Systemic *Conidiobolus Incongruus* Infection and Hypertrophic Osteopathy in a White-Tailed Deer (*Odocoileus Virginianus*)

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What is This?
Systemic *Conidiobolus incongruus* infection and hypertrophic osteopathy in a white-tailed deer (*Odocoileus virginianus*)

Darin M. Madson, Alan T. Loynachan, Subhashinie Kariyawasam, Tanja Opriessnig

**Abstract.** Postmortem examination of a free-range white-tailed deer (*Odocoileus virginianus*) revealed severe emaciation, bilateral firm proliferation of the metatarsal diaphyses, and a large intrathoracic mass associated with the accessory lung lobe. Smaller masses were evident in the abomasum, duodenum, omentum, and the capsular surface of the liver. Microscopically, the masses were similar and were diagnosed as eosinophilic granulomas with intraleisonal fungal hyphae characteristic of *Zygomycetes* spp. Fungal hyphae were identified as *Conidiobolus incongruus* by 18S ribosomal RNA sequencing on fresh lung tissue. Furthermore, the proliferative lesions of the metatarsal bones along with the intrathoracic mass were compatible with hypertrophic osteopathy.

**Key words:** Cervidae; *Conidiobolus incongruus*; hypertrophic osteopathy; *Odocoileus virginianus*; white-tailed deer; zygomycosis.

*Conidiobolus* species are opportunistic fungal pathogens classified in the order *Entomophthorales* of the class *Zygomycetes*. This genus is distributed worldwide and is found in soil and decaying organic material. Three species, *Conidiobolus coronatus*, *Conidiobolus lamprauges*, and *Conidiobolus incongruus* are capable of causing illness and death in humans and animals. In wild or domestic animals, *C. incongruus* has previously been reported to infect sheep and red deer in Australia. In both cases, the fungus was associated with nasal swelling, rhinocerebral mycosis, ophthalmitis, and disseminated disease.

An approximately 2.5-year-old, male, free-range white-tailed deer was observed in Ledges State Park (Boone County, IA) to be in poor body condition and debilitated with swollen hind legs. Upon physical examination, the deer had a dull hair coat, bilaterally sunken eyes, and diffuse firm proliferations along the entire metatarsal diaphyses of both hind legs. Necropsy revealed marked emaciation with scant retroperitoneal fat and a 25 cm ovoid intrathoracic mass associated with the accessory lung lobe. Smaller masses were evident in the abomasum, duodenum, omentum, and the capsular surface near the abomasum. In addition, the abomasal pylorus contained a 4-cm raised, mural, circular, raised mass was also present on the antimesenteric side in the duodenum, approximately 4 cm from the abomasal pylorus. Furthermore, there were two 0.5-cm tan nodules in the liver.

Appropriate tissue sections were placed in 10% phosphate-buffered formalin, fixed overnight, processed, and embedded in paraffin wax. Tissue blocks were sectioned at 4 μm, mounted on glass slides, and stained with hematoxylin and eosin for routine microscopic examination. Pulmonary and abomasal masses were aerobically and anaerobically cultured for bacteria and fungi; additional fresh tissue was stored at −80°C.

Microscopically, the accessory lung lobe was greatly expanded by abundant well-vascularized fibrous connective tissue that replaced normal parenchyma. Intermixed and walled-off from the normal lung parenchyma were multifocal to coalescing irregular inflammatory nodules containing numerous macrophages, eosinophils, nondegenerative to degenerative neutrophils, fewer lymphocytes, and multinucleated giant cells that were frequently centered on flame figures and karyorrhectic cellular debris. Deeply basophilic to nonstaining fungal hyphae were rarely visible. The hyphae were 10–15 μm in diameter, had nonparallel walls, lacked septa, contained occasional 30-μm bulbous dilations of the wall, and exhibited infrequent nondichotomous branching. Rare 30–40-μm sporangia were also present. Similar fungal hyphae and inflammatory cells were present in the abomasal and duodenal lesions. Additional findings included multifocal ulcerations of the mucosa, transmural inflammation, and necrotic adipocytes adjacent to the serosa within the associated mesentery. Fungal hyphae were also occasionally visible within areas of collagenolysis and necrotic debris. Periodic acid–Schiff (PAS) and Gomori methenamine silver (GMS) stains of pulmonary, abomasal, and duodenal lesions revealed numerous fungal hyphae with characteristics as described above (Fig. 3).

