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Case Report

Staphylococcus aureus-induced pyogranulomatous dermatitis, osteomyelitis, and meningitis with Splendore-Hoeppli reaction in a cat coinfected with the feline leukemia virus and *Leishmania* sp.

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Abstract

This report describes a case of a pyogranulomatous dermatitis, osteomyelitis, and meningitis with Splendore-Hoeppli reaction caused by *Staphylococcus aureus* in a sheltered female adult cat coinfected with the feline leukemia virus (FeLV) and *Leishmania* sp. The cat had a mild anemia and marked increased total leukocytes, particularly band and segmented neutrophils. The cat had laboratorial diagnosis of FeLV and *Leishmania* sp. infections. Clinically, the cat had extensive and multifocal areas of ulceration in cranial region. Due to the progression of cutaneous lesions, progressive weight loss, and the risk for other sheltered animals, the cat was euthanized. Microscopically, there was marked pyogranulomatous ulcerative dermatitis, osteomyelitis and meningitis, with multiple large intralesional colonies of Gram-positive cocci associated with Splendore-Hoeppli reaction. Aerobic bacterial isolates were identified as *S. aureus* by MALDI-ToF MS. *Leishmania* sp. DNA sequences were detected in liver and spleen, and amastigotes were demonstrated in skin sections by immunohistochemistry. In conclusion, here we describe a case of *S. aureus*-induced pyogranulomatous meningitis with SH reaction in a cat naturally coinfected with FeLV and *Leishmania* sp.

Key words: granuloma, meningitis, osteomyelitis, immunohistochemistry.

Introduction

Animal shelters are often associated with crowded housing conditions, where cats tend to be exposed to pathogens. In addition, stressful housing conditions may lead to increased susceptibility, favoring transmission of infectious diseases. Furthermore, other factors such as noise, odor, and sight of other cats or eventually dogs as well as conflicts may impair immunity favoring infections (19).

Staphylococcus is one of the most common genera of opportunistic bacteria in animals and humans. In mammals, Staphylococcus spp. are found primarily in the skin, mucous membrane of the nasal cavity, throat, and anus, as part of the microbiota (20, 30). In cats, *Staphylococcus aureus* is an opportunistic pathogen that has been identified as cause of skin and bone lesions associated with Splendore-Hoeppli (SH) reaction (8).

SH reaction is histologically characterized by accumulation of an eosinophilic radiating-shaped material around infectious (fungi, parasites and bacteria) and non-infectious agents (12). SH is usually associated with an inflammatory reaction that is rich in eosinophils, histiocytes, epithelioid macrophages, and multinucleated giant cells. The eosinophilic material in the SH may

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Table 1. Complete blood count (CBC) of the cat.

Parameter	**Results**	**Reference values** (18)
Erythrocytes	4.58 millions/µL	5.0-10,0 millions/µL
Hemoglobin	6.6 g/dL	8.0-15 g/dL
Hematocrit	23.80%	24-45%
Total leukocytes	27,360 / mm^3	5,500-19,500 / mm^3
Band neutrophils	2,189 /mm^3	0-300 / mm^3
Segmented neutrophils	20,794 /mm^3	2,500-12,500 / mm^3
Lymphocytes	3,010 /mm^3	1,500-7,000 / mm^3
Monocytes	1,368 /mm^3	0-850 / mm^3

correspond to the deposition of antigen-antibody complexes (immunoglobulins and major basic proteins) and host cell debris (15). SH has been seen in cases of bacterial pseudomycetoma, a chronic pyogranulomatous skin disease that is uncommon in animals (23). *Staphylococcus* sp., *Pseudomonas* sp., *Proteus* sp., *Streptococcus* sp., and *Actinobacillus* sp. are the agents most commonly isolated from those lesions (1, 23). Another important differential between bacterial agents is *Nocardia* sp., which may be identified by Ziehl-Neelsen and Grocott methenamine silver (GMS) stains (6).

