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Case Reports

Fungal myelitis caused by *Phialosimplex caninus* in an immunosuppressed dog

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A bone marrow infection caused by *Phialosimplex caninus* was diagnosed in a seven-year-old female spayed Cocker Spaniel that was receiving prednisone for autoimmune hemolytic anemia. Histopathologic examination of a bone marrow core biopsy revealed clusters of oval to round yeast-like cells of varying shape and size and occasional irregular hyphae. Culture of a bone marrow aspirate sample yielded a mould initially suggestive of *Paecilomyces inflatus* or *Sagenomella* species but later determined to be *P. caninus*. The dog was treated with itraconazole and amphotericin B, and prednisone was continued at the lowest dose needed to control the hemolytic anemia. The patient died after 18 months of treatment. This is the first detailed clinical report of infection caused by *P. caninus*, a newly described fungus associated with disseminated disease in dogs.

Keywords anemia, bone marrow biopsy, myelitis, *Phialosimplex caninus*, canine

Introduction

Aspergillus, *Paecilomyces*, *Penicillium* and *Geosmithia* are members of the ascomycete family Trichocomaceae and are among the most common moulds associated with opportunistic infections in dogs [1–7]. *Phialosimplex*, a phylogenetically-related genus, was described recently and contains two species, *Phialosimplex caninus* and *Phialosimplex chlamydosporus*, which are also associated with invasive canine infections [8]. All of these fungi are similar in producing conidia in chains from phialides but in contrast to the other genera, *Phialosimplex* species produce conidia from solitary phialides that may be slightly swollen centrally, and conidia are produced in both chains and clusters [8]. Prior to the description of *Phialosimplex*, isolates from cases of canine infection were reported incorrectly as being caused by *Paecilomyces*, *Sagenomella* or *Monocillium* species because each of these genera includes species forming solitary phialides [9–11]. The isolate in the present case was one of six (five canine and one human) that were included in the taxonomic study that led to the description of *P. caninus* and to reclassification of *Sagenomella chlamydospora* as *P. chlamydosporus* [8]. Because clinical details of the isolates were then unavailable, we report here the clinical history and unique aspects of histopathology of the isolate that was selected as the type strain of *P. caninus*.

Case report

A seven-year-old, 14 kg female spayed Cocker Spaniel was referred for evaluation of non-regenerative immune mediated hemolytic anemia. The anemia had been diagnosed by the referring veterinarian three and a half months prior based on a low hematocrit, spherocytosis and reticulocytosis. Antibody titers for *Ehrlichia canis* were negative, and a positive response to therapy with prednisone (1 mg/kg orally PO q12hrs) had initially been observed. At the time of referral, an acute decrease in the hematocrit and a reticulocyte count of less than 1% had been noted.

Upon presentation to Atlantic Veterinary Internal Medicine, physical examination revealed hepatosplenomegaly

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and superficial cervical and popliteal lymphadenomegaly. A complete blood count revealed normocytic, normochromic anemia (hematocrit, 22%; reference interval: 36–60%), neutrophilia (22,962/μl; reference interval: 2060–10,600/μl) with a left shift (band neutrophils 1335/μl; reference interval: 0–600/μl), and 3.6% corrected reticulocytes. Serum chemistry tests demonstrated an increased alkaline phosphatase (745 U/L; reference interval: 5–131 U/L) and results of urinalysis were unremarkable. Serum was negative for anti-*Bartonella vinsonii* antibody (< 1:16), and PCR assays for *Babesia* and *Ehrlichia* genera performed on whole blood were negative. Abdominal sonography revealed hepatomegaly with normal echogenicity of the hepatic parenchyma, and mild intra-abdominal lymphadenopathy. The left limb of the pancreas was heterogeneous and surrounded by hyperechoic fat. Fine needle aspiration and cytologic evaluation of mesenteric and popliteal lymph nodes revealed hyperplasia, with no evidence of neoplasia or infectious organisms.

Histologic examination of a bone marrow core revealed severe chronic granulomatous myelitis with a marked multifocal histiocytic inflammatory infiltrate, normocellular erythroid and myeloid series, normal precursor cell maturation sequence, and adequate megakaryocytes. A Gomori methenamine silver stained preparation revealed prominent clusters of oval to round yeast-like fungal organisms measuring 3–12 μm in diameter (Fig. 1). These cells produced one or more secondary cells suggestive of budding. Occasional fragments of irregular hyphae or pseudohyphae were also noted. Serum antibody titers for *Aspergillus*, *Blastomyces*, *Histoplasma*, and *Coccidioides*, as well as serum *Cryptococcus* antigen titers, were all negative. Six days later, a bone marrow aspiration was performed to obtain a sample for fungal culture. Cytologic evaluation of this specimen again revealed the presence of similar fungal organisms and the remaining sample was submitted to the Pythium Laboratory, Louisiana State University, for fungal culture. A urine specimen collected at the same time was negative for bacterial and fungal growth. Therapy with itraconazole (Sporanox, Janssen Pharmaceutica, Piscataway, NJ, USA) (5 mg/kg PO q24hr) was initiated, and prednisone administration (1 mg/kg orally PO q24hrs) was continued.

