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# Fungal diseases in dogs and cats in Northeastern Brazil[1]

Rodrigo C. Alves[2]*, Yanca G.S. Soares[2], Daniele F.L. Costa[2], Millena O. Firmino[3], João R.C. Brito Junior[2], Almir P. Souza[2], Glauco J.N. Galiza[2] and Antonio F.M. Dantas[2]

**ABSTRACT.-** Alves R.C., Soares Y.G.S., Costa D.F.L., Firmino M.O., Brito Junior J.R.C., Souza A.P., Galiza G.J.N. & Dantas A.F.M. 2023. **Fungal diseases in dogs and cats in Northeastern Brazil**. *Pesquisa Veterinária Brasileira 43:e07169, 2023*. Laboratório de Patologia Animal, Hospital Veterinário, Centro de Saúde e Tecnologia Rural, Universidade Federal de Campina Grande, Campus de Patos, Avenida Universitária s/n, Bairro Santa Cecília, Patos, PB 58708-110, Brazil. E-mail: rodrigo_cruz90@live.com

In this retrospective study, the clinical and anatomopathological findings of fungal diseases in dogs and cats in Northeastern Brazil are described. During the study period, 6,827 histopathological examinations (4,090 necropsies and 2,737 biopsies) of companion animals were reviewed, and fungal infections were diagnosed in 54 cases. Of these, 32 cases were diagnosed in cats and 22 in dogs. Sporotrichosis was the most prevalent mycosis (19/54), followed by dermatophytosis (9/54), cryptococcosis (8/54), candidiasis (7/54), and phaeohyphomycosis (4/54). Other diseases had two cases each, including aspergillosis, mucormycosis, and histoplasmosis, whereas coccidioidomycosis occurred once. Mycoses were diagnosed through microscopic lesions associated with the histomorphological characteristics of the agent, as evidenced by routine and special histochemical stains. They were occasionally confirmed by immunohistochemistry and microbiological cultivation. This retrospective study showed that despite being uncommon, fungal diseases are important causes of death in dogs and cats in the region, with cats being the most affected species and sporotrichosis being the most prevalent mycosis. Clinical signs were correlated with the portal of entry of the infection associated with localized or disseminated superficial and deep skin lesions or affecting parenchymal organs, especially in systemic infections. Microscopic lesions were mainly characterized by pyogranulomatous inflammation associated with fungal structures. In dogs, mycoses occurred mainly owing to an immunological impairment secondary to canine distemper virus co-infection.

INDEX TERMS: Fungal diseases, dogs, cats, company animals, immunosuppression, mycoses, special staining techniques, histomorphological characteristics.

**RESUMO.-** [**Doenças fúngicas em cães e gatos no Nordeste do Brasil.**] Neste estudo retrospectivo, são descritos os achados clínicos e anatomopatológicos das doenças fúngicas em cães e gatos no Nordeste do Brasil. Durante o período de estudo, foram revisados 6.827 exames histopatológicos (4.090 necropsias e 2.737 biópsias) de animais de companhia e diagnosticados 54 casos de infecções fúngicas. Destes, 32 casos foram diagnosticados em gatos e 22 em cães. A esporotricose foi a micose mais prevalente (19/54), seguida pela dermatofitose (9/54), criptococose (8/54), candidíase (7/54) e feo-hifomicose (4/54). Outras doenças tiveram dois casos cada, incluindo aspergilose, mucormicose e histoplasmose, enquanto a coccidioidomicose ocorreu uma vez. As micoses foram diagnosticadas por meio das lesões microscópicas associadas às características histomorfológicas do agente, evidenciadas pelas colorações de rotina e histoquímicas especiais, e ocasionalmente confirmadas pela imuno-histoquímica e cultivo microbiológico. Esse estudo retrospectivo demonstrou que apesar de incomuns, as doenças fúngicas são importantes causas de morte em cães e gatos da região, sendo os gatos a espécie mais afetada e a esporotricose, a micose mais prevalente. Os sinais clínicos estavam correlacionados com a porta de

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[2] Programa de Pós-Graduação em Ciência e Saúde Animal (PPGCSA), Universidade Federal de Campina Grande (UFCG), Av. Universitária s/n, Bairro Santa Cecília, Patos, PB 58708-110, Brazil. *Corresponding author: rodrigo_cruz90@live.com

[3] Instituto Federal do Sertão Pernambucano (IFSertãoPE), Rua Projetada s/n, Bairro Caetano II, Floresta, PE 56400-000, Brazil.

entrada da infecção, associados a lesões cutâneas localizadas ou disseminadas superficiais e profundas ou afetando órgãos parenquimatosos, especialmente em infecções sistêmicas. As lesões microscópicas eram caracterizadas principalmente por inflamação piogranulomatosa associada a estruturas fúngicas. Nos cães, as micoses ocorreram principalmente devido ao comprometimento imunológico secundária a coinfecção pelo vírus da cinomose canina.

TERMOS DE INDEXAÇÃO: Doenças fúngicas, cães, gatos, animais de companhia, imunossupressão, micoses, técnicas especiais de coloração, características histomorfológicas.

## INTRODUCTION

Several studies have highlighted the importance of fungal diseases in humans and animals in recent decades (Guarner & Brandt 2011, Seyedmousavi et al. 2018), especially in companion animals, wherein several fungal species are responsible for causing localized and systemic infections (Galiza et al. 2014). Eventually, mycoses culminate in the death of immunosuppressed individuals (Hargis & Myers 2017) and trigger sporadic disease in immunocompetent hosts (Mauldin & Peters-Kennedy 2016, Seyedmousavi et al. 2018).

In Brazil, despite the increase in fungal infections in dogs and cats in recent years, there is a scarcity of studies related to the characterization of mycoses in these species (Galiza et al. 2014, Castro et al. 2017). However, publications have reported the occurrence of mycoses in the Northeastern region, restricted to the description of a few isolated cases (Uchôa et al. 2012, Souto et al. 2016, Alves et al. 2021, 2022), case study compilations (Souto et al. 2018, Alves et al. 2020a, 2020b), and description of an outbreak (Silva et al. 2018). Therefore, the objective of this retrospective study was to determine the occurrence of fungal diseases in dogs and cats in the region and characterize the clinical and anatomopathological findings.

