NOTE Internal Medicine

Aspergillus niger Pulmonary Infection in a Dog

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ABSTRACT. A 6-month-old male golden retriever was presented with fever, bloody-watery diarrhea and mild cough. Parvovirus and Isospora canis infection was confirmed in a serum examination were detected through a fecal floating test. In addition, parvoviral infection was confirmed and successfully treated. Two weeks later, the dog had severe cough and mucopurulent nasal discharge. Aspergillus niger was cultured from endotracheal washings on blood agar at 37°C. Treatment with itraconazole for about 10 weeks resolved the clinical signs.

KEY WORDS: Aspergillus niger infection, canine, pulmonary infection.

A 6-month-old, 10.5 kg, intact male golden retriever was presented at the Seoul National University Veterinary Medical Teaching Hospital, Seoul, Korea for the evaluation of fever, diarrhea and mild cough of 5-day duration. Hematological abnormalities included a moderate anemia (PCV 28% : reference range, 38–55%) and increased WBC (19,000/μl: reference range, 8,000–17,000/μl). Serum biochemical abnormality included mild hypoalbuminemia (2.5 g/dl: reference range, 3.1–4.5 g/dl). Interlobal fissure between the right mid and caudal lung lobes, and mild and diffuse interstitial lung pattern were observed on radiographs. Isospora canis oocysts and Toxocara canis eggs were detected through a fecal floating test. In addition, parvoviral infection was confirmed in a serum examination with a parvoviral antigen detection kit (IDEXX®, U.S.A.).

The dog patient was treated with intravenous injection of lactated Ringer’s solution, cephradine (15 mg/kg body weight, PO q 12 hr), ranitidine (2 mg/kg body weight, IV q 8 hr), trimethoprim-sulfa (15 mg/kg body weight, IV q 8 hr), Drontal-L® (febantel 150 mg, pyrantel pamoate 2T/day). After treatment, the dog recovered a good appetite and activity with normal defecation, but still had a wet cough and mucopurulent nasal discharge. The dog was presented again for a recheck 2 weeks after it was discharged from hospital. The owner reported that the dog had a good appetite and activity, but coughing continued with worsened nasal discharge. Microscopic examination of the nasal discharge detected no bacteria or fungal cells. PCV was 35% and WBC was 29,000/μl. Alveolar-interstitial lung patterns and air-bronchograms were observed in cranial and caudal lung lobes on the right and left sides, and the diameter of the peripheral bronchi was increased in the lateral view of the radiograph (Fig. 1). Endotracheal washing was carried out to obtain a sample for microbiological examination and for the antibiotics susceptibility test as well as for cytology. Fungal colonies which developed on the blood agar at 37°C in 6 days, were subs cultured on Sabouraud’s dextrose agar at 24°C.

The colony of the clinical isolate from the specimen of endotracheal washings was black-colored after 1 week incubation on Sabouraud’s dextrose agar at 27°C. Microscopic examination of the clinical isolate revealed conidiophores thick-and smooth-walled, reaching about 300 to 500 μm in length. The vesicle was globose, about 60 μm in diameter, and produced brownish metula on its entire surface. The metula developed in doubled series and produced black and globose conidia (4 μm in diameter).

DNAs from the colony on Sabouraud’s dextrose agar were prepared by the method reported previously [6]. The genomic DNA (100 ng) samples were amplified by PCR in a reaction mixture (30 μl) containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 0.001% gelatin, 2.5 mM each of deoxynucleoside triphosphates, 1.0 unit of Taq polymerase and 0.5 μl of a pair of primers. The sequences of the primers for the 25S ribosomal DNA were constracted based on the sequences reported [4, 6]. The sequence of the forward primer S-1 was 5’-GCA TAT CAA TAA GCG GAG GAA CAG 3’ and that of the reverse primer S-2 was 5’-CCT GGT CCG TGT TTC AAG ACG 3’. The PCR amplification was carried out for 35 cycles consisting of template denaturation (1 min, at 94°C), primer annealing (2 min, at 55°C) and polymerization (3 min, at 72°C). The PCR products from the sample was sequenced by the dyeodeoxy chain termination method (Big dye Terminator Cycle Sequencing Ready Reaction Kit; Applied Biosystems, Foster City, CA, U.S.A.) with an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, U.S.A.). Amplification of the sample DNA with 25S ribosomal DNA primers yielded fragments of about 600-bp, consistent with the sizes of 25S ribosomal DNA sequences from fungal species previously reported [4]. Homology relationships among the 25S ribosomal DNA of reference strains of Aspergillus spe-
cies and that of the clinical isolate were examined by FASTA data base analysis in DNA data bank of Japan (DDBJ). The homology analysis of nucleotide sequences of 25S ribosomal DNA between the clinical isolate and a reference strain of *A. niger* (DDBJ accession no. U65306) showed 98%, indicating that they were classified in the same species since their sequence similarity was more than 98% [1].

The clinical isolate was identified as *Aspergillus niger* by analyzing their DNA sequences as well as by mycological examination. Therefore, the dog patient was treated with itraconazole (5.0 mg/kg body weight, PO 12 q hr). After 3 weeks, it had recovered good activity and appetite. The owner reported that coughing decreased and that the nasal discharge had nearly disappeared. Hematology revealed that WBC (17,500/µl) and PCV (38%) were recovering. Radiographs showed a mixed lung pattern on the whole lung field, and mild infiltration on the cranial portion of the left cranial lung lobe, but on the whole, the lung field was less infiltrated than before the administration of itraconazole. After additional 6 weeks of medication, the treatment was discontinued since the clinical signs were completely resolved.

*A. fumigatus* has been isolated in most cases of canine disseminated aspergillosis [5], but *A. niger* has been rarely isolated [5]. The portal of entry of *A. fumigatus* is thought to be via the respiratory tract with subsequent hematogenous spread [5]. In this case first infection was also in the respiratory tract. In human pulmonary aspergillosis, *A. niger* has sometimes been isolated producing calcium oxalate crystals in the lung tissues [8]. Factors predisposing to canine aspergillosis such as immunodeficiency and immune suppression [5], were not detected in this case. The examination of bronchoalveolar washing helped in differential diagnosis of pulmonary aspergillosis from bacterial and viral pneumonia which resembled to this infection in clinical signs.

Amphotericine B and itraconazole have been used in canine disseminated aspergillosis with the unfavorable prognosis [2, 5, 7]. Liposomal amphotericine B as well as intravenous itraconazole and voriconazole have recently been shown to be highly effective in treating invasive aspergillosis in human patients [3], and are promising for use in veterinary fields.

The results suggested that the molecular analysis presented in this study could be helpful for the rapid identification of *A. niger* within 2 days, since conventional mycological methods require about 1 to 2 weeks.

REFERENCE


