

Aspergillus fumigatus Bronchopneumonia in a Hellenic Shepherd Dog

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ABSTRACT

A 3 yr old intact female Hellenic shepherd dog was referred due to depression, partial anorexia, fever, and a mild productive cough of 2 mo duration. Thoracic radiographs showed increased opacity of all of the left lung lobes. Upon bronchoscopy, a sanguineous, purulent discharge was detected in the tracheal lumen with hyperplastic tissue narrowing the left main stem bronchus. Cultures were positive for bacteria (*Bacillus* spp. and *Clostridium* spp.) but negative for fungi. Due to the severity of the lesions, a complete left lung pneumonectomy was performed. Histopathological examination of the excised lung tissues revealed a severe granulomatous bronchopneumonia with numerous alveolar macrophages laden with structures stained positively by periodic acid-Schiff and Grocott stain that had morphology consistent with fungi. PCR and sequencing of internal transcribed spacer regions 1 and 2 from genetic material extracted from paraffin-embedded pulmonary tissue confirmed the presence of *Aspergillus fumigatus*. Itraconazole was administered for 5.5 mo and the dog was clinically normal 26 mo after surgery. (*J Am Anim Hosp Assoc* 2011; 47:e13–e18. DOI 10.5326/JAAHA-MS-5497)

Introduction

Aspergillosis can present as either a localized or disseminated disease caused by filamentous fungi of the genus *Aspergillus*. *Aspergillus* spp. are ubiquitous soil saprophytes. These fungi are regarded as opportunistic pathogens that are widespread worldwide, especially in areas with an appropriate climate, such as those with hot, dry summers.^{1,2}

Dogs developing localized sinonasal aspergillosis are generally of dolichocephalic breeds and are typically infected by *A. fumigatus*.¹ Canine disseminated aspergillosis is a relatively uncommon disorder but has been described (most commonly) in Australia and Northern, Eastern, and Southwestern parts of the United States.² The portal of entry of fungal spores in disseminated disease is thought to be via the respiratory tract, although affected dogs generally lack respiratory pathology.^{3,4} The majority of cases of disseminated aspergillosis are caused by *A. terreus* but

A. deflexus, *A. flavipes*, and *A. fumigatus* have also been reported causes.³

Canine bronchopulmonary aspergillosis, with or without dissemination, is sporadically described in the literature and involves the same range of *Aspergillus* spp. isolated as in disseminated disease. In all three forms of disease (i.e., nasal, bronchopulmonary, and disseminated), a breed predisposition for the German shepherd dog has been described.^{5,6}

The diagnosis of canine *Aspergillus* infection is challenging. Despite the fact that agar gel double diffusion and counter-immunoelectrophoresis are reportedly highly sensitive and specific, serology is of limited value because serum antibodies are readily detected in healthy dogs and some infected dogs are seronegative. Detection of serum antigen is also of limited value in diagnosing aspergillosis in dogs because false-negative and false-positive results have been reported.⁷ Histopathologic examination

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BAL bronchoalveolar lavage; CBC complete blood count; CDV canine distemper virus; CPV canine parvovirus; H&E hematoxylin and eosin; ITS internal transcribed spacer; PAS periodic acid-Schiff; PCR polymerase chain reaction; PCV packed cell volume; PO per os

of affected tissue(s) may enable a diagnosis but it does not always permit identification of the species. Positive culture from affected tissue(s) is currently regarded as the gold standard for diagnosis. Application of newer molecular techniques such as PCR to amplify targets such as the internal transcribed spacer (ITS) region could allow earlier diagnosis and accurate identification of different organisms; however, such techniques have only proven of adjunctive value in the diagnosis of canine sinonasal aspergillosis.^{1,8,9}

Choosing the appropriate antifungal agent to treat pulmonary or disseminated aspergillosis is also difficult. Itraconazole currently appears to be the most effective drug whereas fluconazole and ketoconazole are of limited or no therapeutic value.⁴ Usually, even after prolonged antifungal therapy, the agent involved is not totally destroyed and a relapse can occur weeks or months after itraconazole, fluconazole, or ketoconazole discontinuation.³ Prognosis is guarded, especially in severely affected cases.¹⁰

In Greece, as in other areas of Europe, the incidence of canine disseminated or pulmonary mycosis (regardless of the causative fungi) is lower than the incidence of sinonasal disease. The present report describes what appears to be the first report of canine bronchopneumonia caused by *A. fumigatus*.

