Disseminated Aspergillus citrinoterreus and Concurrent Localized Dermal Phaeohyphomycosis in a Dog

David Sender[1], Benjamin Hulsey[2], Connie Cañete-Gibas[3], Nathan Wiederhold[3], Keun Lee[2], Abigail Finley[2], Catherine Cruz[2], and Mary White[2]

[1]Auburn University College of Veterinary Medicine
[2]Midwestern University
[3]The University of Texas Health Science Center at San Antonio

January 3, 2023

Abstract

A 5-year-old Staffordshire terrier mix being treated for immune-mediated hemolytic anemia was diagnosed with concurrent disseminated Aspergillus citrinoterreus and localized Curvularia lunata infections. This case highlights the potential development of multiple concurrent opportunistic fungal infections, and it is the first reported case of Aspergillus citrinoterreus infection in a dog.

Disseminated *Aspergillus citrinoterreus* and Concurrent Localized Dermal Phaeohyphomycosis in a Dog

David Sender DVM[1], Benjamin Hulsey DVM[2], Connie Cañete-Gibas[3], Nathan Wiederhold[3], Keun J Lee DVM, DACVP[4], Abigail Finley DVM, DACVP[4], Catherine Cruz[4], Mary E White DVM, DACVP[4]

[1]Department of Clinical Sciences, College of Veterinary medicine, Auburn University, Auburn, Alabama, USA.

[2]Department of Clinical Medicine, Midwestern College of Veterinary Medicine, Midwestern University, Glendale, Arizona, USA.

[3]Departments of Pathology and Laboratory Medicine, University of Texas Health Science Center at San Antonio, University of Texas, San Antonio, Texas, USA.

[4]Department of Pathology, Midwestern College of Veterinary Medicine, Midwestern University, Glendale, Arizona, USA.

Correspondence

David Sender, Auburn University

Department of Small Animal Clinical Sciences

1220 Wire Road, Auburn, AL 36832

Email: dzs0103@auburn.edu

Abstract

A 5-year-old Staffordshire terrier mix being treated for immune-mediated hemolytic anemia was diagnosed with concurrent disseminated*Aspergillus citrinoterreus* and localized *Curvularia lunata*infections. This case highlights the potential development of multiple concurrent opportunistic fungal infections, and it is the first reported case of *Aspergillus citrinoterreus* infection in a dog.

KEY WORDS

Veterinary, Canid, Infectious diseases, Emergency medicine

CASE PRESENTATION

A 5-year-old, male neutered, American Staffordshire mix presented to the Midwestern University Companion Animal Clinic (MWU CAC) for evaluation of an acute development of labored breathing after having been previously diagnosed with presumptive immune-mediated hemolytic anemia (IMHA) that was treated with multimodal immunosuppressive therapy. The presumptive diagnosis of IMHA was made at MWU CAC a month prior to the current presentation according to the previously published consensus statement based on the presence of severe regenerative anemia (hematocrit 19%; RI, 36-60) with spherocytosis and a positive saline agglutination test as well as hyperbilirubinemia.[1] His work up at the time had included repeated complete blood counts, biochemical evaluation with serum chemistries, urinalysis, thoracic radiographs, abdominal radiographs, abdominal ultrasound, SNAP 4Dx test (IDEXX Laboratories, Inc., Westbrook, ME), and babesia titers (Protatek, Mesa, Arizona). The IMHA was presumed to be non-associative based on that work up. However, a complete work up based on consensus guidelines to more definitively rule out associative IMHA was not performed. Ongoing medications at the time of his last presentation included oral cyclosporine at 5.4 mg/kg once daily, oral prednisone at 1.1 mg/kg once daily, oral clopidogrel at 1.0 mg/kg once daily, oral clindamycin at 12.3 mg/kg twice daily, and oral enrofloxacin 11.1 mg/kg once daily. Until 8 days prior to the last presentation, the patient had been receiving oral cyclosporine at 5 mg/kg twice daily. He had been receiving oral prednisone at a dose of 1.5 mg/kg daily after initial diagnosis, which, after two weeks of therapy, had been decreased to the 1.1 mg/kg daily dose due to a stable hematocrit and medication side effects. Clindamycin was started 6 days prior to the last presentation after an acute onset of paw swelling and self-trauma to digits 3 and 4 of the right manus. Based on gross evaluation of the digits during physical exam, primary consideration for the digital lesions was given to opportunistic bacterial osteomyelitis and cellulitis secondary to immunosuppression. No abnormalities with his digits had been noted on any examination prior to that time. The day prior to his final presentation, enrofloxacin was added to the patient's antimicrobial regimen due to progression of the digit lesions. Due to rapid clinical decline, further cytological work up of that lesion was not pursued prior to broadening the antimicrobial regimen.