At the Pythium Laboratory, a hyaline mould was isolated on Sabouraud-dextrose agar after 48 h incubation at 37°C. Because a dimorphic pathogen was suspected based on the yeast-like cells that had been observed in tissue, the isolate was forwarded to the Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio, Texas (UTHSC), for further identification, and accessioned into their culture collection as UTHSC 03-1073. AccuProbe DNA probes (Gen-Probe, Inc., San Diego, CA, USA) for *Histoplasma capsulatum* and *Blastomyces dermatitidis* carried out on the isolate were negative. The isolate was subcultured onto potato flakes agar (PFA, prepared in-house) in culture tubes and a PFA slide culture incubated at 25°C for morphological examination. Additional tests included assessment of growth of the isolate at temperatures ranging from 25–40°C and on media containing cycloheximide. Colonies, after 10 days of incubation, were somewhat granular, white to cream with a similar reverse, slow growing with colony diameters less than 15 mm. Upon extended incubation a faint yellow diffusible pigment was evident and the reverse became pale brownish. The yellow diffusible pigment was more pronounced in tubes than on plates. The isolate grew less well at 40°C than at 25, 30, or 35°C. No growth occurred on media containing cycloheximide. Microscopically, globose conidia measuring 2–3 μm in diameter were borne in chains from somewhat inflated mono- and polyphialides. The morphologic characteristics were originally suggestive of *Paecilomyces inflatus* or *Sagenomella* species. The isolate was sent for further evaluation to the University of Alberta Microfungus Collection and Herbarium (UAMH), Edmonton, AB, USA, where it was accessioned as UAMH 10337 (= UTHSC 03-1073) and determined to be *P. caninus* following morphological and molecular comparison with related isolates using sequencing of the internal transcribed spacer region and the small subunit of the nuclear rRNA gene [8].

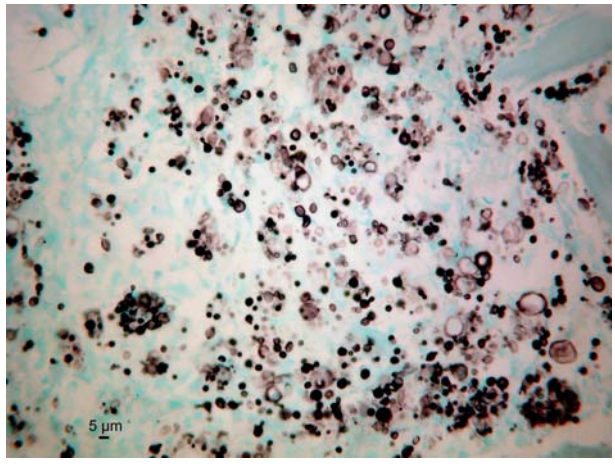

Fig. 1 Section from a bone marrow core biopsy stained with Gomori methenamine silver showing oval to round yeast-like fungal cells of varying size and rare fragments of irregular hyphae. Bar v 5 mm.

On day 16 after referral, treatment with amphotericin B lipid complex (Abelcet, The Liposome Company, Princeton, NJ, USA) (1 mg/ml solution in 5% dextrose, 2.4 mg/kg, administered IV over 90 min three times weekly) was initiated and itraconazole was discontinued. However after the sixth treatment, amphotericin had to be discontinued due to development of azotemia. Urine cultures were negative for bacterial and fungal growth. Itraconazole was restarted at 5 mg/kg PO q24hr and prednisone was decreased to 0.5 mg/kg/day. On days 40, 54 and 76 the hematocrit ranged from

23–27% (reference interval: 36–60%) and the creatinine and BUN were within reference intervals.

Reevaluation on day 105 revealed persistent normocytic, normochromic anemia with minimal regenerative response (1.08% corrected reticulocytes). Bone marrow aspirate cytology was normocellular with a normal erythroid maturation sequence and no evidence of fungal organisms. Fungal culture of the bone marrow sample yielded a mould that appeared morphologically similar to the initial isolate and therefore the isolate was not forwarded for further evaluation.