## MATERIALS AND METHODS

A retrospective study was performed from the records of necropsies and biopsies of dogs and cats at the "Laboratório de Patologia Animal" (Laboratory of Animal Pathology – LPA), University Veterinary Hospital Prof. Dr. Ivon Macêdo Tabosa (HVU) from the "Universidade Federal de Campina Grande" (UFCG), Campus de Patos, Paraíba, from January 2003 to December 2021. Cases of fungal diseases were selected, and the epidemiological data, clinical manifestations, macroscopic and microscopic changes, microbiological examination, and immunohistochemistry (IHC) were evaluated. Subsequently, the histological lesions of all cases were identified and reviewed.

The histological slides of each case were reviewed and, when necessary, new slides were prepared from paraffin blocks, cut into 4-5µm sections and stained with hematoxylin and eosin (HE). Different histochemical techniques were used for the histomorphological characterization of the agents, including staining with Grocott methenamine silver (GMS), periodic acid-Schiff (PAS), Alcian blue (AB), and Fontana-Masson (FM).

## RESULTS

From January 2003 to December 2021, 4,090 necropsies and 2,737 biopsies of dogs and cats were performed; of the 6,827 total samples, 54 (0.79%) cases of mycosis were diagnosed. A total of 4,775 samples of dogs were analyzed, including 2,657 necropsies and 2,118 biopsies, with 22 (0.46%) cases of fungal diseases. In the same period, 2,052 samples of cats were analyzed, with 1,433 necropsies and 619 biopsies, with 32 (1.56%) cases of fungal infections.

Among the diagnosed mycoses, sporotrichosis was the most frequent, followed in descending order by dermatophytosis, cryptococcosis, candidiasis, phaeohyphomycosis, aspergillosis, mucormycosis, histoplasmosis, and coccidioidomycosis. The fungal diseases diagnosed at the LPA/HVU/UFCG are shown in Table 1. Debilitating conditions and/or concomitant diseases in dogs and cats with fungal infections diagnosed at the LPA/HVU/UFCG are shown in Table 2.

### Sporotrichosis

Nineteen cases of sporotrichosis were diagnosed in cats (17 males and two females). Their ages ranged from one to 10 years, with an average of <24 months. Of these, 17 cats lived in a peridomiciliary environment with access to the street, and two were free-ranging. The animals had a history of skin lesions with a variable evolution of 7-90 days. In 11 cases, sporotrichosis was previously diagnosed by cytological examination. Approximately 15 cats died because of the severity of their injuries, and four animals did not respond satisfactorily to their treatment and were euthanized. In these cases, there was no information related to concomitant infections with feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV).

**Table 1. Diagnoses and number of cases of fungal diseases in dogs and cats performed at the LPA/HVU/UFCG from January 2003 to December 2021**

| Fungal diseases | Cats | Dogs | N Total | Necropsies | | Biopsies | | % Total |
|---|---|---|---|---|---|---|---|---|
| | N | N | | N | % | N | % | |
| Sporotrichosis | 19 | - | 19 | 19 | 35.19 | - | - | 35.19 |
| Dermatophytosis | 3 | 6 | 9 | 6 | 11.11 | 3 | 5.56 | 16.67 |
| Cryptococcosis | 6 | 2 | 8 | 6 | 11.11 | 2 | 3.71 | 14.82 |
| Candidiasis | 1 | 6 | 7 | 7 | 12.96 | - | - | 12.96 |
| Phaeohyphomycosis | - | 4 | 4 | 4 | 7.41 | - | - | 7.41 |
| Aspergillosis | 1 | 1 | 2 | 2 | 3.70 | - | - | 3.70 |
| Mucormycosis | - | 2 | 2 | 2 | 3.70 | - | - | 3.70 |
| Histoplasmosis | 2 | - | 2 | 1 | 1.85 | 1 | 1.85 | 3.70 |
| Coccidioidomycosis | - | 1 | 1 | 1 | 1.85 | - | - | 1.85 |
| TOTAL | 32 | 22 | 54 | 48 | 88.88 | 6 | 11.12 | 100 |

Macroscopically, all patients had skin lesions on the face, nasal plane, periocular region, eyelids, base and edges of the ears, neck, thoracic and pelvic limbs, and tail. The lesions consisted of masses, nodules, or reddish ulcerated plaques that were well-circumscribed, circular, and moist (Fig.1), partially covered by crusts. When cut, they were firm with a yellow-white and multilobed surface. In five cases, yellow-white masses were observed in the submandibular, retropharyngeal, superficial cervical, and axillary lymph nodes. Additionally, masses were observed in the tongue and lungs; in the meatus, with compression of the nasal conchae of the nasal cavity; and in the skin of the scrotum extending to the testis and epididymis. In three cases, similar skin lesions were reported in people who were scratched or bitten by the affected animals.

Histologically, the lesions were characterized by granulomatous or pyogranulomatous inflammation, multifocal to coalescing, marked associated with myriads of yeast-like fungi compatible with *Sporothrix* sp. In the skin, there was a marked thickening of the superficial and deep dermis by multifocal to coalescing areas of inflammatory infiltrate consisting of epithelioid macrophages and foamy macrophages surrounded by plasma cells and lymphocytes. Occasional neutrophils were observed around the blood vessels and adnexa. In the epidermis, ulcerated multifocal areas covered by a serocellular crust associated with discrete acanthosis were observed. The fungi were observed in the cytoplasm of macrophages (Fig.2) and dispersed in the tissue amid the inflammation. In the lymph nodes, the lesions were observed in the subcapsular and peritrabecular sinuses and extended to the medullary region, often with multifocal areas of necrosis in the center of the lymphoid follicles. In the nasal cavity, there was a marked pyogranulomatous inflammatory infiltrate associated with myriads of fungi, distending and replacing the submucosa with necrosis of the serous glands and hyaline cartilage, in addition to areas of ulceration of the respiratory and olfactory epithelium. Similar lesions were observed in the lungs, tongue, ependymal intraductal stroma, and around the seminiferous tubules. In HE-stained sections, the microorganisms were characterized by round to oval structures measuring 5-10µm in diameter with a slightly basophilic central nucleus surrounded by a clear halo. Yeasts were strongly stained pink and black by PAS (Fig.2, inset) and GMS staining, respectively.