Results of physical examination included a rectal temperature of 101.4°F, heart rate of 140 beats per minute, resting respiratory rate of 80 breaths per minute, body condition score of 7/9, and quiet to dull mentation. Increased respiratory effort, moderate labored breathing, and muffled heart sounds were also noted. Pain was elicited on palpation of the right manus, which was edematous and erythematous throughout, with open ulceration of digit 3 and mild serosanguineous discharge from nail beds of digits 3 and 4 (Figure 2).

Cytologic smears of the discharge and fine needle aspirates from digits 3 and 4 were evaluated. Nucleated cells were found individually and in intermediate to large aggregates that were often associated with extracellular hyphal structures. The hyphae were irregularly septate, exhibited acute branching and rarely would have large, round, bulbous ends that contained fine green to black pigmentation (Figure 1). The predominant cell population consisted of numerous degenerate neutrophils and fewer moderate to markedly vacuolated macrophages that occasionally exhibited leukophagia and erythrophagia. There were occasional multinucleated giant cells seen on scanning. The digit cytology was interpreted as marked pyogranulomatous inflammation with intralesional dematiaceous fungal hyphae.

A CBC (Advia 2120i Hematology System) from the previous day revealed a hematocrit of 33% (RI, 36-60), platelet count of 338 K/µL (RI, 170-400), an increased mean cell volume of 82 fL (RI, 58-79), a reticulocytosis of 188 K/µL (RI, 0-0), a leukocytosis of 37.4 x 10^3 µL (RI, 4-15.5) characterized by a marked total neutrophilia of 29,546 (RI, 2,060-10,600) with a left shift (bands 5,984/µL, RI, 0-300). No spherocytes were reported at

that time. Serum biochemistry profile through Antech Laboratories from two days prior revealed an increased alanine transferase of 531 IU/L (RI, 12-118), alkaline phosphatase of 4813 IU/L (RI, 5-131), gamma-glutamyl transferase of 212 IU/L (RI, 1-12), a mild hyperbilirubinemia of 0.7 mg/dL (RI, 0.1-0.3), and a mild total hypocalcemia of 8.6 mg/dL (RI, 8.9-11.4).

Thoracic point of care ultrasound revealed bilateral, severe pleural effusion and pericardial effusion. Cytologic samples of the pleural and pericardial fluid showed numerous degenerate to poorly preserved neutrophils. There were moderate numbers of moderately vacuolated macrophages that occasionally contained phagocytized cellular debris. Both the pericardial and pleural fluid contained moderate numbers of extracellular fungal hyphae (Figure 2) that were often surrounded by degenerate neutrophils and fewer macrophages. The hyphae exhibited predominantly tapered ends with occasional end branching. The hyphae were septated and the septations varied in length () and minimal to mild variation of septae width (). The hyphae exhibited acute branching. Given the difference of fungal morphologies between lesions of the digit and chest, concern for a mixed fungal infection was suspected and culture and PCR were recommended for further evaluation.

Given the poor prognosis, the owner elected palliative care, and euthanasia was performed the following day after respiratory signs recrudesced. A necropsy was performed. During gross examination, liver, kidney, lung, pleural and pericardial fluid, and nail bed samples were submitted for fungal culture and mycologic assessment. The thoracic cavity contained approximately 2 liters of serosanguineous fluid (Figure 2B). Affecting approximately 80%, the pulmonary pleura and mediastinum were markedly thickened with multifocal to coalescing, white-tan, firm plaques and nodules. The pericardium was diffusely and markedly thickened with a pyogranulomatous inflammation and contained approximately 110 ml of red serosanguineous fluid (Figures 2C and 3). The epicardium was also multifocally thickened, rough, and irregular with the same white to tan nodules (Figure 2C). Examination of the abdomen showed multiple, fairly demarcated, white, firm nodules ranging from 0.1 to 0.3 cm in diameter and affecting approximately 5% of the renal cortices bilaterally. Examination of the right, distal forelimb revealed markedly edematous, firm, red digits 3 and 4. An open 1.0 x 0.6 cm wound was noted on the medial aspect of the right front 3rd digit with pink, purulent exudation. There was a small amount of tissue loss around the nail bed of the right front 4th digit with a small amount of yellow-white fluid (Figure 2A).

Significant histopathologic findings included severe, fibrinonecrotizing, pyogranulomatous dermatitis with intralesional and intravascular brown-pigmented septate fungal hyphae and conidia, necrotizing vasculitis, and fibroplasia of the nail beds of digits 3 and 4 of the right forelimb. Similar severe, fibrinonecrotizing, pyogranulomatous pneumonia, pleuritis, pericarditis, and nephritis with intralesional fungal hyphae that exhibited different morphology from hyphae seen on digits 3 and 4, fibroplasia, and serosanguineous effusion were also noted (Figures 1 and 2). Fungal cultures were obtained from samples taken from the visceral organs (lung, liver, and kidney) and presumptively identified as *Aspergillus terreus* by matrix-assisted laser desorption ionization time-of-flight (MALDI-ToF MS). Another culture of a dematiaceous fungus was isolated from nail beds of digits 3 and 4 of the right front limb and was identified by MALDI-ToF MS as *Curvularia lunata* . A culture slant of the presumptive *Aspergillus terreus* UTHSCSA DI20-341 isolate obtained from the lung was submitted for species confirmation to the Fungus Testing Laboratory at the University of Texas Health Science Center at San Antonio (FTL).

