single-cell necroses of hepatocytes on histology. The brain was congested with multifocal, mild, mononuclear vasculitis (Fig. 3D). Other organs and tissues showed no gross or microscopic lesions. Inclusions were not detectable in any tissue.

Molecular diagnostic testing. Viral DNA was extracted using the QIAamp cador Pathogen Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The isolated DNA was subjected to nested pan-herpes PCR, as described earlier (VANDEVANTER et al., 1996). The presence of the amplified herpesvirus DNA segment was visualized on a 2% agarose gel and, after visualization, sent to Macrogen, Amsterdam, The Netherlands, for purification and sequencing. Both DNA strands were sequenced with an ABI PRISM 3100 Genetic Analyzer using a BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Sequence alignment and edition were performed with MEGA7 software (KUMAR et al., 2015) using CLUSTAL W. The obtained sequence of 169 nucleotides was 100% identical with the DNA polymerase gene of human alphaherpesvirus 1 isolated in the United Kingdom (GeneBank accession number JN555585). In addition, DNA isolated from swabs and liver was subjected to a second PCR targeting the specific glycoprotein of herpesvirus simplex type 1 (NORBERG et al., 2004). Again, positive results were obtained, and PCR products were sent to Macrogen, Amsterdam, The Netherlands, for purification and sequencing. The amplified 768-nucleotide fragment was identical to the glycoprotein E sequence of UK strain herpes simplex virus type 1 (nucleotide position from 141,358 to 142,125).

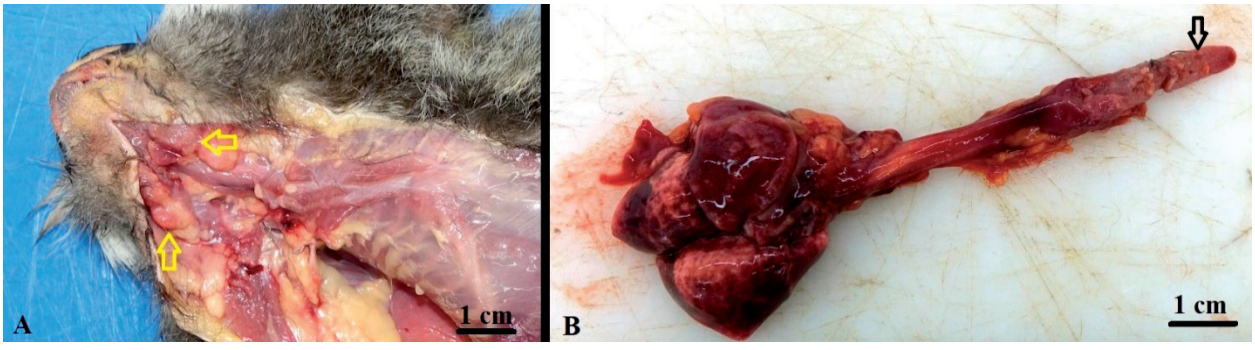

Fig. 2. Gross findings during necropsy

A) Submandibular lymph nodes were enlarged (yellow arrows). B) On the tongue were multifocal round ulcers (black arrow). The lungs were diffusely enlarged with heavy parenchyma due to pulmonary edema