**Dermatophytosis**

Dermatophytosis was diagnosed in nine cases, of which six were dogs aged three months to five years, and three were cats aged 3-5 years. Clinical signs included alopecic skin changes with lichenification, hyperkeratosis, easily epilable hairs, pustules, epidermal collarettes, and pruritus with an evolution of 4-120 days. Three dogs were co-infected with the canine distemper virus (CDV). Of these, one dog was diagnosed with systemic candidiasis and the other with hepatozoonosis. One cat had a notoedric mange on the face and an auricular pavilion.

Macroscopically, the skin lesions consisted of localized or generalized alopecia areas with a grayish irregular surface and raised edges associated with scaling (Fig.3), partially covered by crusts in the regions of the head, thorax, abdomen, back, thoracic, and pelvic limbs, and tail.

Histologically, the lesions were characterized by dermatitis and pyogranulomatous folliculitis associated with various fungi. In the superficial and deep dermis, there were multiple hair follicles with discreet to marked distension and occasional furunculosis. The follicles were filled with keratin debris and hairy stems, often fragmented, surrounded by numerous fungi (Fig.4) associated with an inflammatory infiltrate composed of neutrophils, lymphocytes, plasma cells, epithelioid macrophages, and rare multinucleated giant cells that occasionally infiltrated the panicle. In the epidermis, acanthosis and orthokeratotic or parakeratotic hyperkeratosis associated with serocellular crusts surrounded by a degenerated neutrophilic infiltrate, fungi, and occasional bacterial aggregates were evident. In five cases, the inflammatory infiltrate was discrete or absent. With HE staining, the microorganisms were characterized by rounded and basophilic structures (arthrospores) measuring 2-3µm in diameter that surrounded the hair shaft (ectothrix). Numerous negative tubular images with parallel walls and rare septate (hyphae) measuring 5-7µm in diameter were observed inside the hair shaft (endothrix). The fungi were stained intensely with GMS and PAS (Fig.4, inset).

A case of dermatophytic pseudomycetoma was diagnosed in a Persian cat that presented with a cutaneous nodule in the cervical region with a 5-month evolution. Macroscopically, there was a marked increase in the skin volume with multifocal ulcerated areas measuring 0.1-0.3cm in diameter. On sectioning, a marked and diffuse thickening of the dermis and subcutaneous tissue by a yellowish, multilobulated, granular, friable mass was observed (Fig.5). Microscopically, there was an expansion of the superficial and deep dermis by multiple pyogranulomas, which often coalesced and extended to the panicle. In the center of the pyogranulomas, aggregates of hyphae were observed (Fig.6) with thick, non-parallel septate walls, rare non-dichotomous irregular ramifications, and bulbous

**Table 2. Debilitating conditions and/or concomitant diseases in dogs and cats with fungal infections diagnosed at the LPA/HVU/UFCG from January 2003 to December 2021**

| Mycoses | Debilitating conditions and/or concomitant diseases (number of cases) | |
|---|---|---|
| | Dogs | Cats |
| Dermatophytosis | CDV (3) | Notoedric mange (1) |
| Candidiasis | CDV* (4)/Septicemia (1)/Senility (1) | Mycoplasmosis (1) |
| Phaeohyphomycosis | CDV* (2)/Hemoparasitosis (1)/Postpartum (1) | - |
| Aspergillosis | - | Dystocic delivery (1) |
| Mucormycosis | CDV* (1)/Immunosuppressive drugs (1) | - |
| Histoplasmosis | - | FeLV (1) |
| Coccidioidomycosis | Undifferentiated sarcoma (1) | - |

CDV = canine distemper virus, FeLV = feline leukemia virus.