MYCOLOGICAL METHODS AND RESULTS

Morphology and Phenotypic Assessment. Isolate UTHSCSA DI20-341was three-point inoculated onto Creatinine agar (Crea), Czapek yeast extract agar (CYA), malt extract agar (MEA), yeast extract sucrose agar (YES), and potato flakes agar (PFA) and were incubated in the dark at 25 ^{o}C for 7 d. Growth was also assessed at 30 ^{o}C, 37 ^{o}C, 45 ^{o}C and 50 ^{o}C. Slide cultures were set up and was mounted on lactophenol cotton blue after 7 d. Morphological and microscopic characteristics typical of*Aspergillus citrinoterreus* showed cinnamon-colored colonies on MEA at 7d at 25, 30 and 37 °C (Figure 4 A, B, C) with intense yellow diffusing pigment, globose to subglobose yellowish conidia, and smaller obovoid accessory conidia (Figure 4 D, E). Growth at 25, 30, 37, and 45 °C were observed but not at 50 °C. Sporulation was abundant on MEA

compared to the other culture media.

DNA Sequencing and Phylogenetic Analysis. Genomic DNA was extracted from harvested mycelia on PFA and sequenced using Bt2a and Bt2b primers for the partial beta-tubulin (*BenA*) gene region and CF1 and CF4 primers for the partial calmodulin (*CaM*) gene region as previously described.[2-4] Newly generated sequences were deposited in the GenBank database under the accession numbers MW419109 (*BenA*), MW419108 (*CaM*). BLASTn searches were conducted for presumptive species identification. BLASTn results for*BenA* and *caM* showed 99-100% identity with*Aspergillus citrinoterreus* sequences in GenBank. Many of the*Aspergillus citrinoterreus* sequences in GenBank were identified as *Aspergillus terreus* but were re-identified later as *A. citrinoterreus* .[5]

Based on BLASTn results, the dataset was compiled using the newly generated sequences and publicly available representative sequences of authentic and type strains of species in the *Aspergillus* section*Terrei* . Maximum likelihood trees were generated on individual locus and combined using the IQ-Tree.[6] The best fitting model for each locus and combined was determined by corrected Akaike Information Criterion as implemented in IQ-TREE. Branch supports were estimated by 1000 bootstrap replicates (BS) and Bayesian inference (BI) with MrBayes v.3.2.6 using a Markov Chain Monte Carlo (MCMC) algorithm.[7,8] *Aspergillus fumigatus* NRRL 163^{T} was used as the outgroup species. Phylogenetic analysis of each individual loci and combined showed the isolate consistently grouping with the type and other strains of *A. citrinoterreus* and confirmed the identity of the isolate with high branch support (*caM* = BI/BS = 1.00/88%; *BenA* = BI/BS 0.97/ 94%; *caM* +*BenA* = BI/BS 1.00/ 99% BS) (Figure 5 and Figure 6).

DISCUSSION

This report highlights a case of a dog on multimodal immunomodulating therapy for IMHA that became coinfected with *Aspergillus citrinoterreus* and *Curvularia lunata* . Our case emphasizes the importance of vigilant monitoring for adverse effects related to immunosuppression and opportunistic infections, and it demonstrates the necessity of appropriate and adequate evaluation of lesions that arise after initiation of immunosuppression treatment protocols.

Opportunistic fungal infections in small animals were recently described in a review article by Dedeaux, et al. In that review, they describe how opportunistic mycoses caused by soil saprobes that are typically non-pathogenic to immunocompetent animals have emerged as clinically relevant causes of disease in patients undergoing immunomodulating therapy for immune-mediated diseases. The same review also mentions naturally occurring causes of immunocompromise that can predispose patients to opportunistic mycoses, with examples including*Candida* spp. urinary tract infection in diabetic dogs and cats, disseminated aspergillosis and hyalohyphomycosis in German shepherd dogs with suspected familial immunodeficiency, *Pneumocystis* pneumonia in miniature dachshunds with combined variable immunodeficiency, systemic mold infection in young dogs with hereditary cobalamin deficiency, and phaeohyphomycosis in cats with diabetes mellitus, retrovirus infection, or lymphoid neoplasia.[9]Opportunistic mycoses in animals with naturally occurring causes of immunocompromise are rare and generally well-described. Opportunistic mycoses in patients receiving immunomodulating therapies are more likely to be caused by saprobic organisms that can be more difficult to definitively differentiate.[9] One report describes an incidence of cutaneous opportunistic fungal infection in dogs receiving immunosuppressive therapy of up to 13%.[10]