Feline leukemia virus (FeLV) is one of the most common infectious agents in cats. FeLV is a retrovirus that has been associated with various clinical syndromes. FeLV is linked to one third of all tumor-related deaths in cats. Furthermore, FeLV is very often associated with anemia and secondary infections, which is due to FeLV-induced immunosuppression (10, 16). Therefore, coinfections in cats with FeLV are commonly diagnosed (10).

Domestic cats are susceptible to *Leishmania* sp. infection. Feline leishmaniosis is considered an emergent disease, with increasing number of reports over the last two decades (2, 21, 28). *Leishmania* infection in cats is often associated with multifocal skin nodules that may become ulcerated, with occasional spontaneous healing. Usually, the seroconversion is observed when the size of skin lesions is decreasing. Many domestic cats infected by *L. infantum* do not develop clinical signs, remaining asymptomatic (21). A few cases of coinfection of *Leishmania* sp. and FeLV have been reported (9, 24).

Considering that there are no previous reports of bacterial infection associated with SH reaction in the meninges of cats. Therefore, the goal of this report was to describe a case of pyogranulomatous dermatitis, myositis, osteomyelitis, and meningitis caused by *S. aureus* with SH reaction in a cat coinfected with FeLV and *Leishmania* sp.

Case report

A 1-year-old mixed bred female pregnant cat was rescued from a homeless condition and taken to a shelter in Belo Horizonte (State of Minas Gerais, Brazil). The shelter had a population of 50 cats and five dogs divided in two areas where the animals were distributed according to their friendly coexistence. This cat was in the middle of gestation and gave birth in the shelter. Six months after birth, the cat was submitted to ovarian-hysterectomy. Twenty months after the cat was admitted at the shelter, sheltered dogs and cats were screened for several diseases.

The cat had a history of persistent diarrhea. Thus, a test for direct detection of *Giardia* was performed (Giardia Ag VET FAST - Bioclin, Belo Horizonte, Brazil). The cat tested positive for *Giardia*, and was treated with benzoilmetronidazol, 15 mg/kg (oral route, twice a day, for five days). This treatment protocol was repeated 15 days later. After treatment, a complete blood count (CBC) was performed, indicating a mild hypochromic normocytic anemia, and marked increased total leukocytes, particularly band and segmented neutrophils. Monocyte count was also above reference values (Table 1).

Twenty two months after admission at the shelter, serum samples from this cat were tested for detection of antibodies against FIV and FeLV antigens using a commercially available kit (FIV Ac/ FeLV Ag Combo VET FAST kit - Bioclin) following the manufacturer's instructions. The cat was positive for FeLV and negative for FIV. Serum samples were also tested for detection of anti-*Leishmania infantum* IgG antibodies using the VETLISA Leishmaniose Felina IgG kit (Bioclin) as described by the manufacturer. The cat was strongly positive for *Leishmania infantum* IgG antibodies. One month later, due to a non-healing of cutaneous wound and progressive weight loss, and also considering the health risk for other sheltered