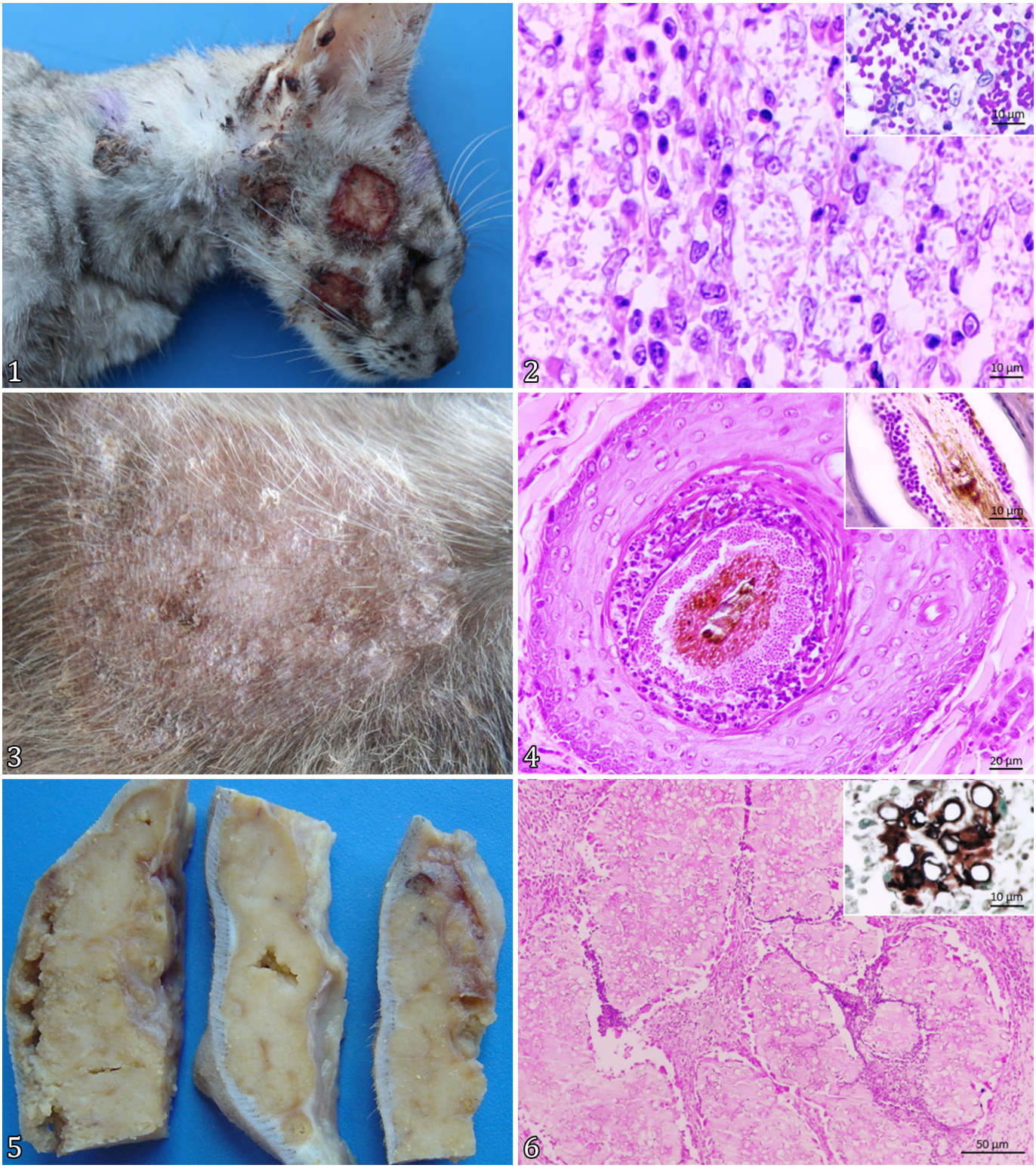


Fig.1-6. Fungal diseases in dogs and cats. (**1**-**2**) Sporotrichosis in a cat. (**1**) Skin of the face with multiple ulcerated plaques, well-circumscribed, reddened, and moist. (**2**) Skin with myriad *Sporothrix* sp. free in tissue and the cytoplasm of macrophages. HE, obj.100x. Inset: yeasts stained in pink. PAS, obj.100x. (**3**-**4**) Dermatophytosis in a dog. (**3**) Skin with a focal area of alopecia with a grayish, uneven surface and raised edges associated with scaling. (**4**) Skin. Hair follicle with multiple negative hyphae within the hair shaft surrounded by basophilic arthrospores associated with neutrophilic inflammatory infiltrate. HE, obj.40x. Inset: arthrospores and hyphae-stained pink. PAS, obj.100x. (**5**-**6**) Dermatophytic pseudomycetoma in a cat. (**5**) Skin of the cervical region. Cut surface with marked diffuse thickening of the dermis and subcutaneous tissue by a yellowish, multilobulated, granular and friable mass. (**6**) Skin. Dermis with multifocal to coalescing pyogranulomas associated with hyphal aggregates. HE, obj.20x. Inset: fungal structures intensely stained in black. GMS, obj.100x.

dilatations measuring up to 24μm in diameter, surrounded by amorphous and eosinophilic material (*Splendore-Hoeppli* reaction). The fungal structures were stained intensely black (Fig.6, inset) and pink with GMS and PAS, respectively.

**Cryptococcosis**

Eight cases of cryptococcosis were diagnosed; six were in cats aged between eight months to three years, and two were in dogs aged 1-8 years. The cats had ulcerated lesions on the nasal plane, lip, and face, with an increase in superficial lymph nodes, occasional symptoms of vomiting, anorexia, apathy, hypodipsia, and breathing difficulty, and a clinical course of 15 days to four months. The dogs had clinical signs, including shock, prostration, breathing difficulty, and nose bleeds. A cat and dog lived among pigeons.

Macroscopically, three cats had systemic involvement, and three other cats had localized skin infections that extended to the nasal region, oral cavity, and regional lymph nodes. The skin lesions were extensive, ulcerated, and depressed centrally with an irregular nodular surface that drained serosanguineous secretion on the face, mucocutaneous region of the upper lip, and the left nostril with the destruction of the nasal plane and facial deformity. Submandibular and retropharyngeal lymph nodes were enlarged in volume and were soft with a diffusely whitish surface when cut. In one case, the lungs were expanded and consolidated diffusely with an irregular yellow-white cut surface. In another case, the kidneys were diminished in size with a whitish, irregular subcapsular surface that was firm to the cut. Among the dogs, one had a disseminated infection, and another had lesions restricted to the lungs. In the lungs, multiple, irregular, reddish, discrete areas were observed on the pleural surface, which deepened on cutting. In the brain, thickening of the leptomeninges by translucent, gelatinous, and slightly granular material was observed in the third ventricle, cerebellum, and cerebral cortex with small irregular cavitations and compression of the thalamus and cortical gray matter (Fig.7).

Histologically, the lesions were characterized by granulomatous or pyogranulomatous inflammation, marked, chronic associated with yeasts that were morphologically compatible with *Cryptococcus* spp. (Fig.8). In the skin, the infiltrate distended the superficial and deep dermis, extending into the panicle and adjacent musculature. In the lymph nodes, the lesions were observed mainly in the subcapsular outer cortex with rare lymphoid aggregates, causing loss of the normal architecture of the organ. In the lungs, inflammation resulted in the thickening of the interalveolar septa and moderate congestion, edema, and rare thrombi. In the kidneys, there were areas of multifocal to coalescing fibrosis surrounded by interstitial pyogranulomatous infiltrate in the cortical and medullary regions. In the brain, the lesions were observed mainly in the leptomeninges of the cerebral cortex, cerebellum, pons, and obex, in addition to the third and fourth ventricles. Fungi were observed to be especially free in the parenchyma and rarely in the cytoplasm of multinucleated giant cells. In HE-stained sections, they were characterized by round to oval structures with basophilic central cells surrounded by a clear halo measuring 5-25μm in diameter, presenting with a "soap bubbles" appearance, often in simple budding. With AB, the capsule was strongly stained blue with a radiated aspect (Fig.8, inset). The structures were stained black and brown, respectively, with GMS and FM, mainly the wall and cell. The cells were stained pink using PAS. The budding cells were best visualized using GMS and PAS stains.