Aspergillus species are saprobic organisms ubiquitously found in the environment and are opportunistic pathogens. *Aspergillus fumigatus* , *A. flavus* , *A. terreus* , *A. niger* , and*A. deflectus* cause disease in dogs. *A. fumigatus* and*A. flavus* usually cause localized disease in the nasal cavity while *A.terreus* is more commonly associated with systemic aspergillosis in dogs. However, *A. deflectus* and *A. niger*have been reported to cause disease in some dogs.[11]

Aspergillus citrinoterreus was first described by Guinea et al as a new species of Aspergillus section Terrei in 2015.[5]It is closely related to *A. terreus* and differs in its colony characteristics and microscopic structure. A clinically significant note is *Aspergillus citrinoterreus* was found to be more susceptible to azole antifungals compared to *A. terreus* , but both species still showed clinical resistance to amphotericin B.[5] Little is known

about the geographical distribution of *Aspergilluscitrinoterreus* . Species in the *Aspergillus* section *Terrei* are reported as common pathogens of invasive aspergillosis in humans.[12-14] Infection with *Aspergillus citrinoterreus* has not been reported in the dog.

Phaeohyphomycosis is an infrequent infection in both humans and animals, though it has been more frequently reported in recent years in immunosuppressed patients (most commonly in solid organ transplant patients).[9,15] It is the name given to cutaneous and systemic diseases caused by black molds that develop dark-walled, septate mycelia in tissue.[16] These infections are usually cutaneous and acquired traumatically, typically affecting exposed areas of the head or upper limbs, though disseminated cases are reported. Phaeohyphomycosis is an overall term used to describe infection from one of any of over 60 genera of dematiaceous (pigmented) fungi. Agents identified to cause infection in veterinary patients include *Alternaria, Aureobasidium, Bipolaris/Curvularia, Cladophialophora, Exophiala, Fonsecaea, Lecythophora, Microsphaeropsis, Moniliella, Mycoleptodiscus* (*Muyocopron*)*, Phialophora, Ramichloridium, Scolecobasidium* , *Scytalidium* , and *Ulocladium* (*Alternaria*).[9,17-19] These fungi are saprobic, widely-distributed organisms found in soil, water, and decaying vegetable matter, which are usually non-pathogenic, but can be devastating to immunocompromised hosts.

The most common clinical manifestations of phaeohyphomycosis in small animals are lesions associated with the digits, pinnae, nasal planum, or the nasal cavity in cats. However, multifocal cutaneous lesions more commonly occur in immunocompromised patients or dogs treated with multiple immunosuppressive agents. Furthermore, the multifocal presentation is even more common when cyclosporine is one of the immunosuppressive agents used.[17] One report describes an odds ratio of 7.1 for patients diagnosed with cutaneous opportunistic fungal infection that were being treated with cyclosporine.[10]

Phaeohyphomycosis in humans is divided into four forms based on the location of the infection and route of inoculation. These include superficial, cutaneous, subcutaneous, and systemic.[16]The most common types of phaeohyphomycosis in veterinary medicine are the subcutaneous and systemic forms.[20] In most of the cases reported in domestic animals, there is no involvement of the epidermis or upper dermis, and traumatic implantation or wound contamination is thought to be the primary mode of infection.[16]

Diagnosis of fungal infections requires microscopic detection of intralesional fungal elements, culture, and often additional diagnostic, such as serology or molecular diagnostics.[21] The utility of cytology is dependent on sampling and processing techniques, exfoliation of representative populations and extracellular elements, and degree of heterogeneity present within the sample, which can limit its accuracy. However, cytology can provide rapid, preliminary information that can be helpful in determination of subsequent diagnostic follow-up; this is especially true for infectious lesions. It should be noted that microscopic evaluation, whether cytologic or histopathologic, is not recommended as a stand-alone diagnostic tool to determine fungal identification. Culture, isolation, and molecular characterization (PCR, genome sequencing) are required for speciation.[21] Morphological diagnosis of fungal species is additionally difficult for reasons such as the presence of cryptic species within different genera, the absence of morphological structures that aid in identification, the lack of expertise in fungal identification in clinical microbiology laboratories and the failure for the fungus to grow from clinical specimens. Molecular testing methods using polymerase chain reaction and comparative sequence analysis enable definitive identification of most clinical isolates.[22]For aspergillosis, diagnosis of fungal infection is achieved through identification of fungal hyphae within tissue samples or urine or by detection of the fungal cell wall antigen galactomannan in blood or urine as well as DNA sequencing of the isolated fungus from specimens.[23]