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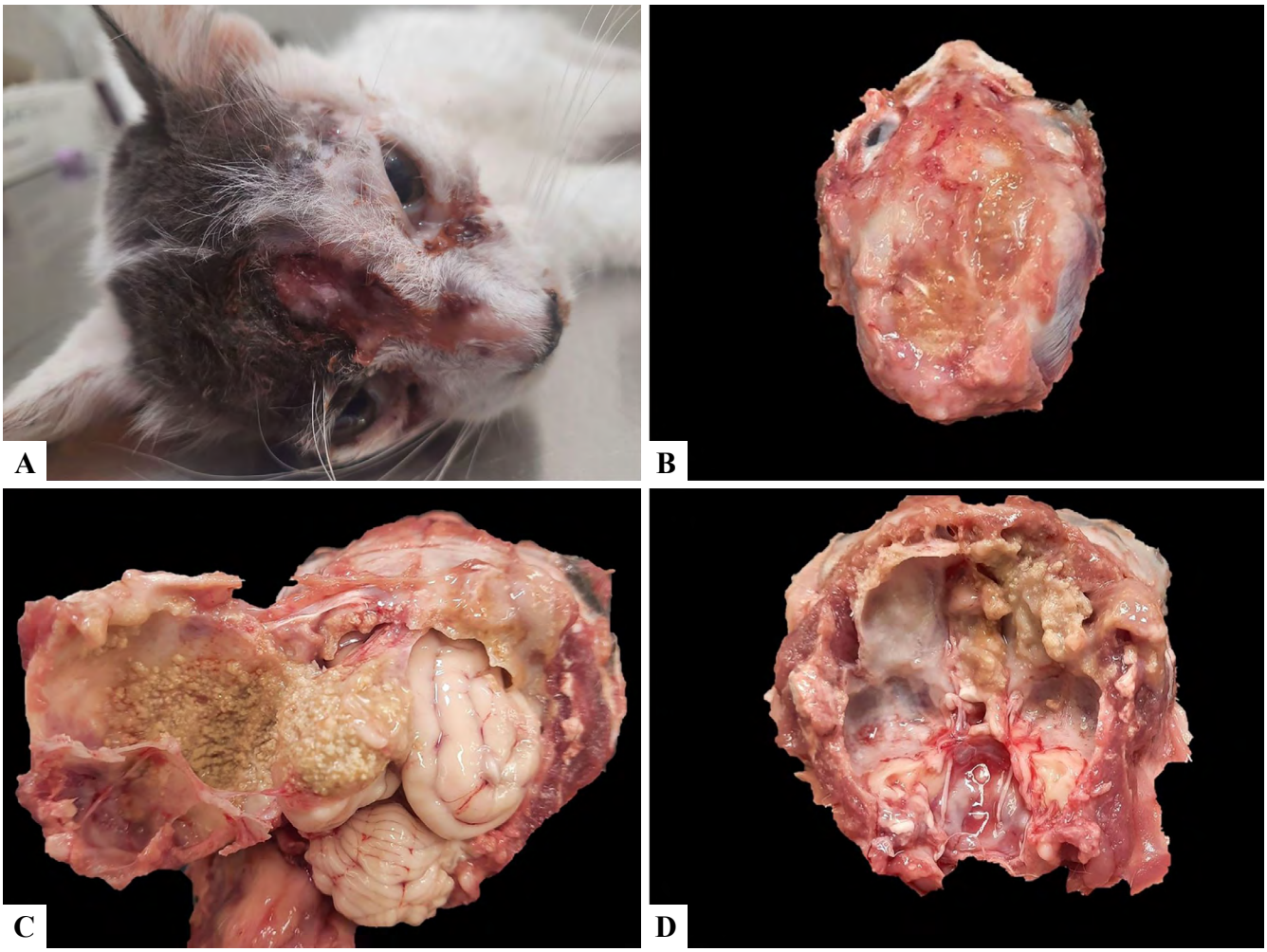

Figure 1. Ulcerative dermatitis, with myositis, osteomyelitis, and meningitis in a cat infected with *Staphylococcus aureus*, and coinfected with *Leishmania* sp. and FeLV. (A) Front lateral view of the head with marked multifocal to coalescent ulcerative lesion. (B) Abundant reddish-yellow viscous exudate in the subcutaneous tissues and adjacent muscles. (C) Focally extensive granulomatous meningitis. (D) Focally extensive area of osteomyelitis.

animals and volunteers, the cat was euthanized, and subjected to necropsy at the Universidade Federal de Minas Gerais (Belo Horizonte, Brazil).

Grossly, there were extensive, multifocal to coalescing profound cutaneous ulceration on the face (Fig. 1A). A reddish-yellow and viscous exudate was observed in the subcutaneous tissue and adjacent muscles, particularly the frontal muscle, also extending to the adjacent osseous tissues, affecting the frontal and parietal bones (Fig. 1B). While opening the skull, the frontal and parietal bones as well as the meninges had a focally extensive area with accumulation of a yellow friable exudate adhered to the meninges and to the surface of the frontal and parietal lobes mostly over the left cerebral hemisphere (Fig. 1C and 1D). The left pinna had mild multifocal cutaneous ulcerations. Additional gross findings included: moderately pale oral and ocular mucosae; moderate pulmonary edema; and severe *Dipylidium* sp. infection.

