Cladosporium cladosporioides-complex infection in a mixed-breed dog

Cladosporium cladosporioides-complex infection in a mixed breed dog

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Abstract
A 3-year-old female mixed-breed dog was referred with a 2-day history of serious dyspnea, coughing, lethargy, anorexia, and a low-grade right anterior lameness. At presentation, the dog had an increased respiratory rate, dull heart and lung sounds, and cyanotic mucous membranes. It was hyperthermic and slightly dehydrated. Laboratory findings showed mild neutrophilia with a left shift, while serum biochemistry variables were in the normal range. However, urinalysis revealed mild proteinuria and rare erythrocytes and leukocytes on sediment examination. Thoracic radiographs showed a diffuse mixed interstitial and alveolar pattern with an air bronchogram, while appendicular radiographs showed a right humeral interrupted brush-like periosteal reaction. Thoracic ultrasonography revealed mediastinal lymph node enlargement. Cytology from a fine-needle aspirate of mediastinal lymph nodes revealed a pyogranulomatous lymphadenitis with numerous fungal hyphae. Culture on Sabouraud dextrose agar isolated dark fungal colonies with microscopic features consistent with Cladosporium spp. Sequencing of the internal transcribed spacer region identified the fungus as a species of the Cladosporium cladosporioides-complex.

KEYWORDS
Canine, lameness, lymphadenitis, phaeohyphomycosis, pneumonia

CASE PRESENTATION

A 3-year-old spayed female mixed-breed dog was referred to the Tibaldi Clinic Milan Italy with a 2-day history of serious dyspnea, coughing, lethargy, and anorexia. The dog had a low-grade right anterior lameness since being adopted 2 years earlier. Prior to referral, the dog was treated intermittently with different antibiotics for 2 months of coughing. On physical examination, the dog was thin with a body condition score (BCS) of 3/9. The dog had an increased respiratory rate, dull heart and lung sounds, and cyanotic mucous membranes. The dog was hyperthermic (40.5°C) and slightly dehydrated (5%). A CBC (ProCyte Dx Hematology Analyzer_ IDEXX, Westbrook, Maine, USA) showed a mildly regenerative mild microcytic, normochromic anemia (HCT 34.2%; RI 37.3%–61.7%, MCV 57.5 fl; RI 61.6–73.5 fL, HGB 12.6 g/dL; RI 13.1–20.5 g/dL, reticulocytes 67.2 9 109/L; RI <60 9 109/L), a mild neutrophilia (20.56 9 109/L; RI 5.3–16.5 9 109/L) with a mild left shift (band neutrophils 0.62 9 109/L; RI 0–0.3 9 109/L), and a mild monocytosis (1.68 9 109/L; RI 0–1 9 109/L). Serum biochemistry was unremarkable. Urinalysis revealed a mild proteinuria (1 + dipstick

method at a refractometric urine specific gravity [USG] 1.035; a urine protein-to-creatinine ratio 0.5), and RBCs and WBCs on sediment examination. Thoracic radiographs showed a diffuse mixed interstitial and alveolar pattern with an air bronchogram (Figure 3 A), and an ultrasonographic evaluation revealed mediastinal lymph node enlargement. Cytology from a fine-needle aspirate of a mediastinal lymph node revealed pyogranulomatous lymphadenitis with numerous fungal hyphae (Figure 1A, B). The hyphae were either free or phagocytized by macrophages and were 2–3 lm thick and sometimes branching. A fungal culture from another mediastinal lymph node aspirate on Sabouraud dextrose agar enriched with chloramphenicol promoted the growth of dark fungal colonies (Figure 2) with microscopic features consistent with Cladosporium spp. (conidia arranged in acropetal chains).

Genomic DNA was extracted from the cultured organism using a commercially available kit (Nucleo Spin Tissue, Macherey-Nagel, D€uren, Germany). To amplify the fungal internal transcribed spacer (ITS) region, PCR using primers ITS5 and ITS41 was performed. Sequencing, using primer ITS4, was performed by a commercial laboratory (Macrogen Europe, Amsterdam). The obtained sequence (GenBank accession number KY411696) was compared with other sequences in BLAST (basic local alignment search tool)2 showing high sequence similarities (99%) with Cladosporium cladosporioides.

