

Case Report

Disseminated aspergillosis in a dog due to *Aspergillus alabamensis*

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ABSTRACT

Disseminated aspergillosis is uncommon in dogs and often associated with *Aspergillus terreus*. A case of disseminated disease in an English springer spaniel is reported from which *Aspergillus alabamensis* was recovered by culture and identified by molecular means suggesting a potential role for this agent as a primary pathogen of dogs.

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1. Introduction

Disseminated aspergillosis is uncommon in dogs and often associated with *Aspergillus terreus* infection [1]; however, *Aspergillus deflectus*, *Aspergillus flavipes*, and *Aspergillus fumigatus* have also been identified in dogs with disseminated disease [2] and there is a recent case report of disseminated aspergillosis due to *Aspergillus versicolor* [3]. German shepherd dogs appear predisposed to disseminated disease [1], although immunosuppression in any breed may increase the potential for infection and disease has been reported in dogs receiving corticosteroids [4–6]. In most cases, the portal of entry is assumed to be the respiratory tract with subsequent hematogenous spread, and organs reported to be affected in 23 cases [1,4–11] of disseminated disease include the kidney, spleen, lymph nodes, bone, liver, heart, lung, eyes, pancreas, bone marrow, brain, urinary bladder, prostate, pleura, adrenal, stomach, uterus, thyroid, and thymus with decreasing frequency.

2. Materials and methods

An ante mortem peritoneal fluid sample and a postmortem liver sample were separately plated onto sheep blood agar, Sabouraud dextrose agar (BD, Sparks, Maryland), Emmons Sabouraud dextrose

agar (BD), and Mycobiotic Agar (Remel, Lenexa, Kansas), and incubated at 25 °C and 35 °C with and without CO₂. First growth was observed at 4th day on both SD and the selective media at 35 °C and appeared as heavy surface growth of buff and white colonies. Colonies of similar morphology were isolated from both clinical specimens and microscopic evaluation revealed septate hyphae; however, initial phenotypic identification was not definitive. A salmon to pale brown-colored isolate demonstrating very tiny colonies with irregular hyphae was submitted to the Fungus Testing Laboratory (University of Texas Health Science Center, San Antonio, Texas) for identification and was accessioned into their culture collection as UTHSC 10-1030. There the isolate was subcultured onto potato flakes agar (PFA) and carnation leaf agar (CLA) [12], both prepared in-house. After 4 weeks incubation at 25 °C colonies on PFA were still only approximately 3 mm diameter, pale pink, and demonstrated a diffusing yellow pigment. Although no diagnostic structures were present on either PFA or CLA, an *A. terreus* was considered at this point. The colonial morphology of the isolate on PFA at 35 °C was similar to that at 25 °C, with no enhanced growth at the higher temperature. A repeat subculture on a 60 mm PFA plate incubated for 16 days at 25 °C, in preparation for deposit of the isolate into the University of Alberta Microfungus Collection, demonstrated more vigorous growth with pinkish-yellow colonies and a more intense yellow diffusing pigment (Fig. 1); however, the colonial size was similar and the isolate remained sterile. As there was no conidial formation after 4 weeks, the isolate was subsequently sequenced under accession number R-4572. Preliminary sequencing of the internal transcribed spacer (ITS) region suggested that the isolate was a member of the genus

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Fig. 1. Macroscopic colonial morphology of the isolated *A. alabamensis* on potato flakes agar after 16 days of growth at 25 °C revealing the presence of yellow diffusing pigment. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Aspergillus; however, for these fungi the ITS sequence is often not specific enough to make a species identification although it can be used for making a species-complex determination [13], and the D1/D2 sequence is even less specific. Instead, sequence from the β -tubulin gene was used to make a species identification due to its greater specificity in the aspergilli [14]. Template DNA was prepared as described [15] and amplified by PCR with the tubulin Bt2a (5'-GGTAACCAATCGGTGCTGCTTTC-3') and Bt2b primers (5'-ACCCTCAGTGTAGTGACCCTGGC-3') as forward and reverse primers, respectively [16] using an annealing temperature of 55 °C and PCR conditions as described [15]. The sequence was then used to search the NCBI Genbank DNA sequence database using the BLASTn search algorithm (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The results were sorted on identity and the closest hits were *Aspergillus alabamensis* [accession #FJ491730.1, identities=498/498(100%)], *A. alabamensis* [accession #FJ491727.1, identities=497/498(99%)], *A. alabamensis* [accession #FJ491726.1, identities=462/463(99%)] and *A. terreus* [accession #EU147716.1, identities=398/401 (99%)].