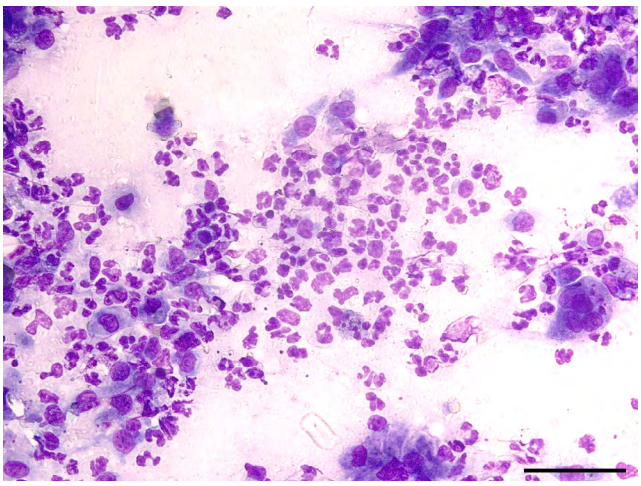

Figure 2. Cytology squash of the ulcerated area of skin showed large numbers of neutrophils, macrophages, and multinucleated giant cells. Rapid Panoptic. Bar = 50 µm.lesion.

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Cytology of skin lesions demonstrated large numbers of neutrophils, epithelioid and multinucleated macrophages (Fig. 2). Samples of skin, cranial bones, meninges, nervous system, lungs, liver, intestine, stomach, kidneys, and spleen were fixed in 10% buffered formalin, processed for paraffin embedding, sectioned in a microtome (4 µm-thick), and stained with hematoxylin and eosin (HE), Gram or Ziehl-Neelsen staining. Histopathology confirmed an infectious and inflammatory process that was characterized by extensive multifocal epidermal ulceration, with abundant neutrophilic infiltrate and cellular debris, and multifocal to coalescing pyogranulomatous inflammatory reaction in the dermis, with many neutrophils and macrophages. In the adjacent subcutaneous tissues, muscles, bone, and meninges there were multiple large bacterial colonies characterized by a granular basophilic material in the center, surrounded by an eosinophilic radiating material in the periphery, morphologically compatible with SH reaction, which was surrounded by numerous neutrophils, macrophages, and giant multinucleated cells (Fig. 3). Gram-stained sections demonstrated a monomorphic population of Gram-positive coccoid bacteria associated with the SH material in all affected tissues including the skin, muscle, bone, and meninges (Fig. 4). No acid-fast organisms were observed in Ziehl-Neelsen stained sections. In addition, the liver had mild random multifocal lymphoplasmacytic hepatitis. In the spleen, there was a moderate diffuse accumulation of plasma cells, with occasional Mott cells.

Samples of the skin and meninges were plated on Mueller Hinton agar (Kasvi, Italy) supplemented with 5% horse blood and MacConkey agar (Kasvi, Italy), and incubated at 37°C for 48 hours under aerobic or anaerobic