The dog was subsequently hospitalized and treated with oxygen therapy through a nasal cannula and crystalloid infusions including itraconazole (10 mg/kg orally every 24 h) (Jannsen-Cilag, Latina, Italy) and ceftriaxone (20 mg/kg intravenously every 12 h) (TEVA ITALIA Srl, Milano, Italy) for 3 weeks, which was followed by daily itraconazole (5 mg/kg once daily) administrations for 4 months. This combination therapy led to complete remission of pneumonia (Figure 3B). Furthermore, the limping and the brush-like periosteal proliferations observed in earlier radiographs of the right anterior limb appeared more solid and regular than before the treatment (Figure 4A, B).

DISCUSSION

Cladosporium cladosporioides is a very common cosmopolitan, saprobic pigmented (dematiaceous) fungus.3 It is an endophytic or quiescent fungus4,5 that has been found in several substrates including air, soil, and textiles.6

The brown–black pigmentation of dematiaceous fungi is due to the presence of melanin in their conidia and hyphae. The presence of melanin allows one to differentiate them from other hyphomycetes (non-dematiaceous or hyaline fungi) that have similar morphology but that lack pigmentation.7–9 Infections caused by melanized fungi are also known as phaeohyphomycosis. Culture and molecular analyses are required for definitive identification.7,10,11 In the past, several distinct taxa were classified as C cladosporioides based on their similarity to the taxon described and illustrated elsewhere.6 Recently, the multilocus DNA sequence typing approach combined

with morphologic analyses12 revealed different taxa that are now called C cladosporioides-complex.3 In people, C cladosporioides occasionally infects lungs, eyes, and brain.13–16 In animals, Cladophialophora bantiana is the most frequently identified fungal agent in systemic phaeohyphomycosis.17–20 Dematiaceous fungi are reported as agents of ocular infections in dogs and cats21 and of dermatitis and panniculitis in dogs.22 Cladosporium cladosporioides has also been identified as the cause of granulomatous encephalitis and nephritis in a German Shepherd dog.8

Here, we report the case of a young dog with disseminated fungal disease involving lungs, lymph nodes, and likely the humerus caused by C cladosporioides-complex.

A bronchoalveolar lavage (BAL) would have been a very valuable diagnostic modality for assuring the etiology of the pulmonary pathology visualized in the thoracic radiographs, but the owner did not consent because of concerns for the considerable anesthesia risk in this dog.

The fungal species involved in this case was identified based on the morphologic and PCR analyses of cultures grown from mediastinal lymph node aspirates. C cladosporioides was recently shown to be a complex that contains several cryptic species, which are morphologically very similar3 and can only be distinguished exclusively by analysis of different DNA regions (ITS region, partial actin, and translational elongation factor 1-a gene sequences) and evaluation of subtle morphologic features (eg, surface ornamentation of conidia). The fungal isolate described in the present study can only be defined as belonging to the C cladosporioides-complex. The radiologic and clinical signs of the pneumonia and the mild neutrophilia with slight left shift and mild monocytosis rapidly returned to the baseline RI after 5 days of antimicrobial and antifungal therapy, suggesting that the neutrophilia and monocytosis were elicited by the fungal and bacterial bronchial pneumonia. The microcytic, normochromic anemia was probably related to the chronicity of the disease (anemia of inflammatory disease).23 The bone lesions that were suspected to be the cause of the lameness could not be definitively diagnosed as the cytology of an attempted fine-needle aspirate was nondiagnostic. However, the positive treatment response to itraconazole suggested that the fungal infection was directly or indirectly etiologic.

As no fungal hyphae were observed in the urinalysis and a urine culture was not performed, proteinuria diagnosed at admission was attributed to direct or indirect inflammation as it disappeared after therapy.

The pathogenesis of the systemic mycosis in this case remains obscure; assuming however that the most probable initiation route and infected tissue site was the respiratory tract.

Immunocompromised individuals are prone to systemic mycosis. 7,9 The history of the dog in this report had no evidence suggesting immune suppression, however an underlying immunodeficiency cannot be excluded.

Twenty-five months after initial presentation, the infection relapsed involving mesenteric lymph node, and the owner requested euthanasia.