Case Report

A 3 yr old intact female Hellenic shepherd dog was referred to the Companion Animal Clinic at the Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Greece in August 2006 for evaluation of depression, partial anorexia, weight loss, productive cough, and intermittent fever of approximately 2 mo duration. The dog's clinical signs were initially observed in the early summer. The dog lived in a fenced yard in an urban agricultural area of Northern Greece and had been vaccinated by the primary veterinarian against CDV, canine infectious hepatitis, CPV, leptospirosis, and rabies and had been treated with antiparasitics^{a,b}. There was no recent travel history.

The referring veterinarian had initially treated the dog with doxycycline^c (5 mg/kg body weight per os [PO] q 12 hr) because the nonspecific clinical signs, in particular the fever, had suggested a preliminary diagnosis of acute ehrlichiosis. Approximately 1 wk after commencing this therapy, the dog's clinical signs had not resolved and the dog developed a productive cough that was precipitated by even mild exercise. A commercial diagnostic test^d was negative for *Dirofilaria immitis*. Radiographic examination of the thorax revealed an area of opacity in the middle lung fields just cranial to the cardiac silhouette on the lateral view. On the dorsoventral view (**Figure 1A**), increased density was identified in the caudal part of the left caudal lung lobe. A second therapeutic trial consisting of enrofloxacin^e (5 mg/kg PO q 24 hr) combined

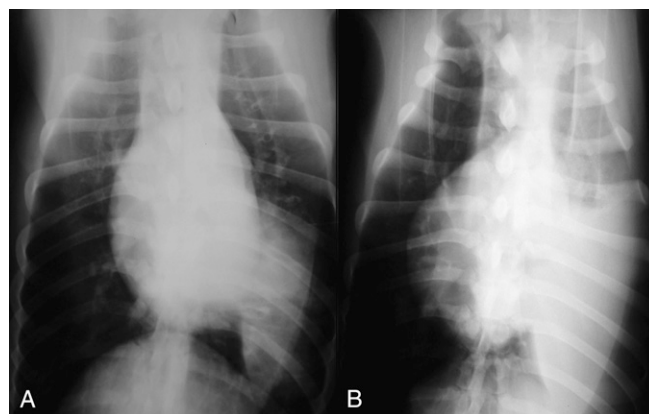


FIGURE 1 A: Dorsoventral radiograph of the thorax 2 mo before admission showing the increased density in the left caudal lung lobe. B: Dorsoventral thoracic radiograph taken at the time of referral showing the increased radiopacity of the entire left lung.

with amoxicillin trihydrate/clavulanate potassium^f (22 mg/kg PO q 12 hr) was initiated because a lobar bacterial bronchopneumonia due to a grass awn was suspected. Further diagnostics were not pursued at that time because equipment for bronchoscopic removal of the awn was not available.

Forty-five days later, the cough occurred even at rest, the dog's body weight had decreased from 46 kg to 38 kg, and the dog was febrile with body temperatures ranging from 39.8°C to 40.1°C. The dog was therefore referred for further evaluation.

On referral, a physical examination revealed depression, pale mucous membranes, an elevated respiratory rate (80 breaths/min), and a mild inspiratory dyspnea that worsened when the dog was forced to stand or walk for even a few meters. A productive cough was elicited on tracheal palpation. Absence of lung sounds and muffled heart sounds were found during auscultation of the left hemithorax. Auscultation of the right hemithorax revealed no abnormalities. Heart rate was 108 beats/min with a sinus arrhythmia; no heart murmur was present.

A CBC showed moderate anemia (PCV=28.6%; reference range, 37–55%), decreased hemoglobin concentration (8.9 g/dL; reference range, 12–18 g/dL), and a mild elevation in leukocytes (20.4/ μ L; reference range, 5.5–16.9/ μ L). The differential white blood cell count showed a neutrophilia with a left shift. Serum biochemistry revealed hypoalbuminemia (1.7 g/dL; reference range, 2.9–4 g/dL) and the urinalysis was normal. Blood gas analysis could not be performed due to equipment failure.

Dorsoventral radiography of the thorax showed progression of the lesions as previously observed by the referring veterinarian. The increased radiopacity now involved the entire left lung but the right lung remained normal (**Figure 1B**).