3. Case

A 5-year-old, spayed female, English springer spaniel presented to the Lloyd Veterinary Medical Center of the Iowa State University College of Veterinary Medicine with a 7 days history of vomiting, inappetence, and lethargy. Four weeks prior to presenting, the patient was diagnosed with masticatory myositis and started on azathioprine and twice daily prednisone at an immunosuppressive dose. Three weeks into this treatment, the patient developed intermittent lameness in the left hind limb and episodic vomiting. The patient's condition continued to worsen despite supportive therapy, and the patient was subsequently referred.

On physical examination, the patient was anxious, its vital signs were within normal limits, and no abnormalities were detected on auscultation. The patient had difficulty rising from the floor, but obvious signs of pain were not elicited on palpation

of the affected limb. The patient vomited once while waiting in the examination room but no other abnormalities were detected during the physical examination. Abdominal radiographs were obtained and revealed mild generalized hepatomegaly and mild abdominal effusion. An ultrasound-guided abdominocentesis was performed and samples of the collected effusion were submitted for cytology and microbial culture.

Cytology of the effusion revealed 96% neutrophils, some of which were degenerate to slightly degenerate, and the remaining cells were macrophages and monocytes. Occasionally there were clusters of neutrophils surrounding septate fungal hyphae and a diagnosis of purulent abdominal exudation with associated fungal hyphae was given. The patient's owners elected not to pursue further treatment for systemic fungal disease and the animal was euthanized and submitted for necropsy.

At necropsy, there was approximately 400 ml of red-brown effusion in each of the pleural and peritoneal cavities, and approximately 200 ml of a similar effusion within the pericardial space. The liver was diffusely pale and there were numerous multifocal to coalescing raised, tan, pinpoint to 0.3 cm diameter foci along the serosal surface (Fig. 2), extending into the cut surface, and throughout the parenchyma. Similar foci were noted in the wall of the gallbladder (Fig. 2), along the serosal surface of the stomach, throughout the walls of the heart, within the visceral pleura, and along both the pleural and peritoneal surfaces of the diaphragm. Both kidneys had widely scattered and variably sized pale foci similar to other organs, and there were multiple areas of infarction, most often at the poles. The pancreas was diffusely thickened with multifocal areas of hemorrhage, there were numerous raised black splenic nodules that extended into the cut surface, and there was marked bilateral atrophy of the masseter and temporalis muscles.

Histologic evaluation revealed disseminated pyogranulomas in the lung, pleura, heart, thyroid, esophagus, diaphragm, liver, gallbladder, stomach, spleen, small intestine, mesenteric lymph nodes, pancreas, kidney, and adrenal gland. Pyogranulomas often contained numerous moniliform hyphae and fewer short, septate, 4.5–5 μ m wide hyphae with parallel walls (Fig. 3A) and occasional laterally branching globose structures consistent with accessory conidia (aleuriospores) (Fig. 3B). Severe segmental necrotizing vasculitis was also identified in the kidney, pancreas, and stomach wall often with evidence of vascular rupture and



Fig. 2. Liver and gallbladder of dog. There are numerous multifocal to coalescing, raised, tan, pinpoint to 0.3 cm diameter foci scattered throughout the hepatic parenchyma and within the thickened and congested gallbladder wall. Bar = 1 cm.