**Candidiasis**

Candidiasis was diagnosed in seven cases, six in dogs aged between four months to 14 years and one in a 5-month-old cat. Clinical manifestations predominantly included neurological signs accompanied by non-specific changes, with a clinical course of 5-30 days. Systemic involvement was observed in four dogs co-infected with CDV and one cat infected with *Mycoplasma* sp. treated with doxycycline for approximately 21 days. Two other dogs had localized infections affecting their brain and stomach and associated with senility and bacterial septicemia.

Macroscopically, the lesions were characterized by yellowish nodules or yellowish-white areas, often surrounded by reddish edges that deepened when we cut into the heart, lungs, kidneys, adrenal gland, liver, submandibular, mediastinal, tracheobronchial, and mesenteric lymph nodes. In one case, a whitish, lumpy material adhered to the epicardial surface, aortic artery, pericardial sac, and spleen, and infarctions were observed in the right testis, hydroureter, pyelonephritis, and hydronephrosis. Another patient had an increase in the right carpometacarpal joint, with reddish and cloudy content. In the brain, there was asymmetry of the telencephalic hemispheres with yellowish, blackened, reddish, and friable areas and partial loss of distinction between gray and white matter.

Microscopically, the lesions in four cases were characterized by necrosuppurative inflammation, multifocal to coalescing, marked, acute associated with hemorrhage, vasculitis, congestion, thrombosis, infarction, and pleomorphic fungi, especially in clusters in the areas of necrosis, thrombi, meninges, and wall and lumen of blood vessels. They were occasionally free in the parenchyma of the heart, lungs, liver, kidneys, left ureter, aorta, pericardial sac, spleen, right testis, and brain. In two other cases, the lesions were characterized by granulomatous or pyogranulomatous and necrotizing inflammation, multifocal to coalescing, marked, chronic associated with vasculitis and fibrinoid necrosis in the walls of blood vessels and occasionally associated with fungal organisms. In one of these cases, the lesions affected the brain, adrenal gland, and submandibular, mediastinal, tracheobronchial, and mesenteric lymph nodes. In another case, the lesions were restricted to the brain. In the case of gastric infection, there was a discrete focal area of mucosal ulceration without inflammation. The fungal structures predominantly had basophilic walls and were occasionally observed as negative images in HE-stained sections. The fungi were intensely stained by GMS and PAS.

Yeasts, pseudohyphae, and hyphae were identified in lesions. Yeasts were characterized by rounded structures measuring 3-8μm in diameter, often in budding. The pseudohyphae had elongated yeasts chains with prominent segmental constrictions measuring 6-10μm in diameter. The hyphae had parallel walls, rarely septate with angular ramifications, and germ tube measuring 4-15μm in diameter. With IHC, six cases were positive for anti-*Candida albicans* polyclonal antibody. Five of these cases have been previously described by Souto et al. (2016) and Alves et al. (2020a).

**Phaeohyphomycosis**

Four cases of phaeohyphomycosis were diagnosed in dogs aged four months to 8 years. Clinical signs included fatigue, ascites, apathy, hyporexia, weight loss, polydipsia, skin lesions with crusts in various regions of the body, hyperkeratosis of the cushions, pale mucous membranes, enlarged superficial lymph nodes, ocular secretion, lateral recumbency, vocalization, myoclonus, diarrhea, and increased volume in the distal portion of the radius and ulna of the right thoracic limb. Clinical evolution was reported in only two cases, between 13 and 30 days. Two animals were infected with CDV; one had pulmonary and cerebral amoebiasis concomitantly. Another dog was being treated for hemoparasitosis, and one bitch had recently given birth.

Macroscopically, the lesions were characterized as multifocal to coalescing, nodular, blackened, or yellowish, rounded areas raised to the surface (Fig.9), occasionally with a depressed greenish center and raised blackened edges measuring 0.2-1.5cm in diameter. On cutting, they deepened into the parenchyma with formations of cystic cavitations filled with a yellowish and pasty content, delimited by a whitish capsule. The affected organs included the liver, spleen, hepatic lymph nodes, kidneys, and testicles. In the brain, blackened, grayish, or brownish multifocal areas were observed on the cortical surface and the cut, associated with asymmetry of the cerebral hemisphere with compression of the lateral ventricle. In one case, *Cladophialophora bantiana* was isolated.

Histologically, two cases had granulomatous and necrotizing inflammation, multifocal to coalescing, marked, chronic, associated with numerous dematiaceous fungi (Fig.10), especially in areas of necrosis in the lumen and walls of blood vessels, associated with fibrin thrombi and vasculitis, and often phagocytosed by giant cells in the liver, spleen, hepatic lymph nodes, kidneys, testes, and brain. In two cases, the lesions were characterized by lymphoplasmacytic and necrotizing inflammation, multifocal, moderate, and chronic, associated with multiple fungi present in the vascular lumen with thrombus formation and the areas of necrosis. One case was restricted to the brain, and the other to the liver.

In HE-stained sections, the fungi presented as tubular structures pigmented brown with thin walls, bulbous dilatations, rarely septate and sparsely branched, measuring 2-5μm in diameter (hyphae) and oval structures arranged in chains measuring 2-10μm in diameter (conidia). The fungi showed brownish pigmentation on unstained histological slides. With FM (Fig.10, inset) and GMS stains, the fungi were stained black and slightly pink, respectively, and were often pigmented brown with PAS stain. Two cases have been described by Uchôa et al. (2012) and Alves et al. (2022).

**Aspergillosis**

Two cases of aspergillosis were diagnosed: a 4-year-old dog and a 3-year-old cat. The dog had breathing difficulty, anorexia, vomiting, diarrhea, and blackened stools. The cat had a history of dystocic delivery and fetal mortality for at least 12 days and had undergone an ovariohysterectomy. The animal died within six days of the development of stump pyometra, peritonitis, and pancreatitis.

Macroscopically, multifocal areas of consolidation of the lung parenchyma were observed in the dog, associated with yellowish content in the lumen of the trachea and bronchi. The heart was globose with pale multifocal areas in the myocardium. In the cat, there was a moderate amount of semi-solid and yellowish purulent exudate in the frontal paranasal sinus.