A bronchoscopic examination revealed hyperemia of the tracheal mucosa with a small quantity of purulent and sanguineous exudate. The left main stem bronchus was narrowed by hyperplastic tissue (**Figure 2**) that exuded a thick, clotted, brown exudate. The bronchoscope could not pass beyond this lesion. Bronchoscopic exploration of the right lung did not reveal any abnormality. Bronchoalveolar lavage (BAL) was performed for cytologic evaluation and bacterial and fungal cultures. Cytologic evaluation of the BAL fluid demonstrated numerous neutrophils (72% of the total cell content) and eosinophils (28% of the total cell content). No microorganisms were identified. Cultures were positive for bacteria (*Bacillus* spp. and *Clostridium* spp.) but negative for fungi. Based on sensitivity tests, doxycycline^c (10 mg/kg PO q 12 hr) and enrofloxacin^e (10 mg/kg PO q 24 hr) were prescribed.

The day after bronchoscopy, the condition of the dog deteriorated rapidly. The oral mucosa was very pale and melena was detected. No petechiae or ecchymoses were found. The PCV was 8% and total serum protein was 4 g/dL (reference range, 6–8.2 g/dL). The plasma was clear and no spherocytes were observed on blood smear evaluation. Anemia secondary to blood loss from the left lung was suspected and 2 units of fresh whole blood were administered. Two days after bronchoscopy and 4 days after admission, a left lung pneumonectomy was performed and the dog made an uneventful postoperative recovery.

Gross inspection and dissection of the lung failed to reveal the presence of a foreign body. Tissue samples were fixed in 10% neutral buffered formalin and submitted for histopathological evaluation. Sections prepared from the lung samples were stained with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), and Grocott's stain. Microscopic examination revealed the presence of a severe granulomatous bronchopneumonia with formation of multiple discrete granulomas (**Figure 3A**). The granulomas consisted of an infiltrate of eosinophils, neutrophils, multinucleated giant cells, and palisading macrophages surrounding a central zone of necrosis. Structures staining positively with PAS and Grocott had the morphologic appearance of fungal hyphae [**Figures 3B and C**]. Vasculitis was also observed within the pulmonary interstitium. The precise identity of the fungi could not be confirmed histopathologically, so DNA was extracted from paraffin-embedded pulmonary tissue sections. PCR using pan-fungal primers (ITS1 and ITS4 which amplify ribosomal DNA regions ITS1 and ITS2) was performed in the School of Translational Medicine at the University of Manchester. The resulting PCR product was gel purified^g and sequenced on an ABI 3730 Genetic Analyzer^h with BigDye Terminator Ready Reaction Mix version 1.1^h in both directions. Sequences were aligned and



FIGURE 2 Endoscopic view of the left main stem bronchus showing hyperplastic tissue narrowing the lumen.

found to be identical. The resulting DNA sequence was compared with both the National Center for Biotechnology Information GenBank and local databases using a basic local alignment search tool (BLAST) search. This methodology identified the organism as *A. fumigatus*.

Treatment with itraconazoleⁱ was initiated immediately to prevent spread of the fungal infection to the right lung. Itraconazole was administered at a loading dose of 5 mg/kg PO q 12 hr for 10 days followed by 5 mg/kg PO q 24 hr for an additional 5 mo. Doxycycline^c and enrofloxacin^e were continued for 2 mo at the same doses described above.

Follow-up radiographs of the thorax at 2, 7, 12, and 22 mo after diagnosing the mycotic bronchopneumonia revealed no evidence of involvement of the right lung. Twenty-six months after admission, the dog was clinically normal.

Discussion

Mycotic bronchopneumonia is usually caused by primary pathogenic fungal organisms (e.g., *Blastomyces dermatitidis*, *Cryptococcus neoformans*, *Histoplasma capsulatum*) and only rarely by opportunistic pathogens such as *Aspergillus* spp., *Sporothrix schenckii*, or *Candida* spp.⁴ Although inhalation of *Aspergillus* spp. spores by a susceptible dog may be accompanied by dissemination of the disease to other body sites such as kidneys, vertebrae, long bones, liver, and spleen, the present report describes a dog with fungal bronchopneumonia caused by *A. fumigatus* in which there was no laboratory or clinical evidence of dissemination.^{12–14} This is an uncommon clinical presentation of infection with *A. fumigatus* as only five other published cases of canine bronchopulmonary