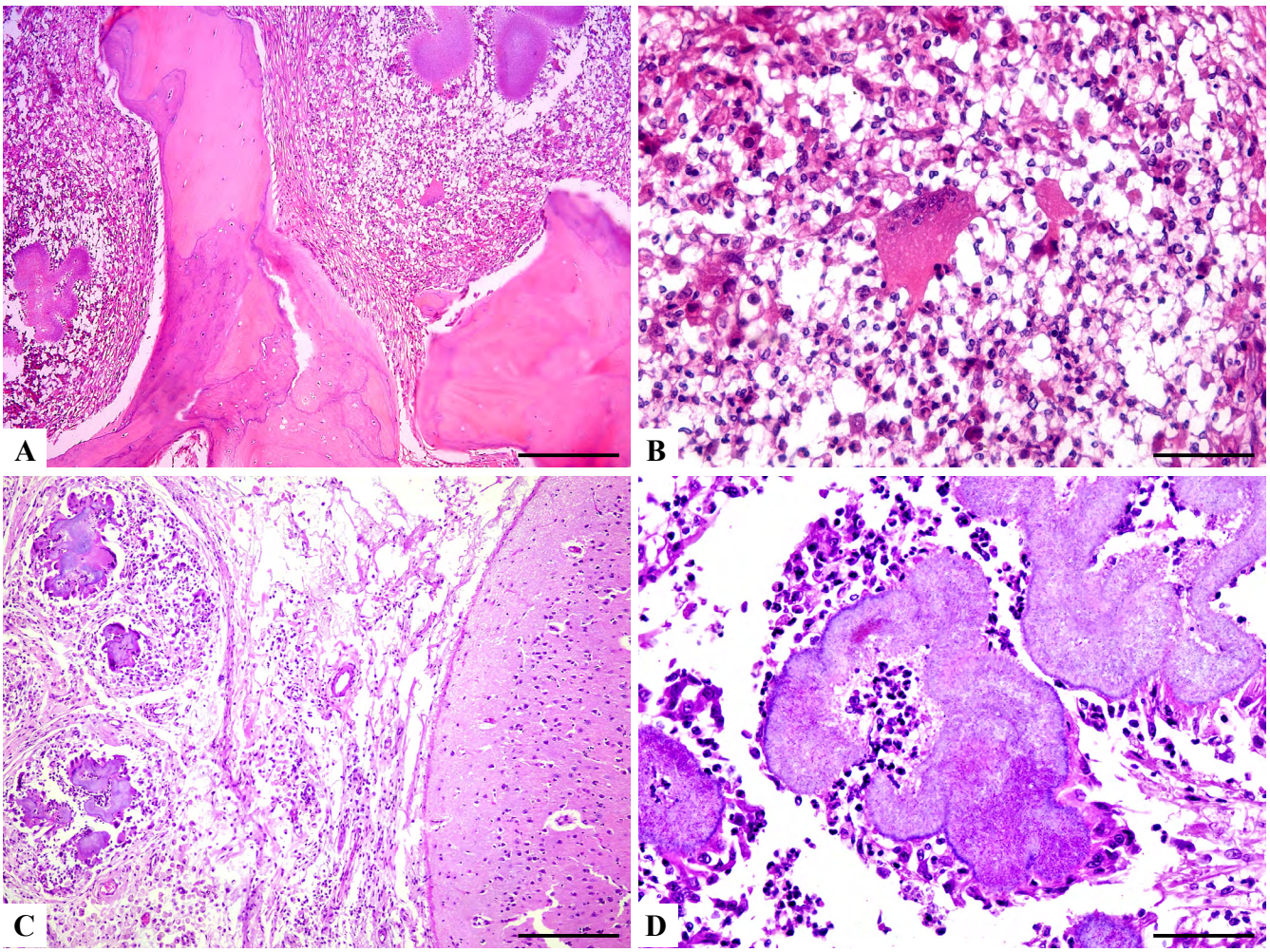

Figure 3. Pyogranulomatous osteomyelitis and meningitis associated to Splendore-Hoeppli in a cat infected with *Staphylococcus aureus*, and coinfected with *Leishmania* sp. and FeLV. (A) Bone; pyogranulomatous osteomyelitis in parietal bone with large multifocal bacterial colonies associated with Splendori-Hoeppli reaction. Hematoxylin and eosin (HE). Bar = 200 µm. (B) Bone; pyogranulomatous inflammatory reaction with neutrophils, numerous macrophages and multinucleated giant cells. HE. Bar = 100 µm. (C) Meninge and cerebral cortex; pyogranulomatous meningitis with focally extensive area of inflammatory infiltrate with large multifocal bacterial colonies associated with Splendore-Hoeppli reaction. HE. Bar = 200 µm. (D) Meninge; large bacterial colonies associated with Splendore-Hoeppli reaction and surrounded by neutrophils and macrophages. HE. Bar = 100 µm.

