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Ulcerative dermatitis caused by feline herpesvirus type 1 in a domestic cat

Dermatite ulcerativa causada por herpesvírus felino tipo 1 em um gato doméstico

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Abstract

A case of ulcerative dermatitis caused by feline herpesvirus type 1 (FeHV-1) in an adult male domestic shorthair cat is reported. The cat was rescued from the streets and presented with ulcerative lesions at the nasal planum and tongue in addition to a history of occasional sneezing. Thirty days after of the first clinical evaluation, the cat died as a result of acute myeloid leukemia. During necropsy, ulcerative lesions were found on the superior lip, the skin of the nasal planum, and at the periorbital region. Ulcerations were also noted on the tongue and hard palate. Histological examination revealed extensive epidermal necrosis, which involved the subjacent dermis and adnexal structures; the inflammatory infiltrate consisted of neutrophils, mast cells, and lymphocytes. Amphophilic intranuclear inclusion bodies were occasionally observed in intact epithelial cells. In the immunohistochemical evaluation, positive intracytoplasmic immunolabeling was detected in the sebaceous and follicular epithelial cells as well as in the bronchiolar epithelial cells. Samples of lymphoid tissue tested positive for the presence of feline leukemia virus and feline immunodeficiency virus by immunohistochemistry. Pulmonary tissue fragments were immunolabeled for feline calicivirus. Samples obtained from a cutaneous lesion were subjected to virus isolation in a cellular culture, which revealed the cytopathic effects characteristic of herpesvirus. FeHV-1 was detected in the samples by polymerase chain reaction.

Key words: Feline. Herpesvirus. Skin. Viral dermatitis. Immunohistochemistry. PCR. Virus isolation.

Resumo

Descreve-se um caso de dermatite ulcerativa causada por herpesvírus felino tipo 1 (FeHV-1), em um gato adulto, macho, sem raça definida. O gato foi resgatado da rua e apresentava uma lesão ulcerativa no plano nasal e língua, além de espirros esporádicos. Trinta dias após o primeiro atendimento, o gato morreu por leucemia mieloide aguda. Na necropsia, o lábio superior e a pele do plano nasal e periorbital apresentaram extensa lesão ulcerativa, além de ulcerações na língua e no palato duro. Histologicamente havia extensa necrose da epiderme, estendendo-se à derme subjacente e estruturas anexas, associada

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ao infiltrado inflamatório, constituído por neutrófilos, mastócitos e linfócitos. Observaram-se ainda, ocasionalmente, em células epiteliais intactas, corpúsculos de inclusão intranucleares anfofílicos. Na avaliação imuno-histoquímica anti-FeHV-1 observou-se imunomarcação positiva intracitoplasmática nas células epiteliais e nas células epiteliais bronquiolares. Amostras de tecido linfoide apresentaram imunomarcação para vírus da leucemia felina, vírus da imunodeficiência felina, além de marcação para calicivírus em fragmentos pulmonares. Fragmentos da lesão cutânea foram submetidos a isolamento viral em cultivo celular, onde foi observado efeito citopático característico de herpesvírus e a amostra foi positiva na PCR para FeHV-1.
Palavras-chave: Felino. Herpesvírus. Pele. Dermatite viral. Imuno-histoquímica. PCR. Isolamento viral.

Introduction

Feline herpesvirus 1 (FeHV-1) is an enveloped DNA virus belonging to the family *Herpesviridae*, subfamily *Alphaherpesvirinae*, and genus *Varicellovirus* (ICTV, 2015). It has a worldwide distribution and is endemic to the feline population (HENZEL et al., 2015). The prevalence of FeHV-1 infection varies according to the population density. Morbidity can reach 100%, especially in large populations of cats. Mortality is highest in newborns, young cats, and immunosuppressed animals (GASKELL; KNOWLES, 1989; GASKELL et al., 2007). FeHV-1 causes infections primarily of the upper respiratory tract, inducing necrotic lesions in epithelial cells. Dermatitis associated with FeHV-1 is an uncommon manifestation of the disease, characterized by erosions and ulcers of the skin in the facial region (GROSS et al., 2009a). This report describes a case of ulcerative dermatitis caused by FeHV-1, diagnosed by anatomopathological, immunohistochemical, virological, and molecular findings in a cat infected with feline leukemia virus (FeLV), feline immunodeficiency virus (FIV), and feline calicivirus (FCV).