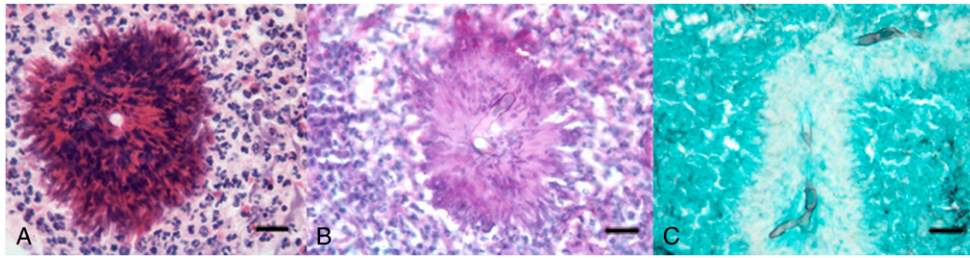


FIGURE 3 A: Histopathologic examination of pulmonary tissues revealed a discrete fungal granuloma within the lung. A central eosinophilic zone of necrosis is surrounded by neutrophils and macrophages (hematoxylin and eosin, bar represents 50 μ m). B: The same granuloma stained with period acid-Schiff revealed the presence of a hyphal element within the central necrotic zone (bar represents 50 μ m). C: Grocott staining of a necrotic focus elsewhere in the lung revealed the presence of branching septate fungal hyphae consistent with the morphology of *Aspergillus* spp. (bar represents 50 μ m).

aspergillosis exist.^{5,6,10,15,16} The species of *Aspergillus* was identified in only two of those cases and were *A. flavus* and *A. niger*.^{15,16} Therefore, this appears to be the first report of canine bronchopneumonia caused by *A. fumigatus*.

It is generally assumed that an underlying local or generalized immunodeficiency must exist in order for opportunist fungal pathogens to infect a host.^{2,4} In addition, the number of spores inhaled and the virulence of the organism may play an important role in the development and clinical manifestation of fungal disease.² Particles of plant origin (e.g., grass awns) have been incriminated as the primary factor that may lead to a breakdown in the normal pulmonary barrier and the subsequent development of local immunosuppression. Once a grass awn is inhaled, it attaches to the mucous membrane and migrates through the lung parenchyma, spurred by respiratory movements. During this migration, clinical signs are mild and are not typically noticed by the owner. If the foreign body penetrates the bronchial tree, a bronchopulmonary abscess may form within 2 wk of inhaling the awn.¹⁷ The dog reported here was not a breed frequently reported to inhale grass awns (e.g., bird hunting dogs); however, other factors (e.g., season, climate, vegetation in the dog's environment) led to a high index of suspicion for grass awn lobar bronchopneumonia despite the fact that no plant material was grossly identified within the lung.¹⁷ Plant material often disintegrates in exudates and may be sporadically identified in necrotic tissue, but there was no microscopic evidence of such material in the lung of the present case.¹⁸ The isolation of bacteria from BAL fluid could have been connected to inhalation of plant material because the foreign body serves as a vehicle for bacterial and fungal agents.¹⁸

Immunosuppression or immunodeficiency are both associated with deep and superficial mycosis in humans and animals.¹ It has been suggested that German shepherd dogs may have an

underlying genetically mediated immunodeficiency that may explain their high susceptibility to aspergillosis. Immunoglobulin A deficiency is often proposed to be an underlying factor; however, definitive evidence for simple deficiency of this immunoglobulin in German shepherd dogs is lacking.¹³ The dog reported herein was a Hellenic shepherd, which may be genetically distant to the German shepherd dog. In humans, local pulmonary defects combined with subtle innate immunity defects in substances such as mannose binding lectin or surfactant, toll-like receptor mutations, and cytokine imbalance, may be responsible for *A. fumigatus* infections in nonimmunocompromised individuals.^{19–21}

Cytologic examination and culture of BAL fluid has a relatively low sensitivity for the diagnosis of fungal disease. A study carried out by Hawkins and DeNicola (1990) revealed that examination of BAL fluid led to a diagnosis of fungal disease in only two of the nine dogs with pulmonary involvement.²² The low sensitivity of BAL fluid cytology for the detection of large *Aspergillus* spp. hyphae necessitates the use of other tests such as culture and/or histopathology of bronchoscopically or surgically obtained tissues. The culture of *Aspergillus* spp. from clinical specimens is not straightforward and may require optimum incubation conditions. One recent study reported that culture of biopsy samples at 37°C increased the sensitivity of fungal cultures.²³ Positive bacterial culture in the present case was probably the result of simultaneous infection or secondary invasion after primary lung injury by the fungal elements, but the bacteria may have had synergistic activity in injuring the lung.¹⁸ On the other hand, bacteria may have been the primary pathogens that caused local barrier breakdown allowing the growth of the fungi and penetration of the lung parenchyma by *A. fumigatus*. Recent advances in real-time PCR may allow for the direct diagnosis on BAL fluid samples.²⁴

