Case Reports

Disseminated pseudallescheriosis in a dog

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A case of disseminated pseudallescheriosis in a German Shepherd bitch is presented. Bones (ilium, a rib and phalanges), joints (elbow and acetabulum) and the surrounding tissues were the principal organs affected. In addition, Pseudallescheria boydii was isolated, in lower numbers, from the eye, kidney, lymph nodes draining the affected regions and urine. The dog was euthanized. P. boydii was identified by morphologic characteristics and molecular techniques (beta tubulin sequence). In addition, an ITS nucleotide sequence analysis showed that this strain differed from another isolate identified as Scedosporium apiospermum that had caused a disseminated infection in another German Shepherd. The importance of the molecular characterization of fungi belonging to the Pseudallescheria/Scedosporium complex, isolated from animals is stressed in light of the ongoing attempts to recharacterize these fungi.

Keywords canine, Pseudallescheria boydii, disseminated, molecular identification

Introduction

Fungi belonging to the Pseudallescheria/Scedosporium complex (PSC) are important emerging human pathogens that have been recently reviewed [1,2] and have had an entire issue of Medical Mycology dedicated to them (volume 47, issue 4, 2009). In the past, Pseudallescheria boydii was defined as the teleomorph of Scedosporium apiospermum. Recently, however, the taxonomy of the PSC underwent significant modifications resulting in the addition of new species and the definition of a new anamorph for P. boydii, i.e., Scedosporium boydii [3] and a new teleomorph for S. apiospermum, i.e., Pseudallescheria apiosperma [4].

Several cases of eumycetoma [5–9], keratomycosis [10] or disseminated infections [11,12] in dogs have been reported to have been caused by the fungi identified morphologically as P. boydii or S. apiospermum. The etiologic agent in one such recent case was identified by molecular methods [13].

Interestingly, while mycetoma cases have been observed in various breeds, often occurring after trauma such as operations or especially in cases involving hysterectomies which were complicated by dehiscence of the sutures, disseminated infections caused by the fungus have been reported only in German Shepherd dogs. Many of the latter, such as the present case, had no apparent portal of entry or predisposing factors. This seems to be in concordance with this breed’s predisposition to disseminated fungal infections, primarily those caused by Aspergillus terreus.

Case report

In mid January 2009, a 2-year-old German Shepherd bitch presented with a three week old limp on the left forefoot and swelling of the elbow and footpad of the leg. Antibiotic therapy was initiated but had no effect. A week later, swelling in the left iliac area and corresponding limping were observed. Radiography of the three sites indicated extensive destruction of bones (Fig. 1a).

Ten days after the first presentation, results of a biopsy from the footpad indicated the presence of fungal hyphae and samples for mycological examination were taken from the elbow and footpad. Itraconazole therapy (30 mg/kg/day) was initiated. The fungus was identified (see below)
as *Sceuporium* spp. and later, after it had developed sexual reproductive structures, as *Pseudallescheria boydii*. At the beginning of February, after the tumefactions increased and the left eye became cloudy, the bitch was euthanized. Throughout this period her temperature was 40–40.5°C and no significant hematological or clinical-pathological abnormalities were observed.

At the post-mortem examination, large irregular necrotic areas of muscular tissue mixed with bone debris due to destruction of the left ilium bone were found (Fig. 1b). The left side of the ilium bone was destroyed. In the region of the left forefoot elbow, there was inflammation of the subcutis and extensive destruction of the joint that was filled with necrotic-hemorrhagic material. On the fifth right rib, two tumefactions were noted, one of which caused a fracture in the rib (Fig. 2a). The prescapular lymph node was enlarged. White nodules measuring between 2 and 5 mm were seen on the spleen and kidneys (Fig. 2b). Mediastinal and pancreatic lymph nodes were enlarged. The left eye was cloudy.

**Fig. 1** Iliac region. (a) Radiography – note extensive bone destruction. (b) Necrotic purulent material around the Ilium.

**Fig. 2** Foci of fungal infection. (a) Rib (arrows indicate lesions). (b) Kidney.
incubation in cultures inoculated with samples from the rib lesions, elbow joint, elbow subcutis, prescapular lymph node, ilium, iliacus muscle abscess, iliac lymph node, kidney, urine and eye but not from the spleen and pancreatic lymph node. Semiquantitative data on the growth on the two media is presented in Table 1.

After a week, the colonies became mouse-gray in the center and long tapering conidiophores bearing a single tear shaped conidium could be observed through microscopic studies. Annelids were present at the tip of the conidiophores and synnemata could be seen. After two weeks, the colonies darkened and cleistothecia, containing round ascii with 8 ascospores were produced. The cleistothecia measured between 100 μm and 175 μm, typical of P. boydii, but smaller than the ones observed for the teleomorph of S. apiospermum [4].