An adult, male, domestic shorthair cat was rescued from the streets in Caxias do Sul, Rio Grande do Sul, Brazil. He was weak and had skin lesions of moderate severity on the nasal planum at the time of rescue as reported by the tutor. Thirteen days later, the cat was referred to the clinic for consultation. On physical examination, the cat was dehydrated with a fever and low body and muscle condition scores. Sporadic sneezing, hyporexia, bilateral purulent nasal discharge, and ulcerative lesions on the nasal planum and tongue were also noted. Twenty-four days after treatment was initiated, skin samples were collected from the nasal planum for histopathology. Extensive epidermal necrosis was observed, which involved the subjacent dermis and adnexal structures. The inflammatory infiltrate consisted primarily of intact and degenerate neutrophils, mast cells and lymphocytes. Fibrin was seen on the surface of the epidermis. In some intact epithelial cells located adjacent to the areas of necrosis, amphiphilic intranuclear inclusion bodies were observed. These were sometimes vitreous in appearance with marginalization of the nuclear chromatin.

Six days after collection of the cutaneous sample, the cat remained weak with no improvement in the skin lesions, and soon died. At necropsy, extensive ulcerative lesions with easily detachable crusts were noted on the upper lip, skin from the nasal planum and periorbital region (Figure 1.A). Multifocal ulcerations were observed at the tongue and hard palate (Figure 1.B). The submandibular lymph nodes were enlarged and congestion. Samples collected from multiple organs were fixed in 10% buffered formalin solution and then processed for routine histopathology. Sections of the lung, trachea, bone marrow, and skin from the nasal planum were submitted to immunohistochemistry (IHQ). Samples were also evaluated via the polymer method (MACH 4 Universal HRP-Polymer, Biocare Medical) was used for FeHV-1 and FCV. The LSAB-AP (streptavidin–biotin–

alkaline phosphatase, Dako) detecting method was used for FeLV and FIV. The samples were counterstained with Harris and Mayer hematoxylin. Positive controls were obtained from previously confirmed cases and were added simultaneously to the slides. Negative controls were created from tissue fragments, such as skin and bone marrow, incubated in phosphate-buffered saline instead of the primary antibody. The primary antibodies and immunohistochemical protocols used in this report are specified in Table 1.

Figure 1. Ulcerative dermatitis caused by FeHV-1 infection in a cat. A. Extensive ulcerative lesions on the upper lip, nasal planum, and periorbital region. B. Multifocal distribution of ulcers on the hard palate (arrow) and tongue (arrowhead). C. Intranuclear inclusion bodies in intact epithelial cells, ranging from a slightly basophilic, vitreous appearance with marginalization of the nuclear chromatin (arrowhead), to homogeneous and eosinophilic (arrow). HE, bar 60 µm. D. Immunohistochemistry anti-FeHV-1 with immunostaining in sebaceous and follicular epithelial cells. 3-amino-9-etilcarbazol (AEC), bar 70 µm.