Tissue biopsy is crucial for establishing the diagnosis of deep mycosis; however, in severely necrotic tissues, it cannot always be

established whether the fungus is the primary cause of necrosis or if the fungal infection is secondary.⁵ In the present case, the histopathologic findings were compatible with mycotic bronchopneumonia and similar to those previously described, but the exact species of organism involved was not identified morphologically.^{2,5,14,15} Instead, in the case described herein, species identification was achieved via PCR by amplifying ITS regions 1 and 2 from DNA extracted from paraffin-embedded pulmonary tissue sections obtained from lung tissues postpulmonectomy. This PCR methodology can distinguish between the various clinically relevant *Aspergillus* spp.⁸ It is often challenging to extract amplifiable DNA from paraffin-embedded samples because of DNA degradation, but the use of relatively fresh samples, as used in this case, may be more amenable to successful DNA amplification.

Successful treatment of mycotic infections is difficult and dependant upon selection of an appropriate antifungal drug as well as the cooperation and financial support of the owner. Selection of an antifungal agent should be based on the results of in vitro susceptibility testing and on the pharmacokinetics of the drug.¹ Such tests were not feasible in this case and the selection of itraconazole was based on its reported efficacy in canine aspergillosis.^{1,4} Management of mycosis is always challenging because an infectious agent that is phylogenetically closer to the host than most bacteria and viruses must be eliminated without excessive risk for the host. At present, the optimum duration of antifungal treatment is unknown and long-term or even life-long administration has been suggested to prevent relapse of infection.^{1,5,10,14,15} In the present case, antifungal chemotherapy was discontinued after 5.5 mo as there was no evidence of infection in the right lung.

Removal of the left lung in this case was deemed necessary due to the severe lung injury, the sudden deterioration of the dog's condition after bronchoscopy, and the possibility of dissemination of the disease to the right lung.²⁵ The sudden deterioration of the dog's condition after bronchoscopy was attributed to acute blood loss caused by this procedure in a severely injured lung. Severe anemia could have been associated with hemolysis or coagulopathy secondary to *Aspergillus* spp. fungal bronchopneumonia, but these causes were excluded on the basis of incompatible clinical and laboratory findings. Excision of the left lung may have contributed to the elimination of the disease and survival of the dog for over 2 yr after diagnosis. Although the involvement of an *Aspergillus* spp. was not known at the time of the surgery, this adjunctive treatment would have been selected because of the need to remove infected tissue.¹ Pulmonary lobectomy for the management of pneumonia in dogs, regardless of the causative agent, is not commonly performed.²⁵

Conclusion

Canine bronchopulmonary aspergillosis is a relatively rare disorder and its diagnosis can be challenging. Aspergillosis should be suspected when there is no response to long-term antibiotic administration even in the absence of a positive BAL fluid fungal culture. Histopathology contributes to the diagnosis of mycotic bronchopneumonia and verification and identification of the *Aspergillus* spp. can be achieved by PCR and sequencing of ITS regions 1 and 2 from paraffin-embedded tissue. Excision of the affected lung and itraconazole administration appears to be effective in the management of bronchopulmonary aspergillosis in dogs. ■

FOOTNOTES

- ^a Drontal; Bayer AG, Leverkusen, Germany
- ^b Frontline; Merial, Lyon, France
- ^c Ronaxan; Merial, Lyon, France
- ^d SnapCanine Heartworm PF; IDEXX, Westbrook, ME
- ^e Enrofloxacin (Baytril); Bayer AG, Leverkusen, Germany
- ^f Amoxicillin trihydrate/clavulanate potassium (Synulox); Pfizer Inc., New York, NY
- ^g Qiaex II Gel Extraction kit; Qiagen Ltd., West Sussex, UK
- ^h ABI 3730 Genetic Analyzer; Applied Biosystems, Carlsbad, CA
- ⁱ BigDye Terminator Ready Reaction Mix version 1.1; Janssen Pharmaceutica N.V., Beerse, Belgium

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