Histologically, pyogranulomatous and necrotizing bronchopneumonia with hyphae were associated with acute myocardial infarction, and pyogranulomatous sinusitis, multifocal, marked and chronic, was associated with fungal structures. The fungi presented as parallel hyphae with weakly basophilic or negative walls and septate with dichotomous ramifications measuring 8μm in diameter also had structures with a vesicle covered by a biseriate layer composed of metulas and hyaline phialides with smooth walls that formed globose phyloconidia arranged radially (conidiophores), and they were positive with PAS and GMS staining. In the case of the dog, there was immunolabeled of the fungi with monoclonal antibody anti-*Aspergillus* spp. in IHC. This case was reported by Souto et al. (2016).

**Mucormycosis**

Two cases of infection by fungi of the order Mucorales were diagnosed in dogs aged 1 and 10 years. Clinical signs included respiratory, neurological, and gastrointestinal changes, with a clinical course of 3 and 19 days, respectively. One dog had a CDV infection, and the other was treated with intensive use of antimicrobials and glucocorticoids.

Macroscopically, one case had irregular, slightly yellowish or reddish, raised areas that deepened at the cut into the lung parenchyma, heart, and brain. In the other case, changes included ruptures of the stomach with borders and gastric mucosa covered by thick, whitish, friable material in addition to multiple extensive nodular areas and deep whitish-yellow cavities on the capsular surface of the liver.

Histologically, in case 1, extensive multifocal areas of necrosis were observed in the lungs, surrounded by a marked pyogranulomatous inflammatory infiltrate, hemorrhage, congestion, and moderate interalveolar edema, associated with multiple hyphae, vasculitis, and thrombosis in vessels containing hyphae. Similar lesions were observed in the heart and brain. In case 2, focally extensive necrosis was observed in the stomach mucosa that extended to the serosa (transmural) and was associated with numerous hyphae. In the liver, multifocal to coalescing areas showed necrosis surrounded by marked inflammatory infiltrates, similar to the stomach.

The hyphae had wide eosinophilic walls or presented as negative tubular images ranging from four to 20μm in diameter, rarely septate and tortuous, with irregular ramifications that occasionally formed dichotomous projections or 90° angles. The fungi were stained black and pink with GMS and PAS stains, respectively. In IHC, the hyphae were immunolabeled with anti-*Rhizopus arrhizus* monoclonal antibody. These cases have been described by Alves et al. (2020b).

**Histoplasmosis**

Two cases of histoplasmosis were diagnosed in adult cats with clinical changes including an increase in volume on the face, lymphadenopathy, epistaxis, gingival bleeding, dyspnea, apathy, fever, anorexia, halitosis, weight loss, and peritonitis, with an evolution of 120 and 150 days, respectively. One animal was co-infected with FeLV.