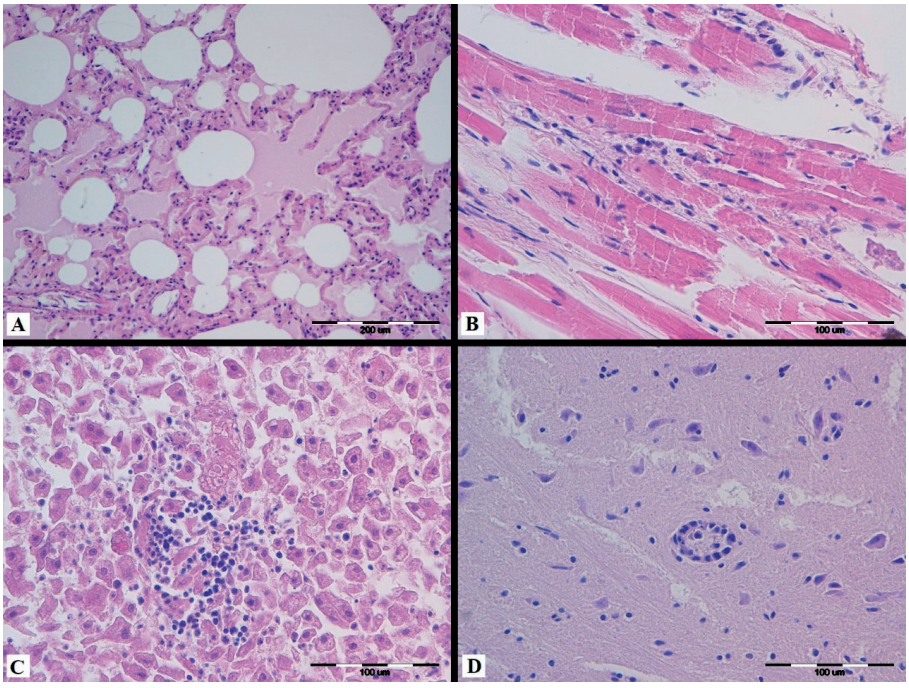

Fig. 3. Histological findings, hematoxylin and eosin stain

A) Protein-rich alveolar edema, objective magnification 20x. B) Necrosis of myocytes with accumulation of a low number of histiocytes, 40x. C) Mononuclear hepatitis with necrosis of single hepatocytes, 40x. D) Mononuclear vasculitis in the brain, 40x

Discussion

Herpesviruses belong to a large family (*Herpesviridae*) of double-stranded DNA viruses that can infect humans and a variety of animals. In general, they are well adapted to their natural hosts, in which they can cause lifelong latency after initial infection. Some of these herpesviruses have the potential to cross the species barrier, and in these cases, they are capable of causing a severe, often fatal disease in susceptible hosts. Sources of these viruses can be both monkeys and their owners. Concerns have been raised about the possible transmission of *Cercopithecine herpesvirus* 1 (CeHV-1) from pet monkeys to humans, in whom it can cause fatal encephalomyelitis (OSTROWSKI et al., 1998). On the other hand, infections of monkeys with herpes simplex, the most common herpesvirus in humans, have also been described (LUDLAGE and MANSFIELD, 2003; MÄTZ-RENSING and BLEYER, 2018).

Herpes simplex virus is divided into 2 types (HSV-1 and HSV-2). HSV type 1 mainly causes oral herpes, and is able to establish latency in sensory neurons and reactivate during periods of stress and immunosuppression (SCHIFFER and COREY, 2015; COHEN, 2020). Both human and non-human primates seem to be susceptible to HSV-1 infection. Infection in Old World primates (gorillas, chimpanzees, gibbons, bonobos, etc.) is mostly comparable to that in humans, with lesions that are localized to skin and mucous membranes (SMITH et al., 1969; EBERLE and HILLIARD, 1989; GILARDI et al., 2014). On the other hand, HSV-1 infection in New World monkeys cause very serious, lethal disease (HUEMER et al., 2002; MÄTZ-RENSING et al., 2003; SEKULIN et al., 2010; CASAGRANDE et al., 2014; IMURA et al., 2014).

HSV-a is transmitted via contact with sores or saliva. The few papers that report the cases of HSV-1

infection in marmosets also discuss possible modes of transmission, ultimately linking them to close contact with an owner or caretaker with active HSV-1 lesions (MÄTZ-RENSING et al., 2003; IMURA et al., 2014). Studies of HSV-1 transmission in humans show that the risk of transmission is highest during the clinical manifestation of the disease, but can also occur during the asymptomatic stage (RAMCHANDANI et al., 2016). As the owner of the monkey in this study indicated that he had had no any visible clinical changes in the previous year, it was assumed that transmission originated from an asymptomatic carrier.