The small discrete tan liver nodules were focal granulomas associated with the capsular surface. The granulomas were delineated from the hepatic parenchyma by small amounts...
Figure 1. Lung, white-tailed deer. Thoracic mass (25 cm × 13 cm × 15 cm) associated with the accessory lung lobe. The mass contains multiple 1–4-cm cystic structures and is firmly adhered to the major vessels leaving the heart, pericardium, adjacent lung lobes, and diaphragm.

Figure 2. Lung, white-tailed deer. The cut surface of the thoracic mass is pale green with interspersed regions that are gritty and light brown with multiple cavernous areas filled with yellow to brown caseous material.

Figure 3. Lung, white-tailed deer. Fungal hyphae are 10–15 μm in diameter, have nonparallel walls, lacked septa, contain occasional approximately 30-μm bulbous dilations of the wall, and exhibit infrequent nondichotomous branching. Periodic acid–Schiff stain. Bar = 50 μm.

Figure 4. Left metatarsal, white-tailed deer. Lateral radiograph. The metatarsal periosteum is diffusely thickened by radiodense perpendicular woven bone.
of fibrous connective tissue and contained eosinophils, flame figures, and fungal hyphae similar to the other lesions. In addition, fungal hyphae and granulomatous inflammation were present at the base of the aorta within the tunica adventitia and tunic media. Bilaterally, metatarsal diaphyses were thickened by woven and trabecular bone that was orientated perpendicular to cortical bone and contiguous with the periosteum. The periosteum was further expanded by mature fibrous connective tissue that extended into and between the new woven and trabecular bone.

No aerobic or anaerobic bacteria or fungal organisms were isolated by routine culture methods, and fresh sections of the associated lung mass were processed for fungal DNA amplification by polymerase chain reaction (PCR). Briefly, fungal genomic DNA was extracted using a commercially available extraction kit according to the manufacturer’s instructions. Primers 18SF (5'-ATTGGAGGGCCAAGTCTGTTG) and 18SR (5'-CCGATCCCTAGTCGGCATAG), binding to highly conserved regions of the fungal 18S ribosomal RNA (rRNA) gene, were used for PCR amplification. The PCR reaction contained 5 μl genomic DNA extract, 5 μl 10 × PCR buffer II, 3 μl MgCl2 (25 mmol/l), 1 μl deoxynucleotide triphosphates (10 mmol/l), 2.5 U AmpliTaq Gold DNA polymerase, 50 pmol of each primer, and sterile double-distilled water for a final volume of 50 μl. The primers amplify a 486 base pair (bp) region of the 18S rRNA gene. The amplification was performed as described, and the PCR product was sequenced twice using BigDye terminator chemistry. The same primer set (18SF and 18SR) used for PCR amplification was used for sequencing. The sequence was then compared with known 18S rRNA gene sequences in GenBank using BLAST (http://www.ncbi.nlm.nih.gov/blast/BlasT.cgi) to confirm the fungal species.

Double-strand sequencing of the 486-bp PCR amplicon confirmed the presence of Conidiobolus spp. in the lung sample. The sequences obtained showed 99% homology to 18S rRNA genes of C. incongruus (GenBank accession nos. EF392543, AF113417, AF113418, AF296753, D29947) and C. firmipilleus (GenBank accession no. AF368507).

To the authors’ knowledge, this is the first report of a domestic or wild animal in North America in which C. incongruus was associated with disease. Previous reports of C. incongruus affecting animals were limited to Australia, although, C. incongruus has been reported as the cause of disease in an immunocompromised human patient in North America. Alternatively, C. coronatus and C. lamprauges have been reported and associated with disease in domestic animals in North America. Similar to C. incongruus, both C. coronatus and C. lamprauges cause respiratory disease and dermal lesions.

In the present case, the gross and microscopic lesions were similar to previous reports. In both sheep with nasal granulomas and in the red deer with pulmonary lesions, the cut surface had a green tinge with areas of necrosis. Microscopically, granulomatous inflammation with neutrophils, eosinophils, and fibroplasia predominated within the lesions; few fungal hyphae were visible. The present case differed from previous reports by the observation of collagenolysis and the absence of a Splendore-Hoeppli response. Conidiobolus spp. are known to produce collage

Sources and manufacturers

a. Soil Master™ DNA Extraction Kit, EPICENTRE Biotechnologies, Madison, WI.
References