Diagnosis of phaeohyphomycosis requires evidence of pigmented fungi in wet mounts or in histologic sections in addition. However, due to similar histologic appearance of many phaeohyphomycotic agents, definitive diagnosis is made by culture and specific identification of the etiologic agent.[16] Local disease may be cured with excision of diseased tissue, but systemic disease can often be deadly as the disease can often be refractory to therapy.[5]

A comprehensive review of literature reporting disseminated phaeohyphomycosis infections in people iden-

tified a total of 72 cases from 1966 to 2002.[24] In that review, the prognosis is noted to be particularly poor with an overall mortality rate of 79%. Within the cases reported in that review, several treatments were attempted, but none were associated with improved survival. There is one report in veterinary medicine of successful treatment of disseminated cutaneous phaeohyphomycosis in a dog caused by *Curvularia lunata* . That case was treated with a combination of amphotericin B and itraconazole. Interestingly, infection in that case was also suspected to be secondary to immunosuppression with a combination of glucocorticoids and cyclosporine.[25]

The patient in the current report was being treated with immunosuppressive doses of both cyclosporine and prednisone for the management of IMHA. It is likely this therapy caused sufficient immune suppression to induce susceptibility to opportunistic fungal infections. The clinical history provided, in combination with laboratory data, and the gross and microscopic disease, suggest the route of infection was likely through the cutaneous wound followed by hematogenous spread to the previously listed organs. Identification of *Aspergillus* in the pulmonary tissue and microscopic findings of pneumoconiosis and eosinophilic edema are suggestive of inhalation and potential for concurrent direct inhalational disease, and further visceral spread cannot be completely excluded.

This report highlights a case of coinfection in a dog with two fungal organisms–*Aspergillus citrinoterreus* and *Curvularia lunata*– likely due to multimodal immunomodulating therapy for presumptive non-associative IMHA. Additionally, this is the first reported case of disseminated *Aspergillus citrinoterreus*infection in a dog in North America to the authors' knowledge. Cases of*Aspergillus terreus* species infections are important to note as they may carry a poorer prognosis due to clinical resistance to Amphotericin B and higher mortality rates.[26] This patient developed a severe disseminated infection of *Aspergillus citrinoterreus* in addition to a localized *Curvularia* infection that both led to considerable morbidity and eventually euthanasia. As the two fungal species were collected from different body sites on this patient, this case also highlights the importance of thorough evaluation for possible coinfections in these immunocompromised patients.

CONFLICTS OF INTEREST

The authors declare no conflicts of interests.

ETHICS STATEMENT

This research did not contain any studies involving animal or human participants, nor did it take place on any private or protected areas. No specific permissions were required for corresponding locations.

AUTHOR CONTRIBUTIONS:

David Sender: Primary author and primary emergency clinician of the patient

Benjamin Hulsey: Contributed to the initial manuscript framework and literature review

Connie Cañete-Gibas: Genetic sequencing and identification of *A. citrinoterreus* and contributed to corresponding sections of the manuscript, Figure 3 and Figure 4.

Nathan Wiederhold: Genetic sequencing and identification of *A. citrinoterreus* and contributed to corresponding sections of the manuscript, Figure 3, and Figure 4.

Keun J Lee: Performed the necropsy and histopathologic evaluation and contributed to those corresponding sections of the manuscript as well as Figure 2.

Abigail Finley: Pathology resident that helped Dr. Lee with necropsy and histopathologic evaluation; contributed to initial manuscript framework and literature review

Catherine Cruz: Grew and isolated both fungal species and contributed to the corresponding sections of the manuscript

Mary E. White: Senior author and evaluated the majority of the clinical pathology-related data of the patient and contributed to corresponding sections of the manuscript and Figure 1.

REFERENCES

1. Garden OA, Kidd L, Mexas AM, et al. ACVIM consensus statement on the diagnosis of immune-mediated hemolytic anemia in dogs and cats. *J Vet Intern Med* . 2019; 33: 313– 334. doi.org/10.1111/jvim.15441

2. Glass NL, Donaldson GC. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes.*Appl Environ Microbiol* . 1995; 61(4): 1323-1330. doi:10.1128/aem.61.4.1323-1330.1995

3. Hong SB, Go SJ, Shin HD, Frisvad JC, Samson RA. Polyphasic taxonomy of *Aspergillus fumigatus* and related species. *Mycologia* . 2005; 97(6): 1316-1329. doi:10.3852/mycologia.97.6.1316

4. Peterson SW, Vega FE, Posada F, Nagai C. *Penicillium coffeae* , a new endophytic species isolated from a coffee plant and its phylogenetic relationship to *P. fellutanum* , *P. thiersii*and *P. brocae* based on parsimony analysis of multilocus DNA sequences. *Mycologia* . 2005; 97(3): 659-666. doi:10.3852/mycologia.97.3.659