The clinical course of HSV-1 infection in marmosets is variable. Erosions and ulcerations of the lips and oral cavity, followed by excessive salivation, anorexia, and depression are the most common clinical findings, and they also occurred in our case. Various neurological deficits in the form of vision loss, ataxia, paresis and seizures, have also been described in most diseased animals (LUDLAGE and MANSFIELD, 2003; MÄTZ-RENSING et al., 2003; SEKULIN et al., 2010; CASAGRANDE et al., 2014; IMURA et al., 2014; MÄTZ-RENSING and BLEYER, 2018). In our patient, severe tremor and tic-like symptoms, in the form of excessive blinking, were observed. Similar clinical findings were also described in one HSV-1 infected marmoset from Japan (IMURA et al., 2014). The presence of other clinical signs is inconsistent. The disease is accompanied by an unfavorable prognosis and lethal outcome, usually within 4 days after the onset of the first clinical signs (MÄTZ-RENSING and BLEYER, 2018).

In previous reports, histopathological findings, immunohistochemistry and molecular investigations describe various lesions and the distribution of HSV-1 over different sites including the skin, mucosae, liver, lungs, spleen and brain (HUEMER et al., 2002; CASAGRANDE et al., 2014; IMURA et al., 2014). In most of the studies multinucleated syncytial cells and intranuclear inclusions, particularly present at the borders of vesicles and ulcers, are described as typical findings (LUDLAGE and MANSFIELD, 2003). In our case, the lesions present on histology were consistent with systemic infection with a viral microorganism (WACHTMAN and MANSFIELD, 2012). Interestingly, typical intranuclear inclusions were not found in any organ, and definitive diagnosis was based on the PCR results and nucleotide sequences obtained.

Natural infection of marmosets with HSV-1 seems to be rare. However, the number of exotic pets (including monkeys) has been increasing recently (SEABOCH and CAHOON, 2021). People usually have a strong social and emotional attachment to their pets (ARCHER, 1997), but often are not well informed about their potential problems and diseases. As the number of people latently infected with HSV-1 is constantly very high, and people's contact with their pets is very close, an increasing number of HSV-1 infections in pet monkeys can be expected.

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SAŽETAK

Ženka običnog marmozeta (*Callithrix jacchus*) u dobi od 19 mjeseci dovedena je na Kliniku za zarazne bolesti Veterinarskog fakulteta Sveučilišta u Zagrebu zbog gubitka apetita i promijenjena ponašanja. Pri zaprimanju uočeni su neurološki znakovi, erozije i ulceracije sluznice usta, jezika i sluznično-kožnih spojeva na temelju čega je postavljena sumnja na infekciju herpesvirusom. Iako je odmah započeta etiološka i simptomatska terapija, životinja je već idući dan uginula. Nakon uginuća obavljena je obdukcija, a dobiveni makroskopski i patohistološki nalazi upućivali su na sistemsku virusnu infekciju. Konačna je dijagnoza postavljena na temelju rezultata molekularnih pretraga kojima je dokazana prisutnost humanog *herpes simplex* virusa tipa 1. Marmozeti su vrlo osjetljivi na infekciju *herpes simplex* virusom tipa 1 koji je široko rasprostranjen u ljudskoj populaciji. Kako bi se smanjio rizik od prijenosa infekcije i spriječila ova bolest, koja je za majmune većinom smrtonosna, kontakt HSV-1 inficiranih ljudi, bez obzira na to je li riječ o klinički manifestnoj ili latentnoj infekciji, i majmuna treba biti ograničen i popraćen odgovarajućim higijenskim mjerama.

Ključne riječi: obični marmozet; *Callithrix jacchus*; *herpes simplex* virus tip 1