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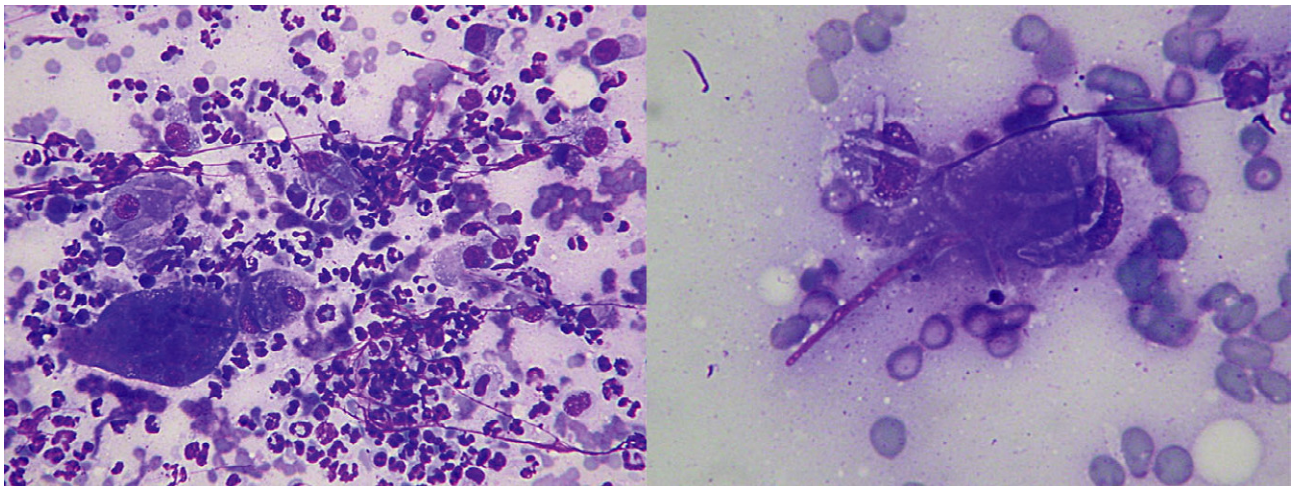
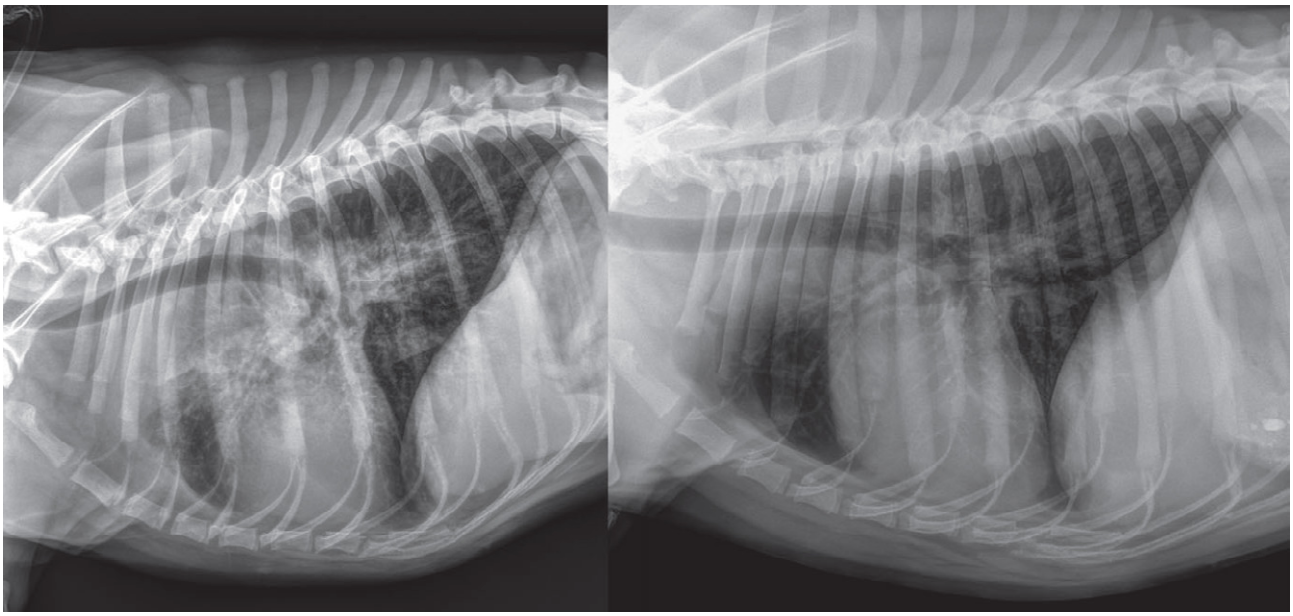
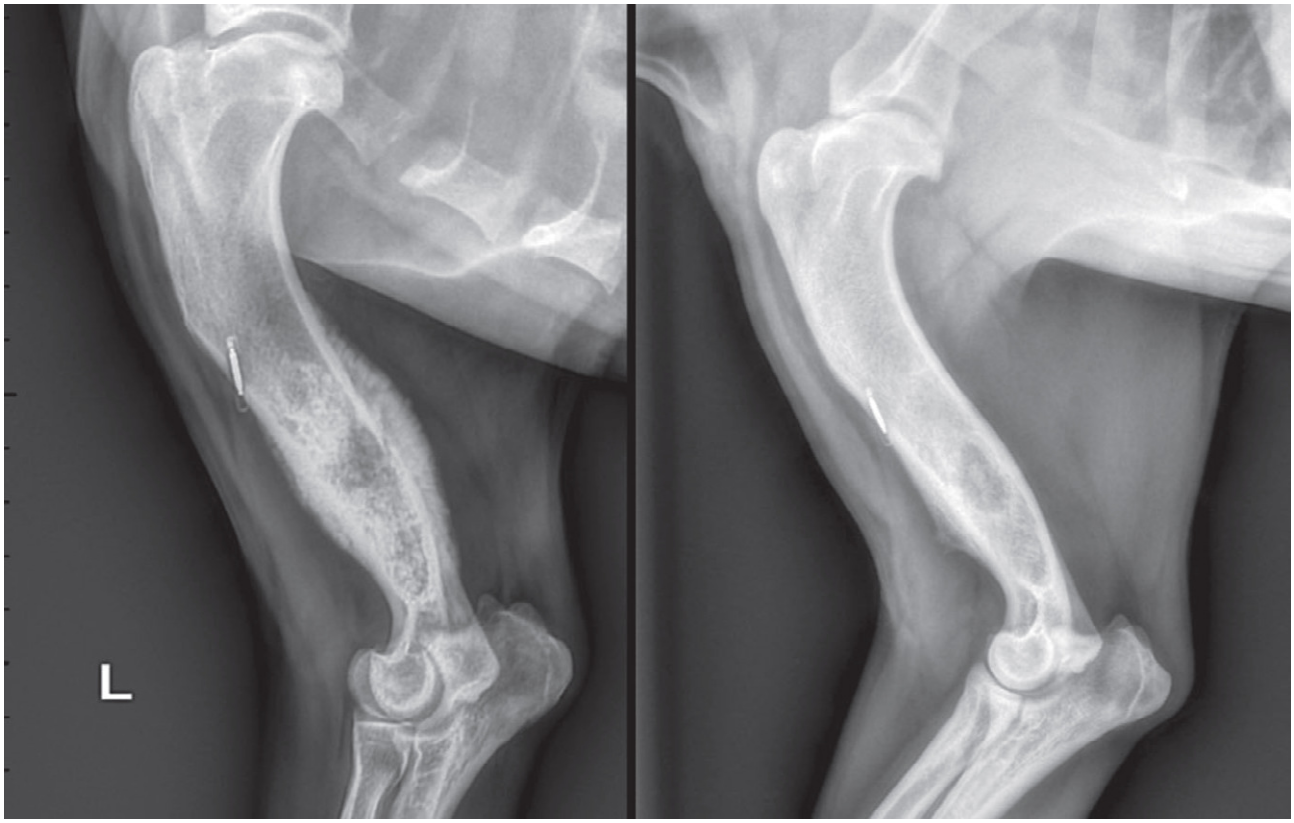


F IGURE 1 . Mediastinal lymph node aspirate cytology of a dog with Cladosporium cladosporioides-complex infection. There is a mixed pyogranulomatous inflammation with multinucleated macrophages and several fungal hyphae Diff-Quick stain. (A), x 40 objective, (B) x 100 objective.



F IGURE 3 . (A) Lateral thoracic radiographic image of a dog with Cladosporium cladosporioides-complex infection before (A) and 2 weeks after treatment (B). A diffuse mixed interstitial and alveolar pattern with an air bronchogram is evident which resolves after therapy



F IGURE 4 . (A) Lateral left-humeral radiographic image of a dog with Cladosporium cladosporioides-complex infection showing interrupted brush-like periosteal reaction in the right humerus causing clinical limping before (A) and resolving and appearing more solid and regular 2 months after therapy (B).