5. Guinea J, Sandoval-Denis M, Escribano P, Peláez T, Guarro J, Bouza E.*Aspergillus citrinoterreus* , a new species of section Terrei isolated from samples of patients with nonhematological predisposing conditions. Warnock DW, ed. *J Clin Microbiol* . 2015; 53(2): 611-617. doi:10.1128/jcm.03088-14

6. Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol* . 2014; 32(1): 268-274. doi:10.1093/molbev/msu300

7. Ronquist F, Teslenko M, van der Mark P, et al. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol* . 2012; 61(3): 539-542. doi:10.1093/sysbio/sys029

8. Geyer, CJ. (1991). Markov Chain Monte Carlo Maximum Likelihood. Interface Foundation of North America. Retrieved from the University of Minnesota Digital Conservancy

9. Dedeaux A, Grooters A, Wakamatsu-Utsuki N, Taboada J. Opportunistic fungal infections in small animals. *J Am Anim Hosp Assoc* . 2018; 54(6): 327-337. doi:10.5326/jaaha-ms-6768

10. McAtee BB, Cummings KJ, Cook AK, Lidbury JA, Heseltine JC, Willard MD. Opportunistic invasive cutaneous fungal infections associated with administration of cyclosporine to dogs with immune-mediated disease.*J Vet Int Med* . 2017; 31(6): 1724-1729. doi:10.1111/jvim.14824

11. Schultz RM, Johnson EG, Wisner ER, Brown NA, Byrne BA, Sykes JE. Clinicopathologic and diagnostic imaging characteristics of systemic aspergillosis in 30 dogs. *J Vet Int Med* . 2008; 22(4): 851-859. doi:10.1111/j.1939-1676.2008.0125.x

12. Baddley JW, Pappas PG, Smith AC, Moser SA. Epidemiology of*Aspergillus terreus* at a university hospital. *J Clin Microbiol* . 2003; 41(12): 5525-5529. doi:10.1128/jcm.41.12.5525-5529.2003

13. Lass-Florl C, Griff K, Mayr A, et al. Epidemiology and outcome of infections due to *Aspergillus terreus* : 10-year single centre experience. *Br J Haematol* . 2005; 131(2): 201-207. doi:10.1111/j.1365-2141.2005.05763.x

14. Blum G, Perkhofer S, Grif K, et al. A 1-year *Aspergillus terreus* surveillance study at the University Hospital of Innsbruck: molecular typing of environmental and clinical isolates. *Clin Microbiol Infect* . 2008; 14(12): 1146-1151. doi:10.1111/j.1469-0691.2008.02099.x

15. Belda B, Petrovitch N, Mathews KG. Sinonasal aspergillosis: Outcome after topical treatment in dogs with cribriform plate lysis. *J Vet Intern Med* . 2018; 32(4): 1353-1358. doi:10.1111/jvim.15219

16. Herráez P, Rees C, Dunstan R. Invasive Phaeohyphomycosis caused by*Curvularia* species in a dog. *Vet Pathol* . 2001; 38(4): 456-459. doi:10.1354/vp.38-4-456

17. Grooters AM. Miscellaneous Fungal Diseases. In: Sykes JE.*Canine and Feline Infectious Diseases* . St. Louis, MO: Elsevier/Saunders; 2014: 660–667.

18. Gannibal PB, Lawrence DP. Distribution of *Alternaria* species among sections. 6. Species formerly assigned to genus *Ulocladium* .*Mycotaxon* . 2018; 133(2): 293-299. doi:10.5248/133.293

19. Hernández-Restrepo M, Bezerra JDP, Tan YP, et al. Re-evaluation of*Mycoleptodiscus* species and morphologically similar fungi.*Persoonia* . 2019; 42(1): 205-227. doi:10.3767/persoonia.2019.42.08

20. Scott DW, Miller WH, Griffin CE. Fungal Skin Diseases. In: Muller GH, Kirk RW, eds. *Small Animal Dermatology* . 6th ed. Philadelphia, PA: WB Saunders; 2001: 379–381. doi:10.3767/persoonia.2019.42.08

21. Sykes, JE, et al. *Greene's Infectious Diseases of the Dog and Cat* . Elsevier, 2023.

22. Bennett PF, Talbot JJ, Martin P, Kidd SE, Makara M, Barrs VR. Long term survival of a dog with disseminated *Aspergillus deflectus*infection without definitive treatment. *Med Mycol Case Rep* . 2018; 22:1-3. doi:10.1016/j.mmcr.2018.07.002

23. Garcia RS, Wheat LJ, Cook AK, Kirsch EJ, Sykes JE. Sensitivity and specificity of a blood and urine galactomannan antigen assay for diagnosis of systemic aspergillosis in dogs. *J Vet Int Med* . 2012; 26(4): 911-919. doi:10.1111/j.1939-1676.2012.00935.x