Antifungal susceptibility testing performed at the Fungus Testing Laboratory using the published reference method for testing filamentous fungi [NCCLS M38-A; 12], indicated minimum inhibitory concentrations (MICs) in μg/ml of 0.03, 0.004, and 0.5 for itraconazole, terbinafine and voriconazole, respectively. Although no defined breakpoints are available for this organism, the isolate appeared to be susceptible to these agents (by human pharmacokinetic standards) as MICs were within achievable serum concentrations of the drugs using standard dosing regimens. An MIC for fluconazole at >64 μg/ml suggested no *in vitro* activity by this compound.

Periodic reevaluation by the referring veterinarian over the next 11 months showed a stable hematocrit. Adding a second antifungal medication was discussed with the client but declined for financial reasons. Itraconazole was continued, and prednisone could not be tapered lower than 0.36 mg/kg orally every other day due to worsening of anemia. At reevaluation 440 days after referral, the dog continued to have a normal activity level, appetite and water intake. Hematocrit was 18.4% (reference interval: 36–60%). Urinalysis showed pyuria and bacteriuria, and urine culture was positive for *Escherichia coli*, which was treated with amoxicillin/clavulanic acid (Clavamox, Pfizer Animal Health, New York, NY, USA) (15 mg/kg PO q12hr). Thoracic radiographs were unremarkable, and systolic blood pressure was normal. Abdominal sonography showed that the entire spleen was effaced with a coarse, complex, heteroechoic architecture. Therapy with itraconazole and prednisone was continued, but at 18 months after referral, the dog died of unknown causes and necropsy was not performed.

Discussion

This is the first report to provide a detailed clinical history of a case of *P. caninus* infection. Among the five cases of *P. caninus* disseminated canine infections known to date [8], this is the only one with evidence of bone marrow infection. Details on the clinical course of infection for the other cases are not available, but one dog was a Vizsla and another was a German Shepherd. Three of the *P. caninus* isolates were obtained from lymph nodes and one from vertebrae. Similarly, discospondylitis in a German Shepherd dog has been caused by the related species *P. chlamydosporus* [9,10].

The role of immunosuppressive therapy in the pathogenesis of infection in this case is not known as it was not determined since infection was present prior to initiation of prednisone therapy. However, given the breed predisposition and initial hematologic characteristics, it seems most likely that the anemia was originally the result of immune-mediated hemolytic anemia, and that infection with *P. caninus* then occurred subsequent to the administration of immunosuppressive therapy. Although *P. caninus* has not yet been isolated from an environmental source, infection is presumed to result from soil or airborne exposure.

The unusual microscopic features, both in tissue and in culture, made the isolate in this case difficult to identify. The presence of numerous oval to round yeast-like cells in tissue and the isolation of a yellowish-white filamentous mould in culture initially led to concern that the organism was a dimorphic pathogen but that diagnosis was ruled out by negative reactions in the AccuProbe tests. The varying shape and size of the fungus and the production of one or more secondary cells suggests that *P. caninus* demonstrates a unique growth form in tissue. Moreover, infections caused by *P. caninus* appear to be characterized by presence of large numbers of pleomorphic forms. Similar observations were reported by Mackie *et al*. in a case in a Rottweiler of granulomatous lymphadenitis and splenitis that was tentatively attributed to *Monocillium indicum* but which was determined by Sigler *et al*. as a probable case of *P. caninus* infection [8,11]. Similarly, a fungus causing disseminated infection in a German shepherd dog was tentatively identified as *Candida* species by Gersehenson *et al*. [13] based on the morphology of yeast-like cells observed in hematoxylin and eosin stained sections, but the fungus was not grown in culture nor definitively identified from tissue by immunohistochemistry or PCR. The description and illustrations of numerous budding oval to round yeast-like cells measuring up to 10 μm in diameter and the invasion of the fungus into lymph nodes, thoracic vertebrae, spleen and other organs is highly suggestive of *P. caninus* infection as described in the present and previous reports and emphasizes the invasive potential of the new agent of canine systemic disease [8,11].

The cultural features that distinguish *P. caninus* include production of conidia from solitary phialides, development of conidia in both chains and clusters, formation of yellowish-white colonies on potato flake agar and production of diffusible yellow pigments (Fig. 2). The phialides are narrow, cylindrical to slightly swollen centrally, tapering at the tip to an indistinct collarette, and are typically monophialidic but sometimes polyphalidic (i.e. having a second opening) (Fig. 3). Conidia are single-celled, smooth, hyaline, and produced in long chains or in clusters. The conidia differ in shape with those borne in chains being subglobose and measuring 2.2–4 μm long by

Fig. 2 *Phialosimplexcaninus* (UAMH 10337) grown on potato dextrose agar at 25°C for 14 days showing yellowish-white colonies and yellow diffusible pigments.

1.8–3.7 µm wide and those in heads being obovoid and measuring 2–4.5 µm long by 1.5–3.2 µm wide.