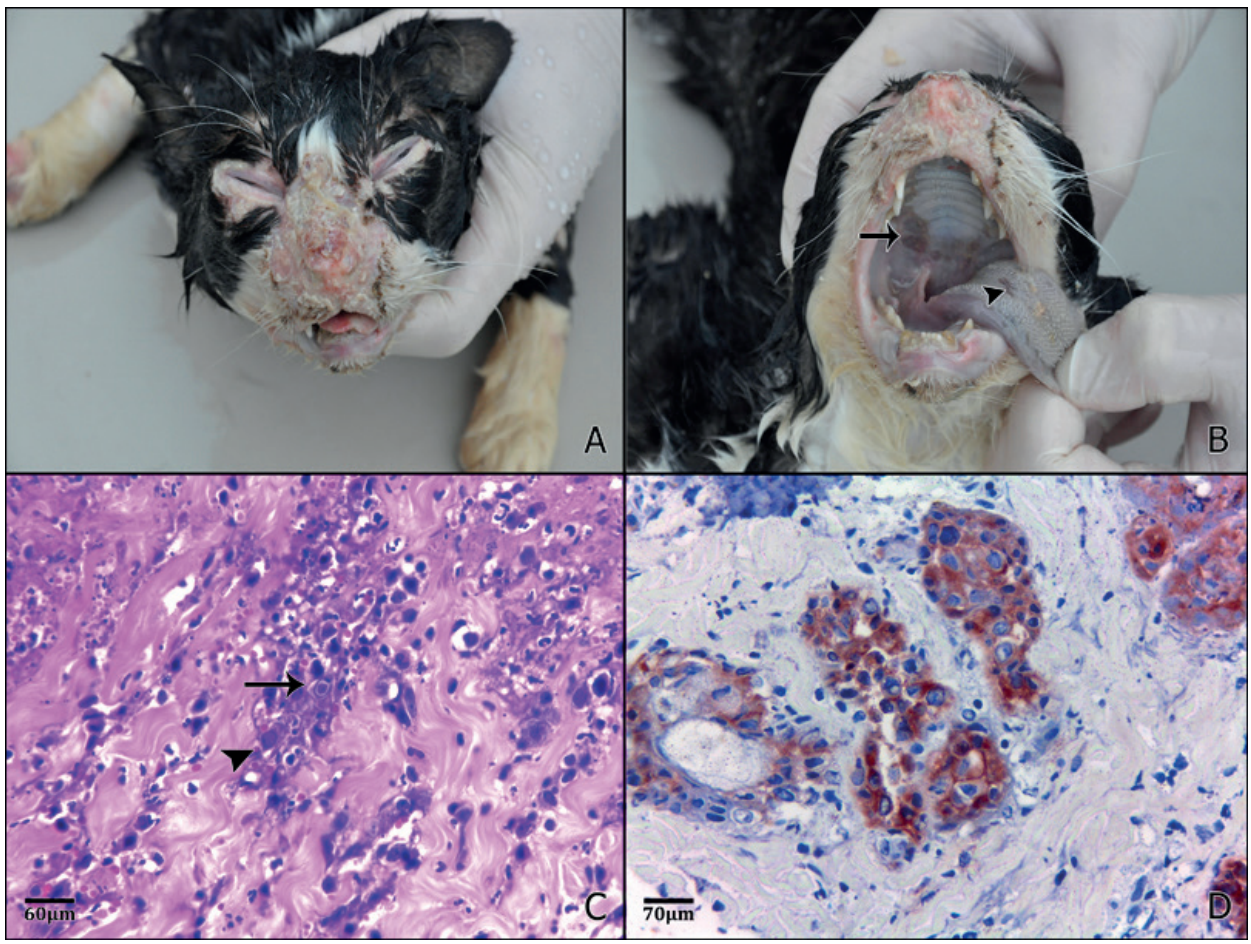

Table 1. The primary antibodies and immunohistochemical protocols used in this case report.