24. Revankar SG, Patterson JE, Sutton DA, Pullen R, Rinaldi MG. Disseminated phaeohyphomycosis: review of an emerging mycosis.*Clin Infect Dis* . 2002; 34(4): 467-476. doi:10.1086/338636

25. Swift I, Griffin A, Shipstone M. Successful treatment of disseminated cutaneous phaeohyphomycosis in a dog. *Aust Vet J* . 2006; 84(12): 431-435. doi:10.1111/j.1751-0813.2006.00068.x

26. Vaezi A, Fakhim H, Arastehfar A, et al. In vitro antifungal activity of amphotericin B and 11 comparators against *Aspergillus terreus*species complex. *Mycoses* . 2017; 61(2): 134-142. doi:10.1111/myc.12716

FIGURE 1: A) Cytopictograph from fine needle aspirate of digit 3: The hyphae are irregularly septate, exhibited acute and perpendicular branching and rarely exhibited large, round, bulbous ends that contained fine green to black pigmentation. Wright Giemsa, 50x

B) Cytopictograph from pericardial effusion. Fungal hyphae exhibited predominantly elongate septae with fewer short, cuboidal to rounded, regular septation that were present centrally.; Wright Giemsa, 100x.

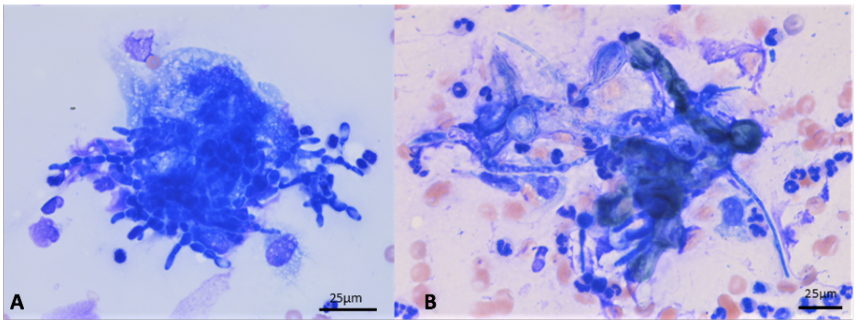
FIGURE 2: A) Digits 3 and 4 of the right forelimb showing an open lesion and swelling of both digits. Localized *Curvularia* infection was suspected to have been acquired traumatically as is most common in veterinary patients. B) Thoracic cavity on necropsy contains approximately 2 liters of serosanguinous fluid. The liver (left) is pale and friable with an increased lobular pattern. C) Heart and pericardium on necropsy examination. The epicardium of ventricles and auricles were multifocally thickened and irregular with fibrous material and white to tan pyogranulomatous nodules.

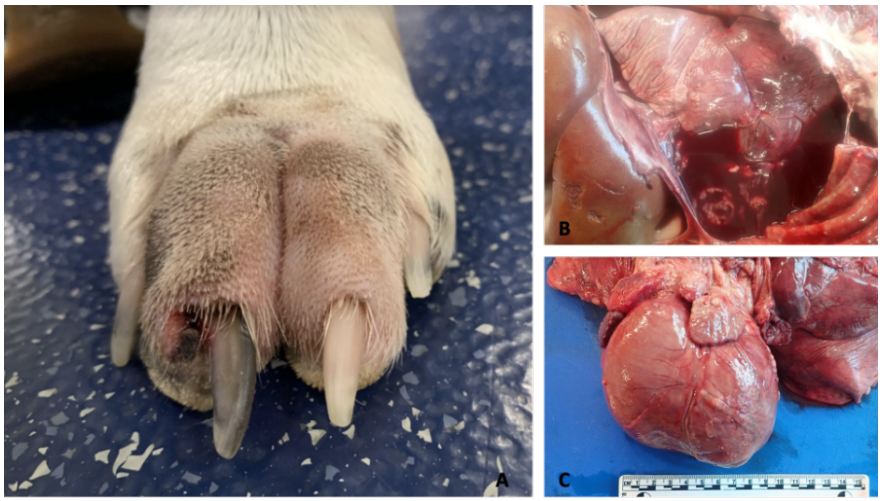
FIGURE 3. Heart: The pericardium is markedly thickened with severe diffuse pyogranulomatous inflammation with multiple GMS (Grocott's Methenamine silver stain)-positive fungal hyphae (insert:*Aspergillus citrinoterreus*).