In addition to providing new information about the clinical course of *P. caninus* canine infection, the case described here is important for other reasons. First, although systemic fungal infection in an immunocompromised host usually carries a poor prognosis, the dog in the present case survived 18 months from the time of diagnosis despite the fact that prednisone therapy had to be continued. It is also important to note that on the second bone marrow aspirate, fungal infection was detected by culture even though no organisms were noted cytologically, suggesting that submission of bone marrow samples for fungal culture should be considered even if no organisms are visualized cytologically or histologically. Second, the isolate appeared susceptible *in vitro* to itraconazole, suggesting that initial treatment with this drug may be useful when infection with *P. caninus* is suspected. However, despite the lack of fungal elements being seen in an aspirate collected during itraconazole therapy, *P. caninus* was presumably still recovered in culture, although not submitted for identification, suggesting suppression of the infection rather than eradication.

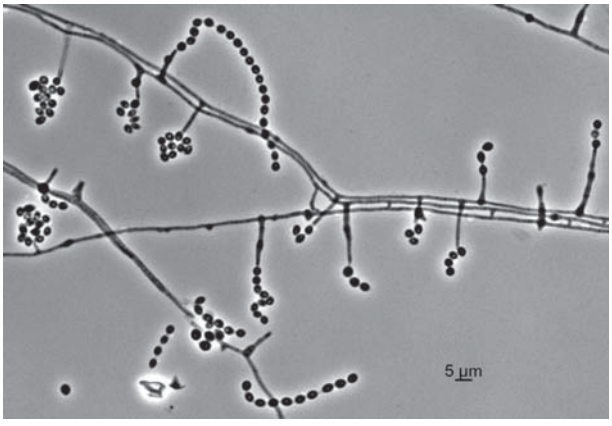

Fig. 3 Microscopic morphology of Phialosimplex caninus (UAMH 10337) showing production of conidia in chains and heads from narrow, simple, centrally swollen or cylindrical phialides. Bar v 5 mm**.**

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References

1 Krockenberger MB, Swinney G, Martin P, Rothwell TR, Malik R. Sequential opportunistic infections in two German Shepherd dogs. *Aust Vet J* 2011; **89**: 9–14.
2 Schultz RM, Johnson EG, Wisner ER, *et al*. Clinicopathologic and diagnostic imaging characteristics of systemic aspergillosis in 30 dogs. *J Vet Intern Med* 2008; **22**: 851–859.
3 Watt PR, Robins GM, Galloway AM, O'Boyle DA. Disseminated opportunistic fungal disease in dogs: 10 cases (1982–1990). *J Am Vet Med Assoc* 1995; **207**: 67–70.
4 Zanatta R, Miniscalco B, Guarro J, *et al*. A case of disseminated mycosis in a German shepherd dog due to *Penicillium purpurogenum*. *Med Mycol* 2006; **44**: 93–97.
5 Booth MJ, van der Lugt JJ, van Heerden A, Pickard JA. Temporary remission of disseminated paecilomycosis in a German shepherd dog treated with ketoconazole. *J S Afr Vet Assoc* 2001; **72**: 99–104.
6 Foley JE, Norris CR, Jang SS. Paecilomycosis in dogs and horses and a review of the literature. *J Vet Intern Med* 2002; **16**: 238–243.
7 Grant DC, Sutton DA, Sandberg CA, *et al*. Disseminated *Geosmithia argillacea* infection in a German Shepherd dog. *Med Mycol* 2009; **47**: 221–226.
8 Sigler, L., Sutton DA, Gibas CFC, *et al*. *Phialosimplex*, a new anamorphic genus associated with infections in dogs and having phylogenetic affinity to the Trichocomaceae. *Med Mycol* 2010; **48**: 335–345.
9 Garcia ME, Caballero J, Toni P, *et al*. Disseminated mycoses in a dog by *Paecilomyces* sp. *J Vet Med Physiol Pathol Clin Med* 2000; **47**: 243–249.
10 Gené J, Blanco J, Cano J, García ME, Guarro J. New filamentous fungus *Sagenomella chlamydospora* responsible for a disseminated infection in a dog. *J Clin Microbiol* 2003; **41**: 1722–1725.
11 Mackie JT, Padhye AA, Sutherland RJ, Lamb WA, Davis S. Granulomatous lymphadenitis and splenitis associated with *Monocillium indicum* infection in a dog. *J Vet Diagn Invest* 2004; **16**: 248–250.
12 National Committee for Clinical and Laboratory Standards. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi, approved standard, document M38-A. Wayne, PA: National Committee for Clinical Laboratory Standards; 2002.
13 Gershenson RT, Melidone R, Sutherland-Smith J, Rogers CL. Abdominal aortic aneurysm associated with systemic fungal infection in a German shepherd. *J Am Anim Hosp Assoc* 2011; **47**: 45–49.