Antibody Monoclonal	Code	Antigenic Recovery	Dilution	Detection method	Chromogen
anti-FeHV-1 (FHV7-5)	FHV7-5[a]	10 min/25ºC Proteinase K[c]	1:100	MACH4[e]	AEC[c]
anti-FCV (FCV2-16)	FCV2-16[a]	10 min/37ºC Protease XIV[d]	1:50	MACH4	AEC
anti-FIV (p24 gag)	MCA 2278	40 min/100ºC, 0,01M, citrate buffer pH 6,0	1:100	LSAB-AP[c]	Permanent Red[c]
anti-FeLV (gp70)	MCA 1897	40 min/100ºC, buffer Tris-EDTA pH 9,0	1:500	LSAB-AP[c]	Permanent Red[c]

Sources of acquisition: [a]Custom Monoclonals International, [b]Serotec, [c]Dako, [d]Sigma, [e]Biocare Medical.

The histopathological findings from analysis of the skin in the nasal region were similar to those described in the biopsy (Figure 1.C); however, no intranuclear inclusion bodies were identified in the samples collected at necropsy. Glossitis, stomatitis, sinusitis, and fibrinonecrotic tracheitis were also observed. There was marked lymphoid depletion in the spleen and lymph nodes, particularly of the submandibular lymph node. The bone marrow was completely obliterated by marked proliferation of myeloid blast cells. Agglomerates of blast cells were also observed in the alveolar capillaries, and a diagnosis compatible with acute myeloid leukemia was established. Immunohistochemistry for FeHV-1 was strongly positive, observed in the cytoplasm of sebaceous and follicular epithelial cells adjacent to areas of skin necrosis (Figure 1.D) as well as in bronchiolar epithelial cells. There was intense immunolabeling for FeLV and FIV in the hematopoietic cells of the bone marrow. Furthermore, a mild immunolabelling for calicivirus was observed in alveolar macrophages from the lung.

Samples of skin from the nasal planum of the affected animal were subjected to viral isolation in cell culture and polymerase chain reaction (PCR). For viral isolation, approximately 10-50 mg of tissue was macerated, resuspended in minimum essential medium, and inoculated into a line of feline kidney cells (CRFK, ATCC CCL 94). After 48 hours, cytopathic effects characteristic of herpesvirus were observed, including cell rounding and lysis. The supernatant from the infected cells and a tissue fragment from an ulcerative lesion were subjected to total DNA extraction. For the FeHV-1 PCR assay, oligonucleotides were used for the glycoprotein E gene (gE), which amplify a 522 pb product (*forward* 5'-3'- ATGCCGATTGGACATCCAG, *reverse* 5'-3'- TCGTCGTTCGATGCGATAC). The PCR reaction was performed using approximately 100 ng of extracted DNA, 0.4 µM of each oligonucleotide, 2.5 mM $MgCl^2$, 10 mM dNTPs, 10% buffer, and 1 U of Taq DNA polymerase. Polymerase chain reaction products were visualized on 1% agarose gel, stained with GelRed® (Biotium, CA, EUA), and analyzed under ultraviolet light. For the PCR reactions, the FeHV-1 SV534/00 isolate was used as a positive control, and ultrapure water was used as the negative control.

The analyzed samples tested positive for FeHV-1 via PCR assay. The amplified product was purified using a commercial kit (PureLink PCR Purification kit, ThermoFisher Scientific, CA-USA) and subjected to nucleotide sequencing (ABI-PRISM 3100 GeneticAnalyzer). Sequences were analyzed by Software Staden (STADEN, 1996) to obtain the consensus sequence, which was deposited in GenBank (accession number KY688067), and that was compared to sequences deposited in GenBank (KR381801, KR381802, KR381803, KR296657,

GU250525, FJ478159, X98449, D4811), showing 99-100% nucleotide identity and 100% amino acid homology with FeHV-1 samples.