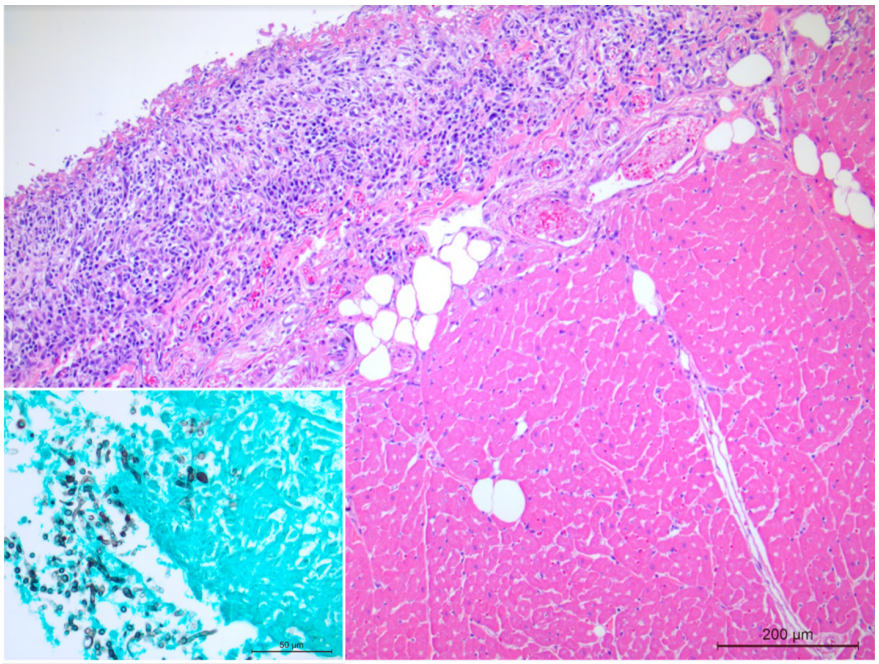
FIGURE 4. *Aspergillus citrinoterreus* (UTHSCSA DI20-341). A-C. Colonies on MEA, 7d at 25, 30 and 37 °C. D-E. Light micrographs of vesicles and accessory conidia at 7d.

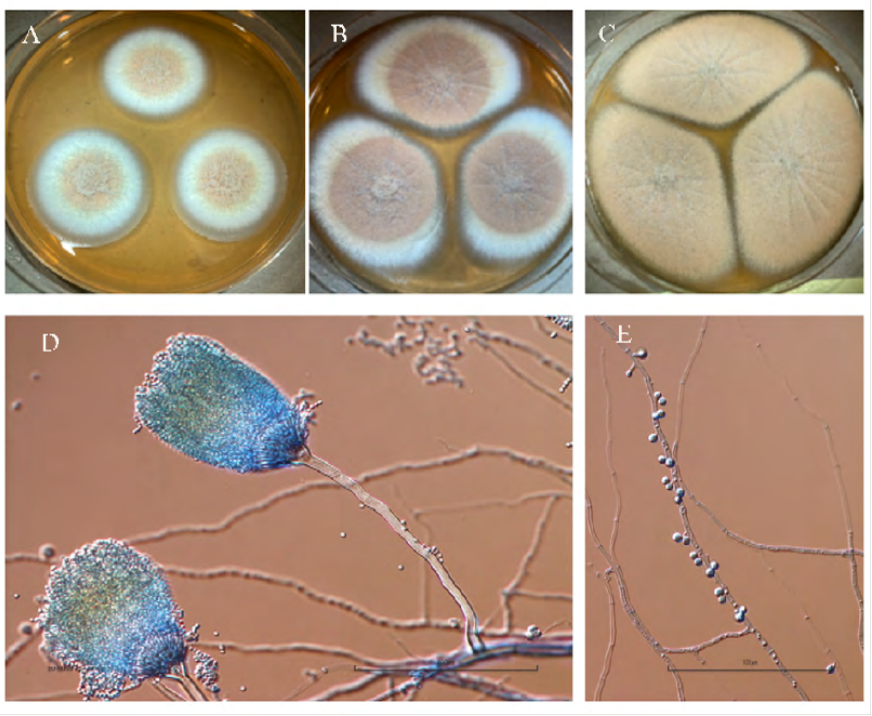
FIGURE 5. A maximum likelihood tree constructed from combined sequences of partial *BenA* and *CaM* showing the relationship of isolate UTHSCSA DI20-341 with representative species in the*Aspergillus* section Terrei. Values at the nodes are Bayesian posterior probability (BI) > 0.95 (right) and bootstrap (BS) $> 70\%$ (left). Ex-type cultures are marked with a ^{T}. Branches with double-bars are truncated two-fold.

FIGURE 6. Maximum likelihood trees from sequences of individual genes*BenA* and *CaM* showing the relationship of isolate UTHSCSA DI20-341 with representative species in the *Aspergillus* section*Terrei* . Values at the nodes are Bayesian posterior probability (BI) > 0.95 (right) and bootstrap (BS)$> 70\%$ (left). Ex-type cultures are marked with a^{T}. Branches with double-bars are truncated two-fold.









BenA + CaM