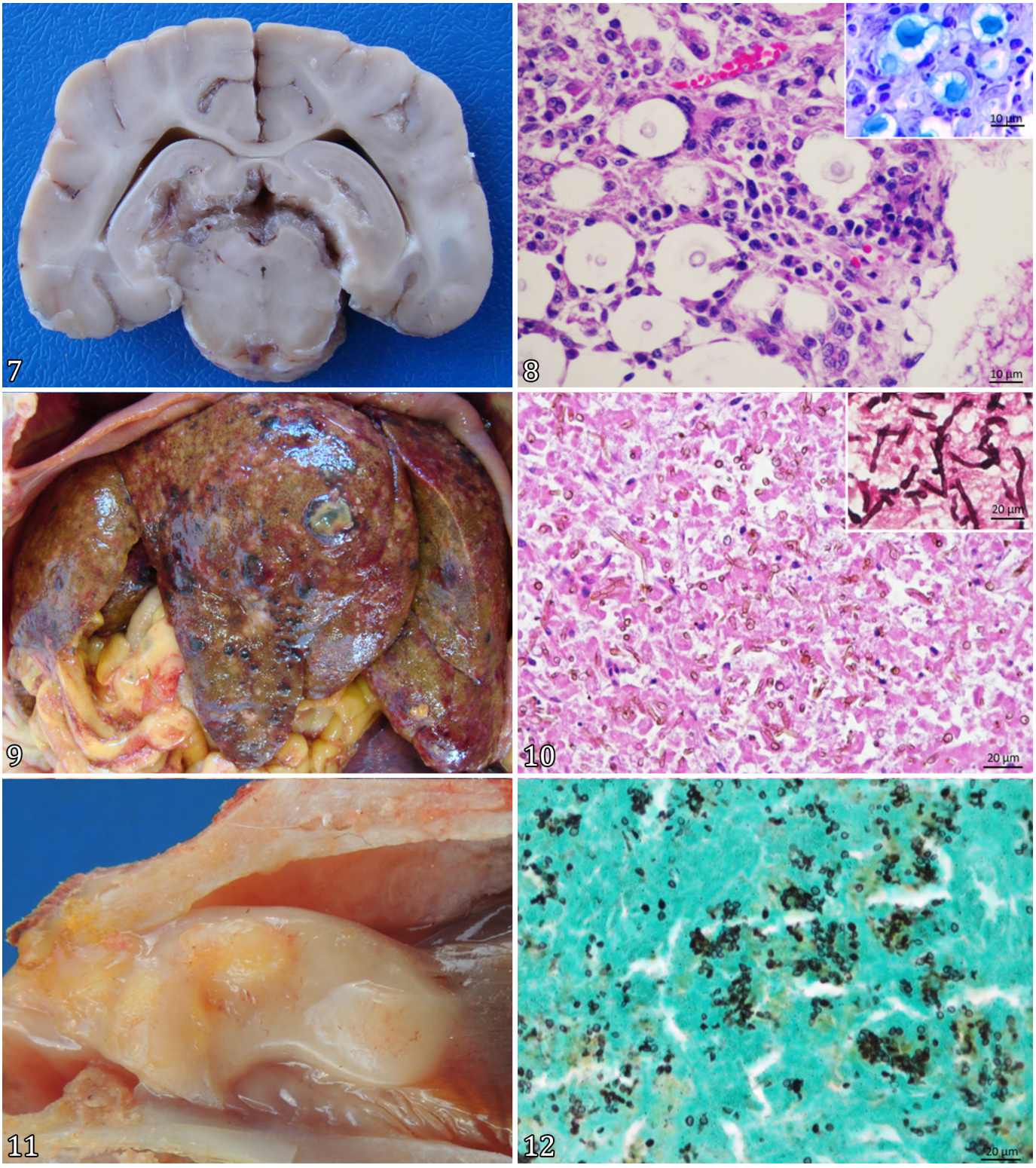


Fig.7-12. Fungal diseases in dogs and cats. (**7**-**8**) Cryptococcosis in a dog. (**7**) Cut surface of the brain with thickening of the leptomeninges by translucent, gelatinous, and slightly granular material in the third ventricle and parietal cortex, with small irregular cavitations and compression of the thalamus and cortical gray matter. (**8**) Brain. Meninges with yeasts *Cryptococcus* sp. in the cytoplasm of a multinucleated giant cell and dispersed in the tissue surrounded by an infiltrate of lymphocytes and plasma cells. HE, obj.100x. Inset: yeasts with the capsule strongly stained in blue with a radiated aspect. AB, obj.100x. (**9**-**10**) Phaeohyphomycosis in a dog. (**9**) Enlarged liver with multifocal to coalescing, nodular, blackened, and yellowish, rounded and raised areas on the capsular surface. (**10**) Liver with an extensive area of necrosis associated with numerous dematiaceous fungi. HE, obj.40x. Inset: fungi intensely stained in black. FM, obj.40x. (**11**-**12**) Histoplasmosis in a cat. (**11**) Sagittal section of the nasal cavity with extensive yellow-white thickening of the vestibule and left ventral nasal concha. (**12**) Nasal cavity with myriads of *Histoplasma* sp. stained in black. GMS, obj.40x.

Macroscopically, multiple nodules on the skin of the frontal and temporal regions of the face, nasal, and gingival mucosa, measuring up to 1.5cm in diameter, were observed. On cutting, they were yellowish, irregular, and firmly adhered to the adjacent tissue. After the sagittal section of the nasal cavity, yellow-white, focally extensive, bilateral thickening was observed in the vestibule extending to the ventral nasal concha (Fig.11). Similar lesions were observed in the submandibular, right axillary, and popliteal lymph nodes, with no distinction between the cortical and medullary regions. The lungs remained expanded, with soft, gray, punctate areas that were discreetly elevated to the pleural surface of the caudal lobes.

Histologically, the lesions were characterized by pyogranulomatous or lymphohistioplasmocytic and necrotizing inflammation, moderate to multifocal, marked associated with fungi morphologically compatible with *Histoplasma* sp. The inflammatory infiltrates distended the superficial and deep dermis, submucosa of the nasal cavity, interalveolar septa of the lungs, spleen, liver, bone marrow, and submandibular, superficial cervical, axillary, popliteal, and mesenteric lymph nodes. In HE-stained sections, the fungi had a small, slightly basophilic, oval shape surrounded by a clear halo measuring 2-4μm in diameter, especially in the cytoplasm of macrophages and amid the necrosis. The fungi were positive with the PAS and GMS stains (Fig.12).

**Coccidioidomycosis**

A case of coccidioidomycosis was diagnosed in a free-ranging adult dog, who presented with clinical signs including apathy, cachexia, anorexia, limited mobility with sternal recumbency, bilateral mucopurulent ocular discharge, dyspnea, pulmonary crepitation, erosive and nodular lesions on the skin, and swelling and stiffness of the left tibiotarsal joint. The dog was concomitantly diagnosed with undifferentiated sarcoma.

Macroscopically, multiple yellow-white nodules were observed in the superficial cervical lymph nodes, intercostal muscles, bronchi, lungs, heart, left tibiotarsal joint, and epididymis. Histologically, an inflammatory infiltrate formed by neutrophils, epithelioid and foamy macrophages, multinucleated giant cells, rare lymphocytes, and plasma cells (pyogranulomatous inflammation) was observed associated with fibrosis and numerous spherules of *Coccidioides* sp. in different stages of maturation. The spherules were stained with PAS and GMS. In IHC, the fungi were immunopositive to anti-*Coccidioides* sp. antibody. This case was reported by Alves et al. (2021).

## DISCUSSION

The diagnosis of fungal diseases was based on microscopic lesions associated mainly with the histomorphological characteristics of the fungal species, observed in routine staining and special histochemistry. In some cases, the etiologic agent was determined through additional examinations, including IHC and microbiological culture. The rare use of microbiological examination may be related to the receipt of samples already conditioned in formalin and sent for histopathological examination, where many mycoses are easily confused with neoplasms (Mauldin & Peters-Kennedy 2016), or because of non-specific changes that make it difficult to suspect a fungal infection during necropsy or biopsy, making it impossible to collect tissue fragments for fungal cultivation and isolation (Galiza et al. 2014, Castro et al. 2017). In this sense, we emphasize the importance of histochemical staining as an aid in diagnosing mycoses when it is not possible to use specific complementary examinations.

Fungal diseases occurred sporadically, mainly in cats, but also in dogs in the region, and most infections were diagnosed in animals subjected to necropsy, represented especially by cases of sporotrichosis. A low incidence of fungal infections was also observed in a survey of mycoses in cats, in which the diagnoses corresponded to 1.33% of necropsies and 2.25% of biopsies (Castro et al. 2017). In another study, the percentage of mycoses diagnosed in domestic animals represented approximately 0.63% and 0.59% of necropsies and biopsies, respectively, with companion animals being the most affected and represented by 92 of the 179 diagnosed cases (Galiza et al. 2014).