FeHV-1 is a pathogen commonly associated with acute and chronic rhinotracheitis, keratoconjunctivitis, and ulcerative stomatitis in cats (GASKELL et al., 2007). Dermatitis is a rare manifestation of FeHV-1 infection, with lesions predominantly found on the face that are characterized by varying degrees of erythema, edema, exudation, erosion, and ulceration (SÁNCHEZ et al., 2012), similar to that observed in this report. Histologically, the cutaneous lesions in cats with FeHV-1 infection are characterized as ulcerative dermatitis, with inflammatory infiltrates consisting primarily of eosinophils and neutrophils, but lymphocytes, macrophages, and occasionally mast cells are also seen (SÁNCHEZ et al., 2012). An additional histological finding facilitating the diagnosis of FeHV-1 infection is the identification of intranuclear inclusion bodies in epithelial cells. These inclusions are seen during the period of active viral replication, which occurs two to seven days after exposure, and are rarely detected after the seventh day of infection (GASKELL; KNOWLES, 1989). This may be the reason why inclusion bodies were detected only in the biopsy specimens and not in the skin samples collected at necropsy in this report.

After a primary infection, FeHV-1 may persist, especially in the trigeminal ganglion, and viral replication may be reactivated in immunosuppressed animals or during periods of stress (GASKELL; KNOWLES, 1989). Cats with FeHV-1-associated dermatitis may have a history of previous or concomitant respiratory disease, and immunosuppression due to prolonged use of glucocorticoids (SÁNCHEZ et al., 2012) or feline retrovirus infection (SUCHY et al., 2000). The factors that likely contributed to FeHV-1 infection in the present report were the viral status of the cat and the fact that he was found on the streets, which presupposes that he was exposed to several stressors and was not subjected to prophylactic measures, as well coinfection with FCV, FIV, and FeLV which presumably led to the death of the cat as a result of acute myeloid leukemia. Frequently, FeHV-1 infection occurs in combination with FCV and/or *Chlamydophila felis*, *Bordetella bronchiseptica*, *Mycoplasma* spp., and *Staphylococcus* spp. causing a respiratory syndrome (THIRY et al., 2009). The presence of ulcers in the oral cavity and/or on the tongue is a common finding with FCV infection (CASWELL; WILLIAMS, 2007); however, a distinction between FCV and FeHV-1 infection based on clinical signs is difficult (SYKES et al., 2001).

Differential diagnoses for ulcerative dermatitis caused by FeHV-1 are eosinophilic plaques, feline indolent ulcers, eosinophilic granulomas, and hypersensitivity to mosquito bites (GROSS et al., 2009a; SÁNCHEZ et al, 2012). The gross findings including ulcerated and crusting lesions on the skin can also be found with fungal dermatitis, such as cryptococcosis and sporotrichosis, as well as neoplasias including squamous cell carcinoma (GROSS et al., 2009b). Eosinophilic plaques exhibit more pronounced spongiosis and mucinosis in the dermis as well as intact superficial hair follicles. Indolent ulcers display eosinophilic degranulation within or just beneath the ulcerated skin. Ulcerative dermatitis caused by FeHV-1 may be indistinguishable from hypersensitivity to mosquito bites (GROSS et al., 2009a). Therefore, immunohistochemistry and PCR assays may assist in the diagnosis, especially when intranuclear inclusion bodies are not identified during histological examination. These are considered useful tools to differentiate herpesvirus-associated dermatitis from other causes of dermatitis (PERSICO et al., 2011). In addition, PCR exhibits high sensitivity and specificity in the detection of FeHV-1 in diseased animals and in those with low viral loads and latent infections, making it an extremely useful tool for diagnostic and research purposes (STILES; POGRANICHNIY, 2008). Virus isolation, as well

as the interpretation of a positive result obtained with PCR, combined with the analysis of clinical signs and histopathological findings, confirmed that the lesion on the nasal planum of the cat in this report was caused by FeHV-1 infection.

This report demonstrates the importance of including facial dermatitis caused by FeHV-1 as a differential diagnosis for facial lesions in this species, especially in those cats affected by viral coinfections. Histopathological evaluation of incisional biopsies has proven to be an efficient method for the diagnosis of this disease. Immunohistochemical staining may allow confirmation of the diagnosis and identification of concomitant infection by FIV, FeLV, and FCV.

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