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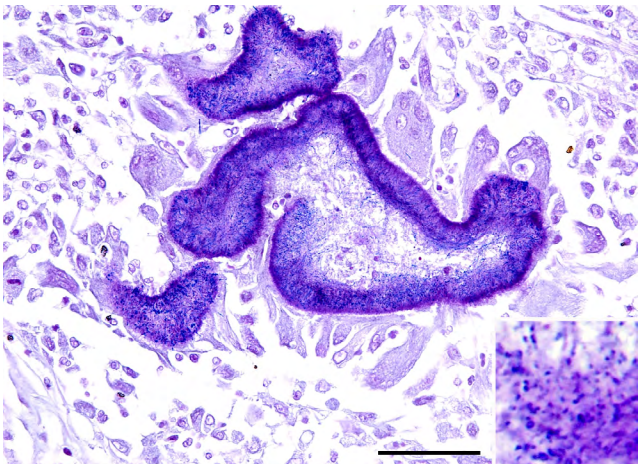

Figure 4. Pyogranulomatous meningitis associated to Splendore-Hoeppli in a cat infected with *Staphylococcus aureus*, and coinfected with *Leishmania* sp. and FeLV. Large colonies of Gram-positive bacteria. Inset: Gram-positive cocci. Gram. Bar = 50 µm.

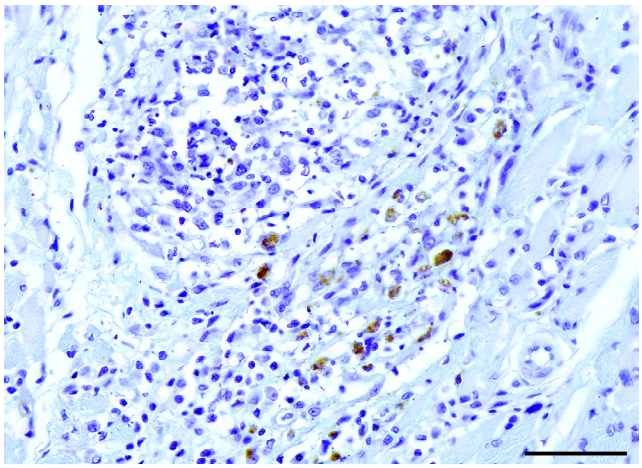

Figure 5. *Leishmania* sp.-associated mild multifocal granulomatous dermatitis. Mild multifocal inflammatory infiltrate composed of lymphocytes, plasma cells, and macrophages, with several immunolabeled *Leishmania* sp. amastigotes. Immunohistochemistry. Bar = 100 µm.

conditions. Isolates were characterized by matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-ToF MS, Bruker Daltonics, Germany), which demonstrated that the isolates had a profile compatible with *Staphylococcus aureus*.

Samples of the liver and spleen were stored at -20°C until DNA extraction, which was performed using the guanidine method as described by Boom et al. (1990) (5). PCR was performed according to Lachaud et al. (2002) (13) for amplification of *Leishmania* spp. (donovani complex) kinetoplast DNA, using the following primers: forward (5'-CTTTTCTGGTCCCGCGGGTAGG-3') and reverse (5'-CCACCTGGCCTATTTTACACCA-3'). PCR reactions contained 23 µL of PCR Supermix (Thermo Fisher Scientific, USA), 1 µL of each primer (25 mM), 2.0 µL of template DNA, and supplementation with 0.5 U of Taq DNA polymerase (Thermo Fisher Scientific, USA). Amplification parameters were 95°C for a 5 min, 40 cycles at 95°C for 1 min, 55°C for 1 min, 72°C for 1 min, and a 5 min final extension step at 72°C. Amplicons were resolved using a 1.5% agarose gel electrophoresis, which demonstrated specific amplicons with 145 bp specific for *Leishmania* sp. amplicons from template DNA samples extracted of the liver and spleen. Positive and negative controls were included in all reactions. No amplification was obtained from negative controls.