Sporotrichosis was the most prevalent disease in this study, with all cases diagnosed in necropsied cats, as observed in a study by Castro et al. (2017). However, mycosis was diagnosed in both biopsy and necropsy materials, representing approximately 57.63% and 29.41% of cases, respectively. These cases, specifically diagnosed through necropsies, were observed in animals with triggered complications that had progressed to death or led to euthanization. It was not possible to determine their viral status, and no other predisposing factors that may have triggered immunosuppression were identified. Infections by FIV and FeLV compromise the immune system of animals, which may favor the proliferation of the agent and severity of the clinical picture of mycosis (Lee et al. 2002, Pereira et al. 2005). Most infections in males verified in this study may be related to the greater access of animals to extradomiciliary environments, with frequent involvement in fights over females or territories (Gross et al. 2009, Schubach et al. 2015). Disseminated cutaneous infections observed in animals are the main clinical form of sporotrichosis (Bazzi et al. 2016). However, the fungus can move from the inoculation site of the dermis or subcutaneous tissue to the regional lymph nodes, characterizing the cutaneous-lymphatic form (Schubach et al. 2015), and rarely, the systemic or extracutaneous infection can occur (Schubach et al. 2002). The contamination of humans by affected animals, observed in some cases, proves to be a public health problem since cats are considered the main transmitters of human infection (Silva et al. 2012).

Dermatophytosis was the disease that most affected the skin of dogs in this study, and in most cases, it was diagnosed as an incidental necropsy finding. This mycosis was the most frequent in companion animals in a retrospective study of mycoses and pythiosis in domestic animals (Galiza et al. 2014). However, the infection was not diagnosed in a survey of mycotic diseases in cats, likely because it is a superficial cutaneous mycosis in which the clinical diagnosis is performed in most cases (Castro et al. 2017). Dermatophytic pseudomycetoma diagnosed in a Persian cat in this study is a rare, atypical form of dermatophytosis associated with *Microsporum canis* infection, with a predominance in Persian cats, wherein infections are associated with a hereditary predisposition (Mauldin & Peters-kennedy 2016, Hargis & Myers 2017). An accurate diagnosis is indispensable to control infections in animals and avoid contagion in humans because of the high zoonotic power of the disease (Galiza et al. 2014, Mauldin & Peters-kennedy 2016).

Cryptococcosis is an important fungal disease in companion animals (Pennisi et al. 2013), as observed in this study, standing out as the most frequent systemic mycotic disease in cats. The mucocutaneous lesion of the nostril with a commitment of the nose cartilage observed in one case characterizes the classic clinical form of the infection called the "clown nose" (Lappin 2015). In dogs, cryptococcosis manifests mainly as neurological and ocular signs (Quinn et al. 2011). In the brain, infection with *Cryptococcus* sp. may cause acquired hydrocephalus secondary to the thickening of the leptomeninges by granulomatous inflammation (Pavarini et al. 2007). However, the lower clinical evolution, in addition to the degree and distribution of the inflammatory reaction observed in the dog, was not significant to the point of obstructing the structures responsible for the drainage of cerebrospinal fluid into the venous circulation. Co-habitation of animals with domestic pigeons (*Columba livia*), as observed in at least two cases, is considered an important epidemiological factor for the infection of animals associated with the inhalation of fungal propagules present in bird droppings (Lima et al. 2015). It is likely that other animals also became infected through contact with a contaminated environment, as the fungus still has eucalyptus trees and decaying plant material as a natural habitat (Hagen & Boekhout 2010).

One study demonstrated that most cases of candidiasis in domestic animals were secondary to primary pathological processes (Galiza et al. 2014). Similarly, all cases of candidiasis in this study were considered secondary infections, with systemic mycosis being more frequent in dogs co-infected with CDV. In the host, the virus causes immunosuppression owing to the impairment of cellular and humoral immune functions, with loss of lymphocytes and leukopenia (Pressler 2015), making the animal susceptible to opportunistic infections (Frade et al. 2015, 2018, Alves et al. 2020a, 2020b). In the cat, prolonged antibiotic therapy favored fungal infection and the clinical triggering of the disease (Giuffrida 2016).

In cases of phaeohyphomycosis, the lesions mainly affected the brain, highlighting the isolated species *C. bantiana* as the main cause of severe brain infections due to its neurotropism, which can rarely cause disseminated infections (Martínez-Lamas et al. 2014, Alves et al. 2022). Among the clinical forms of aspergillosis in companion animals, nasal infection by *Aspergillus* sp., associated with sinusitis, has been the most frequent (Zhang et al. 2012, Barrs & Talbot 2014), according to the clinical signs of a cat observed in this study. However, pulmonary infection identified in dogs is infrequent (Adamama-Moraitou et al. 2011, Souto et al. 2016).

The systemic and gastrohepatic forms of infection by fungi of the order Mucorales seen in dogs in this study are considered extremely rare (Alves et al. 2020a), and they occurred secondary to CDV infection and intensive therapy with antimicrobials and glucocorticoids, which are immunosuppressive factors considered important for triggering them, and predisposing the occurrence of acute and progressive disease in affected animals (Grooters & Foil 2015). Histoplasmosis is one of the most prevalent mycoses in cats (Brömel & Sykes 2005), occurring mainly in immunosuppressed animals owing to FeLV co-infection (Davies & Troy 1996). In this retrospective study, we obtained only two cases of mycosis, with one animal diagnosed with a viral infection.

Infection by *Coccidioides* sp., observed in one dog in this study, is uncommon in Brazil (Alves et al. 2021) and is generally associated with hunting wild animals, especially in the semiarid region of Northeastern Brazil (Wanke et al. 1999, Bosco et al. 2016). Brazil is an endemic region for coccidioidomycosis because of the high incidence of cases in humans (Cordeiro et al. 2009). However, it is rarely diagnosed in animals.

## CONCLUSIONS

This retrospective study showed that despite being uncommon, fungal diseases are prominent causes of death in dogs and cats in the region, with cats being the most affected species and sporotrichosis being the most prevalent mycosis.

Clinical signs were correlated with the portal of entry of the infection, associated with localized or disseminated superficial and deep skin lesions or affecting parenchymal organs, especially in systemic infections. Microscopic lesions were mainly characterized by pyogranulomatous inflammation associated with fungal structures.

In dogs, mycoses occur mainly because of an immunological impairment secondary to canine distemper virus co-infection.

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