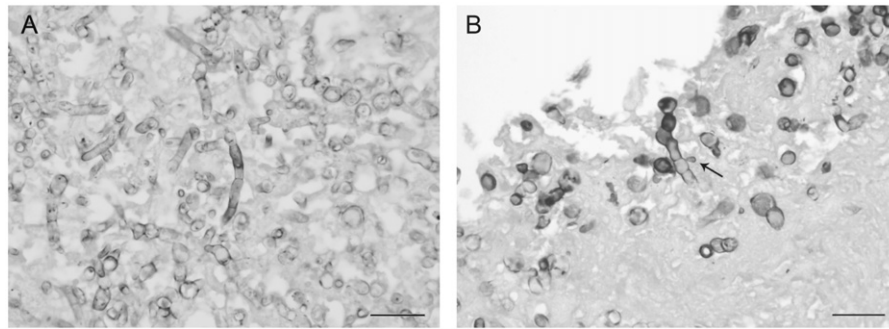


Fig. 3. Pancreas of dog. (A) Photomicrograph from the center of a pyogranuloma revealing numerous fungal elements including many cross and tangential sections of short, septate, 4.5–5 μm wide hyphae with parallel walls. Grocott's methenamine silver. Bar = 20 μm . (B) Photomicrograph demonstrating a septate fungal hypha with a laterally branching accessory conidium (aleuriospore) (arrow). Grocott's methenamine silver. Bar = 20 μm .

associated fungal elements of similar morphology to those described in the pyogranulomas.

4. Discussion

The genus *Aspergillus* contains multiple subgenera, which are in turn divided into sections comprised of related species [14]. *A. alabamensis* is a recently described species within the section *Terrei* that is morphologically similar to *A. terreus* but genetically distinct [17]. While morphology-based identification of *Aspergillus* spp. has been traditionally employed in many laboratories, difficulties with this method exist due to atypical manifestations of clinical aspergilli and overlapping morphological characteristics within members of certain sections [14]. To alleviate these difficulties, molecular identification of *Aspergillus* spp. from clinical isolates using DNA sequence-based analysis is recommended where analysis of the ribosomal internal transcribed spacer region provides identification to the species complex level, and protein encoding genes such as beta tubulin allow identification of species within a given complex [14]. Accordingly, beta tubulin sequence analysis has been reported in the species identification of *Aspergillus* clinical isolates, and was essential for distinguishing several cryptic species [18].

The portal of entry in the case of this report is unknown, although entry through the respiratory or gastrointestinal tract is most likely with hematogenous dissemination. Given the relatively low number of pulmonary lesions in this case and the localization of these inflammatory foci within alveolar septa, it seems likely that these lesions may have developed secondary to hematogenous dissemination and that the gastrointestinal tract may be the most likely portal of entry which is further supported by the significant hepatic involvement in this case. It has been suggested that lesions in disseminated aspergillosis occur with greater frequency in areas with terminal capillary loops or vascular stasis [1], and the frequency and magnitude of the renal, splenic, and lymph node lesions in this case is consistent with this hypothesis. The formation of aleuriospores has been described with *A. terreus* [19], and aleuriospores (aleurioconidia) have been reported to be the agent of hematogenous spread in experimentally infected mice [20]. Dissemination in this case was severe with numerous organ systems affected, and, to the author's knowledge, this is the first report to describe involvement of the gallbladder, diaphragm, esophagus, and small intestine in canine disseminated aspergillosis.

While systemic aspergillosis is most commonly reported in the German shepherd [1,4,7,10], disseminated disease has also been reported in other breeds including the Dalmatian [1], flat-coated

retriever [11], golden retriever [8], Labrador retriever [6], springer spaniel [9], and Weimaraner [11]. The English springer spaniel in this report had received a combination of two immunosuppressive drugs for a 3-week period prior to development of clinical disease, suggesting that immunosuppression may have played a role in disease development in this case; however, colonizing isolates of *A. alabamensis* with decreased susceptibility to amphotericin B have been recovered from immunocompetent human patients [17] suggesting that this species is clinically relevant and a potential primary pathogen. To the authors' knowledge, this is the first report of disseminated aspergillosis in a dog due to *A. alabamensis* and provides further support for the value of molecular methods in the precise identification of agents noted histologically.

Culture collection accession numbers

The case isolate has been deposited into the University of Alberta Microfungus Collection under accession number UAMH 11632. The GenBank accession number is JQ286488.

Conflict of Interest

No conflict of interest is declared.

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