Although no amastigotes were observed in HE-stained sections, immunohistochemistry was performed on sections of skin and axillary lymph node as previously describe by Tafuri et al. (2004) (26). Briefly, endogenous peroxidase was blocked for 30 minutes with 6% hydrogen peroxidase solution. Sections were incubated overnight at 4°C with primary canine anti-*Leishmania* antiserum (1:1000 dilution). Then, sections were incubated for 40 minutes with a secondary antibody and detection system (Dako ENVISION). Diaminobenzidine (DAB) was used as a chromogen. Immunohistochemistry resulted in demonstration of small numbers of immunolabeled amastigotes within macrophages in the skin (Fig. 5), whereas there were no amastigotes in the section of the axillary lymph node.

Discussion

This report describes a unique case of coinfection with FeLV, *Leishmania* sp., and *S. aureus*, affecting an adult female cat, which ultimately developed pyogranulomatous meningitis as an extension of *S. aureus*-infected cutaneous lesions. Cats housed in animal shelters as in this case are usually subjected to stressful and overcrowded environments, which often have inappropriate sanitary conditions, with animals from diverse origins and different ages grouped together. These conditions may contribute to the increased rate of infectious diseases transmission within sheltered animals. Euthanasia in shelters is an extreme approach, which may be justifiable in cases of infections, particularly viral infections such as FeLV and FIV (3, 19, 22). Therefore, shelters may favor the circulation of transmissible diseases due to high population density, direct and indirect fomites, animal stress, and population turnover (22).

Staphylococcus aureus was identified as the cause of inflammatory lesions in the skin, muscle, bone, and meninges, which was further supported by the finding of a monomorphic population of coccoid Gram-positive intralesional bacteria associated with SH reaction. MALDI-TOF MS was employed for identification of the isolates. This technique provides reliable and rapid identification of several bacterial species including *Staphylococcus* spp. (4). Indeed, *Staphylococcus* spp. is one of the pathogens that may be associated with SH as well as other bacteria such as *Pseudomonas* sp., *Proteus* sp., *Streptococcus* sp., and *Actinobacillus* sp. (1, 23). *Staphylococcus* spp. may

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trigger a pyogranulomatous skin or visceral chronic lesion known as botryomycosis (from the Greek *botryo* meaning 'grapes') that affects human and animals (11, 14). Although classic, the term botryomycosis is inappropriate since this is not an actual mycosis. A previous report described SH reaction associated with *Streptococcus*-induced osteomyelitis in a cat (8). However, there are no previous reports of meningitis due to *S. aureus* infection with SH (features of botryomycosis) in a cat.

In this case, the cat was positive for FeLV, but was kept with many other sheltered cats. The exact time and route of exposure in this particular case remained undetermined so this cat may have been a source of FeLV infection to other cats. FeLV infection is associated with increased risk of neoplasia, cytopenia, and immunodeficiency in cases of the progressive form of the disease (10). FeLV transmission generally occurs via the oro-nasal route. FeLV is shed in large quantities in saliva, urine, milk, feces. However, FeLV is unstable in the environment so requires an intimate friendly or aggressive interaction between infected and naive cats (7, 16).

In this case the cat was diagnosed with leishmaniosis based on serologic, PCR, and immunohistochemistry results. Feline leishmaniosis has been described in endemic areas, such as the Americas and the Middle East (2, 21, 28). Leishmaniosis in cats is still poorly understood. Clinical signs are often not clear, but some cats have weight loss, alopecia, micro ulcerations in the pinna and local or generalized lymphadenomegaly. However, some infected cats are oligosymptomatic or asymptomatic, making diagnosis more difficult in this species (2, 21, 28, 29). The role played by cats in the epidemiology of leishmaniosis remains unknown. However, there is evidence that naturally infected cats can transmit L. infantum to sand flies (29). Interestingly, there is serologic evidence that wild feline species are susceptible to *Leishmania* sp. (27).

This report should raise awareness about the role and responsibility of shelters to protect animals, workers, adopters, and potentially immune compromised individuals in any of those groups (25). The cat in this report was in a shelter with other animals and shelter workers for a period of 10 months. Shelters play an invaluable role in protecting abandoned domestic animals, but the epidemiologic situation and sanitary risks must be properly assessed and monitored.

In conclusion, here we describe a case of *S. aureus*-induced pyogranulomatous meningitis with SH reaction in a cat naturally coinfected with FeLV and *Leishmania* sp.

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