For molecular identification, fungal tissue (300 mg) was ground under liquid nitrogen using a mortar and pestle. DNA extraction was than done with DNAeasy plant kit (Qiagen, USA) according to the manufacturer instruction.

Beta tubulin exons 5 and 6 were amplified by PCR using primers 5’ Tub 2 F CTGTCCAACCTCCTTACGC- GGCACCTGACC and primer 3’ Tub 2 R ACCCTCAGC- CAGTATACCAATGCAAAGAAC [14] (Hy Laboratories, Rehovoth, Israel). PCR was performed as previously described [15]. Briefly, the amplification reaction included 100 ng of each primer in a 25 μl solution containing 1 μl template DNA, 300 mM Tris-HCl pH = 8.5, 75 mM (NH₄)₂SO₄, 10 mM MgCl₂, 100 μM Dxtp and 5 units of taq DNA polymerase (AmpliTaq, Applied Biosystems, USA). The PCR products were amplified in a PTC 200 Peltier Thermal Cycler (MJ Research, USA). The reaction consisted of a DNA denaturation step at 95°C for 10 min followed by 30 cycles at 94°C for 30 sec, 50°C for 30 sec and 72°C for 30 sec and a final extension step of 10 min at 72°C. The outcome, visualized on a 1.5% agarose gel after ethidium bromide staining, resulted in a 604 bp fragment.

For the beta tubulin nucleotide sequence analysis, the fragment was purified (GenElute agarose spin columns, Sigma, USA) and sequenced using the two above-mentioned primers in an Automatic Sequencer 3700 DNA analyzer (Applied Biosystems, USA) according to the manufacturer’s instructions. A BLAST search (www.ncbi.nlm.nih.gov/blast/blast.cgi) of the GENBANK and EMBL data bases (accession no. GQ483367) revealed 100% homology between a 506 bp sequence of beta tubulin exons 5 and 6 of the present P. boydii isolate and sequences from the GenBank (accession nos. AJ88997, AJ889993, AJ89980, AJ899997, AM261067, AM261066, AM261065, AM261064, AM261063, AM261062, AM261041) [3,16].

To compare the present isolate with the one reported to have caused a disseminated infection in a German Shepherd [13], the spacer ITS1-5.8S-ITS2 region fragment of...
the rRNA was amplified by PCR using fungal universal primers [17] (Hy Laboratories, Rehovoth, Israel). PCR studies were performed as described above. For the ITS nucleotide sequence analysis, the product was purified (GenElute agarose spin columns, Sigma, USA) and sequenced using ITS4 and ITS5. ITS2 and ITS3 primers [15] resulting in a sequence of 640 bp, out of which a sequence of 526 bp (accession number GQ889500) was compared to that of strain CBS 101723, found to be equivalent to the corresponding sequences of the isolate of Hugnet et al. [13]. Differences in almost 5% (24/526 bp) of the compared sequences were found.

Discussion

Risk factor(s) predisposing to the infection described in this case remain obscure. The bitch underwent no surgical interventions, did not receive hormonal treatments and was not exposed to contaminated water. Although no specific tests were conducted, the fact that the animal suffered from no additional infections seems to indicate that she had no general immunological problems. This does not differ from what is known concerning dogs suffering from disseminated aspergillosis. In addition, no obvious lesions that could act as the portal of entry for the fungus could be found at presentation or, according to the owners, preceding it. However, the fact that the lesions in the pelvic region were significantly more substantial than those observed in other organs and joints, may be an indication that the portal of entry was located in that region.

The prevalence of human infections caused by fungi belonging to the PSC has increased significantly during recent years, making them one of the most important emerging fungal infections. Their prevalence in animals seems to be much lower, although this may be partially the result of their underdiagnosis. In fact, the etiologic agent in this case was initially diagnosed as disseminated aspergillosis based on a biopsy submitted to a laboratory not specialized in mycology. The taxonomy of Pseudallescheria and Scedosporium is currently under molecular scrutiny resulting in a state of uncertainty pending the final classification of the fungi belonging to these genera and their relationships. Consequently, gathering molecular information is of great importance, especially if it originates from strains isolated less frequently such as those causing infections in animals. Among animal isolates, molecular information has been published, excluding this case, only once [13]. The molecular differences between that isolate, identified as *S. apiospermum*, and the one isolated from the present case indicate that, similar to human infections, more then one species belonging to the PSC may be involved in animal mycoses.

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References